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SUMMARY

SOIL SCIENCES

CROP SCIENCES

MISCELLANEOUS

SOIL
SCIENCES

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EVALUATION OF SOIL POLLUTION DEGREE IN THE COPŞA MICĂ AREA (ROMANIA) BY MEANS OF RELATIVE INDICES

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Abstract

Regardless the source, soil pollution is a daily reality resulting from anthropising the environment, especially through industrial explosion, increasing consumption and excessive urbanization. Lead, cadmium and other heavy metals provided by human activities accumulate in the soil by bioaccumulation at concentrations that become dangerously high for the biota living in or on the soil. While getting in permanent contact for a long time with the soil, the inhabitants of the polluted areas are exposed daily to the risk entailed by the toxicity of dangerous concentrations of Pb and Cd. The assessment of the pollution degree with heavy metals of the soil can be accomplished by comparing experimental values with the maximum permissible levels regulated by the regional, national or international legislation or by using relative indices such as the Global pollution index Z. Loading with Pb and Cd of some adjacent areas to the point sources of pollution is conditioned by remoteness from the source, the local orography of the land that dictates the concentration, orientation or, as the case may be, the spreading of air mass polluted with the heavy metal component.

Key words*: heavy metals, historically polluted area, relative indices, geogenic abundance, Copsa Mica area.*

INTRODUCTION

Soil pollution

The discovery and processing of metallic minerals as well as the dispersion in the environment of the waste triggered the pollution and loading over the maximum permissible levels with potentially toxic heavy metals of the soil-component of the terrestrial biotopes that has supported the emergence and development of the human civilization.

What was initially a sporadic phenomenon i.e. the mining-transformed in time, favoured by consequences of the industrial revolution, into a global scale process due to the technological explosion, excessive urbanization and the consumerism the 20th Century and the beginning of the 21st Century (Swartjes, 2011). According to the European Environmental Agency (EEA, 2007) most of the polluted sites are located within or adjacent to the cities.

Soil pollution is a complex phenomenon, the soil being in the position of a polluted system when loaded with polluting substances, or being a polluting agent when it supplies soil particles charged with pollutants or even a depollution system due to its capacity to degrade biodegradable pollutants (Sabău, 2009). The sources of soil pollution can be natural, due to paedogenesis and anthropogenic processes (Galiulin, 2002), generated by human activities. Sabău (2009), Mousavi et al. (2013) presents the causes of the anthropogenic pollution of soils, the main role in this process belonging to the non-ferrous metals processing and mining (including tailings and other residues), the sludge resulting from waste water treatment, irrigation with contaminated water, burning of fossil fuels, treatment of soil with fertilizers, pesticides, exhaust gas from road traffic and waste storage (Popa, 2005). Although it may have further uses, the slag (tailings) resulting from Pb and Zn melting is considered hazardous waste according to European Commission Decision of 18 December 2014.

The causes of heavy metal soil pollution are natural or anthropogenic.

Geogenic contamination sources load the soil in terms of background concentrations (baseline values or Reference Values), which levels are amended with anthropogenic contribution due to the long-distance transport of anthropogenic emissions. (U.S. EPA, 2002; McLaughlin, 2011).

Heavy metal contamination of soil

Metals are natural constituents of soils (Table 1). Chemical elements such as Fe, Mn, Zn, B, Cu, Mo are called micronutrients and are needed for plant growth. Bolan et al. (2008) show that metals accumulate and persist especially in the superficial layers of the soil.

Soil contamination due to emissions from smelters is generally higher near the point source and decreases exponentially with distance (Haugland et al., 2002).

Acid conditions can remove coal fly ash and bottom ash contained in traces of As, Pb, Cd, Cr, Mn, Cu, Zn, Ni, generating the soil source of pollution (Ahmed et al., 2010). In soils not polluted with metals, the concentration of Zn, Cu, Pb, Ni, Cd, Cr ranges between 0.0001-0.065%.

With the exception of Fe, all heavy metals with concentrations in soil above 0.1% become toxic to plants. Soils rich in Zn (0.1-10% Zn) also have high concentrations of Pb, and soils with a Cu concentration of 0.1 to 3.2%, also contain high amounts of Zn, Pb, Co, Ni, Cd (Bothe, 2011).

Table 1. Natural concentrations of Pb and Cd in soil (Franck and Tölgyessy, 1993)

Chemical element	Concentration in soil	
	Characteristic values in ppm	Concentration ranges
Cd	0.06	0.01-7
Pb	10	2-200

The U.S. Environmental Protection Agency (USEPA) encloses the chemical elements Al, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sb, on the major interest index in terms of bioavailability due to the toxic risk they entail to human health (John & Leventhal, 1995).

Soil contamination with Pb

Pb is found in small quantities in the earth's crust and it is a major pollutant of the soil (Gratão et al., 2005). According to Kabata-Pendias (2011) the concentration of Pb is 15 mg/kg, while according to Rudnick and Gao (2003) the ratio is 11 mg/kg. The average Pb concentration in soils according to Fiedler and Rössler (1988) is 15 mg/kg. The global coefficient of geochemical abundance of Pb in soil is 0.94 (Lăcătuşu and Ghelase, 1992). Anthropogenic pollution sources consist mainly by ore extraction and processing (Dunnivant and Welborn, 2019). Kabata-Pendias (2010) reported large amounts of Pb in the proximity of these sources of pollution. Kabata-Pendias & Pendias (2001), indicate very high concentrations of Pb in Europe in the proximity of areas adjacent to mines and industrial ore processing platforms e.g. 21.546 mg/kg (England), 13.000 mg/kg (USA), and 18.500 mg/kg (Greece). In Romania, concentrations of 1.083 mg/kg in Baia Mare, 2.248 mg/kg in Zlatna, 3.550 mg/kg in Copşa Mică were recorded by Răuţă et al. (1992). According to Kloke et al. (1984) the maximum permissible level for the total content of Pb in soil is 100 mg/kg. Moreover, accumulations of Pb in air due to road traffic are sources of soil contamination (Wang & Zhang, 2018).

Increased soil acidity increases Pb solubility but the mobilization is lower than the rate of accumulation in the rich layer in organic matter (Hough, 2010). According to McBride (1994) Pb is distributed and accumulates generally in the surface soil mainly due to adsorption of organic matter, and high acidity conditions can increase the mobility and bioavailability of this chemical element.

Soil contamination with Cd

Cadmium is one of the environmentally most toxic heavy metal present in ecosystems. Cd abundance in the earth's crust is 0.1 mg/kg according to Kabata-Pendias (2011). According to Lăcătuşu & Ghelase (1992), the global coefficient of geochemical abundance of Cd is 2.31, and the average Cd concentration in soil is 0.3 mg/kg (Fiedler & Rössler, 1988). The sources of soil pollution with Cd are the mining and the industry: non-ferrous metals, chemical, and especially phosphatic fertilizers with high Cd content, but also road traffic. Less than 10%

of ambient air Cd concentrations are stored locally, the rest being transported by atmospheric circulation over long distances. Around the decommissioned smelters, concentrations of 100 mg/kg Cd can be measured due to the retention of Cd released over time in the superficial layers of the soil (IPCS INCHEM, 1992).

The objective of this study is to assess the degree of contamination of the soils in the Copşa Mică area affected by historical pollution by a relative index-the Z index.

MATERIALS AND METHODS

The soil sampling was carried out from 15 November to 4 December, 2009, in compliance with soil sampling rules issued by ICPA Bucharest (Methods, Reports, Guidelines, 1981). Between 26 January and 31 March, 2009 the main polluter of the Copşa Mică area i.e. S.C. SOMETRA S.A. ceased its major industrial activity that has not been resumed so far. The soil sampling depths ranged between 0-5 cm, 10-15 cm, and 30-35 cm. For the undifferentiated assessment in depth of the soil variables, weighted average concentrations were calculated. 79 analytical determinations were made on the Pb and Cd content of the soil sampled from 14 sampling areas containing the following soil types: calcareous regosol, marl faeoziom regosol, typical luvisol, reddish preluvosol and argic horizon soil (xxx, 2009; Estefan et al., 2013). A control sample area "SP1", located at the outskirts of the Blaj town, Alba County at a distance of 26.361 km from the main source of pollution, was considered. The location on the map of the sampling plots from which the samples were collected and subjected to analytical determinations, and having the Copşa Mică industrial platform as focal point of reporting, is presented in Figure 1.

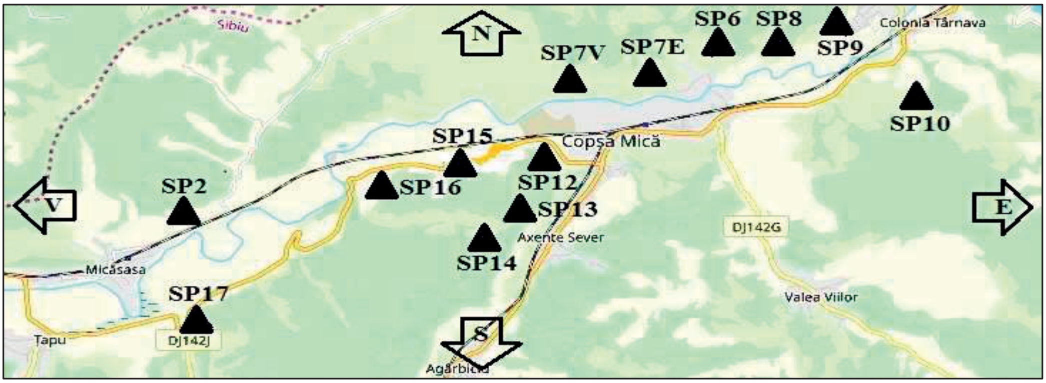


Figure 1. Distribution of the sampling plots in the Copşa Mică area (Romania), from which soil samples were subjected to analytical determinations

Soil sample was homogenized and dried at 105°C. The mineralization of the soil sample and the extraction of the total heavy metal forms studied was carried out by mineralization method using an oxidizing mixture composed of strong acids: HNO_3, $HClO_4$, and H_2SO_4. In order to quantify the total Pb and Cd content of the soils sampled from sampling areas, we used the flame atomic absorption spectroscopy (FAAS) technique, by the Analyst 200 Atomic Absorption Spectrometer from Perkin Elmer with laminar flame generated by the mixture between pure C_2H_2 and air, using the lamp specific to the chemical element to be determined, thanks to its high sensitivity, low detection levels, good accuracy, sound and improved methodology, and its relatively low costs (Franck & Tölgyessy, 1993; Csürös & Csürös, 2002). For calibration, we made use of a standard solution (Pb, Cd, Zn, Cu,) of 1000 mg/l (Merck) concentration for calibration purpose.

Dry ashing was done with strong acids: HNO_3, $HClO_4$, H_2SO_4 in a Berzelius glass that contains 1 g of fine soil mixed in a mortar with pestle were added 15 ml of oxidant mixture

HNO_3: $HClO_4$: H_2SO_4 in a volumetric volume of 2: 1: 0.2 and evaporated to dryness (on a sand bath) (Bader, 2011). After cooling the obtained residue was treated with 5 ml HCI concentrated, the bath sand operation being repeated.

The resulted residue was treated with 0.5 HCl for dissolution, the filterer being passed in a balloon of 50 ml, that was brought to sign with a sol of HCl 0.5 N. The solution that was obtained was aspirated into Perkin Elmer Analyst AA200 spectrometer with the laminar flame generated by the combination of pure C_2H_2 and air, using the lamp specific to the element to be determined. Main parameters of FAAS technique are presented in Table 2. All chemicals used were of analytical reagent grade. Double distilled water obtained with a water purification system (Millipore® Direct-Q, Millipore Corporation, Bedford, MA, USA) was used for all preparations. The glassware and polyethylene containers used for analysis were washed with tap water, then soaked overnight in 10% HNO_3 solution and rinsed several times with double distilled water.

Table 2. FAAS instrumental analytical values of investigated trace elements Pb and Cd

Parameters	Pb	Cd
Wavelength (nm)	217	228.8
Lamp current (HCL) (mA)	9.5	4.0
Flame type	Aer-acetylene	
Air flow rate (l/min)	4	4
Slith width (nm)	0.7	0.7

Concentrations of the Pb and Cd in the soil samples were calculated using the following formula: Concentration (mg/kg) = (Concentration (mg/L)x V)/W (Uwah et al., 2012),

where:

V = final volume (50 ml) of solution;

M = initial weight (1g) of sample measured.

Calculation of the Z index for ranking the pollution degree of the soils sampled from the Copşa Mică area

The assessment of the global pollution with Pb and Cd of the surveyed soils was possible by calculating the Z index based on dedicated literature (Lăcătuşu et al., 2012), using Geogenic abundance (AG) through the equation below:

$$Z = \sum_{i=1}^{n} \left[AG_i - (n-1) \right]$$

where AG_i - geochemical abundance.

Geogenic abundance (AG) is the ratio between the content of the chemical element in soil (C_i) and the clark value (Cl_i), and represents the abundance of the elements in the soil in comparison with that of the lithosphere (Table 3). Clark represents the average content of a chemical element in the lithosphere (Cl), and is calculated as follows:

$$AGi = \frac{C_i}{Cl_i}$$

Table 3. The Clark values, mean content in soil and global geochemical abundance ratio of elements (mg/kg soil), (Lăcătuşu & Ghelase, 1992).
Clarck values (after Fiedler & Rösler, 1988)

Chemical element	Clark's value	Mean content in soil	Global geochemical abundance ratio
Pb	16.0	15.0	0.94
Cd	0.13	0.3	2.31

To calculate the Z index value, only the AG_i values ≥ 1.5 are added (n-number of chemical elements surveyed). According to the Z index value, the following levels regarding the degree of soil pollution were established:

Z<8 - Minimum pollution;
8<Z<16 - Low pollution;
16<Z<32 - Average pollution;
32<Z<64 - Heavy pollution;
64<Z<128 - Very strong pollution;
Z≥128 - Maximum pollution.

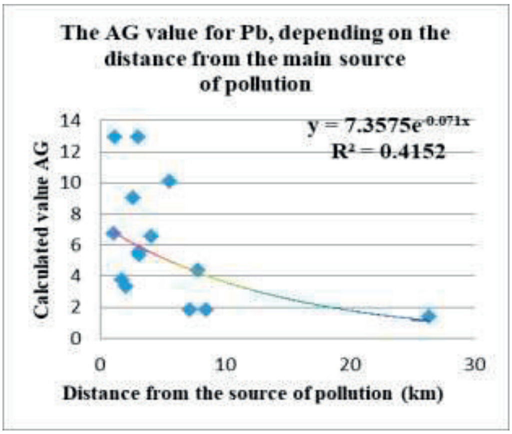
RESULTS AND DISCUSSIONS

The Z index values calculated based on the values of the concentrations of Pb and Cd determined experimentally are presented in Table 4.

Table 4. Z and AG index value, calculated on sampling areas depending on the remoteness from the main source of pollution

Sampling surface SP	Remoteness from the main source of pollution (km)	Chemical element determined quantitatively	Geochemical abundance (AG)	Z index calculated value	Z index interpretation
SP1	26.361	Pb	1.44	2.54	Minimum pollution
		Cd	3.54		
SP2	7.818	Pb	4.42	54.88	Very strong pollution
		Cd	52.46		
SP6	2.621	Pb	9.06	78.75	Very strong pollution
		Cd	71.69		
SP7E	1.708	Pb	3.75	177.90	Maximum pollution
		Cd	176.15		
SP7V	1.035	Pb	6.74	24.74	Average pollution
		Cd	20		
SP8	3.045	Pb	5.59	299.20	Maximum pollution
		Cd	295.61		
SP9	5.510	Pb	10.14	137.06	Maximum pollution
		Cd	128.92		
SP10	7.156	Pb	1.87	6.18	Minimum pollution
		Cd	6.31		
SP12	1.145	Pb	12.99	91.14	Very strong pollution
		Cd	81.15		
SP13	2.022	Pb	3.35	33.27	Heavy pollution
		Cd	31.92		
SP14	3.090	Pb	5.40	90.02	Very strong pollution
		Cd	86.62		
SP15	2.994	Pb	13.00	70.08	Very strong pollution
		Cd	59.08		
SP16	4.052	Pb	6.60	73.29	Very strong pollution
		Cd	68.69		
SP17	8.413	Pb	1.85	8.31	Low pollution
		Cd	7.46		

The dispersion of the calculated values of the AG geochemical abundance for Pb and Cd in the soils from the investigated sample surfaces is shown in Figure 2 and shows the tendency of exponential decrease of the pollution with Pb and Cd reflected in the AG calculated for the soils from the investigated sample surfaces.



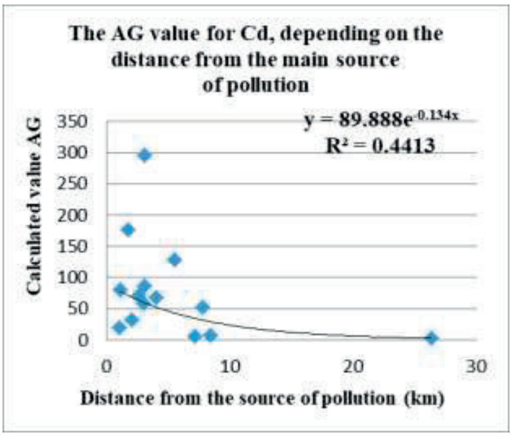
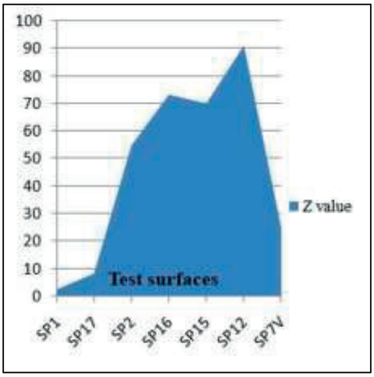


Figure 2. Reporting the distribution of AG values at a distance from the main polluting source

According to the multi-element global pollution index Z, almost half of the soil of the sample surfaces surveyed are **very heavily polluted**, and the sample surfaces with **maximum polluted** soil reaching 20%. The table above indicates that the soil in the control sample area falls into the **minimum pollution** category.

In order to highlight the dynamics of multi-element soil pollution, the distances between the test surfaces and the main source of pollutant emission dispersion located on the Sometra S.A. platform were taken into consideration, as well as the direction of spreading air masses loaded with metal pollutants. The sampling surfaces covered with soils subjected to research are located in the Târnava Mare valley, upstream (eastward) and downstream (westward) from the town of Copşa Mică, to the left or right bank of the river. Exception is SP1 which is considered as a control sample area located near the town of Blaj. The Z index value measured at the point of reference considered westward show a decreasing trend between SP12-SP1, the control sample area SP7V being located at a distance of 1.034 km from the source of pollution.

Z-index interpretation of the dynamics of global multi-elemental soil pollution on the emission source-westward direction is as follows: average pollution/very-strong pollution/very-strong pollution/very-strong pollution/very-strong pollution/low pollution/low pollution/ minimum pollution (SP7V-SP12-SP15-SP16-SP1-SP2-SP17). Increasing the distance of the test surfaces from where soil was samples eastwards causes a sharp decrease in the Z index values between SP8-SP10 whose interpretations show the following trend: maximum pollution-maximum pollution-minimum pollution (Figures 3 and 4).



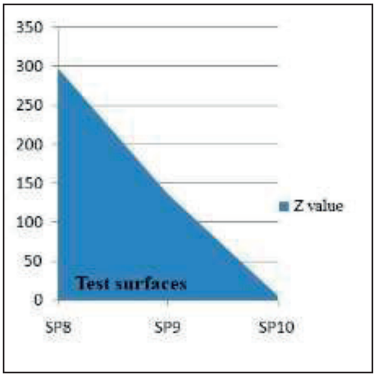


Figure 3. The dynamics of global multi-element pollution of the studied soils according to the index Z and considered cardinal points (W and E)

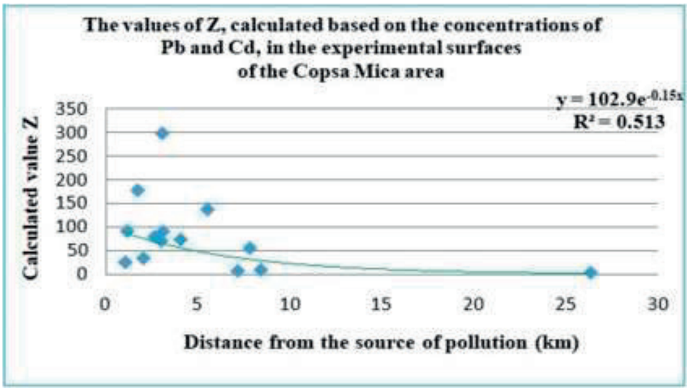
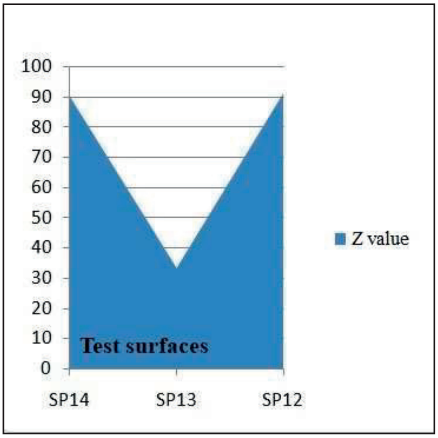


Figure 4. Distribution of the values of Z in the soils of the experimental sample surfaces

When reporting the calculated values of Z index obtained from the sampled soils oriented on the NE and SV direction as against the source of pollutant emissions, a decrease in the pollution degree of the soils was observed from the maximum pollution level (SP7E) to a very strong pollution level in SP6, while increasing distance towards NE. The SV direction determines the following trend of the multi-element global pollution degree of soils: very strong pollution- strong pollution-very strong pollution (Figure 5). Failure to observe a downward trend in the Z index calculated value in SP13 is due to the location of the test surface outside the Visa valley, compared to the other surfaces exposed more strongly to the pollutants that are located in the afore mentioned valley.



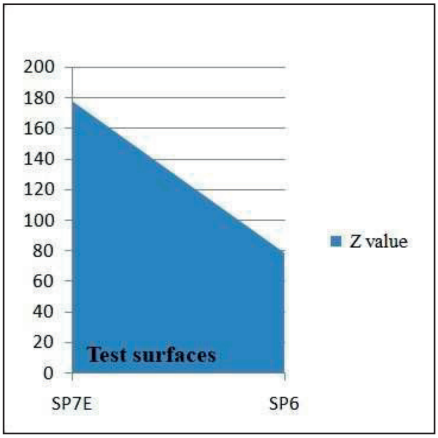


Figure 5. The dynamics of global multi-element pollution of the studied soils according to the index Z and considered cardinal points (NE and SW)

CONCLUSIONS

The calculation of the global multi-elemental pollution index Z allows the classification of soils according to the degree of pollution thereof.

The analysis of the Z index values shows that 20% of the surveyed soils fall into the maximum pollution category, 46% in the very strong pollution category, 7% in strong pollution category, 7% in the average pollution category, 7% in low pollution category, and 13% falls in the minimum pollution category.

It has been determined that increasing the distance from the main source of pollutant emissions westward and eastward is a determining factor in decreasing the degree of global multi-elemental pollution of the surveyed soils.

Besides the dynamic distance, the global pollution of the soils in the Copşa Mică area, the terrain orography and the local conditions of microclimate are factors that can disturb the trend imposed by the distance or geographical orientation.

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RESEARCH OF THE PHYSICAL AND CHEMICAL PROPERTIES OF THE AUTOMORPHIC CHERNOZEM SOLONETZS ON THE TERRITORY OF THE REPUBLIC OF MOLDOVA

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Abstract

In condition of the Republic of Moldova activities for improvement of the automorphic chernozem solonetzs and secondary solonetzized soils by irrigation meet severe difficulties related to the lack of gypsum in the republic. In such a situation, there appeared a need to use and test new amendments obtained basing on industrial waste which contains components of calcium and acid pH. Automorphic chernozem solonetzs and complexes of soils with different degree of solonetization are spread in all soil provinces of the Republic of Moldova, but the most important areas are located in Ciuluc Hills steppe, on the periphery of Codri Plateau as well as in Southern Moldova Plain. Automorphic chernozem solonetzs subtype was formed and has evolved on deposits of salty Neogene clay which contain 1-2% of soluble salts with sodium sulfate predomination. It is important to note that parental rocks of automorphic solonetz are situated at a shallow depth of 50-100 cm. Halogenesis processes from the soil of the republic are caused not only by natural factors such as soil formation rocks with high content of soluble salt or highly mineralized groundwater. With the beginning of chernozems irrigation process, soils salinization and more frequently, secondary solonetization of zonal soils was recorded. Zonal soil alkalization is highly intensified as a result of irrigation with water from local sources such as inland rivers, ponds and reservoirs. These are characterized by high degree of mineralization, alkaline pH and unfavorable chemical composition. Sodium compounds predominate in soluble salts composition.

Key words*: automorphic chernozem solonetzs, Codri Plateau, irrigation, secondary solonetzized soils.*

INTRODUCTION

Automorphic chernozem solonetzs subtype was formed and has evolved on deposits of salty Neogene clay which contain 1-2% of soluble salts with sodium sulfate predomination. It is important to note that parental rocks of automorphic solonetz are situated at a shallow depth of 50-100 cm.

In the geomorphological and, especially, lithological conditions of the republic, the spatial distribution of the automorphic alkaline soils has a diffuse, insular character, with formation of well-defined areas within the zonal soils, predominantly chernozems and greyzems. In areas with high share of solonetzs, crop productivity decreases by 40-60%.

Halogenesis processes from the soil of the republic are caused not only by natural factors such as soil formation rocks with high content of soluble salt or highly mineralized groundwater. With the beginning of chernozems irrigation process, soils salinization and more frequently, secondary solonetization of zonal soils was recorded. Zonal soil alkalization is highly intensified as a result of irrigation with water from local sources such as inland rivers, ponds and reservoirs. These are characterized by high degree of mineralization, alkaline pH and unfavorable chemical composition. Sodium compounds predominate in soluble salts composition.

In condition of the Republic of Moldova activities for improvement of the automorphic chernozem solonetzs and secondary solonetzized soils by irrigation meet severe difficulties related to the lack of gypsum in the republic. In such a situation, there appeared a need to use and test new amendments obtained basing on industrial waste which contains components of calcium and acid pH.

MATERIALS AND METHODS

The research started with the assessment of natural conditions and delimitation of soil units borders. For mapping the comparative-geographical method of V.V. Dokuchaev was used, which allows establishing the natural links between soils and soil formation factors (Составление и использование почвенных карт, 1987). Utilization of geographic information system (GIS) and mapping modern equipment during research activities were carried out within the territory of the pilot areas. The researches were conducted in 2016-2017 by the working team.

For study development, there were used the following materials: maps of territorial organization of communes from the pilot areas at scale of 1:10000; soil maps at 1:10000; topographic plans at 1:10000; digital map of soil cover of Moldova at 1:10000; remote sensing materials "ortophoto" at 1:5000 of Land Relations and Cadastre Agency (LRCA); "Digital Elevation Model (DTM)" of LRCA.

Mentioned materials were connected to the national reference system MoldRef-99. Subsequently thematic layers ("Hydro", "Terrain", "Settlements", "Soil", etc.) as well as structure of the database parameters were formed. Next step consisted in digitization of soil and territories with vineyards and orchards plantations polygons that can be used in irrigation. A digital model of the elevation was developed at a scale of 1:1000 and attributive information for each area was introduced. There were performed field research activities for actualization of soil, infrastructure and land degradation polygons which were estimated digitally in the office from geospatial remote sensing materials. The works were made in the MapInfo (2D) and ArcGIS (3D) software and in accordance with "Instructions on soil survey to attribute of land for state and public needs" approved in 1991 (Instrucţiunile privind …, 1994) "Regulation on the content of general Land Cadastre documentation" approved by the Republic of Moldova Government Decision no. 24 of 11.01.1995 (Regulamentului cu privire la ... 1995).

RESULTS AND DISCUSSIONS

For assessment of automorphic solonetzs amendments and fertilization efficiency, field experience was established which included following variants: 1. Reference (control) soil (no amended and no fertilized soil); 2. Defecation sludge; 3. Defecation sludge + manure; 4. Defecation sludge + manure + NPK; 5. Defecation sludge + NPK. Organic fertilizers were administered with a dose of 60 t/ha. The applied amount of chemical fertilizer (NPK) was equivalent to the nutrient content of 30 t of manure. In the mixed fertilizers version, the organic fertilizer dose was 30 t/ha. Some of the main criteria for solonetzs quality assessment during improvement period are the content and composition of the soluble salts. The experimental field soil is of sulphate salinization. The soil profile is clearly distinguished by the total content of soluble salts. Thus, the superficial horizon is characterized by a low degree of salinization, the central part of the profile is included in moderate salinization values class, and the parental material is moderately to strongly salinize. Analysis of obtained material of chemical composition and soluble salt content demonstrates that the predominant component is sodium sulphate. In soil upper horizons Na_2SO_4 is about 49%, and in the underlying and the parental material this component accounts up to 85% of the total amount of salts.

Regarding the impact of amendment and ameliorative fertilization on the soluble salt composition, an increasing content of calcium bicarbonate and calcium sulphate, which are harmless to agricultural plants, is observed. The calculations show that in the horizon treated with defecation sludge and fertilizers, the sum of toxic salts is 59-76% of the soluble salt total content, and in the reference (control) soils this index reaches values of 85-90%. The soil in the variants treated with calcium waste and fertilizers is determined as slightly solonetzized (6-9% Na^+), and the one from the unfertilized variant is included in the moderately solonetzized category (11% Na^+). Exchangeable Ca^{2+} content has increased in comparison with reference (control) soil with 4-6 me/g soil, which is equivalent to 72-78% of bases sum. They show that the coarse and medium sand total content is insignificant and makes 0.02-0.18%. A higher percentage is characteristic for fine sand. There statistically significant difference in the silt fraction content. Thus, coarse silt prevails with a share of 23-30%, followed by fine (17-24%) and medium silt (8-11%). A high content of fine clay is characteristic for automorphic chernozem solonetz texture. In distribution of particles with a diameter of less than 0.001 mm within the soil profile, there was recorded an increase of 6-13% in the 30-50 cm layer which includes in its composition the biggest part of sodium clay-illuvial horizon (Btna). The character of physical clay distribution is identical to that of fine clay, but in this case it is observed only an increasing trend of the fractions with a diameter of less than 0.01 mm in the Btna horizon. According to the content of this fraction and Kacinski classification, the granulometric composition of automorphic chernozem solonetz is finely clay (Ursu, 2011).

Characteristics of soil layer. According to pedo-geographical regionalization, study area is included in First pedo-geographical Zone of Hilly Silvosteppe of Northen Moldavian Plateau, district II of chernic and leached chernozems of Northen Plain Steppe, rayon III of Steppe of Balti Plateau, subrayon III a of Steppe of Ciuluc Hills with chernic and solonetized chernozems (Instrucţiunile privind cercetările pedologice …, 1987; Урсу, 1980).

For the study area, soil cover map at 1:10000 scale was developed in digital format. In the limit of the area there were found 135 polygons covering an area of 2576.44 ha. The structure of the soil cover is formed by about 64% chernozems where the calcic prevails with 28.4%, widespread at altitudes of 67-257 m and the average slope of 6.07º. Ordinary chernozem with a share of 28.1% are located within altitudes of 63-224 m with an average slope of 4.4º. Chernic chernozem can be found between altitudes of 78 m to 252 m, with the average slope degree of 4.58º and with area 7.8% of total soils area (Tables 1 and 2).

On the second place solonetzs and saline soils are situated (14.8%). Chernozem-like soils occupy about 11%. Share of alluvial soils is 7.8%, landslides constitute 2.1%. Weighted average bonitet (system of land fertility appraisal) is 62 points.

Types of soil degradation are very different in the catchment area. Approximately 32.6% (841.07 ha) of land are subject to halomorphic processes (Table 3). Water erosion extends on about 25% of the total area. Chernic, ordinary and calcic chernozems are eroded at 55.7%, 41.7% and 23.8%, respectively.

Leached chernozem-like soils are eroded at about 87%. The total area of soils with different erosion degrees is 643.68 ha.

Slightly eroded soils prevail with a share of 59% formed at an average altitude of 116 m, with average slope degree of 5.75º. Moderately eroded soils with a share of 37% are located at an average altitude of 137 m, with average slope degree of 7.85º. A strongly eroded soil occupies only 3.6% and spread at an average elevation of 171 m and slope degree of 9.44º. Soils subject to hydromorphic processes occupies 31.15 ha or 1.21% of area.

Within the catchment are at here are highlighted 590.93 ha (22.94%) of soils formed on Neogene salty parental rocks which generates formation and developments of solonetzs and salty soils (Sandu, 1994).

Total area of different types of degraded soils makes up 1459.05 ha of the total area equal to 2576.44 hectares, which constitute 56.6%.

Table 1. Geomorphometric characterization of soils

Soil name	**Area, ha**	**Elevation, m**			**Slope, degree**			**Aspect**
		min.	**max.**	**average**	**min.**	**max.**	**average**	
Chernic chernozem	200.80	77.77	252.23	108.20	0.00	24.57	4.58	South
Ordinary chernozem	724.50	63.49	224.27	120.82	0.00	21.54	4.41	South-East
Calcic chernozem	730.58	67.34	257.04	136.91	0.00	33.33	6.07	South
Typical moor soil	5.02	160.03	209.41	186.70	5.14	11.29	8.16	South-West
Leached chernozem-like soil	62.57	94.32	235.00	139.24	0.64	25.15	9.18	East
Chernic chernozem-like soil	220.22	65.76	233.08	110.95	0.00	22.47	4.02	South
Solonetz molic	369.50	68.46	207.49	128.56	0.00	28.02	5.75	South
Gleic solonetz	0.27	64.94	70.61	67.89	1.00	5.67	4.47	South-West
Gleic solonceak	10.31	94.89	113.35	102.39	0.02	7.66	3.02	South
Mollic alluvial soil	41.81	86.01	123.48	99.67	0.00	16.84	2.10	South-East
Alluvial stratified soil	142.44	65.84	174.49	88.73	0.00	14.87	2.86	South
Gleic alluvial soil	15.54	59.22	70.03	62.93	0.01	11.31	1.86	South
Active landslides	52.87	94.80	244.29	174.95	0.05	35.67	11.89	South-West
TOTAL	**2576.45**							

Table 2. General characteristics of soils

Soil name	Number of polygons	Area, ha	% of total area	Subtype bonitet	Calculated bonitet
Chernic chernozem	8	200.80	7.79	100	89
Ordinary chernozem	39	724.51	28.12	82	69
Calcic chernozem	32	730.58	28.36	71	58
Typical moor soil	1	5.03	0.20	25	25
Leached chernozem-like soil	3	62.57	2.43	85	62
Chernic chernozem-like soil	17	220.21	8.55	85	84
Solonetz molic	24	369.50	14.34	34	34
Gleic solonetz	1	0.27	0.01	34	34
Gleic solonceak	2	10.31	0.40	10	10
Mollic alluvial soil	2	41.81	1.62	85	85
Alluvial stratified soil	1	142.43	5.53	80	80
Gleic alluvial soil	2	15.54	0.60	25	22
Active landslides	3	52.87	2.05	5	5
TOTAL	**135**	**2576.44**	**100**	**-**	**62**

Table 3. Soil degradation types

Soil name		Soil area, ha							
	Total	Eroded				Hydro-morphic	Halo-morphic	Neogene rocks	Degraded
		slightly	moderately	strongly	sum				
Chernic chernozem	200.80	105.54	6.39	-	111.93	-	-	-	111.93
Ordinary chernozem	724.51	149.74	15.23	7.45	172.43	-	208.50	124.71	364.29
Calcic chernozem	730.58	125.44	163.86	15.65	304.95	-	184.42	129.53	468.98
Typical moor soil	5.03	-	-	-	-	5.03	-	-	5.03
Leached chernozem-like soil	62.57	-	54.37	-	54.37	-	54.37	54.37	54.37
Chernic chernozem-like soil	220.21	-	-	-	-	-	6.65	6.65	6.65
Solonetz molic	369.50	-	-	-	-	-	369.50	268.46	369.50
Gleic solonetz	0.27	-	-	-	-	0.27	0.27	-	0.27
Gleic solonceak	10.31	-	-	-	-	10.31	10.31	7.21	10.31
Mollic alluvial soil	41.81	-	-	-	-	-	-	-	-
Alluvial stratified soil	142.43	-	-	-	-	-	-	-	-
Gleic alluvial soil	15.54	-	-	-	-	15.54	7.05	-	15.54
Active landslides	52.87	-	-	-	-	-	-	-	52.87
TOTAL	**2576.44**	**380.72**	**239.86**	**23.10**	**643.68**	**31.15**	**841.07**	**590.93**	**1459.05**
% of total		**59.15**	**37.26**	**3.59**	**24.98**	**1.21**	**32.64**	**22.94**	**56.6**

According to texture composition, clayey loam soil predominates with a share of 50.6%. On the second place loamy clay soils are situated with 45.7%, followed by fine and average clays with about 3.7% (Table 4).

Eluvial-delluvial clayey loam (with 40.59% prevails in parental rock structure. Neogene clay deposits of (Na) ranks on second place with 22.94%, followed by eluvial-colluvial clays with 18.66% (Table 5). Alluvial-delluvial sediments (Ad) for 7.9% and alluvial - 7.75%. Complex of sands and clay under landslide is 2.05%. Structural distribution of parental material is reflected in Figure 1.

Intrazonal subtype of (steppe) chernozem-like automorphic solonetzs and zonal soils complexes with different degrees of alkalinity, in general chernozems, are widespread in all pedogeographic provinces of Moldova. It should be mentioned that spatial distribution of these soils is very scattered and is determined by lithological and geomorphologic conditions. An important role in formation, development and spatial distribution of chernozem-like automorphic solonetzs is played by soil formation rocks (Andrieş, 2007).

Table 4. Soil texture

Soil name	Soil area, ha			
	Total	texture		
		clay	loamy clay	clayey loam
Chernic chernozem	200.80	-	9.54	191.26
Ordinary chernozem	724.51	18.58	310.34	395.59
Calcic chernozem	730.58	57.80	213.96	458.82
Typical moor soil	5.03	-	5.03	-
Leached chernozem-like soil	62.57	8.20	54.37	-
Chernic chernozem-like soil	220.21	-	131.66	88.56
Solonetz molic	369.50	4.66	357.19	7.66
Gleic solonetz	0.27	-	0.27	-
Gleic solonceak	10.31	7.21	3.09	-
Mollic alluvial soil	41.81	-	22.91	18.91
Alluvial stratified soil	142.43	-	-	142.43
Gleic alluvial soil	15.54	-	15.54	-
Active landslides	52.87	-	52.87	-
TOTAL	**2576.44**	**96.45**	**1176.77**	**1303.22**
%		**3.74**	**45.67**	**50.58**

Table 5. Soils formed on different parental rocks

Soil name	Soil cover surface, ha						
	Total surface	Parental rocks					
		Na	eda	edtc	Ad	Al	At
Chernic chernozem	200.80		9.54	191.26	-	-	-
Ordinary chernozem	724.51	124.71	204.22	395.59	-	-	-
Calcic chernozem	730.58	129.53	142.23	458.82	-	-	-
Typical moor soil	5.03	-	5.03	-	-	-	-
Leachedchernozem-like soil	62.57	54.37	8.20	-	-	-	-
Chernic chernozem-like soil	220.21	6.65	9.95	-	203.61	-	-
Solonetzmolic	369.50	268.46	101.05	-	-	-	-
Gleicsolonetz	0.27	-	0.27	-	-	-	-
Gleicsolonceak	10.31	7.21	3.09	-	-	-	-
Mollic alluvial soil	41.81	-	-	-	-	41.81	-
Alluvial stratified soil	142.43	-	-	-	-	142.43	-
Gleic alluvial soil	15.54	-	-	-	-	15.54	-
Active landslides	52.87	-	-	-	-	-	52.87
TOTAL	**2576.44**	**590.93**	**483.58**	**1045.67**	**203.61**	**199.78**	**52.87**
%		**22.94**	**18.77**	**40.59**	**7.90**	**7.75**	**2.05**

The largest areas of automorphic solonetzs and the largest participation in the composition of the soil cover, is recorded in Ciuluc-Soloneţ Hillsand Central Moldavian Plateau. These soils are widespread in the Codri borders, in Southern Moldavian Plain and the Tigheci Hills.

According to soil survey study results, total area of chernozem-like automorphic solonetzsis 26.9 thousand hectares. In most cases these soils are located on the territories of eluvial and trans acumulative (transition) landscapes. It is widely accepted that in mentioned landscapes, predominantly located in interfluves and related hillsides with gently undulating slopes, a regime of slowed migration of several elements and substances in the soil, including soluble salts is installed.

Chernozem-like automorphic solonetzs is formed and evolved in these types of landscape only in conditions when the soil formation rocks were presented by salty clay deposits, generally of Neogen, located in small depth of 0.5-1.0 m. These clays contain considerable amounts of soluble salts (1.5-2.5%), sulfate prevailing in their composition; they are characterized by fine texture with predomination of clay with a share of 40-47%in its composition.

On watersheds, there can be often meet solonetzs with crust or columns at the top of the surface. Solonetzs with medium depth columns occupy slopes with south, southwest and west aspect. Automorphic solonetzs with great depth columns are located on gently undulating slopes of northern, northeastern and eastern aspect. In trans accumulative landscapes automorphic solonetzs often form complexes with semi-hydromorphic solonetzs.

Main peculiarity of chernozem-like automorphic solonetzs distribution consists of formation of small island areas, distinctively outlined. These soils, diffused in the highly productive terrains, substantially increase the complexity of soil cover and reduce their productive capacity by 40-60%. A significant participation of automorphic solonetzs both as number of contours as well as surface share (5-8% of arable land) is recorded in the districts located in the Ciuluc-Solonetz Hills. The largest share of solonetzs is located in Singerei district, this constituting 8.49% or 4634 ha.

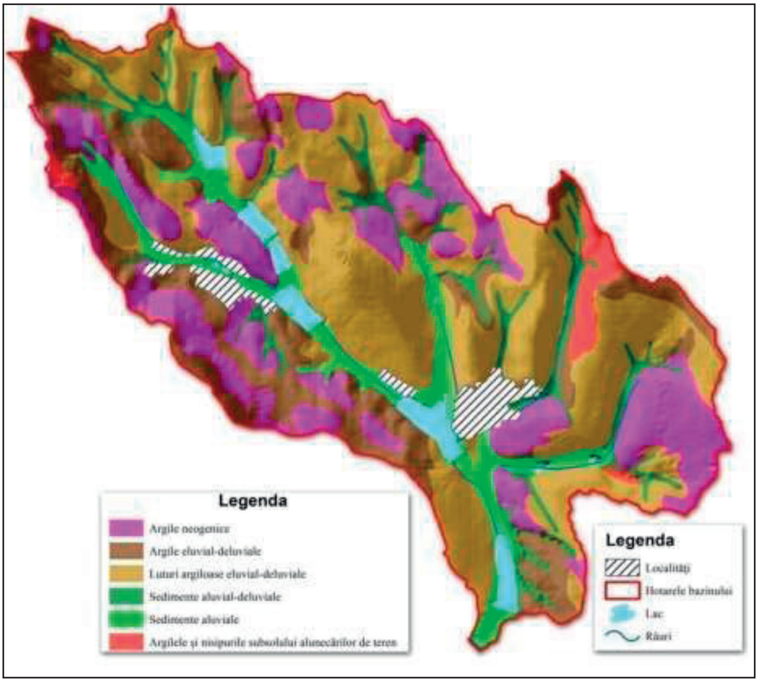

Figure 1. Parental material

For assessment of ameliorative state of salty soil in terms of application of agropedoameliorative works soil profiles were placed on chernozem-like automorphic solonetzs from where samples for chemical and physical analysis were taken. Within field and laboratory work, physical hydrological, physico-chemical and physico-mechanical properties of soil were determined. Soil profile morphology and morphometry are characterized below.

Description ofchernozem-like automorphic solonetzs profile:

Ah (0-30 cm) - arable humic layer, low solonitized, darc-grey, compact, masive structure, fine pores, cracks, plant debris, horizons clearly distinguished.

Btna (30-50 cm) - sodium clay-iluvial horizon, dark-gray with brown hues, massive structure with prismatic slitizate (vertic) formations, very compact, fine pores, semi-decomposed small roots, fine clay, and horizons clearly distinguished.

Bkz (50-70 cm) - transitional horizon, yellow with brown hues, nonhomogeneous, humus tongues, unstructured, compact, fine pores, veins and crystals of gypsum with carbonate, fine clay, gradual transition.

BCkz (70-100 cm) - horizon of transition to soil formation rock, yellow with humus accumulation through the cracks, unstructured, compact, weak gleyzation, stagnant, rare beloglasca, fine pores, spots of carbonates and gypsum crystals, fine clay.

Table 6. Chemical proprieties of chernozem-like automorphic solonetzs

Horizon	Depth, cm	Hygroscopic water	Humus	$CaCO_3$	$N\text{-}NO_3$	P_2O_5	K_2O
		%			mg/100 g soil		
Ah	0 -30	4.95	4.79	-	0.57	2.04	57.1
Btna	30 - 50	5.50	3.26	0.5	0.39	0.76	38.0
Bkz	50 - 70	4.79	1.51	4.5	0.31	0.67	35.9
Bckz	70 - 100	4.10	0.90	7.1	0.25	0.57	33.2

Performed researches showed that the experimental field soil is characterized by "great" share (4.79%) of organic matter on 0-30 cm depth. Sodium clay-iluvial horizon (Btna) contains 3.26% of humus and falls in the "middle" class. The underlying horizons amount of humus is "low" to "very low" (Table 6).

Table 7. Salt content, pH and ionic composition of water content of chernozem-like automorphic solonetzs

Horizon	Depth, cm	Dry residue, %	pH	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	Toxic salts, %
			me/100 g soil							
Ah	0-30	1.170	7.90	0.32	0.02	2.02	0.32	0.08	1.96	5.2
Btha	30 - 50	0.248	8.1	0.35	0.03	3.10	0.32	0.20	2.96	6.5
Bkz	50 - 70	0.464	8.25	0.72	0.10	5.76	0.49	0.74	6.05	12.3
Bckz	70 - 100	0.763	8.75	0.42	0.17	10.28	1.90	0.75	8.22	29.7

Distribution of organic matter within soil profile is characteristic for given subtype of soil and is manifested by a sudden reduction of its content from the upper to deeper horizon. Thus, the difference in humus content in the topsoil and subsoil constitutes 1.53%.

In automorphic solonetzs improvement practice content of $CaCO_3$ and maximum accumulation depth play an important role. From presented data it can be seen that amount of calcium carbonate is insignificant in the first 50 cm. In the subsoil and parental material $CaCO_3$ content increases significantly (7.1%) and is included in the "middle" class. Great depth location of carbonic acid salts excludes the possibility of agro-biologicum provement which consider involving calcium compounds reserves in Btna horizon through soil improvement works.

The content of nitrate nitrogen is reduced, the maximum concentration of $N\text{-}NO_3$ in the soil profile (0.57 mg/100 g soil) was found on surface (Table 6). Chernozem-like automorphic solonetzs has a "moderate" content of phosphorus. P_2O_5 content at the surface is 2.04 mg/100 g soil. In underlying layer it is reduced to 0.76 mg/100 g soil. Republic soils, including the alkali ones, are well supplied with exchangeable potassium, whose content is determined by the mineralogical composition peculiarities. The degree of assurance of chernozem-like automorphic solonetzs with K_2O is "very high". Arable layer contains 57.1 mg/100 g soil K_2O. Profile distribution of exchangeable potassium shows a sharp differentiation (Table 6).

Some of the main criteria for assessment of solonetzs quality state in terms of their improvement is the content and composition of soluble salts. Experimental field soil is of sulphate class of salinization with ratio Cl: SO_4 between 0.01:1 and 0.02:1. Top horizon is characterized by a low degree of salinity, the central part of the profile consists of values of moderately saline class, and parental matter is moderate to highly salinized. From data presented in Table 7, there can be observed increased levels of dry residue in depth from 0.170% to 0.763%. The anionic composition of water content is dominated by sulfate with participation of 2.02 to 10.28 me/100 g soil, and the cation one by sodium content of 1.96 to 8.22 me/100 g soil. HCO_3^- and Cl^- anions and divalent cations Ca^{2+} and Mg^{2+} occupy subordinate positions. Value of pH in top horizon is 7.90 reaching in depth 8.75 units.

Table 8. Composition of soluble salts in chernozem-like automorphic solonetzs

Horizon	Depth, cm	$Ca(HCO_3)_2$	$Ma(HCO_3)_2$	$CaSO_4$	$MgSO_4$	Na_2SO_4	$MgCl_2$	Toxic salts, %
		me/100 g sol						
Ah	0-30	0.32	-	-	0.06	1.96	0.02	85
Btha	30-50	0.32	0.03	-	0.14	2.96	0.03	60
Bkz	50-70	0.72	-	0.07	0.64	5.05	0.10	86
Bckz	70-100	0.42	-	1.48	0.58	8.22	0.17	82

Table 9. Composition of exchangeable cations in chernozem-like automorphic solonetzs

Horizon	Depth, cm	Ca^{2+}	Mg^{2+}	Na^{+}	Suma	Ca^{2+}	Mg^{2+}	Na^{+}
		me/100 g sol				% from sum		
Ah	0-30	22.00	13.00	7.74	42.74	52	30	18
Btha	30-50	16.00	14.01	8.70	38.71	41	36	23
Bkz	50-70	15.06	15.32	8.96	39.34	38	39	23
Bckz	70-100	14.44	15.00	9.52	38.96	37	39	24

The composition of the soluble salts is shown in Table 8. Analysis of the obtained material shows that the predominant component is sulphate sodium. In the upper layers of soil Na_2SO_4 constitutes about 49% and the underlying horizons and parental material this compound is up to 85% of the total salts sum.

For emphasize the value of alkali soils it is necessary to know the content and ratio of exchange cations. The obtained results show a differentiated distribution of alkali and alkaline-earth bases on solonetz profile. Thus, the superficial soil horizons contains 22.00 me/100 g calcium soil; in underlying horizons and parental material this element does not exceed 16.00 to 14.44. A reverse distribution of adsorbed of sodium is recorded within soil profile. Contents of exchangeable Na^{+} increases from 18% in eluvial horizon to up to 24% in soil formation rock. Character of cation Mg distribution is more uniform (Table 9).

Granulometric (textural) composition is one of the intrinsic soil properties, practically unchanged by anthropogenic intervention and also by improvement activities. Decisively, texture determines water and air regime as well as physical, chemical and mechanic properties. In Table 10 there are showed the results of estimation of study area soils textural composition. They show that the content of coarse and medium sand is insignificant and is less than 0.2%. A higher percentage is characteristic for fine sand. From presented data, a statistically significant difference in content of silt fractions can be seen. Thus, coarse silt predominates with a share of 27-30%, followed by fine (17-24%) and middle silt (8-11%).

A high content of fine clay is characteristic for chernozem-like automorphic solonetzs texture. In distribution of particle with diameter ≤ 0.001 mm in the soil profile, there was recorded an increase up to 13% in clay-iluvial sodium horizon. Distribution of physical clay (silt and clay) is identical to fine clay, but in this case there was identified a growing trend of fractions with a diameter less than 0.01 mm in Btna horizon.

Considering this fraction content, and according to Kacinski classification, textural composition of chernozem-like automorphic solonetzs is fine clay.

The content of peptized fine clay in 0-30 cm layer makes up 10.5%, increasing to 14.0% in the underlying layer (Table 11). Dispersion factor in the surface layer makes up 15 to 16%. High salt content in the Bkz salt horizon and soil formation rock lead to suspension clotting, making it impossible to determine the micro-aggregate composition.

Table 10. Textural composition of chernozem-like automorphic solonetzs

Horizon	Depth, cm	Content of fractions (%) with diameters (mm)						
		> 0.25	0.25-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	<0.01
Ah	0-30	0.2	4.5	29.6	11.5	22.1	32.1	65.1
Btna	30-50	-	3.0	27.1	7.5	17.0	45.4	69.9
Bkz	50-70	-	3.4	28.0	9.4	18.0	41.2	68.6
BCkz	70-100	-	0.9	28.5	10.0	20.0	36.6	66.6

Table 11. Micro-aggregate composition of chernozem-like automorphic solonetzs

Horizon	Depth, cm	Content of fractions (%) with diameters (mm):							Fd, %
		>0.25	0.25-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	<0.01	
Ah	0-30	0.3	8.8	44.2	14.5	21.7	10.5	46.7	33
Btna	30-50	0.2	7.0	40.3	15.8	22.5	14.0	52.3	31

Table 12. Physical proprieties and hydro-physical indicators of chernozem-like automorphic solonetzs

Horizon	Depth, cm	D	DA	PT	PD	PU	PI	CH	CO	CC	CU
		g/cm^3		% v/v			%				
Ah	0-30	2.61	1.24	52	19	13	20	11.5	16.54	26.8	10.4
Btna	30-50	2.65	1.45	45	11	9	24	11.9	16.9	23.4	6.5
Bkz	50-70	2.69	1.53	43	8	10	24	11.2	15.9	22.7	6.8
BCkz	70-100	2.71	1.58	42	11	5	26	11.4	16.2	19.5	3.3

Basic physical proprieties which determine the state of soil alignment are shown in Table 12. The results show that the soil solid phase density is characterized by homogeneity with insignificant increase from 2.61 g/cm^3in surface layer to 2.71 g/cm^3 the soil formation rock and due to decrease of humus content by 3.84%.

The apparent density in the arable layer is 1.24 g/cm^3, growing in depth, being in soil formation rock up to 1.58 g/cm^3. The total porosity is a function of solid phase density and the soil bulk density. Arable layer has an average PT of 52%, which decrease to 42% in the parent rock, creating restrictions to aeration process. Aeration porosity indicates free pore volume at soil moisture equal to field capacity. It is the "average" (19%) at the surface and sharply decreases with depth up to 8-11%, indicating deficient aeration propriety of soil. Studied soil is characterized by high values of inactive porosity (20-26%).

Hygroscopic coefficient is between 11.2 to 11.9% and closely correlates with fine clay content. Wilting coefficient represents the lower level of plant available water. According CO data in automorphic solonetzs it is 15.9-16.9%, falling within class values "high" to "very high".

One of main hydro-physical indicators is the field capacity. CC is the upper level of plant available water. In terms of hydro-physics, chernozem-like automorphic solonetzs profile is distinctly differentiated. Its upper layer is characterized by "high" values of CC (26.8%), the transition horizons have an "average" field capacity (22.7 to 23.4%) and in parental material CC is "low" (19.5%).

Water capacity corresponds to useful water reserve that the soil can yield plants. Those measurements show that the shallow soil horizon is "low" (10.4%). The soil and rock underlying horizons have with "very small" (3.3-6.8%).

Wilting capacity (Plant available water) corresponds to water resources that soil can yield to plants. The measurements show that in surface layer of soil horizon CU is "low" (10.4%). Underlying horizons and soil formation rock have CU "very low" (3.3-6.8%).

It is known that alkali soils are characterized by a defective structure. In the unchanged alignment, automorphic solonetzs structure is strictly differentiated in genetic horizons. Eluvial horizon has lamellar structure with reduced hydrostability. Structure of clay-iluvial sodium horizon (Btna) is prismatic-column. Structural aggregates have an extremely compact alignment, and their porosity is low and fine. The underlying soil horizons, soil is nonstructural with massive clod formations.

CONCLUSIONS

Studying automorphic solonetzs and secondary solonetized chernozems through irrigation was performed in subdistrict of Ciuluc Hills, respectively Southern Bessarabian Steppe district.

There were characterized in detail the natural conditions of the study areas, in particular climate, topography, soil formation rocks, soils and hydrographic network.

In field and laboratory conditions there were determined morphological and morphometric parameters, studied the physical, chemical and hydric proprieties of automorphic solonetzs. A particular attention was given to saline indicators required for argumentation of improvement works.

There were determined and assessed the state of deep-plowing ordinary chernozem quality irrigated with water from local sources. Also there were determined the chemical composition and quality indicators of water used in irrigation. Basing on performed research there will be calculated the need for amendments to restore degraded chernozem fertility.

Using remote sensing material there were developed maps of irrigation fund at 1:1000 scale of Lebedenco commune. The works were done in MapInfo (2D) and Arc GIS (3D) software.

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INFLUENCE OF THE DRIP IRRIGATION AND PLASTIC MULCH ON THE WATER DISTRIBUTION AND COMPACTNESS STATE OF GREENHOUSES SOIL WITH MEDIUM TEXTURE

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Abstract

The goal of our investigation is to established the influence of the drip irrigation associate with plastic mulch on the state of soil compactness, especially on the surface of the ground on the paths. In present, the aspect of soil surface (smooth or rough), the presence of small irregularities, the rill on the paths, the cracks formation after water evaporation and soil drying are the most useful indicators for farmers in order to assess soil degradation by compaction. To all these indicators, we added hysteresis of soil-water characteristic curves as indicator of compactness state of soil and quality yield. Hysteresis behavior of water is a soil characteristic curve and can be used by engineers in charge with design of irrigation system to avoid the negative effects of drip irrigation associate with plastic mulch technology. For avoid the soil degradation from greenhouses or solarium, we proposed a set of indicators for farmers to achieve first steps of soil degradation and for improve the irrigation management.

Key words*: soil compactness, plastic mulch, water hysteresis, soil wetting pattern.*

INTRODUCTION

Due to the compulsory location imposed by the existence of water sources many greenhouses or solariums were placed on soils considered with a low capability but then through the application of land improvement works satisfactory results have obtained (Canarache, 1998).

The soil with first class capability in greenhouses must have in 0-50 cm layer a humus reserve higher than 300 t/ha, clay 12-20% (maximum 25%) contented, slightly acid reaction and a low content of soluble salts and sodium cations exchangeable capacity less than 5% (Florea et al., 1987).

Especially in greenhouse, it’s used drip irrigation method which presents many advantages in gardening as low water and energy consumption. In last years, drip irrigation is associated with plastic mulch method in order to have better horticulture results. The plastic mulch has the advantage that limited water loss by evaporation and maintained a higher temperature on soil surface. However, these cumulated methods cause rapid soil degradation by local compaction between plant rows (on paths) and a high salinization at the wetting front was observed (Filipov et al., 2013).

Certainly, the soil degradation processes occur even in soils belongs to first class capability from greenhouses but using plastic mulch increase the compaction of the soil. The soil compaction on paths between rows of plants is pronounced by water circulation in soil, but, also, in the small space between ground and plastic wrap. In greenhouses or solariums water is provided by drip irrigation and water circulation has certain peculiarities. In soil wetting pattern, the wetting front is move downward in coarse texture soil (sandy) and extends horizontally in fine texture soil (clay) (Filipov & Bodale, 2018). For a soil with medium texture (loam-clay), as in our case, the wetting pattern in profile of soil is circular (Figure 1b).

We are study the soil degradation based on complex approach that take into account soil characteristics observation, physical properties determination in lab and, also, by simulations.

The simulations of soil water characterization curves (SWCC) is useful method for understanding the water movement in different types of soils. SWCC is described by water potential in soil at given water content (Hong et al., 2016), where the water content (θ) is define as the ratio of water volume to total volume of soil. The main soil water characterization curves are wetting and drying curves.

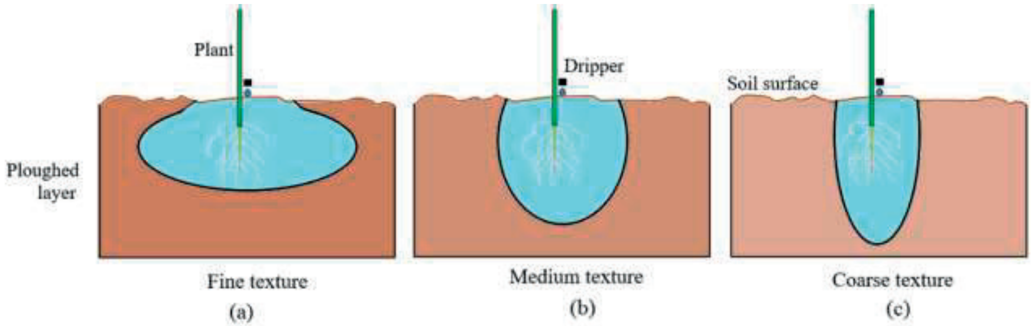

Figure 1. Wetting pattern in ploughed layer of soil with different textures: (a) for fine, (b) for medium and (c) for coarse textures of soil

The wetting and drying processes has a hysteresis behavior cause by a sum of factors as irregular space geometry, different contact angles at the upward and downward of water, the entrapped air inside the soil, shrinking and swelling of pores and thermal effects (Bodale & Stancu, 2020; Nimmo, 2006; Ward et al., 2000). The hysteresis is characterized by a relative large difference between the main drying and wetting soil water retention curves (Bodale & Stancu, 2020).

The infiltration is the move downward of water from the soil surface inside the soil due gravity and diffusion. The wetting pattern depends by hydraulic properties of soils. For example, in sandy soil deep infiltration is favorite, instead in clay the horizontal infiltration is dominate.

In Green-Ampt model (Witelski, 1998) the horizontal infiltration is describe by below equation:

$$\frac{\partial\theta}{\partial t} = \frac{\partial}{\partial x} D \frac{\partial\theta}{\partial z} \quad (1),$$

where: D is water diffusion in the soil; $\partial\theta/\partial t$ is infiltration rate; x is distance on horizontal direction; z is the deep. Water diffusion in the soil depends by soil hydraulic conductivity (K) that in clay is 0.03 cm/h.

MATERIALS AND METHODS

The study of soil degradation in gardens, greenhouses and solariums was performed on field observations, laboratory determinations and SWCC simulations.

The investigations were conducted in some high plastic tunnels from Vegetable Research Stations in the Eastern part of Romania, especially from Bacau, Targu Frumos and Spataresti greenhouses. In present study, the representative soil type is *Cambic Cernozem* in Romanian System of Soil Taxonomy (Florea, 2012) or *Haplic Chernozems* in World References Base of Soil Resources (FAO, 2015). On the other side, from geological point of view, the soils from this study were developed as alluvial deposits or like loess deposits.

In order to highlight the associated effect of soil mulching with plastic foil and drip irrigation was initiated a study of pedo-morfological indicators in both variants; without and with plastic mulch film. Some pedo-morfological indicators such as the aspect of soil surface (smooth or rough), the presence of small irregularities, the rill on the paths, the cracks formation after water evaporation and soil drying are useful indicators for approximate assessment of the compactness state of soil.

In the field, we sampled undisturbed samples from 10 to 10 cm up to the depth of 50 cm in order to analyzed the bulk density, instead the size particles were determined in the laboratory on crushed soil samples from profiles.

Theoretical approach was used to simulate the wetting and drying water retention curves in aggregated and compacted samples. These soil proprieties were described by a set of analytical solution of van Genuchten-Mualem equation (Arrey et al., 2017):

$$\theta(h)=\begin{cases}\theta_r+\dfrac{\theta_s-\theta_r}{[1+|\alpha h|^n]^{1-1/n}}, h<0\\ \theta_s,\ h\geq 0\end{cases} \quad (2),$$

where: θ_s is saturated water content; θ_r - residual water content; h - pressure head; α - air trapped coefficient; n - pore size distribution. The simulated results of soil water retention curves and pedological aspects were used to determine the soil degradation.

RESULTS AND DISCUSSIONS

The crops with (1) and without (2) plastic mulch film (Figure 2) were analyzed. In Figure 2, (a) and (c) were used to note the plants rows, respectively (b) and (d) for the intervals between plants rows (paths).

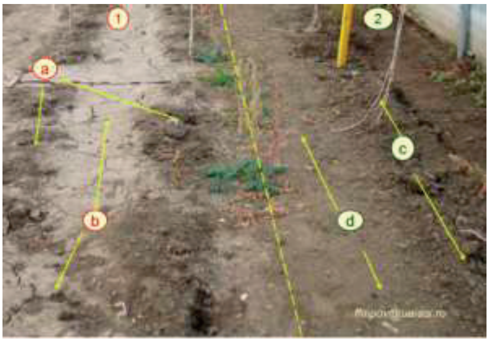

Figure 2. The surface of ground after harvested in Bacau plastic tunnel: (1) is the ground surface after plastic mulch film was used; (a) represents where the rows of plants were; (b) was the interval between plants rows (paths); (2) is the soil surface without plastic mulch film; (c) rows of plants; (d) paths

From pedological point of view, soil compactness state is evidenced by some aspects of soil surface such as the presence of cracks on the smooth surface. The cracks can be seen on rows that were covered with (1) plastic foil (a from Figure 2), also, for covered rows a more pronounced convexities were observed on paths between plants rows (b from Figure 2) than when no foil was used. In garden layouts without plastic mulch film (2) can be seen the greater surface roughness (c from Figure 2) and the absence of the convexity on the interspace between plants rows (d from Figure 2).

Compactness state of soil with medium texture is also evidenced by the distribution of plant roots. The presence of compacted layer, described by plowpan, does not allow penetration of plant roots (Figure 3A-1). In this case, a slows water infiltration occur and favors accumulation of excess water at the base of ploughed layer. In the plowpan there are only few roots, especially on the earthworm channels (Figure 3A-2) or on the path of the cracks (Figure 3B) formed by soil drying. The difference between clod of strong compacted and slight compacted was evidenced (Figures 3C and 3D).

The soil samples, from 0 to 90 cm depths, analyzed in laboratory had medium loam texture with a content of clay range between 21.3% and 27.7%. The soil layers under ploughed layer had values of bulk density higher than density of restricting rooting. Values of packing density range were between 1.46 g/cm^3 up to 1.79 g/cm^3. The limit of packing density value was 1.4 g/cm^3 to separate non-compacted soil by moderately compacted soil and 1.7 g/cm to separate moderately by strong compacted soils.

The aspect of the upper part of soil profile is shown in Figure 4. The lateral development of roots plant (1 from Figure 4) is due to high state of plowpan compactness which prevents the penetration of plant roots or allows roots to penetrate only in the cracks resulted after soil drying (2 from Figure 4). Also, in the strong compacted layer, roots were found (3 from Figure 4).

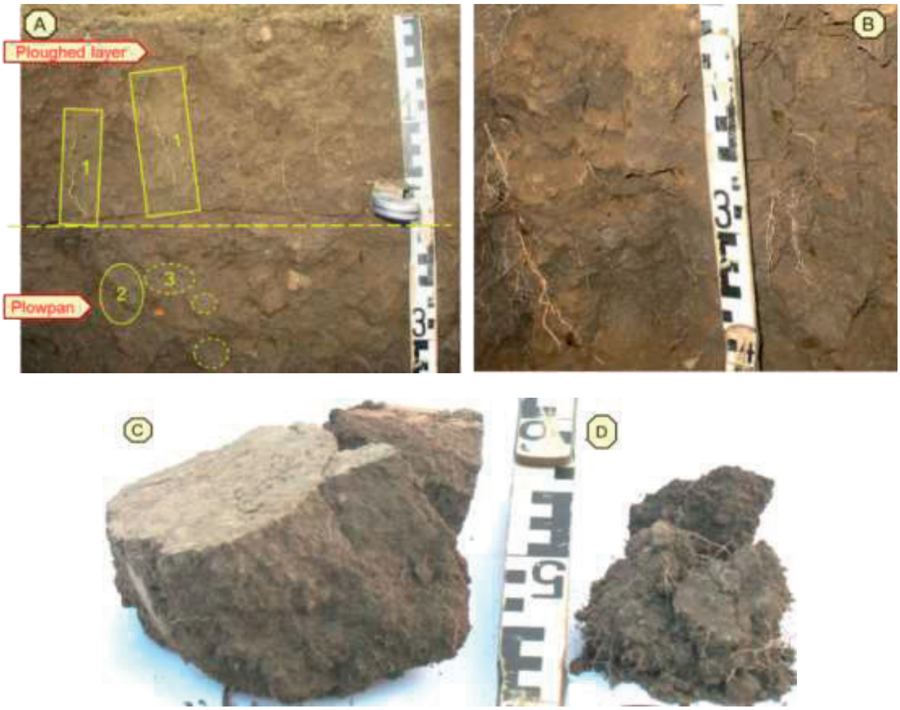

Figure 3. Soil profiles in Bacau plastic tunnel: A - Slight compacted soil in ploughed layer; the presence of plant roots (1) in the ploughed layer and locally earthworm channels (2) & (3) in the plowpan; B - Preferential root distribution in the areas that delineated by cracks; C - Compacted soil from paths; D - Slight compact soil on the plant rows

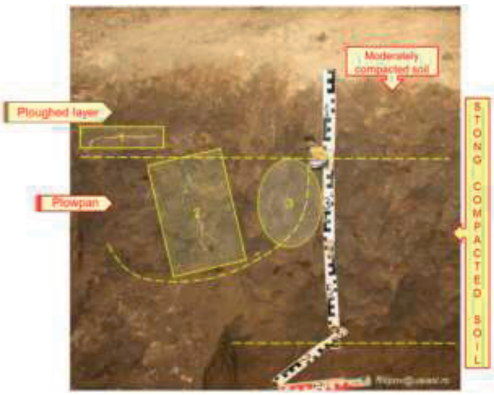

Figure 4. Compactness state of medium texture soil after harvested and removed the mulching foil in Bacau plastic tunnel: 1 - the preferential lateral development of the roots above the compact soil layer; 2 - preferential development of roots on the walls of fine cracks; 3 - inactive roots

In gardening, the water drops from drip irrigation fall on plants row under plastic foil (Figure 5). The wetting pattern shows a bulb in ploughed layer which elongated down in medium texture soil (loam). Furthermore, the plastic foil maintains a temperature higher that in air which favors evaporation of water from moisture surface (Croitoru et al., 2013). Especially in the night, water condense on plastic foil when the dew point is reached and the drops moisten the whole surface of the ground including the paths (Figure 5a). This moisten ant the traffic causes the compaction of ground surface on paths (Figure 5b).

The plowpan act as a barrier against move downward of water because there is a difference in capillarity. When the wetting front reaches at plowpan a lateral extension takes place (Figure 5b). This horizontal moisture under paths has major contributions on soil degradation.

We developed a model based on van Genuchten-Mualem equation which was able to simulated the wetting and drying curves in samples with different compaction (Arrey et al., 2017; Bodale & Stancu, 2020). The relationship between water content and potential by linear semi-logarithmic coordinate (Burton et al., 2015).

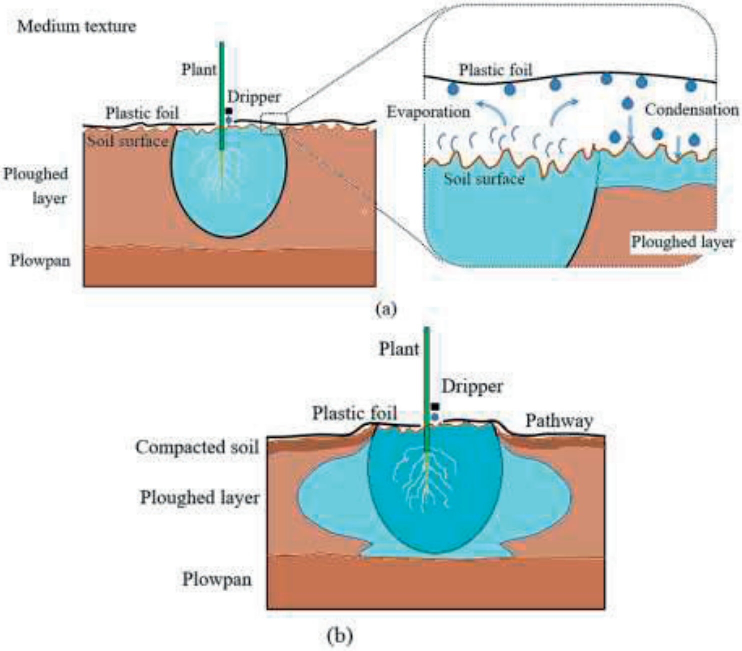

Figure 5. The wetting pattern in medium texture soil with plastic mulch. Evaporation and condensation of water under the plastic mulch foil (a). Lateral water movement caused by plowpan which favors the compaction of soil on paths between plants rows (b)

The simulation of hysteresis loop of aggregated soil from ploughed layer (Figure 6) showed a high water content than in compacted soil (Castendyk & Eary, 2009). This difference is given by variation of the capillary rise when water is move from one layer to another.

The field capacity point is almost the same for the two samples but the slopes of SWCCs of compacted soil decrease slower than aggregated soil. This variation makes difficult the identification of the residual water content point.

The quantity of the water content in aggregated soil is bigger than in compacted soil because the porosity decrease at compaction (Figure 6).

Available water for plant is more clearly defined in aggregated soil where find most of the plant roots.

The morphological indicators that can be used to evaluate the state of soil compactness are represented by the smoothness of the soil surface on the interval between rows of plants, the formation of cracks due to the decrease of soil moisture, the presence of small convexities in the central part of the interval between rows as a result of the decrease of the total porosity of the upper part of the soil, decreasing the frequency of plant roots and preferential distribution of roots on the faces of structural aggregates.

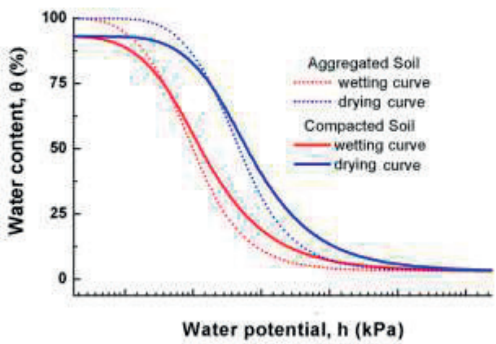

Figure 6. The water hysteresis loops aggregated and compacted soil. Wetting (red shapes) and drying (blue shapes) curves simulated for aggregated (dots line) and compacted soil (solid line)

The local compaction of the under ploughed layer is highlighted, during the growing season, by extending the width of the wet strip on the soil surface. This indicator could be used if the plastic foil does not cover the entire surface of the ground. The mentioned indicators can be easily observed by farmers and use to avoid the soil degradation.

Strong compactness of the soil in the area of the interval between plants rows allows us to recommend avoiding plastic mulch on the intervals between plant rows.

We recommend to use of plastic foil only on the row of plants. This will allow farmers to avoid oversized irrigation by following the wetting strips on the surface of the uncovered soil.

CONCLUSIONS

Plastic mulch on the entire surface of soil from high plastic tunnel favors soil compaction even using drip irrigation. Strong compactness of the soil in the area of the interval between plants rows allows us to recommend farmers to avoid plastic mulch over the whole surface of the plastic tunnel.

Indicators of strong compacted soil, easily distinguishable by farmers, are the smooth soil surface, the presence cracks after soil drying and the presence of pronounced convexity on paths between plants rows.

Plants roots distribution could be used as the additional indicators that confirm strong compactness of the soil.

The slopes of wetting and drying curves simulated for compacted soil decrease slowly which shows that plants extract harder water from compacted soil. In this case, the residual water content accentuates degradation of soil and should be taken into account as a technical indicator of compactness when design the irrigation systems.

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PLANT - SOIL FAUNA INTERACTION - BIOINDICATORS OF SOIL PROPERTIES IN AGROECOSYSTEMS

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Abstract

Agricultural systems have a great diversity of functional traits of plants and soil biota. Invertebrates play a key role in determining soil sustainability and crop health. Many species of invertebrates are important in soil fertility and play a vital role in the production and maintenance of healthy soils. Soil fauna have several roles in the functioning of ecosystems: they influence plant productivity, regulate nutrient mineralization, allow decomposition and act as a buffer. The functional groups of invertebrates in relation to plants are reliable bioindicators, as they provide information on soil quality, ecological services and the functioning of the ecosystem as a whole. The conceptual approach of plant-soil interaction research has shifted from plant strategies to more quantitative approaches using plant-specific functional traits and soil food web characteristics. Because plants use nutrients in inorganic form, they depend on the rate of mineralization in the soil. It has often been assumed that nutrient mineralization is mainly the result of soil microflora activity. The appearance of soil fauna population increases the release of nutrients by fragmenting waste, grazing microflora.

Key words*: agroecosystems, bioindicators, plants, relationship, soil fauna.*

INTRODUCTION

Soils are living environments with a great diversity of plant species and invertebrates. The interaction between plants and invertebrates has an impact both in soil fertility and in the provision of ecosystem services. Soil functions as a favourable habitat for many invertebrate species, and invertebrate communities are involved in geochemical cycles (Lemanceau et al., 2014). Soil benefits from a very rich food web on which many of the terrestrial species depend directly or indirectly (Stork & Eggleton, 1992). Climate and land use changes cause modifications to all ecosystems, including to the structure and distribution of invertebrate communities and species distribution. Some studies predict a lack of self-regulation of soil caused by the disturbances of key links in food webs, caused by rising temperatures, loss of humidity, pollution, etc. In addition to changes in the dynamics of the invertebrate community, changes also occur in terms of functional features of plants (Ulrich et al., 2020). Soil fertility represent its capacity to supply the nutrients (including water) to plants (Culliney, 2013) taking into account the optimal ecological requirements of them (Boháč, 1990). A fertile soil can be defined either on the basis of its own properties or according to the production and productivity of plants. Soil fertility is determined by several physical properties (i.e. texture, structure, profile depth, water retention capacity, drainage capacity), chemical (pH, amount of essential elements available for plants, ion exchange capacity, organic and mineral matter content) and biological (soil organisms, abundance-dominance ratio, intraspecific and interspecific relations) (Chiriac et al., 2020). Crop productivity and soil fertility are steadily declining due to the lack of these essential elements in agroecosystems. Sustainable soil management not only improves crop productivity but also increases soil fertility and sustainability (Khalid et al., 2019). Agricultural systems must sustain stable crops and save energy, inputs and natural resources. This is a goal of new and modern agricultural systems that have very few inputs and are more environmentally friendly providing the expected ecosystem services (Lemanceau et al., 2014). The main purpose of this study is the critical analysis of the literature on the relationship between plant traits and functional groups of invertebrates, a relationship used as a bioindicator of soil fertility in agricultural systems.

MATERIALS AND METHODS

A literature search was conducted using Google Academic, Research Gate and Web of Science. In the present study, no restriction on the publication date was set and we considered original studies and reviews. To identify relevant publications about the relationship between soil invertebrates and plants for the management of agroecosystems, we used different combinations of the search terms "soil" and "soil functions" or "soil quality", "soil health". Other keywords used in conducting the critical analysis of the literature were: springtails and collembola, earthworms, earthworms functions, nematoda, nematoda functions, bacterivores species, fungivores species, agroecosystems, characteristics of agroecosystems, biodiversity role in agriculture, relationship between plants and invertebrates in agricultural systems, invertebrates in agriculture, soil - plants - invertebrates, plant - soil feedback in agriculture, using plant - soil feedbacks, plant and invertebrates as indicators. In this study, we focused only on three groups of invertebrates in the soil: springtails (Collembola), earthworms (Lumbricidae) and nematodes (Nematoda). We analysed 140 scientific papers, but we considered to include in the present publication only 77 papers comprising our interest.

RESULTS AND DISCUSSIONS

Due to the accelerated growth of soil degradation in recent decades, it has been necessary to identify methods to define not only soil quality but also, in a holistic approach, soil health (Menta & Remelli, 2020). Soils are living environments in which particularly abundant and diverse microbiome and fauna are involved. The resulting biological functioning has a direct impact not only on soil fertility but also on a series of ecosystems services (Lemanceau et al., 2014). Soil is one of the most heterogeneous ecosystems on the planet. It plays an irreplaceable role in the biosphere: it governs plant productivity and allows organic matter degradation and nutrient cycles (Santorufo et al., 2012). Soil quality represents the ability to function within the limits of the ecosystem, to support crop and animal productivity, to maintain or enhance environmental sustainability, and to improve human health worldwide (Yang et al., 2020). Invertebrates in soil can be defined as all the ontogenetic stages of organisms that live permanently or temporarily in the soil or on its surface. Soils provide goods and services (production services) that the human population can benefit (Lavelle et al., 2006). Soils provide goods and contribute to ecosystem services, having important functions in the supply of food and fiber (Cornu et al., 2020). It functions as a support for agro-forestry-pastoral ecosystems. It also has a role in regulation services (climate regulation by controlling the flow of greenhouse gases, carbon sequestration, flood control). It also contributes to cultural services, but in a lesser extent due to society's low interest in the sustainable use of this key resource (Lavelle et al., 2006). Soil invertebrates can play two roles in the soil food web: shredding and wetting ingested plant debris thus improving the substrate for bacterial decomposition and ecosystem engineers (organisms that physically modify the habitat by establishing the availability of resources for other species) (Culliney, 2013). Soil invertebrates have a great importance in soil quality and considering their size, can be divided into microfauna (nematodes in general), mesofauna (not large enough to change soil structure; mites, collembolans, enchitraeids, small diplopods) and macrofauna which change the soil structure by their movements (isopods, eartworms beetles, dipterans, ants). The advantage of studying these organisms is that they benefit from a great diversity, appear in large numbers, are easy to take in every season (Stork & Eggleton, 1992; Behan-Pelletier, 1999; Manu et al., 2015). All these groups of soil fauna presented above have an essential role in the soil processes. They improve microbial activity by accelerating decomposition and mediating soil transport processes (Stork & Eggleton, 1992; Stone et al., 2020). Soil invertebrates are critically important for human well-being, contributing to soil function by regulating key ecosystem services such as litter decomposition, nutrient cycling, plant nutrient uptake and climate regulation such as CO_2 fluxes (Bastida et al., 2020).

Invertebrates in the soil, taxonomy and ecology

Individual species have functional traits than can be grouped together to form functional groups. Functional groups create the biophysical traits of ecosystems, through interactions between ecosystems, communities and individual species traits (Zoeller et al., 2020). Regarding the ecology of functional group of invertebrates, they have a global distribution depending on certain factors. Abiotic factors, as altitude and latitude are the most important, determining both the composition and the structure of the trophic networks of invertebrates. In addition to these aspects, anthropogenic activity has a major impact; for instance, low concentrations of heavy metals are not toxic for plants, invertebrates and animals, but they are indispensable for multiple soil functions (Stork and Eggleton, 1992; Manu et al., 2017; Inobeme, 2021). Many soil functions (litter decomposition, nutrient cycling) are influenced by the relationship between the biological activity of soil non-vertebrates and the processes above the soil. Moreover, invertebrates are also used as soil quality indicators (Lavelle et al., 2006; Fiera et al., 2020). The majority of invertebrate groups are sensitive to environmental changes. They present a fact response to soil management and are influenced by environmental characteristics: refuge, breeding or feeding habitats. For this reason they function as a tool for monitoring the quality of the environment (Paoletti et al., 2009; Manu et al., 2019; Manu et al., 2020). Some soil invertebrates can be used as indirect indicators (for the entire soil community), as well as direct indicators (for the provision services of soil ecosystems) (Domínguez et al., 2018).

Collembola (springtails)

Collembolans play an important role in decomposition process. Larger species accelerate the mineralization process while smaller species help the soil humidification (Stork & Eggleton, 1992). Some characteristic Collembola species had been identified in agroecosystems where they accelerate decomposition, influence microbial activity and nutrient cycling. Phytophagous species influence the distribution of highly mobile metals (i.e. potassium) and detritivore species accelerate the mineralization rate of less mobile elements (i.e. phosphorus and calcium) (Mulder, 2006; Fiera et al., 2020). Low numbers of collembolans in an ecosystem stimulate the growth of bacterial activity, but when they multiply excessively they affect fungal populations and thus can reduce the process of soil humidification (Stork & Eggleton, 1992). Springtails are known to have a great impact on above and below ground ecosystems (Baird et al., 2019).

Lumbricidae (earthworms)

The role of earthworms in agricultural ecosystems is also increasingly recognized (McTavish & Murphy, 2020). Soils in which earthworms are present have many properties: larger volume pores, higher water retention capacity and infiltration rate and higher nutrients retention rate (Stork & Eggleton, 1992). Earthworms (also termites and ants) have been identified as the most important soil engineers (Spurgeon et al., 2013). In essence, "soil engineer" means the ability of organisms to build organo-mineral structures with certain physical, chemical and microbiological properties through their movements. These structures can be of several types: casts, mounds, fungus-comb chambers and so on (Jouquet et al., 2006). Earthworms can actively or accidentally ingest seeds as they move through the soil (McTavish & Murphy, 2020). Thus, they have an important role in the processes that take place in the soil establishing its quality, influencing the decomposition of organic matter, nutrient cycles, soil structure, and being key species in food webs (Ezeokoli et al., 2021).

Nematoda (nematodes)

Nematodes are the most abundant organisms. Like the other invertebrates, they provide many ecosystem services and maintain the stability of food webs by ensuring the nutrient cycles (Neher, 2001; Zhang et al., 2020). Nematodes are heterotrophic, primary consumers (parasites of plants), secondary consumers (predators) and consumers of decomposers (bacterivores and fungivores) (Wasilewska, 1997; Bonkowski et al., 2009). Nematodes are biological components of multiple functional groups of the soil food web, and can be used as indicator of ecological processes (the maintenance of nutrient cycling and soil suppressiveness against pest or invasive species) (Sánchez-Moreno & Talavera., 2013).

The main function of bacterivores in the food web is to improve the mineralization of immobilized nutrients in organisms contributing more than 80% to nitrogen mineralization (Ferris et al., 2004). Bacterivorous nematodes represent the first group of invertebrates reacting to environmental changes; for example the introduction of a quantity of nitrogen can change this group structure in 24 hours (Wasilewska, 1997). The increase in the number of fungivores offers information about an augment in soil acidity caused either by the excessive use of mineral fertilizers or by an acid rain. For this reason, nematods are considered a good indicator group that can highlight changes occurring in soil characteristics. The increase in the abundance of plant parasites is correlated with the processes of environmental damage caused by excessive use of nitrogen-based fertilizers, intensification of agricultural activities, contamination by acid rain, drying of swampy soils. Predatory nematodes represent the highest trophic level in the soil microfauna. The potential to use this group as a bioindicator is low due to low numbers of individuals, especially in agricultural lands. Increasing the abundance of predatory nematodes may be an indicator of an unaffected environment (Wasilewska, 1997). In food webs, the basal entities usually are not biological species but feeding groups (guilds, trophic species) consisting of groups of species which are assumed to be functionally equivalent (i.e. depending on similar prey and therefore exert similar top-down forces on prey populations) (Scheu, 2002).

The characteristics of an agroecosystem

An agroecosystem consists of land cultivated with cereals or other plants that depend on fossil fuels and agricultural products. Agroecosystems totally depend on human input and are functional units of the man-made biosphere (dos Santos et al., 2021) covering as much as a quarter of the global land (38% of global land use) located near human settlements (Rapidel et al., 2015). These simplified systems with low diversity and simplified trophic network (mono-specific crops) may be more complex systems (pastures, poly-specific crops and agroforestry systems) with the ability to support greater biodiversity (Estrada et al., 2012). Agricultural ecosystems are anthropogenic systems (ecological systems modified by humans), their origin and maintenance are associated with the activity man, who transformed nature to obtain mainly food and fibres (Altieri, 2002; Sans, 2007) and other agricultural products (dos Santos et al., 2021). Agricultural systems also produce other goods and services essential for human beings (climate regulation, flood protection) (Rapidel et al., 2015). In agroecosystems, soil health can change due to anthropogenic activities, such as intensive cultivation practices and land use management, which can have an additional impact on soil functions (Manu et al., 2018; Yang et al., 2020).

Soil functions important for agro-ecosystems

The nature of the soil is determined by its chemical, physical and biological properties. It plays a key role in setting growth, productivity and reproductive success of plants (van der Putten et al., 2013). The quality of a soil can be defined as its metabolism, the ability to support plant growth and animal productivity, to maintain or improve water and air quality (Maikhuri & Rao, 2012), participates in nutrient cycling through mineralization, has a role in energy transfer and functions as a buffer (Neher, 2001). The function of soil production has long been recognized but others that support the provision of ecosystem services such as water purification, pathogen control, nitrogen fixation and biodiversity conservation have been discovered more recently (Maikhuri & Rao, 2012).

Soil invertebrates can participate both directly and indirectly in processes of decomposition and its genesis and can influence soil characteristics (porosity, aeration, fertility, infiltration) (Manu et al., 2019). Soil quality determines the sustainability of agriculture and the quality of the environment in general (Maikhuri & Rao, 2012).

Soil food web

A food web is a map of the feeding relationships between species in which energy flows and dynamics are highlighted. The trophic level represents the energy flow that occurs when one organism feeds on another organism (Walter et al., 1991; Neher, 2010). The structure of trophic interactions determines the sensitivity of food webs to perturbations, but rigorous assessments of plant diversity effects on network topology are lacking (Giling et al., 2019).

The food web can also be defined as an ecological network whose nodes are the species in the ecosystem and in which the links are the prey-predator relations. The direction of the connection represents where the energy comes from and where it is going. This network illustrates the complete architecture of the ecosystem functioning as a whole (Conti et al., 2020). The researches on the plant-invertebrate relationship in soil has changed to a more quantitative approach that uses functional features of the species and characteristics of the food web related to the provision of ecosystem services (Mariotte et al., 2018). An important step in this approach is the inclusion of energy flow between species in the food web (i.e. the construction of an energy flow network) (Scheu, 2002). The flow of the energy network can provide clues about the control between species (prey - predator). Some invertebrate species are flexible in their diet. It is difficult to place a certain species in a certain trophic category (Scheu, 2002). Depending on the resources available, some invertebrates (i.e. mites) can eat algae, fungi, detritus or other invertebrate species (Scheu, 2002; Manu et al., 2013). Plants and organic waste provide habitat for soil invertebrates. Plants influence the soil biota directly by providing organic matter and indirectly through the physical effects of shading, soil protection, water and nutrients supplies. Plant energy and nutrients are incorporated into the detritus that provides the resource base of a complex food web (Neher, 2001). Soils host an unprecedented diversity of organisms that are interconnected through numerous trophic bonds and complex trophic networks (Brose & Scheu, 2014; Erktan et al., 2020). In the soil, the basic structure of the food web contains primary producers, consumers and detritivores. The number and biomass of organisms are correlated with the volume of soil and decrease from the bottom up in the food web. Unlike surface networks, soil networks have a longer length and complexity (Neher, 2001). In communities with many species as in the case of those in the soil, the formation of trophic groups is very likely to happen (Scheu, 2002). Trophic interactions are essential for soil functioning and are considered key factors in biogeochemical cycles. Trophic interactions play a major role in waste decomposition and the C and N cycles (Erktan et al., 2020). Mesofauna is found in all levels of the soil trophic network and directly influences both primary production by feeding the roots and indirectly by their contribution to the decomposition and nutrients mineralization (Neher, 2001). Although mites are essential in the food web, enchytraeids are probably "key elements" for the decomposition process. All of these invertebrates are dependent on each other and compete with each other (Mulder, 2006; Manu et al., 2016). Soil organisms play an important role in the dynamics of the above-ground community and the functioning of the terrestrial ecosystem. However, most studies considered soil biota as a black box or focused on specific groups, while little is known about entire soil networks (Morriën et al., 2017).

Plants - invertebrates - soil triangle

Many functions of the ecosystem are based on interactions between primary producers and other trophic levels, such as pollinators, soil decomposers and herbivores. For example, the nitrogen cycle involves complex interactions between plants and soil biota and between plants and herbivores (Moretti et al., 2016). Recent reviews reveale the genetic basis to plant–soil linkages that are critical to demonstrating their evolution due to plant - soil feedback (PSF). Considering plant-soil linkages only at plant species level, had been demonstrating that their feedbacks influence many aspects of plant communities, including invasion by exotic species, plant competitive interactions, and successional dynamics (Schweitzer et al., 2018). PSF can bring improvements to plant succession, invasion and coexistence, processes that have consequences on the nutrient circuit and other ecosystem services (van der Putten et al., 2016). Based on oscillations of structural and chemical root traits, it is demonstrated that plants can accumulate rhizosphere specific soil communities', including mutualist groups, (mycorrhizal fungi and plant growth-promoting bacteria), and antagonist groups (microbial pathogens and nematodes). These organisms affect the performance of their hosts and neighbouring plants, but also of subsequent generations of plant individuals through a mechanism known as plant-soil feedback (Wilschut & Kleunen, 2021). The underground and aboveground communities have most often been studied separately, but lately the vision has changed to a more integrative and holistic (Scheu, 2003). Researches on food webs have been focused only on "who eats whom?" as feeding relationships, but no attempt has been made for extensive studies of the entire network and functional traits of the species as network nodes (Moretti et al., 2016). Soil fauna has an essential role in the homogenization and decomposition of plant residues in the soil, which changes the physical and chemical composition and accessibility of organic matter (Bray & Wickings, 2019). The roots have several roles: anchorage the plant in the soil, transfer nutrients from the soil to the upper parts through the roots, nutrient storage, etc. For this reason they are attacked by herbivores. To defend themselves, they developed a whole arsenal of direct defence compounds, such as terpenoids, as well as indirect tools, involving communication strategies to interact with soil fauna, soil microorganisms and other plant roots to prevent attack (Bonkowski et al., 2009). Detritivorous species can alter the food web, providing large amounts of nutrients through decomposition, accelerating plant growth and performance. There are several mechanisms through which they can do this: mineralization of organic matter, stimulation of microbial activity, pest control and changes in soil porosity (Scheu, 2003; Johnson et al., 2011). Soil invertebrates affect directly or indirectly the decomposition of organic matter, maintain soil structure and can exert a direct influence on plant communities through selective feeding on roots, leaves or seeds (Cifuentes-Croquevielle et al., 2020). Changes in the density and composition of species of mites affect the rate of transformation of soil organic matter and soil fertility (Manu et al., 2018). By affecting plant features, the effect can cascade to higher trophic levels and affect the functions of the above-ground ecosystem (i.e. plant productivity and resistance to pests) (Wurst et al., 2018). Underground communities regulate plant growth and community composition; plants themselves regulate the quantity and quality of resources available for soil biota (Wardle et al., 2004; Wurst et al., 2018). Plant species and their composition change local environmental conditions: light, temperature, soil moisture and chemical quality of the substrate. Plant diversity is positively correlated with invertebrate diversity (Cifuentes-Croquevielle et al., 2020). Plants can influence soil and its properties by the input of chemical compounds and organic matter, by impact on hydrological processes as well as by ensuring habitats and/or resources for microscopic and macroscopic organisms (van der Putten et al., 2013).

Functional features of plants have a strong effect on the rate of soil communities' decomposition. Consequently, the composition of plant species and soil communities are closely linked; any change in plant species communities lead to changes in soil communities and viceversa (Cifuentes-Croquevielle et al., 2020). The functional features of plants influence soil organisms and the functional features of soil organisms influence the direction and power of plant feedback (Mariotte et al., 2018). The approach based on functional features has extended to the study of plant-soil relationships (Bardgett, 2018).

Differences in soil invertebrate communities could be explained, at least in part, through the soil properties, such as soil nutrient content and water infiltration rates, thus revealing a functional link (Cifuentes-Croquevielle et al., 2020).

Using the plant - soil feedback (PSF) relation as a bioindicator

The experience gained from natural systems where there is a great diversity of plant and invertebrate species supports a sustainable management necessary in agricultural systems (Mariotte et al., 2018). Bioindicators are taxa or functional groups that reflect the state of the environment (Manu et al., 2021). They are divided into few groups: *environmental indicator* - reacting rapid (early warning) at any change in the local environment and indicate levels of taxonomic diversity in a site; *ecological indicator* - monitoring a particular ecosystem stress. Invertebrates are commonly used as bioindicators and may accurately reflect certain environmental trends (Borges et al., 2021). Invertebrates, as bioindicators of biodiversity, can reflect trends in species richness and community composition more accurately than vertebrates, having a simpler body structure, being more diverse and abundant. Invertebrates can be used as bioindicators due to their small size making them sensitive to local conditions, while their mobility allows them to move in response to changing conditions. It is also important that they have a short life cycle. Because invertebrates are easy to collect and are very abundant, they are ideal for studies focused on species richness (alpha-diversity), species cycling (beta-diversity) and on comparison of different communities from many ecosystems using similarity indices (Moscatelli et al., 2005; Gerlach et al., 2013).

The main factors that influence PSF are: soil microbial pathogens, herbivorous nematodes, insect, other invertebrate larvae, mycorrhizal fungi, non-mycorrhizal endophytic fungi, endophytic bacteria, nitrogen - fixing microorganisms and decomposers. They can influence plant growth directly and indirectly by influencing the physicochemical properties of the soil, such as pH, organic matter content, water retention capacity, soil temperature and structure (van der Putten et al., 2016). Earthworms influence plant growth through the physical, chemical and biological changes that they produce in the soil. Scheu (2003) reviewed more studies and over 75% of them showed an increase in plant biomass in the presence of earthworms. The author mentioned that earthworms influence plant productivity through both direct and indirect effects. Direct effects include, for example, plant root feeding and seed transport, while indirect effects include impact on soil structure, mineralization processes, dispersal of microorganisms and hormone-like effects (Wurst et al., 2018). Although there are many reasons to use invertebrates in different studies that highlight the plant-invertebrate relationship, also there are some disadvantages (i.e. taxonomic challenges: relatively small proportion of species are known or described taxonomically) (Gerlach et al., 2013).

Limits and future research perspectives

With all these aspects presented above, there is still a lack of an explicit understanding of the mechanisms that regulate the relationship between plants - invertebrates - soil, especially in the context of global environmental changes. Solving these problems will improve the predictions about the changes that await us in the future (van der Putten et al., 2016). The mechanisms that manage the relationship between biodiversity and ecosystem functioning are still poorly understood, despite growing evidence of the importance of trophic interactions. Plant chemical composition and the soil community are known to influence litter and soil organic matter decomposition. Although these two factors are likely to interact, their mechanisms and outcomes of interaction are not well understood (Carrillo et al., 2011). There are scarce studies considering multitrophic systems taking into account species diversity rather than functional components of biodiversity (Moretti et al., 2016); the ecosystem services provided by a wide range of organisms whose effects are still relatively little explored, especially for smaller taxonomic groups. There are limited knowledge about soil nematodes, largely due to their microscopic size, distribution, and trophic variation (Lavelle et al., 2006). Few expertises yet exist about the complex interactions of the community and the trophic relationships between soil organisms. The use of plant-invertebrate soil feedback improves agricultural sustainability, increases resource efficiency, reduces the amount of applied fertilizer and fights against pests and diseases (Mariotte et al., 2018). A quantitative and explicit analysis of the physical structure of the soil in the ecology of the soil food web is needed. An interdisciplinary study opens the perspective to understand the structure and functioning of underground systems allowing a more holistic understanding of terrestrial ecosystems (Erktan et al., 2020). Soil biodiversity represents a major part of global biodiversity, but its drivers, threats, and possible future changes are still untapped and not well understood (Phillips et al., 2020). Another big question in soil ecology is whether the food web of an ecological system is regulated by resources (bottom-up) or predators (top-down) (Neher, 2010). Although there are a large number of publications that highlight aspects related to PSF, few of them have been focused on agricultural systems. Trait-based crop rotation could improve the efficiency of land use and thereby promote sustainable agriculture by reducing the excessive use of fertilizers and pesticides (Mariotte et al., 2018). Further future research is needed on the functional features of plants that could expand knowledge about how plants adapt to declining invertebrate density within and between species and how it can shape ecosystem functions (Ulrich et al., 2020). To predict the consequences of global change on multitrophic interactions in terrestrial ecosystems, more studies are needed. Another research challenge is controlled or small-scale laboratory studies that have shown that soil macrofauna, such as earthworms, can mobilize or transfer substantial amounts of nutrients to plants. One limitation is that the information we have about key organisms or soil biota groups that contribute to the nutrient cycle and crop production in different sets of management practices is limited. Future research should focus on their impact in a broader context, for example at the level of the agricultural field. Regarding the growing demand for sustainable agricultural practices, more knowledge is needed on the functional role of soil macrofauna in agroecosystems (Wurst et al., 2018). The challenge is to conduct field studies to show where, when and how PSF influences the real world to explain and predict community and ecosystem responses to a constantly changing world (van der Putten et al., 2016). The need to discover new methodologies to characterize and evaluate the performance of agroecology in a holistic way is a reality and a challenge for all, especially due to the multidimensional nature of agroecology (Petersen et al., 2020).

CONCLUSIONS

For a better understanding of the plant-soil invertebrate relationship, it is necessary to detail: the role and importance of soil invertebrates, the interactions and synergistic effects of soil invertebrate populations on specific diversity and biomass of soil surface plants, and the influence of plant species on the diversity and biomass of soil invertebrates. Also, the structure and functions of invertebrate communities are of particular importance because they can vary considerably, both inside (i.e. in urban areas: lawns, gardens, open land, green roofs) and between different habitats. The soil invertebrates communities can be also used as assessment and prediction tools of ecosystem services. The diversity of soil invertebrate is influenced by the specific diversity of soil surface plant communities, soil type and natural or anthropogenic environmental factors. In terms of human well-being, soil invertebrates are very important because they contribute to soil functions by regulating key ecosystem services: litter decomposition, nutrient cycling, nutrient uptake by plants and climate regulation (CO_2 flows).

In agriculture, the result of the expected research will lead to: reducing the artificial entry of nutrients into the soil (reducing the use of chemical fertilizers), increasing the availability of nutrients; increasing crop resistance to agricultural pests, increasing crop production and productivity. Success in healthy development of a crop requires knowledge from farmers preparing the soil, selecting nutrient inputs, planting the crops, managing the crops from emergence to harvest. The farmers should use the basic principles of soil fertility and plant nutrition that change over time, for instance, procedural practices should adapt to new products, the system of crop management procedures and plant genetics.

An accentuated economic problem in recent years is represented by invasive species and the result of our research might contribute to the development of new methods for eradicating them.

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RESEARCH OF CO_2 EMISSIONS IN THE REPUBLIC OF MOLDOVA AND IN SOIL UNDER DIFFERENT FIELD CROPS

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Abstract

The research object of this study is the agricultural soils of the Republic of Moldova. From an economic point of view, they are attributed to the field of phytotechnics and soil resources in the agricultural economic sector. Greenhouse gas emissions (GHGE) from agriculture have three major sources of origin: enteric fermentation, manure management (both in the livestock sector) and agricultural soils (in the sector of plant and soil resources). The 2010 year was determined as the reference year for projections of CO_2 emissions/seizures from agricultural soils in the Republic of Moldova. For modelling future emissions, the results of national inventory of GHG emissions for 1990-2012 were used as a basis. The projections of CO_2 emissions from groundwater were developed for 2015, 2020, 2025 and 2030 after some scenarios (IPCC, 2016). With respect to green fertilizers (autumn vetch as an intermediate crop), the following basic parameters were taken into account: average green weight - 80%; average nitrogen content in the green mass - 0.8%; average productivity - 20 t/ha; 1.4 (or, in other words, 1 tonne of green lemon meal equivalent to 1.4 tonnes of manure). It is planned to sow autumn meadows as an intermediate crop used as a green fertilizer, and crop rotation will be as follows: autumn wheat or autumn barley - vetch as intermediate crop - corn or sunflower. The introduction of intermediate crops as a green fertilizer will be carried out in parallel with the implementation of the farming conservative system ("No-Till" and "Mini-Till"). Changing the use of agricultural land and soil management practices can greatly influence the organic carbon reserves in the soil. Carbon of organic origin and nitrogen are closely related to the organic matter (humus) content of the soil. Carbon leakage through the oxidation process due to land use changes and soil management practices are accompanied by the co-mineralization (biochemical decomposition) of the humus nitrogen. In the case of soil carbon losses, mineralized nitrogen is considered as an additional source of nitrogen available to convert to direct GHG emissions.

Key words: *crop rotation, greenhouse gas emissions, soil carbon leakage, Republic of Moldova.*

INTRODUCTION

The carbon cycle has a decisive role in the global changes in the environment with which the rest of the cycles are closely linked, as well as the climate-dependent climate change.

The carbon cycle in terrestrial systems is determined by the balance between the carbon dioxide stored in the vegetal carbon and the amount of CO_2 emitted mainly through the respiration process of the soils. Soil respiration is the most important source of CO_2 and other greenhouse gases. This can be illustrated by the fact that only 10% of total CO_2 emissions are responsible for industrial CO_2 emissions, while the rest of the bio-systems, with the predominance of soils, is 90% (Заварзин, 1993).

Organic matter, stored in humus and dead biomass in the planet's soils, contains three times as much carbon as all terrestrial vegetation (Sundquist, 1993). Each year soil releases 4-5% of its carbon in the atmosphere by transforming organic matter into CO_2 and other compounds due to biochemical mineralization processes.

Soil utilization and exploitation in agriculture contributes to accelerating processes of decomposition of accumulated organic matter and consequently to carbon loss. This process has a universal character and occurs at increased rhythms, especially in the early years after land reclamation. The soils of Canada after 100 years of exploitation lost 25% of the original carbon (Jauzen et al., 1998).

This phenomenon is characteristic for other countries as well as for the Republic of Moldova. Research carried out by various authors has shown that chernozems of Moldova 100 years after recovery have lost as much as 25% of the CO_2 - accumulated carbon dioxide (Крупеников, 1967, 1989; Загорча, 1990) as well as Canada's soils. It is obvious that human activity related to the exploitation of fertility contributes to a large extent to the wider opening of the most important carbon deposits on the terrestrial surface, represented by the organic matter of the soils. Changes that take place in the carbon circuit under the influence of human activity have many aspects, including the greenhouse effect.

The United Nations Framework Convention on Climate Change only takes into account climatic changes that are directly or indirectly conditioned by human activity through global changes in the atmosphere. From this point of view, the appreciation of CO_2 emissions in agricultural land is of particular importance due to the enormous amount of carbon stored in the humus of the soils that undergo the essential transformation under the anthropic factor.

To achieve this goal, it is important to make a clear target of total CO_2 emissions from soils resulting from soil respiration and emissions that contribute to climate change by increasing the CO_2 content in the atmosphere. Here, it must be taken into account that the soils cover is an open thermodynamic unit, the balance of which is determined by the amount of accumulated indoor energy, the trophic links in the soils and the biogenetic circuit. The dominant factor determining the functioning of this system is the carbon flux.

In steady state, the amount of carbon released from the soil through ”output” CO_2 emissions is equal to the amount of carbon (stored) "input" carbon. In cases where the "entry" becomes steadily less than the "exit", the soil balance deterioration occurs resulting in its degradation and fertility decrease. The amount of carbon captured (deposited) in the soil ”input” depends on the mass of organic matter produced by photosynthesis, and the amount of carbon lost "output" is determined by the intensity of respiration of the soil biota.

The dynamics of input and CO_2 emissions in soils changes according to external factors such as temperature, humidity, biogenic ratio, environmental response, etc. External factors change your microbiological activity level, but initially the value of this process is actually a function of the amount of carbon entering the soil.

Soils of natural phytocoenoses are characterized by a high bioenergetic level and are still in a steady state due to equal amounts of organic matter consumed and entrained. Therefore, CO_2 emissions from soils occupied by natural phytocoenoses are compensated for by carbon stored in the soil as a result of biochemical processes of humming of dead vegetation.

This is very important for the correct assessment of the changes in human activity in the balance established in the untapped natural soils and the consequences related to the greenhouse phenomenon.

Multiple researches carried out abroad and in the Republic of Moldova found that the exploit-tation and exploitation of soils in agriculture necessarily lead to the gradual decrease of the microbiological activity of the soils and consequently to the reduction of the CO_2 emissions from the agricultural lands (Бабьева et al., 1989; Лебедев, 1988; Cornfield, 1961; Herrmann, 1993; Witkamp, 1966; Мехтиев, 1961, 1963; Маринеску, 1992).

Based on this postulate, in fairly frequent cases, wrong conclusions are made regarding the contribution of agricultural land to the greenhouse effect. The error lies in the fact that the demining of CO_2 emissions from soils harvested in relation to those covered with natural vegetal carpet is interpreted directly, without taking into account the second part of the carbon circuit - the quantity returned to the soil “input”.

Due to the fact that the “input” of carbon into the soil with vegetal residues is affected from a qualitative and quantitative point of view in a much larger proportion of the land, a negative carbon balance is established.

Namely, the value of the CO_2 balance in arable soils considered their contribution to the increase in the atmosphere of this gas and its contribution to the phenomenon called greenhouse effect.

MATERIALS AND METHODS

Until now, no valid method has been developed to determine greenhouse-gas emissions from arable land that could be applied on large areas. Research carried out in the Republic of Moldova and other countries determined that the CO_2 emissions in the soils used are 2.0-2.5 times lower than those covered with natural vegetation.

Hence, agricultural land, unlike land with natural vegetation, is characterized by a negative carbon footprint, which is why it can be seen as a source of CO_2 with the contribution to greenhouse effect and climate change.

Measuring CO_2 emissions from agricultural soils that lead to anthropogenic interference in the atmosphere is of particular importance for predicting climate change in view of the enormous amount of carbon stored in the organic matter of the soils.

From a methodological point of view, this is very difficult because the carbon circuit in agrofitocenoses is influenced by multiple natural and anthropogenic factors, often very variable in space and time.

In the country and abroad literature, a large amount of data on soil respiration has accumulated according to the most diverse natural and anthropogenic factors, but there is virtually no research that would highlight carbon emissions that remain uncompensated.

Here we have to mention that a universal method in this sense usable for all possible cases cannot be elaborated because the factors that influence the carbon circuit are multiples and quite frequently have a regional or even local character.

On a major scale the problem is reduced to solving two main tasks. The first is to give a satisfactory assessment of soil carbon losses over a certain period of time, and the second is to determine the amount of carbon returned to the soil within the same time limits.

The end result of the processes that influence the carbon circuit is reflected in the changes in soil humus content over time and also gives the answer to the question of greenhouse gas emissions. It is also worth mentioning that changes in humus in the ground where carbon is deposited are slow, that the changes produced can be measured significantly with a long period of time (5-10 years). An additional problem in this respect is the natural variation of the organic substance in the soil cover, which often exceeds the changes found in periodically harvested samples.

The extensive use of the method based on direct assessments of carbon content change in soils for the purpose of measuring greenhouse gas emissions is limited by two key factors. The results obtained are valid only for areas with uniform soil cover. Large areas (the arable land area of a country) would require an enormous number of such measurements, which is impossible for economic reasons even for well-developed countries.

The method is similarly appreciated by Canadian researchers, although this country has a network of 15 000 polygons that carries out carbon monitoring in soils (Jauzen et al., 1998).

The possibility of using the nitrogen exported from the soil to the agricultural plants for the appreciation of the humus consumed was based on the academic I.V. Tiurin (Тюрин, 1965), then the idea was concretized by A.M. Lâcov (Лыков, 1979). Consideration has been given to the close link between CO_2 emissions and the amount of nitrogen released from soils as a result of the biochemical decomposition of organic matter. The content of carbon and nitrogen in humus is stable, with slight variations within the pedogeographic areas. In the soils of Moldova, the ratio of carbon and nitrogen in humus is equal to 10.7, ranging from 10.1 to 11.3 (Крупеников, 1967; Крупеников et al., 1984). This ratio is characteristic of the upper soil layer and slightly decreases to greater depths.

The elaborated methodology follows the purpose of assessing the greenhouse gas emissions from agrofitocenoses taking into account the agricultural lands of the Republic of Moldova. Data from the universal and local scientific literature, including recent information (Rusu et al., 2005; Унгурян et al., 1997; Боинчан, 1999), were used in the development.

The field works were carried out according to the methodology of pedological field research.Laboratory analyzes were performed according to classic methods and GOST.

The carbon balance is determined for the area occupied by each crop.

$$B \pm = (V - C) * S \qquad (1)$$

B - carbon balance;

V - carbon entering the soil by humification of vegetable residues and organic fertilizers;

C - carbon released from the soil through CO_2 emissions as a result of humus mineralization;

S - the area occupied by the crop, ha.

The amount of carbon entering the soil (V) is determined according to the equation:

$$V = V1 + V2 \qquad (2)$$

V1 - carbon entering the soil with vegetal remains;
V2 - carbon entering the soil with organic fertilizers.

The amount of carbon entering the soil with vegetal debris (V1) is equal to the result obtained from the multiplication of the basic crop with the accumulation and humification coefficients of the vegetable residues divided by the coefficient of 1.724 for the transition from humus to carbon. For this purpose, the data in the annex is used.

The amount of carbon entering the soil with the applied organic fertilizers (V2) is equal to the result obtained from the multiplication of the dose with the respective humification coefficient (appendix) and divided by the coefficient 1.724 for humus to carbon.

The sum of the results (V1 + V2) considered your carbon bound (entrained) by the soil into humus (V).

The amount of carbon released from the soil is estimated by the equation:

$$C = [Er - (Em+Eo+ Ev+ Es)]\cdot r_1\cdot r_2\cdot 10.7 \qquad (3)$$

Er - the amount of nitrogen exported with the crop production (main and secondary) is determined by multiplying the main crop of the crop by that coefficient in the Annex;
Em - the amount of nitrogen exported from the chemical fertilizer account. It shall be determined taking into account the amount of fertilizer applied, the nitrogen content of the fertilizer and the nitrogen content of the fertilizer (Annex);
Eo - the amount of nitrogen exported from organic fertilizers. Determine in the same way as nitrogen from chemical fertilizers, using the appropriate information in the Annex;
Ev - the amount of nitrogen used in plant debris. It is determined, taking into account the calculated mass of vegetable residues, the nitrogen content of the vegetal debris and the coefficient of its use (Annex);
Es - the amount of symbiotic nitrogen exported from the soil. Depending on the harvest, the nitrogen fixation coefficient and its use for harvesting shall be determined in accordance with the Annex;
r_1 - the coefficient expressing the dependence of humus mineralization on the soil granulometric composition (appendix);
r_2 - the coefficient expressing the dependence of the cultivation of humus mineralization (appendix);
10.7 - the nitrogen passage in carbon.

RESULTS AND DISCUSSIONS

The imbalance in agrofitocenoses between soil mineralization processes and those responsible for the synthesis of humus is well demonstrated by the data presented in the fundamental work “The Basics of Soil Science”, the author of which is V.A. Covda (Ковда, 1973). In natural steppe phytocoenoses annual phytomasium production accumulates 10 t/ha of carbon, of which 1.04 t/ha is stored in soil humus as a result of humification processes. In agrofitocenoses, the amount of carbon in the crop production reaches only 2 t/ha, of which 0.16 t ha is stored in humus. The author believes that under specific conditions these data may vary widely, but the difference between natural and agricultural phytocoenoses regarding the ratio between loss and accumulation of carbon in the soil is preserved. The annual amount of carbon stored in the humus "input" in natural phytocoenoses is 6.5 times higher than agrofitocenoses.

In soil, along with the processes of organic matter mineralization and CO_2 release, the synthesis processes take place with its storage in humus. Changes in carbon content over time (difference between initial and final concentrations) is the balance of the carbon content for that time period.

The balanced balance (without change) is evidence of a lack of greenhouse gas emissions as well as a positive balance showing that the soil has accumulated more carbon than it lost through CO_2 emissions.

The negative (carbon footprint) in the measured soil considered the contribution of the soil to greenhouse emissions.

In Tables 1 and 2, prospects for nitrogen, organic and green chemical fertilizers (sidereal crops) are submitted to 2030.

In the case of SLB, the prospecting was carried out on the basis of the information available in the New Land Recovery and Soil Fertilization

Program (Part II: Increasing Soil Fertility). For MS, the prospecting was based on the Soil Fertility Conservation and Enhancement Program for 2011-2020, the National Development Strategy "Moldova 2020" and the National Strategy for Agricultural and Rural Development for the years 2014-2020. With reference to SMA, the recommendations of good practices on sustainable development of the agricultural sector as well as the draft version of Moldova's low-emission development strategy were taken into account until 2020 (Cerbari et al., 2010; 2012).

Table 1. Prospects regarding the application of natural nitrogen and organic chemical fertilizers in the Republic of Moldova in the period 1990-2030, thousands tons N

Name	1990	1995	2000	2005	2010	2011	2012	2015	2020	2025	2030
	SLB (baseline scenario)										
Chemical fertilizers, nitrated, FSN	92.10	10.51	10.24	16.10	20.63	24.99	34.05	55.00	59.40	90.00	99.90
Natural organic fertilizers, FON	54.54	9.96	0.47	0.25	0.10	0.18	0.13	0.28	0.84	2.52	4.20
	SM (scenario with measures)										
Chemical nitrate fertilizers, FSN	92.10	10.51	10.24	16.10	20.63	24.99	34.05	45.00	49.50	77.50	86.00
Natural organic fertilizers, FON	54.54	9.96	0.47	0.25	0.10	0.18	0.13	0.49	1.68	5.04	10.08
	SMA (scenario with additional measures)										
Chemical nitrate fertilizers, FSN	92.10	10.51	10.24	16.10	20.63	24.99	34.05	37.50	42.50	70.00	80.00
Natural organic fertilizers, FON	54.54	9.96	0.47	0.25	0.10	0.18	0.13	0.56	2.52	6.72	11.76

Table 2. Prospecting for Green Fertilizer Applications in the Republic of Moldova 1990-2030, thousands tons N

Name	1990	1995	2000	2005	2010	2011	2012	2015	2020	2025	2030
	SLB (baseline scenario)										
Areas where green fertilizers will be applied - autumn vetch, thousands of ha	0	0	0	0	0	0	0	0	25	50	75
Ground green vetch embedded in the soil, thousands of tons	0	0	0	0	0	0	0	0	500	1000	1500
Green fertilizers transferred to equivalent organic fertilizers, thousands of tons	0	0	0	0	0	0	0	0	700	1400	2100
Green fertilizers – F SIDERAL thousands of tons N	0	0	0	0	0	0	0	0	3.92	7.84	11.76
	SM (scenario with measures)										
Areas where green fertilizers will be applied - autumn vetch, thousands of ha	0	0	0	0	0	0	0	25	50	75	100
Ground green vetch embedded in the soil, thousands of tons	0	0	0	0	0	0	0	500	1000	1500	2000
Green fertilizers transferred to equivalent organic fertilizers, thousands of tons	0	0	0	0	0	0	0	700	1400	2100	2800
Green fertilizers – F SIDERAL thousands of tons N	0	0	0	0	0	0	0	3.92	7.84	11.76	15.68
	SMA (scenario with additional measures)										
Areas where green fertilizers will be applied - autumn vetch, thousands of ha	0	0	0	0	0	0	0	50	75	100	150
Ground green vetch embedded in the soil, thousands of tons	0	0	0	0	0	0	0	1000	1500	2000	3000
Green fertilizers transferred to equivalent organic fertilizers, thousands of tons	0	0	0	0	0	0	0	1400	2100	2800	4200
Green fertilizers – F SIDERAL thousands of tons N	0	0	0	0	0	0	0	7.84	11.76	15.68	23.52

With respect to green fertilizers (autumn vetch as an intermediate crop), the following basic parameters were taken into account: average green weight - 80%; average nitrogen content in the green mass - 0.8%; average productivity - 20 t/ha; 1.4 (or, in other words, 1 ton of green lemon meal equivalent to 1.4 tons of manure).

It is planned to sow autumn meadows as an intermediate crop used as a green fertilizer, and crop rotation will be as follows: autumn wheat or autumn barley - vetch as intermediate crop - corn or sunflower.

The introduction of intermediate crops as a green fertilizer will be carried out in parallel with the implementation of the farming conservative system ("No-Till" and "Mini-Till").

Table 3 presents the prospecting on the areas where the agricultural conservative systems will be applied in the Republic until the year 2030 in Moldova (Cerbari et al., 2010; 2012; IPCC, 2006).

We plan to perform the following tasks: sowing in April of the basic crop (corn or sunflower); determination of changes in the characteristics of the arable layer as a result of the incorporation into the soil of two vines (June); appreciation of crop harvest of basic crops; the appreciation of changes in soil characteristics after harvesting the first basic crop; calculating the carbon balance in the soil after the first basic crop.

In case of extension of the project during the next years, the research polygon will ensure the planned crop rotation: bushy vines → maize → autumn wheat → autumn barley → sunflower. Under these systems all plant residues from the basic crop are to remain in the field for mulching (Cerbari, 2010). The area of implementation of the agricultural conservative system is expected to be twice as high as the area of the intermediate crops, as they only resemble the grain crops of autumn (wheat and barley) and in the second year after the incorporation of the broom into soil as green fertilizers, these areas will again be used in the farming conservative system, already under the crops of sunflower and corn.

Table 3. Prospects for the application of nitrogen fertilizers in the Republic of Moldova in the period 1990-2030, thousands tons N

Name	1990	1995	2000	2005	2010	2011	2012	2015	2020	2025	2030
	SLB (baseline scenario)										
Areas where agricultural conservative systems will be applied, thousands ha, including:	0	0	0	0	0	0	0	0	50	100	200
wheat autumn	0	0	0	0	0	0	0	0	20	40	70
autumn barley	0	0	0	0	0	0	0	0	5	10	30
maize	0	0	0	0	0	0	0	0	20	40	70
sunflower	0	0	0	0	0	0	0	0	5	10	30
	SM (scenario with measures)										
Areas where agricultural conservative systems will be applied, thousands ha, including:	0	0	0	0	0	0	0	50	100	200	300
wheat autumn	0	0	0	0	0	0	0	20	40	70	90
autumn barley	0	0	0	0	0	0	0	5	10	30	60
maize	0	0	0	0	0	0	0	20	40	70	90
sunflower	0	0	0	0	0	0	0	5	10	30	60
	SMA (scenario with additional measures)										
Areas where agricultural conservative systems will be applied, thousands ha, including:	0	0	0	0	0	0	0	100	200	300	400
wheat autumn	0	0	0	0	0	0	0	40	70	90	120
autumn barley	0	0	0	0	0	0	0	10	30	60	80
Maize	0	0	0	0	0	0	0	40	70	90	120
Sunflower	0	0	0	0	0	0	0	10	30	60	80

In the case of SLB, the prospecting was carried out on the basis of the information available in the Program for Land Reclamation and Soil Fertility Enhancement (Part II: Increasing Soil Fertility), in the "Moldovan Village" National Program (2005-2015), in the National Strategy for Sustainable Development of the Agro-Industrial Complex of the Republic of Moldova (2008-2015). For MS, the prospecting was based on the Soil Fertility Conservation and Enhancement Program for 2011-2020, the National Development Strategy Moldova 2020 and the National Strategy for Agricultural and Rural Development for the years 2014-2020. With reference to SMA, the recommendations of good practices on sustainable development of the agricultural sector as well as the draft version of Moldova's low-emission development strategy were taken into account until 2020.

In spite of all these shortcomings, this method is recognized by most researchers as a benchmark method for checking other less sophisticated methods.

In the Republic of Moldova, 3 rounds of agrochemical cartoons were carried out, covering virtually all arable land with a period of 5 years. However, the data obtained cannot be used to determine carbon balance in soils, and is in many cases contradictory (Andrieș, 1999). The main cause is that the samples were not collected from fixed points and the variability of carbon in the arable layer in many cases exceeded its changes over time.

Therefore, the method of assessing the carbon balance and CO_2 emissions of agricultural land by periodic measurements of soil content cannot provide the desired results at country level. For this purpose, it can be used to verify data obtained by other methods with priorities and shortcomings of another order.

A network of long-lasting field experiences has been established in the Republic of Moldova for the development of advanced cultivation technologies for field crops, which studies the evolution of soil fertility according to various fertilization systems. Experiences are 35-55 years old and cover all the country's pedoclimatic zones. The data on carbon evolution in the main soils can be used for the purpose indicated above.

Another way to measure CO_2 emissions from greenhouse soil would be by measuring carbon dioxide exchange between the vegetation mat and the atmosphere using detectors placed on special stationary towers, or by using mobile means such as aviation. In this case the carbon dioxide shift is determined within the field surface area with one crop or another. The measurements are carried out continuously for several months. The method has been tested in Canada and has been found to be expensive and difficult to use for large areas of land. Another shortcoming mentioned by the authors is related to the impossibility of interpreting the data obtained for a longer period of time.

In recent years, attempts have been made to solve the problem of greenhouse gas emissions from soils by applying various mathematical models using computers. The data obtained in this way are qualified as very approximate and must be compared with direct estimates of the carbon circuit parameters. The advantage of mathematical models is that they can be applied on large surfaces and thus meet the greenhouse gas emission assessment requirements.

Satisfactory results have been obtained by applying the "Century model" model in Canada which has allowed the humic evolution of soils to develop over the course of 100 years (Jauzen et al., 1998). Improving mathematical models along with the quality of the initial data introduced into computers makes it possible to use them more widely in assessing the carbon footprint and greenhouse-gas emissions of agricultural land.

Taking into account the stable ratio of carbon to nitrogen in soil organic matter, and with the export of nitrogen from the soil to the vegetal production (primary and secondary crops) it is possible to calculate the amount of carbon released from the soil simultaneously with the nitrogen, the carbon released from the soil CO_2 emissions.

When making calculations, consider that part of the nitrogen used by the plants may have a different origin than humus. Of those from the total nitrogen exhaust, the nitrogen bound by the leguminous crops, that is used by plants from industrial and organic fertilizers, vegetable debris is reduced. An insignificant amount of nitrogen enters the soil with atmospheric precipitations (7 kg/ha), by nesting (5 kg/ha). The nitrogen coming from these sources corresponds to losses by denitrification and leaching and is not taken into account.

For assessing carbon balance and assessing CO_2 emissions from greenhouse soil, the amount of CO_2 input and land in the soil must be determined with the non-alien plant material and organic fertilizers applied. Other carbon inputs into the soil, such as seed carbon and carbon-bound blue algae, are not considered to be insignificant.

The amount of carbon entering the soil is determined taking into account the humification coefficients of vegetable residues and organic fertilizers, as well as the carbon content in the humus formed.

The difference between ground and inbound carbon (the balance sheet) considered the greenhouse gas emissions in the case of dominating the mineralization processes over the humification.

The principles outlined have been used by several authors to determine the balance of humus in agricultural soils and to develop measures to preserve and increase fertility (Ţurcan et al., 1994; Лыков, 1979; Дьяконова et al., 1984; Дьяконова, 1990; Лозановская *et al*., 1987; Попов et al., 1987). The achievement of satisfactory results is conditioned by the specification of the parameters of the indices used at local and regional level in relation to their variation according to the pedoclimatic factors.

The application of the methodology allows to assess the greenhouse gas emissions of agricultural land with reduced time and financial means. Emissions can be determined for one year and for longer periods of time. With the help of the methodology, the monitoring of CO_2 emissions in soils can be organized, the prognosis of the evolution of this phenomenon and the elaboration of the control measures.

The shortcomings to be taken into account the variability of the coefficients are related to the use for the evaluation of emission (humification of plant residues coefficients for use of nitrogen in fertilizers, fixation of atmospheric nitrogen). These indices need to be specified at the pedagogic and agricultural level.

Given the approximation of the results obtained by applying the calculation method, they should be compared with the data obtained in the long-term experiments by direct estimation of the parameters of the carbon circuit in the agrofitocenoses.

It should be noted that for the conditions of the Republic of Moldova the methodology developed can provide satisfactory results.

CONCLUSIONS

Soils with natural vegetal carpet (natural phytocoenoses) cannot contribute essentially to the greenhouse effect due to a balanced carbon balance.

Soil valorisation leads to deterioration of carbon balance and the dominance of decomposition processes with organic matter compared to humification following the alienation of plant production on the field and the intensification of biological processes. The phenomenon is experiencing increased rhythms especially during the first years of fertility exploitation.

The imbalance that is set in the carbon balance in the soils, along with other consequences, causes the carbon dioxide to rise in the atmosphere.

Measurement of CO_2 emissions contributing to the greenhouse effect can only be achieved through the balance sheet approach taking into account all carbon inputs and outputs of the soil.

Total CO_2 emissions from soils from the breathing process cannot be considered as greenhouse emissions because a considerable part of the carbon lost by respiration returns to the soil with vegetal residues being mobilized by photosynthesis.

Increasing CO_2 emissions from soils exploited in agriculture to natural phytocoenoses can be qualified as anthropogenic contributing factor to the greenhouse effect.

So, the remediation of the quality and the increase of the production capacity of the studied soil is possible only by increasing the flow of organic matter into the arable layer. The use of the vetch as a green fertilizer is an effective way of achieving this genre.

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SLIME MOULD - SWEET POTATO ASSOCIATION ON SANDY SOILS OF ROMANIA

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Abstract

Myxomycetes are naturally occurring organisms with habitats from tropical to temperate area with preference for humid and diverse ecosystems. Yet, an association of myxomycetes - sweet potato in Romania, on sandy soils has been detected in 2020. Sweet potatoes are cultivated at Dabuleni Research Station in mixture of forest top soil, sand and peat under greenhouse conditions. Relative humidity and temperatures are high throughout the season with highest values at the end of summer, favouring myxomycetes organism to complete its life cycle. Several stages of plasmodium and sporangia were observed in the field. The crop was not affected by the colonization of myxomycetes. Morphological identification lead to a species of Stemonitidales. This is the first report in Romania of myxomycetes-crop association.

***Key words**: myxomycete, slime moulds, Protista, ecology.*

INTRODUCTION

Slime moulds were previously considered fungi, but presently have joined the Protozoa group, otherwise named paraphyletic kingdom Protista. Once classified as fungi, they have been booted out of that kingdom due to their lack of chitin and their feeding by engulfing food (Glime, 2019). They are now considered Protista due to their motile stages that look and behave like protozoa. The slime moulds are comprised of more than 1000 species from all seven continents (Glime, 2019). The fascinating life cycle of myxomycetes involves two main stages, one consisting of uninucleate amoebae and the second a multinucleate plasmodium, which under favourable conditions develops into spore-containing fruiting bodies (Rikkinen et al., 2019). When slime mould spores germinate, amoeba-like cells form. These are typically haploid, can move and feed on bacteria. If these amoebae encounter the correct mating type, they can mate to form zygotes that develop into plasmodia. The protoplasm within the plasmodium can stream at speeds up to 1.35 mm per second, the fastest rate known for any organism (Glime, 2019). The motile amoebae feed on bacteria and divide until they give rise to the plasmodium. The plasmodium, which also moves, feeds by engulfing surface bacteria, fungal spores, yeasts and algae. The plasmodia have many nuclei with no dividing cell membranes and can form a plasmodial mass that may be several meters in size. The amoebae can avoid adverse conditions by transforming into dormant microcysts, and the plasmodium can convert into a hard, dormant sclerotium which sometimes resembles the slime left by a slug. When this sclerotium once again becomes moist, it returns to the active plasmodium state. Microcysts and sclerotia can withstand drought and cold, and spores may remain viable for years or even decades (Rikkinen et al., 2019). When food becomes limiting, the plasmodium moves to the surface and begins to form its rigid fruiting bodies – sporangia. It is this stage that caused us to originally think they were fungi, but it lacks the chitin that is present in fungi. The life cycle is completed when these sporangia produce spores, usually by meiosis, for the next generation of amoebae (Alexopoulos, 1959). Myxomycetes are ubiquitous in most vegetated, terrestrial ecosystems. Their diversity tends to be higher in communities with higher diversity and biomass of the vascular plants, which support the microorganism substrates upon which the amoebae and plasmodia feed. They are most diverse in tropical and temperate forests, and least in boreal forests, arctic or alpine ecosystems. In tropical forests, myxomycetes seem to produce fruiting bodies more readily in relatively dry habitats (Rikkinen et al., 2019). Schnittler et al. (2015) concluded that corticolous Myxomycetes are some of the most drought-tolerant organisms in that habitat. They are opportunistic, permitted by their ability to survive in a dormant state for decades and to complete their life cycles in a few days of appropriate conditions (Glime, 2019). Identification of species can be difficult for a number of reasons. Not only are there different colour phases during the development of the sporangia, but there are different sexual strategies within currently perceived species (Clark & Haskins, 2015; Steven L. Stephenson & Schnittler, 2017). Within *Trichia varia* "species" there are three distinct sexual biospecies that are reproductively isolated from each other with distinct genotypes but equal phenotypes (Feng & Schnittler, 2015). The class Myxomycetes is comprised of sporocarpic Eumycetozoa with fruiting bodies containing numerous spores and usually a persistent peridium around the sporotheca (taxa with evanescent peridia lack the covering in mature fruiting bodies). The subclass Columellomycetidae comprises the dark-spored myxomycetes with a capillitium connected to a true columella. The superorder Stemonitidia have spores appearing usually darken mass, with order Stemonitales having an epi-hypothallically developed stalk that usually extends into a true columella (extension of the stalk inside the sporotheca), and an evanescent peridium (a usually persistent covering that surrounds the spore-containing sporothecae of fruiting bodies). The central synapomorphy of the newly circumscribed order Stemonitidales is the fugacious peridium, i.e., the mature sporothecae of these myxomycetes lack peridia. The family Stemonitidaceae is characterized by a branching and anastomosing capillitium arising from the columella and with capillitial threads usually forming a surface net. The genus *Stemonitis* unites the myxomycetes that produce cylindrical sporothecae and have a richly branched and anastomosed capillitium that forms a pronounced surface net. Identification of extant myxomycete species is based almost entirely on morphological characteristics of sporocarps and spores. The genus *Stemonitis* currently includes around 20 accepted species. Many of them are common worldwide, but also morphologically plastic (Strelow et al., 2020).

The present study is a first report of a Stemonitidales member in association with sweet potato crop in a greenhouse at the Dabuleni Research Station, in the south of Romania. Dabuleni Plain is characterized by a landscape of dunes, sandy soils, poor in organic matter prolonged heat, heavy rainfall falling at long intervals of time, long periods of drought, thin and discontinuous snow, low fertility of sandy soils and deflation (Simulescu & Zamfir, 2015).

MATERIALS AND METHODS

Collecting place and agro-technical data

Dabuleni city (43 ° 48′ 18.4 ″ N, 24 ° 05′ 33.4 ″ E) is located in the south of Oltenia, 8 km-close to the Danube, in an area with sandy soil, also called "Sahara Olteniei". The relief of the region consists of plains and meadows covered with sand dunes. It has a continental climate with slight Mediterranean influence. Thus, the region has a period of severe drought in July-September and a regular amount of rainfall in May and June. The average annual temperature is 11°C. Precipitation reaches an annual average of 548 mm, is unevenly distributed throughout the year and can have significant variations from one year to another. The wind in the area causes temperature drops in winter, melting snow in spring and scattering sand in spring and summer. Winds still play an important role in shaping the current relief due to the steppe regime, with low rainfall, especially in dry years. Semi-consolidated and mobile dunes are continuously subjected to the action of winds. The woody vegetation specific to the region is represented by acacia (*Robinia pseudoacacia*), and in the ditches on the bank of the Danube white poplar (*Populus alba*), willow (*Salix alba*) and oak (*Quercus robur*). Nearby Research & Development Center for Agricultural Plants on Sands Dabuleni were planted protective curtains of *Robinia* *pseudoacacia* to protect vegetable crops from the action of spring-summer winds. The sandy soils in the area are characterized by a high content of coarse sand (50-70%), little clay and dust (2-8%). Sweet potato tubers were planted in a mixture of forest top soil, sand and peat (1:1:1) at the end of March, in greenhouse. A soil sterilizer (Basamid) with nematocidal, insecticidal, fungicidal and herbicidal action was applied 25 days before. To stimulate the herbicidal action and to retain the sterilizing gas for as long as possible in the soil, the treated soil surface was covered with PE foil until the end of March. After four days sweet potato tubers were planted. In greenhouses, registered average values of parameters were: air temperature 20-28°C, soil temperature 15-25°C and relative humidity 65-75%. To keep temperature below 30°C, ventilation and irrigation with sprinklers were applied.

Myxomycetes isolation and characterization

Sample collection and field observations were carried out at 2 sampling points - greenhouses, from late May until mid-September. In addition to direct observation of sweet potato crop - myxomycete association, samples were collected at different stages of life cycle in Petri plates, kept at 5°C until laboratory further manipulation. The fruiting bodies and microscopic structures were examined by light microscopy. Sporotheca and spores were mounted in water with Tween80 (0.005%) for observation. Observation and measurement of sporocarp morphological characteristics were carried out with Olympus SZ61 stereomicroscope with an attached Olympus SP350 camera. Sporotheca and spores were observed using optical Motic B series microscope with an attached microQ UCMOS series Toup Up camera. ToupView software was used for image manipulation and measurements.

RESULTS AND DISCUSSIONS

In May 2020, the first author observed and collected an unidentified myxomycete on sweet potato crop in greenhouse at Dabuleni Research Station. The specimen was sent to the correspondent author, who identified it as a member of Stemonitidales. During August and September, several observations were made in the field and multiple samples were collected at different stages of the life cycle.

The specimen was observed in small clusters on living plants of sweet potato - shoots, leaves and petioles. Different stages of plasmodium and fruiting bodies were observed (Figure 1). Initially, the plasmodium is slender-veined and transparent (Figure 1, a), followed by a phase in which the extensive reticulum is withdrawn into a dense, cream-coloured strands forming a coarse network. The veins appear thick and opaque. The veins condense further to form a coralloid mass. The formation of the white coralloid structure (Figure 1, b) marks the change from assimilative to the reproductive phase (Indira, 1971).

Figure 1. From left to right: a) early stage of plasmodium just prior to fruiting, condensing into thick veins; b) prefructification coralloid plasmodium (fruiting creamy white knobs, the sporangial initials - sporotheca formation) typical for *Stemonitis* with a clustered fruiting body; c) coralloid stage on leaves; d) mature plasmodium on soil

Next, it condenses and forms a thick uniformly flattened mass at the surface of which appear minute knobs, the sporangial initials (Figure 1, b). The coralloid stage (Figure 1) may last for varying periods for 10-15 day, depending on the conditions in the greenhouse. During the observations temperature ranges between 28-30°C, micro-sprinkler irrigation was applied every two days and relative humidity was 75%. In the present study no intermediate phases between the initial sporocarps and the fully formed ones were detected. Intermediate phases are described in detail elsewhere (Dai et al., 2020; Indira, 1971). Yet, coralloid plasmodium was detected at different stages up to decay (dessication) possibly due to stress caused by the environmental factors (Figure 2). Dark-grey sclerotia were also observed in collected samples (Figure 2, e).

Figure 2. Plasmodium phases from up to bottom, left to right: a) plasmodium development: coralloid form of mature plasmodium on debris; b) coralloid form of mature plasmodium on petiole of sweet potato; c) decaying coralloid form of mature plasmodium on debris; d) decaying coralloid form of mature plasmodium on sweet potato leaves; e) sclerotium formation

The fruiting body (Figure 3) is represented by reddish-brown sporangia (sporocarps), in clusters supported on slender stalks. Fructification sporocarps, aggregated, stipitate, in clusters of approximately 50, dark brown, acute or sub-acute apex. Sporocarps tufted, 10-15 mm total height, in clusters of approximately 25. Sporotheca dark brown with a reddish tinge with height of 2.26-2.89 mm. Sporocarp with solid, black, not hollow stalk with height of 1.31-1.42 mm.
Stemonitida is a morphologically homogeneous order, characterized by a unique feature - the stalk is internally secreted, slender, regular and smooth and it extends itself into the spore mass (Fiore-Donno et al., 2012; Kalyanasundaram & Paramasivan, 1993).
The typical stalk, the dark spore colour and the absence of lime deposits make members of the Stemonitida easily recognizable when collected in the field.
In the traditional classification Stemonitida includes only one family and 16 genera (Poulain et al, 2011).

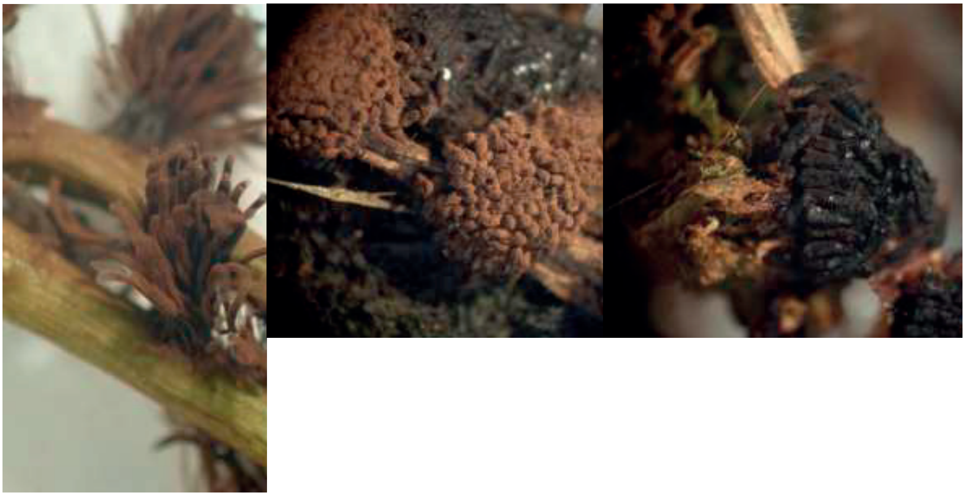

Figure 3. Fruiting body development from left to right: a) gregarious mature stalked sporocarps with powdery spore mass on sweet potato leaves; b) decaying gregarious mature stalked sporocarps on debris; c) dried, decayed sporocarps on debris

The hypothallus was membranous and red brown. The peridium was brown and persistent. The capillitium was densely reticulate, arising from the entire columella. The spores were warted, globose spores of (7.5-) 9 (10) µm diameter, SD = 0.61 µm, pale purple brown in colour (Figure 4).

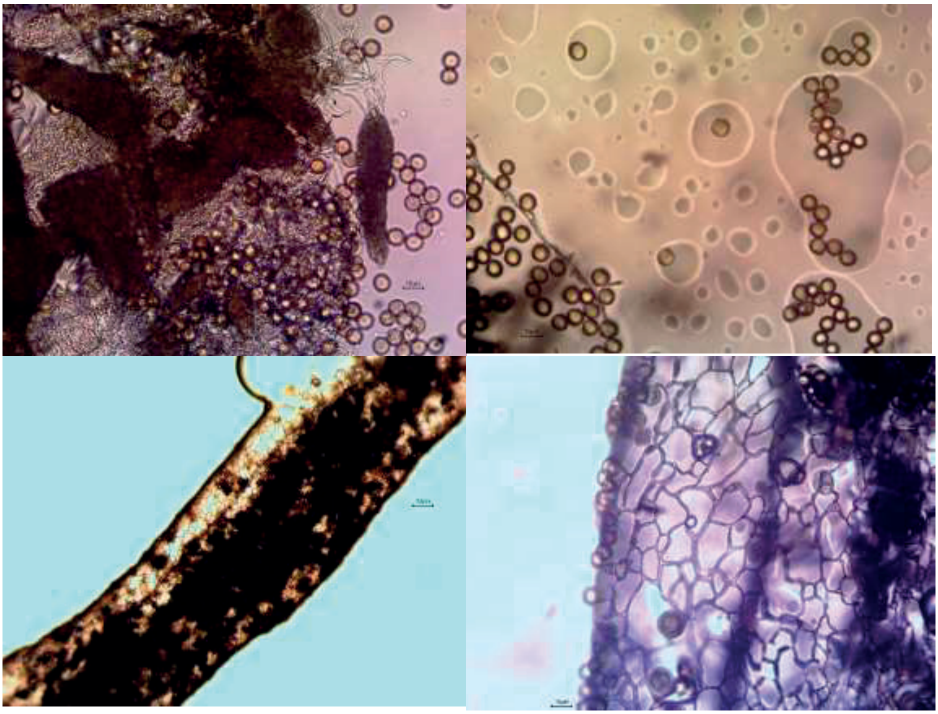

Figure 4. Up: Reticular spores released after crushing sporotheca (x 40); Down from left to right: Capilitium of a sporotheca (10x); Capilitium - the mesh of internal network holding spores (40x)

There are only subtle differences between the four genera *Stemonitis*, *Stemonaria*, *Stemonitopsis* and *Symphytocarpus*. Strelow et al., (2020) hypothesized that spore ornamenttation may constitute a more meaningful characteristic than the capillitial network to reveal affinities between species in *Stemonitis* and the genera segregated from it. Their phylogenetic analyses suggest that spore ornamentation is a major, hitherto neglected feature essential to delimitate genera in *Stemonitis* and allied genera. The two *Stemonitis* clades are characterized by spores either warted or reticulated (Strelow et al., 2020).

Our specimen presented warted spores, therefore preliminary analysis indicates it as a member of this genus. Also, several species were discarded according to ornamentation and size of spores: *S. lignicola* (Poulain et al., 2011), *S. fusca* and *S. typhina* (Dai et al., 2020), *S. herbatica* (Indira, 1969), *S. planusis* (Bo & Yu, 2017). Ground litter (consisting mostly of dead leaves) on the forest floor is one of the primary substrates for myxomycetes (Martin & Alexopoulos, 1969; Stephenson & Stempen, 1994).

Studies on this substrate have indicated that ground litter in both temperate and tropical forests supports a diverse assemblage of these organisms (Harkönen, 1981; Stephenson, 1989; Stephenson & Stempen, 1994). Presumably, when a leaf dies and falls to the forest floor, it acquires airborne spores of myxomycetes (Rojas, 2020).

Sweet potato crop includes adding peat in the substrate composition each year. It comes easy to speculate that the most probable route for the detected specimen of Stemonitidales in the greenhouse, was via top soil forest used in the substrate mixture. Another way of entrance would have been anemophily from the nearby forests. In any case, the important fact is that this organism was able to colonize beside debris, living plants of sweet potato, completing its life cycle by producing fruiting bodies.

Myxomycetes species are known to prefer certain substrates for fruiting as *Dictydium cancellatum* sporangia always can be found on rotten pine logs, *Physarum cinereum* fruits on living St. Augustine grass and *P. bivalve* prefers living herbaceous plants (Townsend et al., 2005).

It comes at hand to speculate that Myxomycete specimen completed its life cycle due to combined factors of water input (micro-sprinkling irrigation), infiltration rates, high temperature and relative humidity. These might have been similar to its natural habitat (hot and humid forests nearby). It is noteworthy to mention that plants thrive in association with the Myxomycete, the organism did not attack the plant, but only used it as physical support, which indeed might have as effect a lower photosynthetic surface. Several water showers were used to “wash away” the “invading” organism.

The presence of fruiting structures (sporangia) is considered as dependent on the arrival of rain after a prolonged warm period, making their presence most common in autumn in temperate regions (De Holanda et al., 2016).

So, the agro technical method against the colonization of the myxomycetae might have act as propitious to complete its life cycle - sporocarps with spores which theoretically germinate and a new cycle is on the road. Myxomycete perform important functions in many different terrestrial ecosystems. At the amoebae stage feed mainly on bacteria but their plasmodia feed on a wider variety of organisms, including fungal fruiting bodies and spores, algae and possibly lichens. Some appear to produce the enzymes necessary to break down dead plant tissues. Soil nutrients are largely immobilised in microbial biomass. Nutrients are released through the feeding activities of bacterivores like myxomycetes, making them responsible for mediating the flow of nutrients first to the surrounding substrates and then to higher trophic levels, including plants and animals (Stephenson & Rojas, 2017).

Eliasson & Lundqvist (1979) noted that species with large plasmodia typically are rare under arid conditions. This would suggest that the slime molds on bryophytes are the larger species in most habitats because of the moisture-holding capacity of the bryophytes. Yet, *Didymium wildpretii* was found in arid zones - Canary Islands and Chihuahuan Desert Mexico (Beltrán-Tejera et al., 2010).

A particular anatomical adaptation of *Stemonitis* which might have allowed them to colonize arid zones is the stalk of sporocarps which elevates spores above the substrate, allowing them to dry out and become airborne (Rikkinen et al., 2019). The authors proposed this characteristic as evidence of strong environmental selection favouring the maintenance of adaptations that promote wind dispersal in this lineage of myxomycetes.

CONCLUSIONS

This first report of myxomycete - crop association in Romania reveals another example that naturally occurring species in forests may shift to man-made monoculture ecosystems and complete their life cycle without harming the associated plants. Further molecular identification is required as well as field observations during present year to determine if myxomycete has established in the soil where sweet potatoes are planted.

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INFLUENCE OF DRIP IRRIGATION ON THE CHEMICAL PARAMETERS OF TYPICAL CHERNOZEM

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Abstract

Research studies have proven that results obtained with LAQUAtwin pocket meters are strongly correlated with those of traditional laboratory analyses. The LAQUAtwin pocket meters allow direct measurement of micro-volume sample (as low as 0.1 ml) and deliver results in just a few seconds. These advantages enable to make fertilization and irrigation decisions quickly. Regular monitoring of nutrient levels such as sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) in plant petioles, soil solution, irrigation water produces not only good yield and fruit quality, but also reduces fertilizer cost and mitigates environmental hazards. The LAQUAtwin pocket meters are the perfect tools for testing as they directly measure samples and provide results in just few seconds allowing growers to identify and correct any nutrient deficiency or excess immediately.

Key words: *chernozem, irrigation, chemical parameters.*

INTRODUCTION

Agriculture analysis is a very important aspect to crop growing. To increase quality and yields, it is crucial to understand the current nutrient levels of the soil to be able to ascertain which areas require improvement (Greig, 2009).

Soil and plant tissue testing is a valuable tool for determining the fertilizer needs and maximizing the fertilizer efficiency.

Soil test is especially useful early in the growing season when plants are too small to collect tissue samples (Hoeft, 2001). Following soil indicators are used more often: soil pH, conductivity, the content of calcium, sodium, potassium, nitrate in soil solution.

Plant tissue test for nitrogen (N) and potassium (K) levels during the growing season provides information for diagnosing problems.

It is preferred than soil test as the results of which can change quickly by rain or irrigation (Campbell, 2000).

Plant sap testing offers advantages over the conventional dry tissue testing being carried out in laboratories.

Aside from the lower cost it requires, plant sap testing can be easily done in the field and the results can be obtained quickly which is important in making fertilization decisions (Horneck, 1998).

Plant sap analysis has been used by growers to manage crop nutrient content and fertilization strategies.

A plant sap test gives a view of the nutrients available for the plant, at that time, for growth or development. It directly shows if a crop suffers from a nutrient deficiency or an excess.

Soil solution analysis is also important for growers. It gives a view of the nutrient and salinity levels in the soil and nutrient leaching past the root zone.

Soil solution is the water held in the soil, which contains a mixture of nutrients taken up by roots (Bottoms, 2013). All plants need calcium rich soil to grow. The calcium is used by the plant in developing the plant cell walls and membranes. Furthermore, it is a non-leaching mineral (it will stay in the soil) and will improve water penetrability and reduce soil salinity. It is thus helpful to determine the amount of calcium contained in soil (Vardar, 2015). The use of accurate calcium ion testing in controlling the calcium content of soil ensures that the plants which are grown in the soil are given the necessary minerals and can easily absorb water.

Sodium is a mineral constantly present in soil, but an excess of it can cause the yield to dwindle. Thus, it is beneficial to measure the salinity of soil on which crops are grown.

Strawberry production requires a root environment with good availability of essential nutrients to achieve optimum plant growth, fruit quality, and yield.

Soil pH influences on the availability of nutrients such as nitrate (NO_3^-), potassium (K^+) and calcium (Ca^{2+}) ions, which have specific roles in strawberry fruit quality. For example, NO_3^- determines the size, K^+ influences the taste, and Ca^{2+} affects the firmness.

The nutrients should be present in a right balance to avoid competition or improper plant uptake. Soil pH is a measure of the acidity or alkalinity in soils. In the pH scale, pH 7.0 is neutral. Below 7.0 is acidic and above 7.0 is basic or alkaline. Soil pH affects nutrients available for plant growth.

In highly acidic soil, aluminum and manganese can become more available and more toxic to plant while calcium, phosphorus, and magnesium are less available to the plant. In highly alkaline soil, phosphorus and most micronutrients become less available (Casteel, 2004).

The desirable soil pH range for optimum plant growth varies among crops. Generally, soil pH 6.0-7.5 is acceptable for most plants as most nutrients become available in this pH range.

Soil pH can be determined by mixing soil sample with water (in a ratio 1:5) and then measuring the resulting aqueous solution.

By the way, conductivity should be monitored due to the strawberry plants do not tolerate high conductivity value (Hicks, 2015).

Soil solution analysis can help growers make decisions about their irrigation and nutrient management program (Casteel, 2004). It should be used to enhance nutrient management and not as a measure of overall soil nutrient status. Nitrate and salinity levels are the key focus of soil solution monitoring. As nitrate is a highly mobile nutrient, soil solution can help identify incorrect nitrogen application, which could result in nitrate accumulation within the root zone and nitrate leaching below the root zone (Bottoms, 2014). Potassium and calcium are bound to soil and their levels in soil solution do not provide a reliable indication of their supply and availability to the crop. However, potassium, calcium, and sodium levels in soil, irrigation water, and drain water should be checked regularly.

Salinity levels measured in soil solution allow growers to perform corrective action (e.g., increase irrigation to maintain salinity levels) before symptoms appear on leaves, yields or crop quality.

Overwatering leaches fertilizer out of the root zone and results in environmental hazard (e.g., groundwater contamination).

Soil solution analysis can be tested throughout the growing season (Baliuk, 2016).

Fertigation management requires rapid and accurate methods to determine nutrient concentrations in soil solution and plant sap (Ancay, 2014). Folegatti et al. (2005) found that the concentrations of Ca^{2+}, K^+, and Na^+ in soil solution determined by LAQUAtwin ion pocket meters showed good correlations with those obtained in soil solution determined by standard methods in laboratory, and concluded that LAQUAtwin ion pocket meters are useful low-cost tools in fertigation management.

The aim of our study was to investigate changes in the chemical parameters (calcium, sodium and potassium content) of Calcic Voronic Chernozem under the influence of drip irrigation by using LAQUAtwin devices.

MATERIALS AND METHODS

Our field site was the Research Field of the Study, Research and Production Centre (SRPC) in Kharkiv District that was laid out in 1946 under Professor OM Grinchenko. The soil is thick Calcic Voronic Chernozem (typical heavy loamy chernozem on the loess-like loam) that has been tilled for more than a century. Since 1946, some has been set aside as natural grassland, most is still cultivated under arable rotations.

The experiment for strawberry garden variety ‘Roxana’ was laid in the fall of 2017 on an area of 0.3 hectares. Planting was carried out by comb technology using mulching film and drip irrigation. The precursor for strawberries was black couples (Figure 1).

In the experiment, nitroammophoska $N_{16}P_{16}K_{16}$ and half-roasted manure were used for fertilization. Strawberries were planted in a checkerboard pattern in two strips with a distance between plants of 25 cm with rows of 130 cm. Irrigation was carried out as needed to ensure a constant soil moisture within 75%, which was measured with a field moisture meter. The technology of the cultivation system provides for the use of chemical plant protection products against pests and non-root fertilization in the flowering phase.

Figure 1. General view of a strawberry field

For research in the field where grown strawberry were selected following variants (4 rows in each variant):
- Variant 1 - control (without fertilizers);
- Variant 2 - mineral system ($N_{64}P_{64}K_{64}$);
- Variant 3 - organo-mineral system ($N_{64}P_{64}K_{64}$ + manure 50 t/ha);
- Variant 4 - organic system (manure 50 t/ha).

Additional variants in 2020 year for research were selected:
- Variant 5 (black couples) - field crop rotation (more than 100 years) without irrigation;
- Variant 6 (fallow) - herbaceous vegetation over 70 years old.

Chemical parameters were studied in Calcic Voronic Chernozem samples, which were taken from the surface layer of the soil (ridge - in the experiment with growing garden strawberries), and then every 10 cm to a depth of 50 cm in these variants of the experiment.

Method for determining chemical parameters. Take a soil simple to measure, at the roots depth in order to get a precise idea of the nutrient levels at the root's systems depth. Take various samples to have a homogeneous representation of the culture. Pass the soil simple through a sieve to remove stones and organic matter. Weigh 10 grams of soil samples. Prepare a 1:1 dilution with distilled water (10 ml). Mix the soil and water for 2 minimum and let the paste settled for 5 minutes. Place the paste obtained on the LAQUAtwin sensor after calibrating the meter. For the ions, multiply the Reading per the dilution factor (x2) to calculate the real concentration of nutrient in soil.

RESULTS AND DISCUSSIONS

The amount of water-soluble salts in irrigated soils depends on the quality of irrigation water and the use of fertilizers (organic, mineral, organo-mineral).

In the control variant with the use of irrigation without fertilizer in the first year of the experiment (2018) the amount of water-soluble sodium decreased sharply from 360 ppm in the ridge to 40 ppm in the layer of 40-50 cm. Compared to the following year (2019), the amount of sodium decreased more than 2 times in the ridge and more than 1.5 times in the layer 0-10 cm.

The following studied layers have almost no significant fluctuations compared to the previous year of sampling. The third year of research shows a slight decrease in water-soluble sodium by 34 ppm in the ridge and a sharp increase in the lower investigated soil thickness to 200 ppm (Table 1).

The amount of water-soluble calcium on the contrary increases from the upper part of the profile to the lower during three years of research. However, in the third year of research, its number begins to decrease sharply in the ridge and subsequent layers. This leads to a sharp decrease in the ratio of Ca:Na. Fluctuations in calcium content occur in the range from 88 to 110 ppm, which is 1.5-2.5 times less than in previous years. In addition, the water-soluble calcium content of the 30-40 and 40-50 cm layers is increased to 340 and 420 ppm.

Other variants of the experiment have a similar tendency in the distribution of water-soluble sodium and calcium content. Thus, under the mineral system of fertilizer, the sodium content gradually decreased from 300 ppm to 28 ppm in the 40-50 cm layer, and from 320 ppm to 158 ppm, then increased to 200-280 ppm of water-soluble calcium occurs in the first year of study. The following year, the amount of water-soluble sodium ranges from 114-142 ppm from the top to 30 cm and from 78-102 ppm from 30 to 50 cm.

Table 1. Dynamics of water-soluble sodium and calcium content (ppm)

Variant	Depth, cm	Na, ppm			Ca, ppm			Ca:Na		
		2018	2019	2020	2018	2019	2020	2018	2019	2020
Control	ridge	360	172	138	220	166	106	0.6	1.0	0.8
	0-10	280	150	152	240	198	88	0.9	1.3	0.6
	10-20	120	126	186	186	200	90	1.6	1.6	0.5
	20-30	134	132	192	168	280	110	1.3	2.1	0.6
	30-40	80	98	200	320	280	340	4.0	2.9	1.7
	40-50	40	88	174	340	360	420	8.5	4.1	2.4
Mineral system	ridge	300	116	144	320	360	126	1.1	3.1	0.9
	0-10	200	142	154	220	200	120	1.1	1.4	0.8
	10-20	158	142	176	158	200	90	1.0	1.4	0.5
	20-30	108	114	220	200	360	240	1.9	3.2	1.1
	30-40	96	102	176	220	340	260	2.3	3.3	1.5
	40-50	28	78	154	280	360	280	10.0	4.6	1.8
Organo-mineral system	ridge	164	200	148	380	360	142	2.3	1.8	1.0
	0-10	192	178	164	240	170	118	1.3	1.0	0.7
	10-20	92	136	178	260	280	94	2.8	2.1	0.5
	20-30	94	102	170	300	340	84	3.2	3.3	0.5
	30-40	130	88	158	340	380	104	2.6	4.3	0.7
	40-50	116	38	180	320	480	260	2.8	12.6	1.4
Organic system	ridge	142	166	124	280	154	118	2.0	0.9	1.0
	0-10	170	134	136	380	186	94	2.2	1.4	0.7
	10-20	52	154	168	320	200	92	6.2	1.3	0.5
	20-30	80	164	184	400	300	106	5.0	1.8	0.6
	30-40	68	144	220	400	320	170	5.9	2.2	0.8
	40-50	18	122	200	420	340	220	23.3	2.8	1.1

At the same time, the decrease in calcium content is observed at depths of 0-10 and 10-20 cm to 200 ppm, and other layers had slight fluctuations from 340 to 360 ppm. Some increase in sodium to 100 ppm was observed in the third year of the experiment in all soil layers. In addition, the calcium content decreases sharply in the third year of the experiment to a depth of 20 cm and, as a consequence, the ratio of calcium to sodium is in the range of 0.5-0.9.

Slightly lower values of water-soluble sodium were recorded in the variant with organo-mineral fertilizer system up to 164 ppm in the ridge and 192 ppm in the 0-10 cm layer. Unlike previous variants, the increase in sodium content occurs in the second year of research in the ridge to 200 ppm, and then its amount gradually decreases. Practically the highest indicators of water-soluble sodium from 148 to 180 ppm were obtained for the third year of the experiment. The amount of water-soluble calcium in the first year of research was the highest in the ridge - 380 ppm, and then decreased to 240 ppm, and increased to 320-340 ppm. Mediocre values were obtained for the second year, and the lowest (from 84 to 142 ppm) - for the third, where the ratio of Ca:Na was the highest in the upper part of the soil (1.0) and gradually decreased to 0.5 (Table 1).

The organic fertilizer system is characterized by the redistribution of the resultant values according to the content from the ridge (142 ppm) to the lower thickness of 40-50 cm (total 18 ppm). In the second year of research, the amount of water-soluble sodium is slightly increasing, both in the upper part of the profile and especially in the lower part. A sharp increase in sodium content compared to the first year occurs from a depth of 10-20 cm from 168 ppm to 200-220 ppm at a depth of 30-50 cm. Also, this variant revealed the highest content of water-soluble calcium in almost the entire study thickness, and especially at a depth of 40-50 cm, where the figure reaches 420 ppm. The Ca:Na ratio in the studied soil reached the level of the variant without irrigation and fallow - 23. But, next year the amount of calcium decreases sharply by 1.5-2.0 times to a depth of 10-20 cm. Even more significant changes compared to the first year of research can be traced to the third year. Thus, the calcium content decreases by another 2.0-2.5 and 3.5-4.0 times.

Thus, we have practically the highest content of water-soluble sodium in the variant of control and mineral system in the ridge part of the soil in 2018. Indicators in the ridge part of the soil in 2019 are slightly decreasing, but are the largest in the control variant, organo-mineral and organic system. In 2020, the highest values were recorded in the lower part of the profiles of control variant and the organic system. The lowest values are observed during the first two years of all variants of the experiment at depths of 30-40 and 40-50 cm, and for the third year - in the upper layers (ridge).

In contrast, the highest content of water-soluble calcium, within three years, is in 30-40 and 40-50 cm thick of all research variants. The lowest calcium content for three years on the control variant from the ridge to 10-20 cm.

None of the analyzed variants revealed water-soluble potassium content, except for single samples. Thus, the insignificant potassium content was 8 and 10 ppm on the control variant and organo-mineral fertilizer system for the first year of research. A slightly higher figure was obtained in the variant of fallow, where the water-soluble potassium content was 24 ppm.

Based on the dynamics of the obtained values during the three years of research on the content of water-soluble sodium and calcium, we observe that the sodium content gradually increases in all variants of the experiment, and the amount of calcium decreases. As a result, the ratio of Ca:Na decreases. Therefore, the use of irrigation for three years promotes a marked transformation of the content of water-soluble salts.

In addition to these data, in the variant without the use of irrigation, the amount of water-soluble sodium varies slightly from 6 to 12 ppm at all depths studied. The content of water-soluble calcium reaches the level of 200-380 ppm. As a result, the ratio of calcium to sodium is 22-60. In the fallow variant, the sodium content also fluctuates slightly in the range of 8-12 ppm, and the amount of calcium in the upper thickness of 0-10 cm reaches 300 ppm. The ratio of calcium to sodium (Ca:Na) is about 25 (Table 2).

So, by comparing the variants to drip irrigation and without drip irrigation, we can state that drip irrigation during strawberry growing contributes to a significant increase in water-soluble sodium and a decrease in water-soluble calcium in Calcic Voronic Chernozem of all studies. Irrigation of mineral water is the cause, that are confirmed further analysis of the electrical conductivity (soluble salt content) of the irrigation water.

Table 2. Water-soluble sodium and calcium content in 2020 (ppm)

Variant	Depth, cm	Na, ppm	Ca, ppm	Ca:Na
Without irrigation	0-10	8	200	25.0
	10-20	10	220	22.0
	20-30	12	380	31.7
	30-40	10	340	34.0
	40-50	6	360	60.0
Fallow	0-10	12	300	25.0
	10-20	10	240	24.0
	20-30	10	260	26.0
	30-40	10	240	24.0
	40-50	8	240	30.0

For drip irrigation, when watering is carried out often enough, we can assume that the soil solution and irrigation water are identical. Theoretical maximum values of electrical conductivity at which plants cannot grow have been established for crops. To determine the theoretical reduction in yield from the use of a particular irrigation water determine the electrical conductivity of water, which is much easier to do than to determine the electrical conductivity in the soil. Next, compare the electrical conductivity of water with the limit values. If the electrical conductivity of water is less, it is concluded that the reduction in yield due to salinization is not expected. Otherwise, they talk about lower yields.

Thus, the water used for irrigation has the following characteristics: electrical conductivity - 1142 µs/cm; total mineralization - 757 ppm; salinity - 570 ppm; the aqueous pH is 6.88.

According to the literature, the threshold value of electrical conductivity for irrigation water of loamy soils is 900 µs/cm. Accordingly, in our case, the exaggeration is 242 µs/cm, which can negatively affect the yield of strawberry plants.

In general, the five levels presented in the table 3 are set for the assessment of irrigation water by electrical conductivity.

After determining the actual salinity of irrigation water make a conclusion about the feasibility of growing this crop or choose another crop that can grow in these conditions without reducing yields.

Therefore, the level of soluble salts of irrigated water can be classified as medium saline.

Changes in pH are one of the main indicators of salinity. The water pH of the control variant increases with depth from the ridge to 50 cm. Thus, the upper part has a value of 7.35 and 0-30 cm thickness is characterized by fluctuations in pH within the smallest significant difference. From a depth of 30-40 cm, the pH of the water rises by 1.50 units compared to the upper soil to 8.81 units (Figure 2).

Table 3. Classification of irrigation water by electrical conductivity

Electrical conductivity of water, µs/cm	Classification of irrigation water by mineralization (the level of soluble salts)
<650	low
650-1300	medium
1300-2900	high
2900-5200	very high
>5200	extremely high

A variant of the mineral fertilizer system recorded a gradual increase in pH from 7.15 to 7.81 to a depth of 20-30 cm inclusive. Then the figure increases and is at the level of 8.96.

Within the smallest significant difference, up to a depth of 20 cm, are the water pH on the variant of simultaneous application of organic and mineral fertilizers. From a depth of 20-30 cm there is a decrease in the water pH first to 7.40 units, and then to 7.21 at a depth of 30-40 cm. Compared with the upper studied thickness (ridge and 0-20 cm), the water pH decreases by 4-5% in the lower part (20-50 cm), which is not typical for the previous two variants of the experiment.

In the variant of the organic fertilizer system, the highest pH value is at a depth of 10-20 cm - 7.48, which is larger than the ridge, 0-10 cm and a thickness of 20-40 cm. The soil layer of 40-50 cm is characterized by a decrease in water pH to 6.87.

Minor fluctuations in the 0-20 cm thickness were detected in the variant without the use of drip irrigation - 6.63-6.48 units. From a depth of 20-30 cm, the pH level rises to 8.41, and then to 8.76 (30-40 cm), which is 25-30% higher compared to the upper layers.

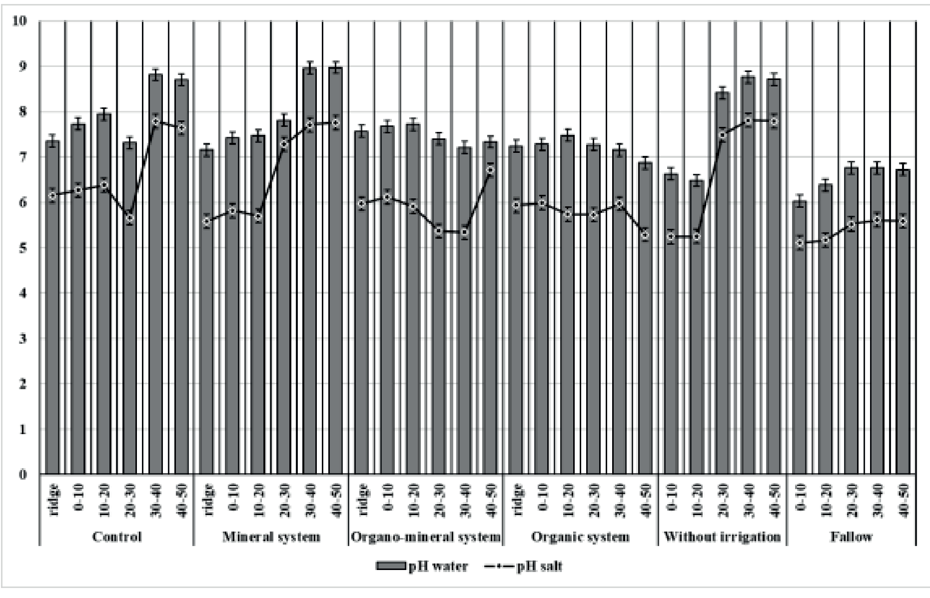

Figure 2. pH water and pH salt of the research variants

The fallow variant of use has a natural increase in water pH from 0-10 cm to 40-50 cm. The values gradually increase from 6.03 to 6.72.

Therefore, the highest values of water pH were recorded in the lower parts of the 30 to 50 cm variant of the control, mineral system, as well as from 20 to 50 cm variant without irrigation.

The lowest pH at all depths in the variant of fallow use, the upper part of the variant without irrigation and 40-50 cm thickness of the variant with organic fertilizer system.

All other indicators can be attributed to the average values ranging from 7.15 to 7.94 (Figure 2).

The pH of the salt of all studies ranges from 5.11 to 7.81 units, corresponds to the reaction of the soil solution from the mild acidic to the medium alkaline.

Thus, the control variant has an increase in the pH salt from 6.15 at the depth of the ridge to 6.38 by layer of 10-20 cm.

The thickness of 20-30 cm is characterized by a decrease in pH to 5.66, and 30-50 cm - a sharp increase to 7.64-7.79, which is 1.5-2.0 units higher than the previous values.

The variant of the mineral system according to the obtained values of the pH salt can be divided into two parts: upper - from the ridge to a depth of 20 cm, where the pH ranges from 5.58 to 5.82 and the bottom - from 20 to 50 cm with a pH of 7.28-7.76.

The ambiguity of the obtained values was recorded on the organo-mineral fertilizer system. The pH salt from the ridge to a depth of 20 cm varies within the smallest significant difference - 5.91-6.12.

At depths of 20-30 and 30-40 cm, the values are almost the same - 5.34-5.37 units, and the depth of 40-50 cm is characterized by an increase in the pH of salt to 6.71, which is the largest study in this case.

The soil using organic fertilizer system in terms of salt pH also has a different distribution. The highest pH is in the ridge (5.93), as well as layers of 0-10 and 30-40 cm (5.99 and 5.97), and the lowest - 5.29 at a depth of 40-50 cm.

The average pH salt is from 10 to 30 cm with a value of 5.74.

In the variant without irrigation, we have the same salt pH indicators in 0-20 cm soil thickness - 5.25, and then there is a sharp increase this indicator about in 40% (2.24 units). At the depths of 30-40 cm, the figure increases slightly and reaches a value of 7.81.

Similarly, to the previous described, in the variant of fallow use, the pH salt of the upper 20 cm thickness is the smallest - 5.11-5.17 units. Further, at the studied depth there is some increase first to 5.52 and then to 5.61 at a depth of 30-40 cm. The pH salt of 40-50 cm thick is within the smallest significant difference with the previous figure.

Thus, most of the obtained pH salt values belong to the neutral and close to neutral reaction of the soil solution, slightly less than the analyzed samples show a weakly acidic reaction, and a small amount of it slightly alkaline.

Neutral and close to neutral have the upper ridges and soil thickness from 0 to 20 cm of all variants with the cultivation of garden strawberries. The average alkaline reaction is at depths of 30-40 and 40-50 cm of control variant, mineral system and variant without irrigation. Slightly acidic pH salt in the lower part of the soil of the organo-mineral system and, conversely, in the upper parts of the variants without irrigation, as well as the fallow variant.

CONCLUSIONS

According to the dynamics of the obtained values during the three years of research on the content of water-soluble sodium and calcium, we can state that the sodium content gradually increases in all variants of the experiment, and the amount of calcium decreases. As a result, the ratio of Ca:Na decreases. Therefore, the use of irrigation for three years leads to a significant transformation of the content of water-soluble salts in Calcic Voronic Chernozem of all research variants. None of the analyzed variants revealed water-soluble potassium content, except for single samples.

According to scientific research, higher indicators of electric conductivity for irrigating water loamy soil for strawberry growing is 900 μs/cm. Studies have shown an exaggeration of the electrical conductivity of irrigation water by 242 μs/cm, which can be some negative impact on the yield of strawberry plants.

The use of irrigation also affects soil pH. Most of the obtained pH salt values belong to the neutral and close to neutral reaction of the soil solution, slightly less than the analyzed samples show a weakly acidic reaction, and a small amount is slightly alkaline.

The highest values of water pH were recorded in the lower parts from 30 to 50 cm variants of control and mineral system. The lowest pH in the 40-50 cm thickness of the variant of the organic fertilizer system. All other indicators can be attributed to the average values ranging from 7.15 to 7.94.

Therefore, irrigation with medium saline water for three years during the cultivation of strawberries does not cause a significant accumulation of salts in the soil. However, it caused an increase in water-soluble sodium, a decrease in calcium and a change in pH.

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Equisetum arvense L. AS A BIOINDICATOR OF ACID SOILS

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Abstract

Bioindicator plant species are useful in deciding which species or cultivars of plants can be grown, which soil analysis should be performed and how soils with some limiting characteristics for plant growing can be improved. In agricultural practice Equisetum arvense is known as a bio-indicator species for acid soils with excess moisture. Diminution of acid soils can be achieved by liming. In such cases, the incorporation into the soil of calcium carbonate to diminish the acidity is not always justified. In order to make a correct decision, it is necessary to perform soil analysis. Equisetum arvense is frequently found on soils with a compacted plow pan. It is not justified in this case to carry out drainage works. We consider the results obtained from the studies carried out regarding the characteristics of the habitats of this species will be useful in order to take sustainable measures for the management of soil resources.

Key words: *horsetail, compacted soil, wide pH amplitude.*

INTRODUCTION

According to Wikipedia the free Encyclopedia, *Equisetum* (horsetail, snake grass, puzzlegrass, candock, scouring-rush) is the genus of plants belonging to the the living genus in *Equisetaceae*, a family of vascular plants that reproduce by spores rather than seeds of *Equisetaceae* family.

The term, *Equisetum* derives from the latin words *equus* and *seta* which means the *horse* and respectively *bristle* or *hair*, i.e. horsetail (Chifu, 2006).

In Romania, are known eight plant species belonging to the genus *Equisetum* (E): *E. arvense, E. pratense, E. palustre, E. ramosisisma, E. fluviatile, E. syilvestris, E. maxima, E. hyemale* (Grintescu et al., 1952).

The genus *Equisetum* can be considered cosmopolitan because missing in some region such as Antarctica, Australia and New Zealand (https://en.wikipedia.org/wiki/Equisetum).

The requirements of *Equisetum* species are very different from ecological factors such as light, temperature, soil moisture, soil reaction and content of nutrients.

The species *Equisetum fluviatile* with the highest requirements for soil moisture is followed by species *E. telmateia, E. variegatum* that have moderate to high requirements for soil water content (Chifu et al., 2006).

After Atland (2003) *Equisetum fluviatile*, is an emergent aquatic, rooted in water with shoots growing into the air. The stalks arise from underground rhizomes that are difficult to dig out (https://en.wikipedia.org/wiki/Equisetum).

In some publications it is mentioned that *Equisetum arvense* is a plant bioindicator of moisture excess (waterlogging) and in other more recent publications it is considered that this species has moderate water requirements.

Knowing the water regime in the soils in which horsetails are abundant is important in order to establish the correct improvement measures.

Berca (2004) noticed that *Equisetum arvense* is found in all areas of the country, on different types of soil in annual or perennial plant crops, as well as in rural places. It does not tolerate cold, frost and shade, elements that can be used in weed control.

Some species such as *Equisetum ramosissimum*, *E. telmateia* and *E. variegatum* are common on slight alkaline soil. Another species such as *E. ramosissimum* are common on a soil with a high level of acidity.

The shade sensitivity of *E. arvense* can partly be explained by its small, scale-like, non-functional leaves at the nodes (Holm et al., 1977).

E. arvense has a high potassium demand, especially at high nitrogen levels.

E. arvense is tolerant of low levels of nitrogen but will be overtopped by fast-growing species

when competing for increased supplies of nitrogen. (Andersson and Lundegårdh, 1999).
Most of the publications that address *E. arvense* mention that this plant is bioindicative to acid soils and therefore it is recommended to apply calcium carbonate amendments to reduce soil acidity and thus reduce the degree of infestation of this agricultural weed (Ionescu-Şişeşti et al., 1958; Budoi et al., 1994; Gus et al., 1998; Lăzureanu, 1994).
Studies conducted by Chifu et al. (2006), showed that *E. arvense* has a wide ecological amplitude for pH, it develops on both acidic and neutral or slightly alkaline reaction soils.
In these cases, the application of limestone to reduce soil acidity would not be justified in all habitats with *Equisetum arvense*.
Field horsetail can be very competitive in crops for light, water and nutrients as its rhizomes can form an underground dense network during the growing season, especially summer and autumn growing seasons (James and Rahman, 2010). The plant can regrow from very small pieces of rhizome remaining in the soil (Chirilă, 2001).
The correct identification of *Equisetum* species and the knowledge of the requirements for ecological factors is useful in establishing the most appropriate measures to reduce weeding.
In this paper we intend to come up with additional data on soil characteristics in which the presence of this species has been found.

MATERIALS AND METHODS

Investigations were conducted in the agricultural area from the north-eastern part of Romania.
In our investigations have been taken many pictures with digital camera and have been collected samples of biological material consisting of mature plants of *Equisetum* sp.
The obtained images in the field were stored, analysed and processed on the computer. Biological collected material was used in order to establish genus and species of plants. The binomial nomenclature of plant species was done on behalf of the rules of the International Code for Botanic Nomenclature reviewed in the latest taxonomy works (Ciocârlan et al., 2004).
Diagnosis and name of the soil was done according to new Romanian Sol Taxonomy System known as STRS 2003 (Florea & Munteanu, 2012; WRB-World Reference Base for Soil Resources, 2014).
Soil samples were taken from each pedogenetical horizon in order to conduct laboratory analysis such as particle size, calcium carbonate, pH, content of organic carbon, cation exchange capacity).
The analysis was performed according to the standard methods presented in the current methodology (Dumitru et al., 2009; Lăcătușu et al., 2017).

RESULTS AND DISCUSSIONS

In order to establish control measures of horsetail weeds, it is necessary to correctly identify the species, the knowledge of the biology and the ecological requirements for environmental factors such as light, temperature, soil moisture, content of the nutrients etc.
Many authors noted that green stems of *Equisetum arvense* may be easy mistaken with other species such as *E. pratense, E. palustre, E. sylvaticum*, *E. pratense* Ehrh. may be distinguished by the presence of long thin silica spicules on the ridges of the middle and upper internodes of the main stem (Cody & Wagner, 1981).
E. sylvaticum L. may be easy distinguished by secondary branching of the branches. *E. palustre* L. (Figure 1) may be distinguished by the first branch internode being shorter rather than longer than the subtending stem sheath.
In Romania, *E. palustre* is the most toxic species belonging to the family Equisetaceae (Anghel et al., 1972) and the Equisetum is spread over large areas, starting with the forest-steppe zone to the forest area with spruce forests. It is found in cereals, corn, potatoes, meadows and even in plastic tunnels used predominantly for growing vegetables.
Our studies conducted in poly-tunnels located in the northeastern part of Romania have highlighted the strong infestation of soils horticultural substrata with *Equisetum arvense* and *Equisetum pratense* (Figure 2).

Figure 1. Fertile shoots of *Equisetum palustre*

Figure 2. Strong infestated soil with horsetail from plastic tunnels in Targu Frumos (foto: F. Filipov, 2014)

Special attention must be paid to the weeds of *Equisetum arvense* because they may expand rapidly after they have established themselves, and may subsequently dominate for years.
Horsetail is a very competitive species, online there are images available with the asphalt traversed by plant developed from rhizomes (https://www.vc77botany.org/equisetum-species-horsetails).
Even if it seems impossible, we also noticed that asphalt path can be traversed by plant roots or runners that develop from buds on roots or rhizomes of couch grass. The land infestation with couch grass caused gradual and continuous degradation of asphalt paths around (Filipov, 2013).
Field horsetail can also expand the rhizomes that grow in the cracks resulting from drying of compacted soils.
Such a case was reported by us in the pathway in the vicinity of the plastic tunnel located on a Phaeozems with a compact under ploughed layer that can be defined as plowpan.
Following some brief analysis performed on the plowed layer, it was shown that the reaction is slightly acidic (pH = 6.4), clay loam texture (clay = 34.8%) and well supplied with humus and nutrients of nitrogen, phosphorus and potassium.
The sterile stems grown from the rhizomes in the cracks of the strongly compacted soil on the access alley in the plastic tunnel can be seen in Figure 3.

Figure 3. *Equisetum arvense* grown from rhizomes extended into cracks in compact soil

In our studies, we identified weeds of horsetail that perforated the black plastic foil (Figure 4) used as mulch in polytunnels from Dumbrava, Neamt county.
Highly heated foil during the day can be perforated by sterile growing horsetail stems.
The strong heating of the foil takes place during the summer and corresponds to the intense growth and expansion of the surfaces infested with horsetail.

Figure 4. Perforation of black plastic foil by *Equisetum arvense* (foto: F. Filipov, 2012)

We also noticed that horsetail infestation occurs on Aluviosol with slight alkaline soils (Figure 5) with pH of 7.8.

Figure 5. *Equisetum arvense* on the slight alkaline alluvial soil

Horsetail infestation was also noticed on soils with good external drainage, but with a strong hardpan (Figure 6).
Following the description in the field, soil was named as Faeoziom cambic (after Romanian Soil Taxonomy System-2012) or haplic Phaeozems (after WRB-2014).
Haplic Phaeozems (Figure 7A, Figure 8) is strong compacted in the upper part of soil profile, over the depth between 0 and 35 cm. The strong compaction of the soil is evident both in the plowed layer and in the underlying horizon (Figure 7B) and is due to poor agricultural practices.

Figure 6. Infestation with *Equisetum arvense* of sloping ground with good external drainage

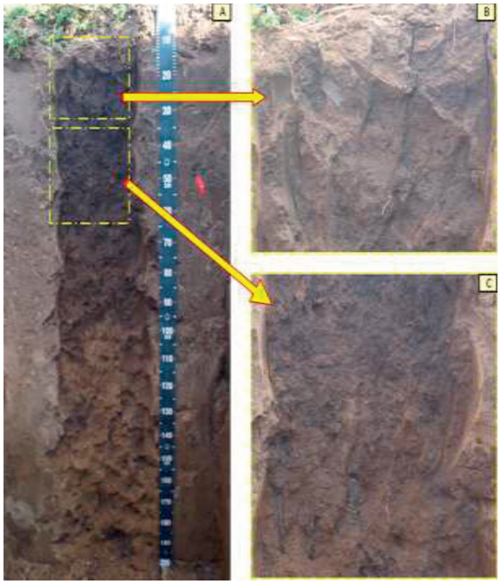

Figure 7. Soil profile of haplic Pheozems (A); Strong compacted upper part of soil (B); Frequent galleries of earthworms (C)

Strong soil compaction favors soil erosion that is easily observed in the field, especially on the tracks of farm machinery wheels.
The soil layers located at a depth higher than 35 cm is maintained loose (Figure 7C).

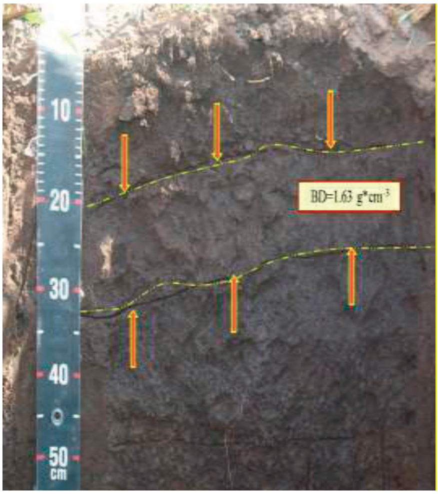

Figure 8. Soil strongly compacted on the depth of 20-32 cm

The presence of earthworm galleries indicates that in this soil the biological activity was intense. Earthworm species are a very good bio indicator of soil health. Earthworm populations have declined considerably with soil degradation through compaction and due to using high doses of the chemical fertilizers.
We consider that the compaction of the upper part of the ash, which favored the retention of a larger amount of water, the soil tillage frequencies allowed the multiplication of the horsetail and the extension of the weeding.
Some soil properties are presented in Table 1. In the ploughed horizon is registered pH value of 7.47.

Table 1. Some properties of Phaeozems infested with horsetail

Depth cm	Clay %	pH	$CaCO_3$ %	T me/100 g	OC (%)
0-30	32.50	7.47	-	19,73	1.7
30-55	33.71	6.95	-	20,3	1.5
55-75	29.58	6.61	-	19,7	--
75-115	28.93	7.58	2.63	18.38	-
115-155	28.11	823	4.61	16.31	-

OC-organic carbon; T cation exchange capacity

We mention that in our studies the most common horsetail infestations were found on acid soils. As weakly alkaline soils infested with *E. arvense* were also noticed, we recommend that the establishment of the opportunity to decrease soil acidity should be done following the laboratory analysis.
The competitiveness of horsetails to infest agricultural land is also due to its biological characteristics.
The experiment conducted by the Cody and Wagner (1981) highlighted that, in growth room, a rhizome of horsetail with length of 10 cm has produced a total of 64 m of rhizome in a year. Some biological characteristic that favor the spread of this species are summarized in Figure 9.
Several authors (James and Rahman, 2010) has noted that in the agricultural land where horsetail is well established and has an extensive root system, effective control weeds with herbicides is very difficult due to the biological characteristics of *Equisetum arvense*.

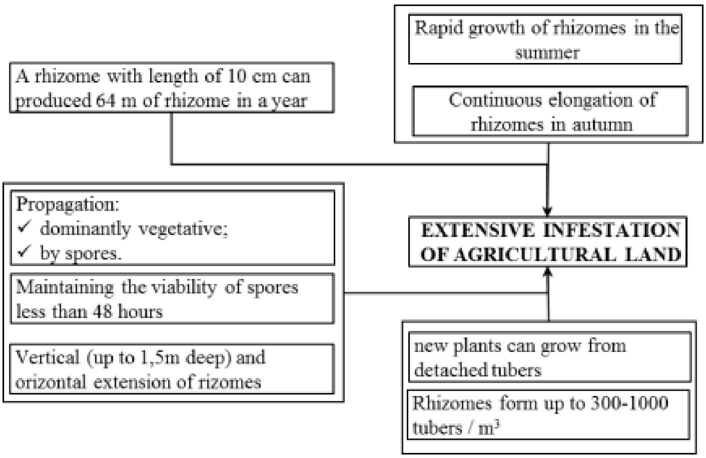

Figure 9. Diagram with some biological characteristics of *Equisetum arvense*

CONCLUSIONS

The genus *Equisetum* includes several species of plants with different ecological requirements.

Equisetum arvense is frequently spread on soils with compacted under ploughed layer. In this case, it is necessary to loosen the soil in order to increase the draining porosity from the upper part.

Underground drainage is not justified when the soil is loose under the layer of plough pan.

Equisetum arvense is a plant species with wide ecological amplitude for soil reaction. The application of amendments containing calcium carbonate is not always justified.

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HUMIFICATION OF PLANT RESIDUES UNDER OPTIMAL CONDITIONS

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Abstract

Humus as a specific organic part of the soil is involved in the formation of soil fertility. Humus is formed as a result of humification of decomposition products of organic residues. This process varies depending on temperature and humidity. Plant residues of different crops are subject to the process of humification in different ways. This became a question of our research. The article presents the data of humification of plant residues: winter wheat, barley, corn for grain and sunflower.

***Key words**: humus, humification, plant remains.*

INTRODUCTION

The main task of modern agriculture is to create food production on the basis of scientifically sound systems of agriculture and comprehensive growth of fertile soils.

Currently, the basic law of agriculture is rarely supported - the law of return, in accordance with all substances, felt with the harvest of crops, must be returned to the soil in excess, which will increase the process of humus mineralization and dehumidification as a result.

In the soil, the processes of mineralization and humification occur in parallel with each other. As a result of mineralization, carbon dioxide, mineral compounds of nitrogen, phosphorus, potassium and other macro-microelements are released. During humification, a complex biological and chemical process of transformation of high-molecular decomposition products of organic substances into a special class of organic compounds - humic acids takes place.

The intensity of the humification process depends on the amount of newly formed humus, and the soil fertility, which depends on the quantity and quality of humus in the soil. One of the the most fertile soils is considered to be typical chernozem (Fedaka, 2011). The area of chernozems in the world is 314 million hectares, or 2.4% of the area of all soils in the world. Ukraine occupies a leading position among the countries in which chernozems are widespread. The area of typical chernozems in Ukraine is 6.2 million hectares (Pozniak, 2016)

The views of scientists on the process of humus formation and humification are formulated in the following sequence: from chemical to microbiological and later biochemical positions (Kostychev, 1886; Kononova, 1963; Aristovskaya, 1980; Orlov, 1990). However, among the average representatives of the theory of the last confirmed experimental experiment does not exist, and since then until the final understanding of this process, scientists seek to the present day, so this question has not lost relevance in modern soil science.

Most scientists (Alekseev et al., 1970; Tittarelli et al., 2002; Dovban, 2009; Antonets, 2014; Shuvar et al., 2015; Balaev and Pikovskaya, 2016; Vitvitsky, 2016; Segnini et al., 2017) believe that in modern conditions, the main source for improving the fertility soil, increasing the higher yields of crops, as well as replenishment of organic matter in the soil is straw with the crop residues in combination with green manure. They proved that it promotes the activation of the processes of humification of organic matter.

Building on a of scientists (Jones et al., 2012; Adamenko, 2014; Reshetchenko et al., 2015), which indicates that over the past 35 years, the average air temperature rises at 1 ° C and there are significant changes in the distribution of precipitation during the growing season, which makes it a priority to study the processes of intensity of humification of crop residues in climate change.

MATERIALS AND METHODS

The research was conducted on the territory of the state enterprise of educational and research farm "Dokuchaevske" within the training, research and production center "Experimental Field" of Kharkiv National Agrarian University named after V.V. Dokuchaev. The soil was used for research: chernozem typical heavy loam on loess-like loam of the fallow area, which was withdrawn from agricultural use in 1956.

Plant material - surface post-harvest residues of winter wheat, corn, sunflower, barley.

Soil samples of typical chernozem were taken from the arable layer with a depth of 0-30 cm. Determination of mobile phosphorus and potassium by the modified Chirikov method. The method is based on the extraction of mobile phosphorus and potassium compounds from the soil with a solution of acetic acid with a concentration of $c(CH_3COOH) = 0.5$ mol/dm^3 at a soil to solution ratio of 1:25. And the next determination of phosphorus in the form of a blue phosphorolibdenum complex on a photoelectrocolorimeter and potassium - on a flame photometer. Determination of alkaline hydrolyzed nitrogen by the Cornfield method. The method is based on the hydrolysis of organic soil compounds with an alkali solution of molar concentration $c(NaOH) = 1$ mol/dm^3 in a thermostat at a temperature of (28 ± 5)°C in a Conway cup with a lid. After hydrolysis, ammonia is quantified by titration with a solution of sulfuric acid of molar concentration $c(1/2\ H_2SO_4) = 0.02$ mol/dm^3. Determination of total humus by the Turin method. The method is based on the oxidation of soil organic matter by a solution of potassium dichromate in sulfuric acid, followed by determination of organic carbon content through the determination of potassium dichromate after oxidation by titrometry. Dry matter and moisture by weight were determined. Gravimetric method. Soil samples are dried to constant weight at (105 ± 5)°C. And determination of total soil moisture by saturation in cylinders. The principle of the method is based on determining the amount of moisture in soil samples when all pores and voids are completely filled with water.

Calculation and preparation of plant material and soil for composting was performed according to the method of Chesnyak. Plant residues are selected a week before harvest. The rests are selected manually, the crop rests are crushed to the size of 0.5 cm and in a shade bring to an air-dry condition. The weight of the soil in terms of a completely dry sample for each repetition is equal to 100 g. The selected general soil sample to grind it better with plant residues is ground, sifted through a sieve and visible plant remains are selected. Two days before the start of the experiment in the general soil sample determine the moisture content and the content of total humus.

In the laboratory, "composting" of plant samples with soil was performed for 40, 50 and 60 days at optimal languages, namely at a temperature of 26-28°C and at a humidity of 60 % of the total moisture content.

Statistical data processing is performed in Microsoft Excel.

RESULTS AND DISCUSSIONS

Typical heavy loam chernozem on loess-like loam was used for research. With initial data: total humus - 6.24%; light hydrolysis nitrogen - 214 mg/kg of soil; mobile phosphorus - 294 mg/kg of soil; exchangeable potassium - 282 mg/kg of soil.

For calculations and laboratory analysis, the total soil moisture capacity content was determined - 64%. And soil moisture - 4.36%.

Composting was performed in a thermostat for 60 days with stepwise selection for 40, 50 and 60 days.

The results of composting, namely the dynamics of the content of total humus in the soil and the change in the amount of plant material of the surface crop residues of winter wheat are presented in Tables 1, 2.

During the first 40 days of composting, the weight of plant material decreased sharply by 44.3% and amounted to 4.75 g (Table 1). During this period, the content of total humus (Table 2) in the studied soil increased by 0.26% and amounted to 6.50%. Over time, during composting, a sharp decrease in the mass of plant debris grows into a moderate one. Thus, during the 50-day period of composting of plant material of winter wheat, the mass of plant residues decreased by only 0.22 g from the previous 40-day period (Table 1), while the content of total humus, on the contrary, increased sharply and amounted to 6.83%, which is 0.33% higher than the previous value (Table 2).

The tendency of moderate decrease in the mass of surface crop residues of winter wheat is observed in the version for 60 days of composting. The difference between the previous version is only 0.06 g. In this sample, there is also a slowdown in the accumulation of total humus. The difference between the options of 50 and 60 days of composting is only 0.10%.

Table 1. Change in the mass of plant material during the composting period

Composting time, days	Mass of plant material before composting, g	Mass of plant material after composting, g	Change in the mass of plant material after composting
40		4.75 ± 0.09	3.77* / 44.3
50	8.52	4.53 ± 0.09	3.99 / 46.8
60		4.47 ± 0.09	4.05 / 47.5

*Above the line - the difference in weight of plant material after composting, g; under the line - reduction of plant residues, %

Table 2. Change in the content of total humus in chernozem typical for the composting period

Duration of compostin g, days	The content of total humus in the soil before composting, %	The content of total humus in the soil after composting, %	Increase in total humus in the soil after composting, %
40		6.50 ± 0.21	0.26* / 104.2
	6.24	6.83 ± 0.21	0.59 / 109.5
50		6.83 ± 0.21	0.69 / 111.1

*Above the line - the increase in the content of total humus in the soil after composting, %; Under the line - % of the control content of humus in the soil

The increase in the content of total humus in the soil, compared with the control, for 40 days of composting was 0.26%, and the weight loss of plant residues was 3.77 g. For 50 days of composting, the increase in total humus content is 0.59%, and the decrease in the mass of plant material of the surface crop residues of winter wheat 3.99 g. During the 60-day period of composting, the increase was 0.69%, and the decrease in the mass of plant material by 4.05 g.

Thus, studies have shown that during the first 50 days of composting in optimal environmental conditions there is an intensive loss of mass of plant material, and there is an intensive humification.

Thereafter, it shall moderate, which is due to a decrease in the activity of microorganisms, as stressed in his writings Aristovs`ka.

Against the background of increasing the content of total humus, the mass of surface crop residues of winter wheat decreased. The intensity of this decrease was proportional to the intensity of humification (Figure 1).

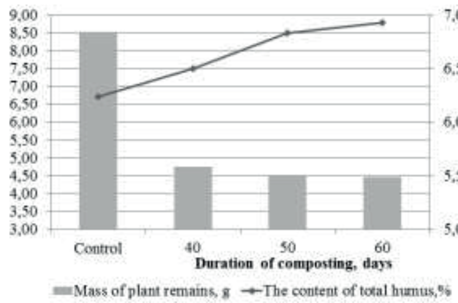

Figure 1. Dynamics of total humus content and mass of plant residues

Confidence interval for changes in total humus content and mass of plant residues of winter wheat in the composting process For total humus content in soil it is ± 0.21%, for plant residues - ± 0.09 g. Therefore, the difference in total humus content in soil between control and all the presented options is significant because it exceeds the icon by 0.21%. In comparison with the different options between each other, that after 40 days and 50 days of the composition is clearly significant difference, while the difference between the 50 and 60 days of the composition is less than 0.21% and is not significant, as defined above, due to the slowdown of the humification process.

Changes in the mass of plant substances containing winter substances between the control and the studied variants are significant. This makes it possible to ensure a continuous process of conversion of plant material during the study, but its intensity decreases over time.

It is established that the humification process is continuous. The content of total humus with the winter period of composting in optimal conditions increases. The intensity of the increase in the content of total humus, as well as the process of humification, varied. Time distribution of humification intensity after the following character: return → peak → recession. When using the humus content, the mass of winter wheat residues decreases. The intensity of this reduction is proportional to the intensity of the humification process.

Dynamics of nutrient content in the version with winter wheat.

Figure 2 shows the dynamics of the content of light hydrolysis nitrogen in terms of composting. As can be seen from the graph, the increase in light hydrolysis nitrogen has an increasing rate. For the first 40 days the increase is 11.7%, for 50 days - 15.4% and for 60 days the increase from control is 24.3%

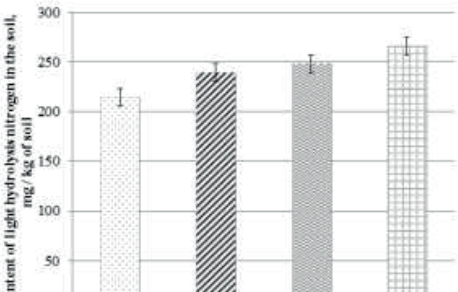

Figure 2. Dynamics of light hydrolysis nitrogen content in soil

The dynamics of mobile phosphorus in the studied variant was as follows (Figure 3). As can be seen from the graph for the period of composting, a significant difference in the content of mobile phosphorus was observed only at 50 days of composting and amounted to 275 mg/kg of soil.

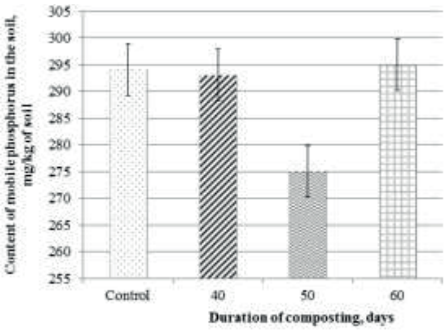

Figure 3. Dynamics of mobile phosphorus content in soil

The dynamics of the content of exchangeable potassium is shown in Figure 4. From this graph we see that from the first 40 days of composting in the studied variant there is a significant increase in potassium and lasts up to 50 days and is 478 and 542 mg/kg of soil, respectively. Starting from 60 days, there is a moderate decrease in the content of metabolic potassium and is 532 mg/kg of soil.

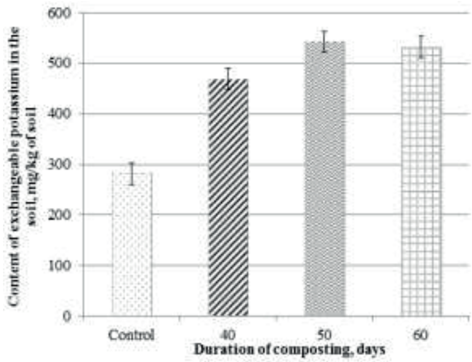

Figure 4. Dynamics of metabolic potassium content

The dynamics of the content of total humus in the soil and the change in the amount of plant material of the surface crop residues of barley are presented in Tables 3 and 4.

During the first 40 days of composting the mass of plant material decreased by 2.79 g (Table 3), during this period the content of total humus (Table 4) in the studied soil increased by 0.11% and amounted to 6.35%. The change in weight over the next 10 days was 1.55 g, and the increase in humus during this period was only 0.10%. In the next 10 days there is a decrease in the intensity of the transformation of surface plant residues. The change in weight during this period is 0.6 g, and the intensity of humus accumulation increases, the difference is 0.32%.

Table 3. Change in the mass of plant material during the composting period

Composting time, days	Mass of plant material before composting, g	Mass of plant material after composting, g	Change in the mass of plant material after composting
40	7.94	5.15 ± 0.11	2.79 * 35.1
50		3.60 ± 0.11	4.34 54.7
60		3.00 ± 0.11	4.94 62.2

*Above the line - the difference in weight of plant material after composting, g; under the line - reduction of plant residues, %

The increase in the content of total humus in the soil, compared with the control, for 40 days of composting was 0.11%, and the weight loss of plant residues was 2.79 g. For 50 days of composting, the increase in total humus content is 0.21%, and the decrease in the mass of plant material of the surface crop residues of barley 4.34 g. During the 60-day period of composting, the increase was 0.53%, and the decrease in the mass of plant material by 4.94 g.

Table 4. Change in the content of total humus in chernozem typical for the composting period

Composting time, days	The content of total humus in the soil before composting,%	The content of total humus in the soil after composting, %	Increase in total humus in the soil after composting, %
40	6.24	6.35 ± 0.06	0.11 * 101.8
50		6.45 ± 0.06	0.21 103.4
60		6.77 ± 0.06	0.53 108.5

*Above the line - the increase in the content of total humus in the soil after composting,%; Under the line -% of the control content of humus in the soil

Thus, studies have shown that during the first 50 days of composting in optimal environmental conditions there is an intensive loss of mass of plant material.

Against the background of increasing the content of total humus, the mass of surface crop residues of barley decreased. The intensity of this decrease was proportional to the intensity of humification (Figure 5).

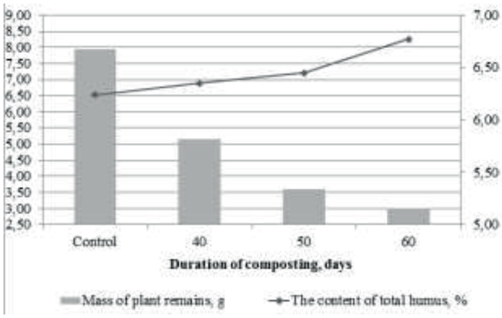

Figure 5. Dynamics of total humus content and mass of plant residues

Confidence interval for changes in total humus content and weight of barley crop residues during composting. For the content of total humus in the soil it is ± 0.06%, for the amount of plant residues ± 0.11 g. Therefore, the difference in the content of total humus in the soil between the control and all the presented options is significant because it exceeds the mark of 0.06%.

The change in the mass of barley crop residues between the control and the studied variants is significant. This makes it possible to argue about the continuous process of transformation of plant material in the process of the experiment, but its intensity decreases over time.

It is established that the humification process is continuous. The content of total humus increases with increasing composting period in optimal conditions.

Dynamics of nutrient content in the version with barley.

Figure 6 shows the dynamics of the content of light hydrolysis nitrogen in terms of composting. As can be seen from the graph, the increase in light hydrolysis nitrogen is oscillating. For the first 40 days, the content of light hydrolysis nitrogen was 203 mg/kg of soil, which is 6% less than the control. Over the next 10 days, the increase in light hydrolysis nitrogen in the studied variant was 34 mg/kg of soil, and over the next 10 days the content decreased by 31 mg/kg of soil and amounted to 206 mg/kg of soil.

The dynamics of mobile phosphorus in the studied variant was as follows (Figure 7). As can be seen from the graph during the composting period, the dynamics of the content of mobile phosphorus has a gradually increasing rate. The increase in composting days was 4.8%, 7.1% and 18.7% of control.

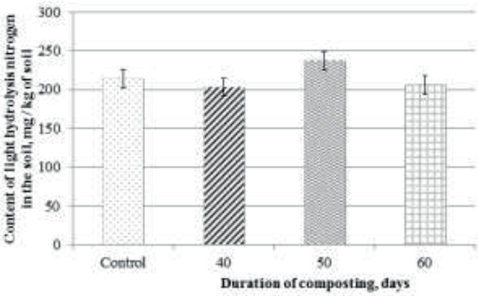

Figure 6. Dynamics of easily hydrolyzed nitrogen in the soil

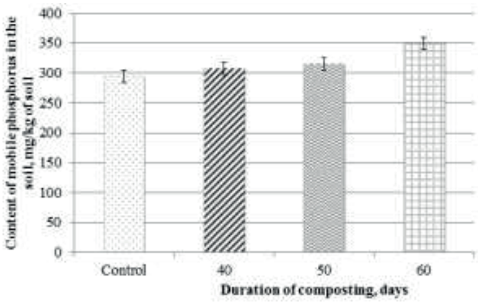

Figure 7. Dynamics of mobile phosphorus in soil.

The dynamics of the content of exchangeable potassium is shown in Figure 8. From this graph we can see that from the first 40 days of composting in the studied variant there is a significant increase in potassium and is 558 mg/kg of soil.

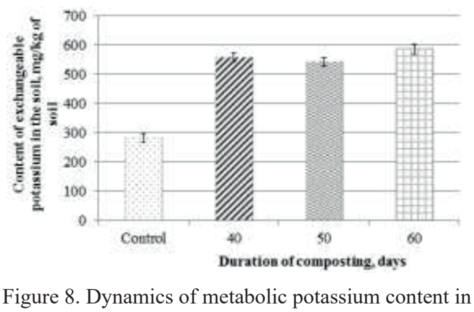

Figure 8. Dynamics of metabolic potassium content in soil

Slightly lower content of metabolic potassium is observed in the studied variant at 50 days, its content is 541 mg/kg of soil. From 60 days there is an increase in the content of metabolic potassium and is 586 mg/kg of soil.

The dynamics of the content of total humus in the soil and the change in the amount of plant material of the surface crop residues of corn for grain are presented in Tables 5 and 6.

During the first 40 days of composting the mass of plant material decreased by 4.26 g (Table 5), during this period the content of total humus (Table 6) in the studied soil was 6.28%. The change in weight over the next 10 days was 1.22 g, and the increase in humus during this period is 0.17%. In the next 10 days there is a decrease in the intensity of the transformation of surface plant residues. The change in weight during this period is 0.67 g, and the intensity of humus accumulation increases, the difference is 0.48%.

Table 5. Change in the mass of plant material during the composting period

Composting time, days	Mass of plant material before composting, g	Mass of plant material after composting, g	Change in the mass of plant material after composting
40	8.28	4.02 ± 0.20	4.26 * / 51.4
50		2.80 ± 0.20	5.48 / 66.2
60		2.13 ± 0.20	6.15 / 74.3

*Above the line - the difference in weight of plant material after composting, g; under the line - reduction of plant residues,%

Table 6. Change in the content of total humus in chernozem typical for the composting period

Composting time, days	The content of total humus in the soil before composting, %	The content of total humus in the soil after composting, %	Increase in total humus in the soil after composting, %
40	6.24	6.28 ± 0.08	0.04 * / 100.6
50		6.45 ± 0.08	0.21 / 103.4
60		6.93 ± 0.08	0.69 / 111.1

*Above the line - the increase in the content of total humus in the soil after composting, %; Under the line - % of the control content of humus in the soil

The increase in the content of total humus in the soil, compared with the control, for 40 days of composting 0.04%, and the weight loss of plant residues was 4.26 g. For 50 days of composting, the increase in total humus content is 0.21%, and reduction of the mass of plant material of surface crop residues of corn per grain of 5.48 g. During the 60-day period of composting, the increase was 0.69%, and the decrease in the mass of plant material by 6.15 g.

Thus, studies have shown that during the first 50 days of composting in optimal environmental conditions there is an intensive loss of mass of plant material.

Against the background of increasing the content of total humus, the mass of surface crop residues of corn for grain decreased. The intensity of this decrease was proportional to the intensity of humification (Figure 9).

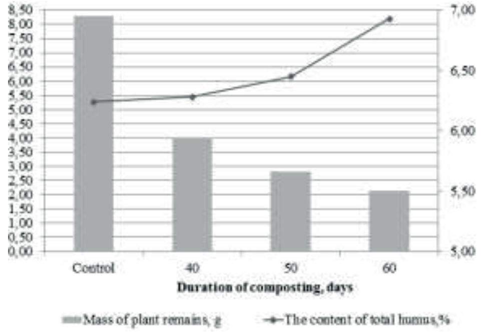

Figure 9. Dynamics of total humus content and mass of plant residues

Confidence interval for changes in the content of total humus and the mass of crop residues of corn for grain during composting. For the content of total humus in the soil it is ± 0.08%, for the amount of plant residues ± 0.20 g. Therefore, the difference in the content of total humus in the soil between the control and all the presented options is significant, except for the difference between the control and the option of 40 daily composting because it does not exceed the mark of 0.08%.

The change in the mass of crop residues of corn for grain between the control and the studied options is significant. This makes it possible to argue about the continuous process of transformation of plant material in the process of the experiment, but its intensity decreases over time.

It is established that the humification process is continuous. The content of total humus increases with increasing composting period in optimal conditions.

Dynamics of nutrient content in the version with corn for grain.

Figure 10 shows the dynamics of the content of light hydrolysis nitrogen in terms of composting. As can be seen from the graph for the first 40 days of composting, the content of light hydrolysis nitrogen decreases to the mark of 164 mg/kg of soil. Over the next 10 days, the decrease in the content of easily hydrolyzed nitrogen is 9 mg/kg of soil. In another 10 days the content of light hydrolysis nitrogen slightly increases to the level of 167 mg/kg of soil.

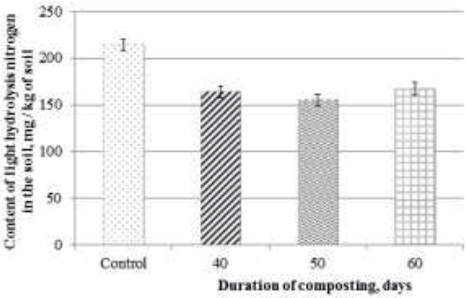

Figure 10. Dynamics of light hydrolysis nitrogen content in soil

The dynamics of mobile phosphorus in the studied variant was as follows (Figure 11). As can be seen from the graph during the composting period, a significant difference in the content of mobile phosphorus was observed during 40 days of composting. During the following periods of composting there is an accumulation of mobile phosphorus where the increase is 18 and 27 mg/kg of soil according to the terms of composting.

The dynamics of the content of exchangeable potassium is shown in Figure 12. From this graph we see that from the first 40 days of composting in the studied variant there is a significant increase in potassium and goes to growth up to 60 days. Thus, as a percentage of control, the increase in potassium exchange in terms of composting is 50.4%, 64.9% and 79.8%, respectively.

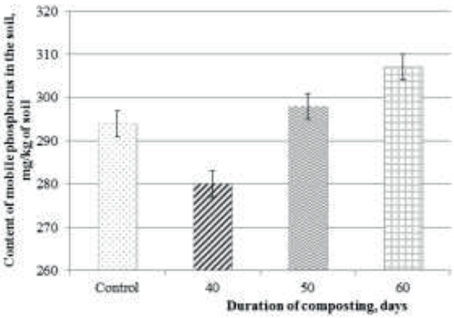

Figure 11. Dynamics of mobile phosphorus in soil

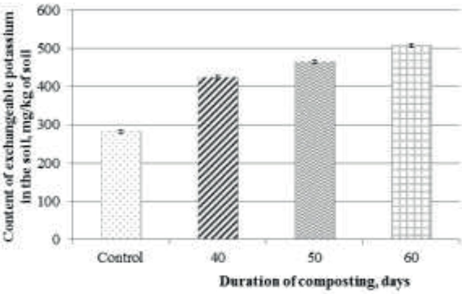

Figure 12. Dynamics of potassium content in soil

The dynamics of the content of total humus in the soil and the change in the amount of plant material of the surface crop residues of sunflower are presented in Tables 7 and 8.

During the first 40 days of composting, the weight loss of plant material was 3.56 g and amounted to 5.63 g (Table 7), during this period the content of total humus (Table 8) in the studied soil was 6.38% the increase is equal to 0.14%.

Table 7. Change in the mass of plant material during the composting period

Composting time, days	Mass of plant material before composting, g	Mass of plant material after composting, g	Change in the mass of plant material after composting
40	9.19	5.63 ± 0.45	3.56 * / 38.7
50		3.85 ± 0.45	5.34 / 58.1
60		2.70 ± 0.45	6.49 / 70.6

*Above the line - the difference in weight of plant material after composting, g; under the line - reduction of plant residues,%

The change in weight over the next 10 days decreased by 58% and amounted to 3.85 g, which is 1.78 less than the 40-day period of composting, and the increase in humus during this period is 0.38%. During the entire composting period, the weight loss was 6.49 g, which is 1.15 g less than 50 and 2.93 g less than 40 days of composting. As a percentage, the weight loss in 60 days of composting was almost 71%. The intensity of humus accumulation during this period increases, the increase was 14.6%, the difference is 0.91% of the total humus in the soil.

The increase in the content of total humus in the soil, compared with the control, for 40 days of composting 0.14%, and the weight loss of plant residues was 3.56 g. For 50 days of composting, the increase in total humus content is 0.52%, and decrease in the mass of plant material of surface crop residues of sunflower 5.34 g. During the 60-day period of composting, the increase was 0.91%, and the decrease in the mass of plant material by 6.49 g.

Table 8. Change in the content of total humus in chernozem typical for the composting period

Composting time, days	The content of total humus in the soil before composting, %	The content of total humus in the soil after composting, %	Increase in total humus in the soil after composting, %
40	6.24	6.38 ± 0.16	0.14 * / 102.2
50		6.76 ± 0.16	0.52 / 108.3
60		7.15 ± 0.16	0.91 / 114.6

*Above the line - the increase in the content of total humus in the soil after composting, %; Under the line -% of the control content of humus in the soil

Thus, studies have shown that during the first 50 days of composting in optimal environmental conditions there is an intensive loss of mass of plant material.

Against the background of increasing the content of total humus, the mass of surface crop residues of sunflower decreased. The intensity of this decrease corresponded proportionally to the intensity of humification (Figure 13).

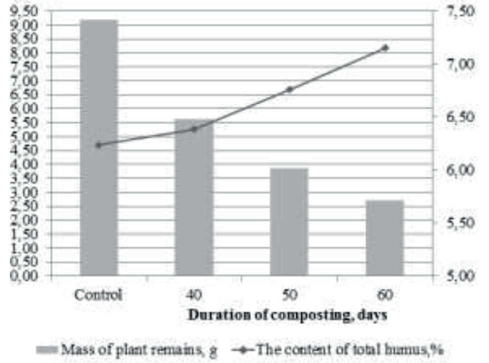

Figure 13. Dynamics of total humus content and mass of plant residues

Confidence interval for changes in total humus content and mass of sunflower crop residues during composting. For the content of total humus in the soil it is ± 0.16%, for the amount of plant residues ± 0.45 g. Therefore, the difference in the content of total humus in the soil between the control and all the presented variants is significant, except for the difference between daily composting because it does not exceed the mark of 0.16%.

The change in the mass of plant residues by sunflower between the control and the studied options is significant. This makes it possible to argue about the continuous process of transformation of plant material in the process of the experiment, but its intensity decreases over time.

Thus, studies have shown that during the first 40 days of composting in optimal environmental conditions there is a moderate process of humification of plant material, when as for the next 20 days the intensity of humification increases.

Dynamics of nutrient content in the version with sunflower.

Figure 14 shows the dynamics of the content of light hydrolysis nitrogen in terms of composting. As can be seen from the graph for the first 40 days of composting, the content of light hydrolysis nitrogen decreases to the mark of 164 mg/kg of soil. Over the next 10 days, the increase is 15 mg/kg of soil. In another 10 days the content of easily hydrolyzed nitrogen decreases to the level of 160 mg/kg of soil.

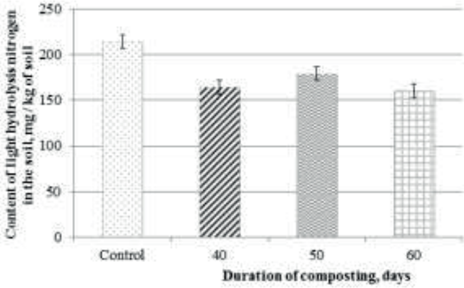

Figure 14. Dynamics of light hydrolysis nitrogen content in soil

The dynamics of mobile phosphorus in the studied variant was as follows (Figure 15). As can be seen from the graph during the composting period, a significant difference in the content of mobile phosphorus was observed throughout the composting period. However, the lowest amount of mobile phosphorus is observed in 40 days of composting on foot and is 270 mg/kg of soil. During the following periods of composting there is an accumulation of mobile phosphorus.

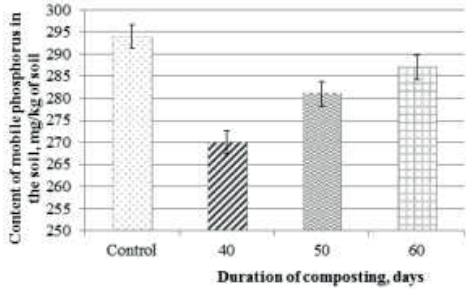

Figure 15. Dynamics of mobile phosphorus content in soil

The dynamics of the exchangeable potassium content is shown in Figure 16. From this graph we see that from the first 40 days of composting in the studied variant there is a significant increase in potassium, for 50 days its content decreases slightly to the mark of 334 mg/kg of soil. Starting from 60 days, there is an increase in the content of metabolic potassium and is 458 mg/kg of soil.

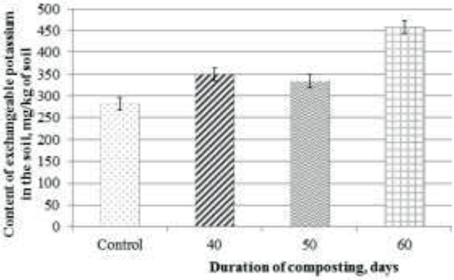

Figure 16. Dynamics of metabolic potassium content in soil

CONCLUSIONS

Thus, based on the above research results, the following conclusions can be drawn:

The intensity of humification as a link in humus formation depends on the climatic factor, this was talking in your time by Vasily Vasilyevich Dokuchaev.

It is established that the humification process is continuous. The content of total humus with the as the length period of composting in optimal conditions increases. The intensity of the increase in the content of total humus, as well as the process of humification, varied.

Amid increasing the humus content, the mass of winter wheat residues decreases. The intensity of this reduction is proportional to the intensity of the humification process. The increase in humus in the laboratory for the full period of composting is 0.69%, and the decrease in plant material for this period is 4.05 g, the increase in humus in the laboratory for barley during the entire period of composting is 0.53% with a decrease in weight for this the same period is 4.94 g. The increase in humus in the laboratory for sunflower during the entire period of the composting is 0.91% for this weight loss for the same period is 6.49 g. Such a high increase in humus in the due to structure of plant residues. The increase in humus in the laboratory for corn for the whole time of composting will be 0.69% for this weight loss for the same period is 6.15 g

The dynamics of the content of nutrients in the soil varies from the options and timing of the analysis.

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INFLUENCE OF SEED-PROTECTANT PESTICIDES ON SOIL AZOTOBACTER AND CLOSTRIDIUM NITROGEN-FIXING BACTERIA

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Abstract

This study was designed to evaluate the influence of five seed-protectant pesticide (fluxapiroxad, triticonazol, tetraconazol + tiofant metil, difenoconazol + fludioxonil and teflutrin + fludioxonil) at recommended concentration (for wheat seed treatment) on the numerical concentration in soil of Azotobacter spp. and Clostridium spp. nitrogen-fixing bacteria. The control variant was represend by soil sample where no seed treatment was applied. Soil samples were taken periodically and analyzed for the numerical determination of nitrogen-fixing bacteria of the genera Azotobacter and Clostridium.

***Key words**: Azotobacter spp., Clostridium spp., nitrogen-fixing bacteria, soil, pesticides.*

INTRODUCTION

Pesticides are substances widely used in agriculture to control harmful organisms in crops (Johnsen et al., 2001). The most common use of pesticides is as plant protection products, which in general protect plants from damaging influence such as weeds, fungi, or insects (Adams, 2017). Pesticides can be classified by target organism (e.g., fungicides, insecticides, herbicides, rodenticides, bactericides etc.), chemical structure (e.g., organic, inorganic, synthetic, or biological), and physical state (Adams, 2017).

The use of plant protection products has been recognized and accepted as an essential ingredient in the modern agriculture for the control of pests, which damage crops and as result, they produce a severe loss in food production. However, the extended use of pesticides, together with the inadequate behaviours of prevention and use of basic protection requisites will increase the probability of accidental intoxication in a notorious manner.

Impact of pesticides on agronomic yield and profit margin makes them significant component of modern agricultural practices. However, the indiscriminate use of pesticides leads to the degradation of soil's microbial ecosystems.

Pesticides are often applied several times during one crop season and a part always reaches the soil. The wide use of pesticides has created numerous problems, including the pollution of the environment. The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides (Arora et al., 2016; Sethi et al., 2013).

The application of pesticides starts from the pre sowing stage. Different treatments include soil application, seed treatment, foliar spray, etc. Repeated applications of pesticides contaminate the soil. Soil is the most important site of biological interactions. The indiscriminate use of pesticides disturbs the soil environment by affecting flora and fauna including microflora of soil, and also the physico-chemical properties of soil like pH, salinity, alkalinity leading to infertility of soil (Sarnaiket et al., 2006).

When pesticides are applied, the possibilities exist that these chemicals may exert certain effects on non-target organisms, including soil microorganisms (Simon-Sylvestre and Fournier, 1980).

The microbial biomass plays an important role in the soil ecosystem where they fulfil a crucial role in nutrient cycling and decomposition (De-Lorenzo et al., 2001).

Microorganisms are the primary soil decomposers driving key ecosystem processes such as organic matter decomposition, nutrient cycling and thereby, plant productivity (Devareet et al., 2007; Pandey and Singh, 2004).

In the last few decades, many soil microorganisms have been shown to have a positive effect on plant development. In addition to well-known symbiotic bacteria, free nitrogen-fixing organisms in the rhizosphere (*Azotobacter*) can stimulate plant development or limit damage caused by soil-borne plant pathogens, which is why it has been used as a possible nitrogen fertilizer to increase crop yields (Askar et al., 2013).

The aim of this study was to determine the effect of a number of five pesticides used for the treatment of wheat seeds on the numerical density of bacteria of the genera *Azotobacter* and *Clostridium* during the vegetation period of the winter wheat crop.

MATERIALS AND METHODS

Pesticides

Pesticides used for treatments in the research are commonly used for winter wheat seeds treatment, both by farmer's in Romania and by those in the European Union (ISSN, 2020). Pesticides used in the research were Fluxapyroxad (Systiva 333 FS), Triticonazol (Premis), Tifofant-metil + Tetraconazol (Biosild Top), Difenoconazol + Fludioxonil (Difend Extra), Teflutrin + Flodioxonil (Austral Plus) (Table 1). To compare the effect of pesticides on the numerical density of soil bacteria of the genera *Azotobacter* and *Clostridium*, the control of the experiment was represented by the variant where winter wheat seeds were not treated.

Table 1. Pesticide treatments under the study

S. No.	Pesticide used		Mode of application	Dosage
	Trade Name	Active ingredient (technical name)		
1	Systiva 333 FS	Fluxapyroxad 333 g/l	Seed application	150 ml/100 kg seeds
2	Premis	Triticonazol 25 g/l	Seed application	150 ml/100 kg seeds
3	Biosild Top	Tiofanat-metil 350 g/l Tetraconazol 20 g/l	Seed application	100 ml/100 kg seeds
4	Difend Extra	Difenoconazol 25 g/l Fludioxonil 25 g/l	Seed application	200 ml/100 kg seeds
5	Austral Plus	Teflutrin 40 g/l Fludioxonil 10 g/l	Seed application	500 ml/100 kg seeds

Field experience

Field plot experiment was carried out at Ezareni Farm - Iasi Didactic Station of "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine.

The experiment was monofactorial, studied factor was represented by seed-protectant pesticides. The design of the experiment was done according to the "randomized block method" in four replications.

Winter wheat crop technology was represented by classical technology. Wheat crop was sown after *Lupinus* sp. and was fertilized with moderate doses of fertilizer: 120 kg N/ha^{-1} and 45 kg P_2O_5/ha^{-1}.

It should be noted that no phytosanitary treatments were carried out during the growing season.

The sowing took place on October 22, 2019 and was done with the help of the Wintersteiger seed drill, and the harvesting took place on June 20, 2020 and was carried out with the Wintersteiger combine harvester.

Wheat variety

Wheat variety *Triticum aestivum* L. *cv. Glosa* was used in the experiment.

Total bacterial count

Soil sample were taken from each plot at 20 (11.XI.2019), 41 (02.XII.2019), 170 (08.IV.2020) and 184 (22.IV.2020) days after sowing (DAS). The number of *Azotobacter* and *Clostridium* population of wheat rhizosphere was determined by counting the colonies formed on solid Ashby medium.

Soil samples from experimental plots at 10 cm depth were collected randomly from five places from each plot and composited. 10% (w/v) suspensions of soil samples in sterile distilled water were serially diluted for determining total count of bacteria using plate technique.

For enumeration of nitrogen fixing bacteria solid Ashby medium containing: glucose 20 g,

KH_2PO_4 0.2 g, K_2SO_4 0.2 g, agar 15-18 g, $MgSO_4$ 0.2 g, NaCl 0.2 g, $CaCO_3$ 5 g, in one litre distilled water was used.

Soil dilutions

From the collected samples, successive dilutions were made in sterile water, using a dilution coefficient in the rate of 10 (dilutions 10^{-1}, 10^{-2}, …,10^{-6}). By this technique a series of dilutions are obtained in which the number of germs decreases in arithmetic progression. To prepare these dilutions, 9 ml of double-distilled water sterilized at 120°C for 30 minutes was distributed in sterile tubes of 30 ml capacity. Weighed 1 g of soil onto a sterile watch and placed it in the first dilution tube. After vigorous stirring for five minutes a 10^{-1} (1/10) dilution was obtained. From this dilution was taken with a sterile graduated pipette, 1 ml of suspension and transferred to another test tube with 9 ml of sterile water, obtaining the dilution 1/100 (10^{-2}). In the same way the other dilutions were obtained: 1/1000 (10^{-3}), 1/10000 (10^{-4}), 1/100,000 (10^{-5}) and 1 / 1,000,000 (10^{-6}). Under aseptic conditions, seedings were made from the obtained dilutions, introducing 1 ml of suspension from each dilution into a Petri plate. The plates were incubated at 28^0C up to 5 days.

Determination of the number of microorganisms in the soil

After incubation period at the thermostat, the colonies were counted with the help of Wolfhügel plates. With the help of the Wolfhügel plate, the colonies of bacteria from 15-20 surfaces of one cm^2 taken on the two diagonals of the Petri plate were counted. To calculate the number of colonies on the entire surface of the plate, the average number determined per cm^2 was multiplied by the surface area of the plate in cm^2. To determine the number of bacteria in a gram of soil, multiply the number of colonies in a Petri dish by the inverse value of that dilution. The result of the count is related to the dilution used, and the final result is expressed in colony forming units (CFU) per 1 g of soil.

Statistical analysis was performed using the SPSS program (IBM SPSS Statistics 20).

RESULTS AND DISCUSSIONS

Some studies on the action of pesticides on soil microorganisms have shown that some of the pesticides used to protect plants have a harmful effect on soil microbial growth, survival, and activity (Meena et al., 2020; Milenkovsky et al., 2010; Cycon et al., 2006; Chen et al., 2001).

Effect of seed protectant pesticides on *Azotobacter* spp.

Analyzing the results, it is observed that during the vegetation period, under the influence of applied pesticides on winter wheat seed, it was observed that the numerical density of bacteria of the genus *Azotobacter* decreased in the case of variants where the winter wheat seed was treated with pesticides. In the case of the control (no seed treatments) the number of bacterial colonies was higher. In the second part of the vegetation period, it is observed in the case of all the studied variants that the number of bacteria has increased, reaching close to the value registered by the control (Figure 1).

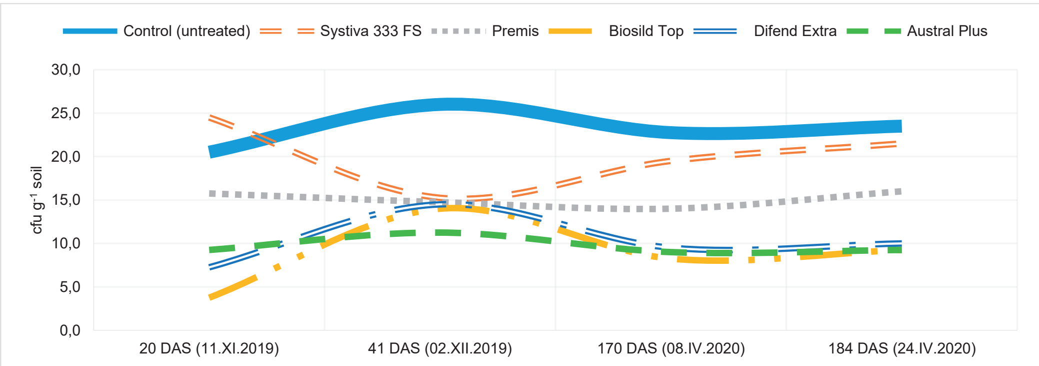


Figure 1. Numerical evolution of bacteria of the genus *Azotobacter* spp. during the vegetation period of winter wheat crop under the influence of seed-protectant pesticides (DAS = days after sowing)

Following the influence of seed-protectant pesticides on the numerical density of the genus *Azotobacter* (Figure 2) it is observed that at 20 days after sowing the lowest values were recorded when wheat seed was treated with Biosild Top, Difend Extra, and Austral Plus. The difference recorded compared to the control was statistically ensured. At the second observation, at 41 days after sowing, a balance of numerical density was observed between the researched variants, but compared to the control, in all cases the difference was statistically distinctly significant.

In the second part of the vegetation period, only the variant represented by Systiva 333 FS registered values of numerical density close to the control, in the other cases, the differences were major, being statistically ensured.

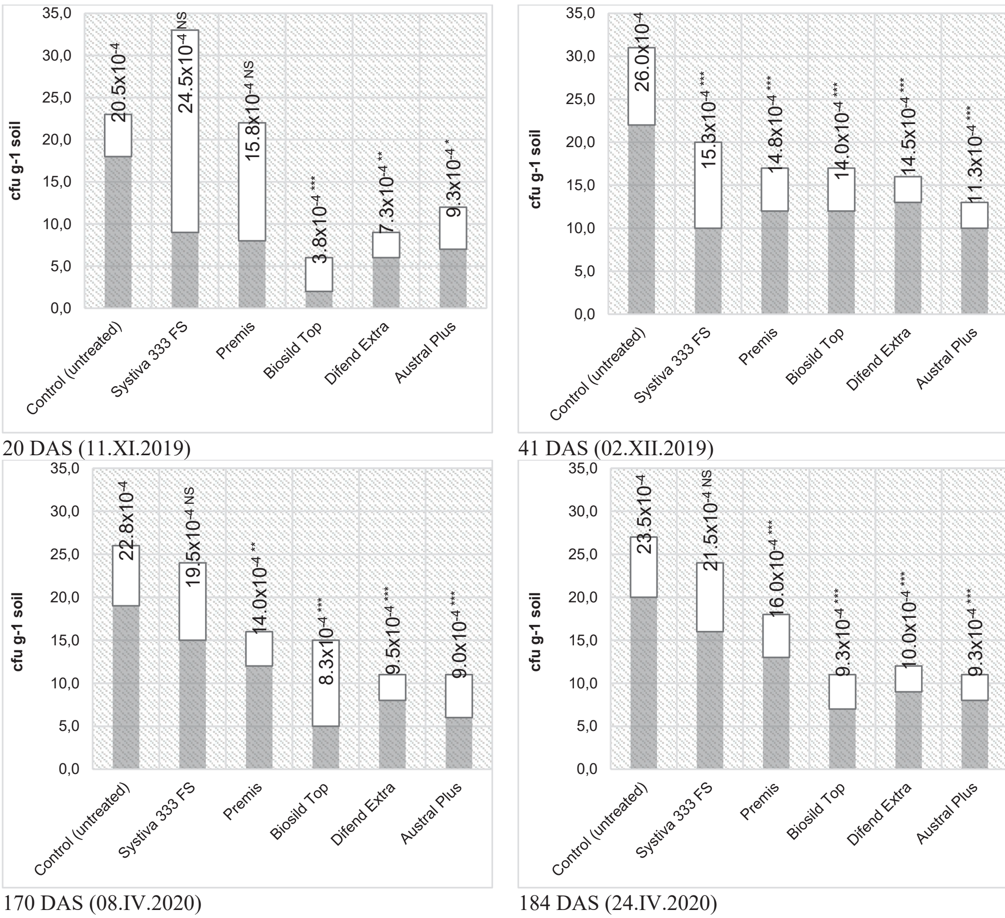


Figure 2. Effect of seed-protectant pesticides on *Azotobacter* spp. during winter wheat growing season (DAS = days after sowing) [NS = not significant; *not significant; **significant; ***distinctly significant]

Effect of seed protectant pesticides on *Clostridium* spp.

Analyzing the results, it is observed that during the vegetation period, under the influence of applied pesticides on winter wheat seed, it was observed that the numerical density of bacteria of the genus *Clostridium* decreased in the case of variants where the winter wheat seed was treated with pesticides. In the case of the control (no seed treatments) the number of bacterial colonies was higher and presented close values throughout the study period. In the second part of the vegetation period, it is observed in the case of all the studied variants that the number of bacteria has increased, reaching close to the value registered by the control (Figure 3). Following the influence of seed-protectant pesticides on the numerical density of the genus *Clostridium* (Figure 4) it is observed that at 20 days after sowing the lowest values were recorded when wheat seed was treated with Biosild Top and Austral Plus.

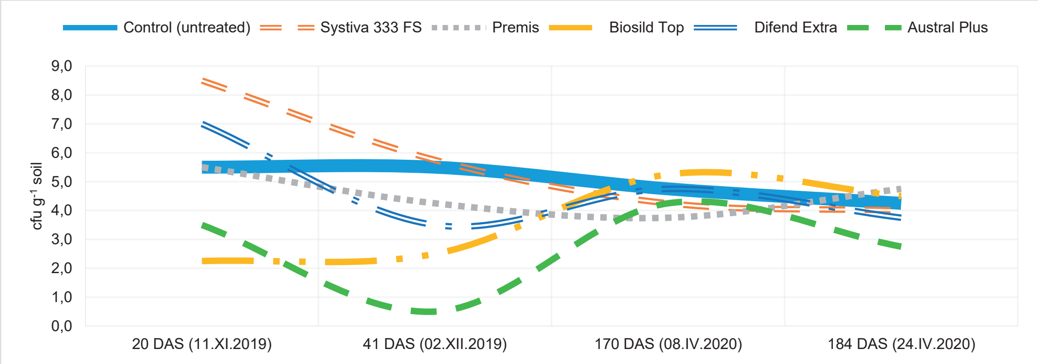
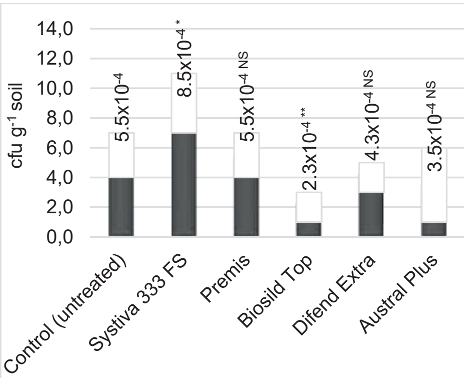
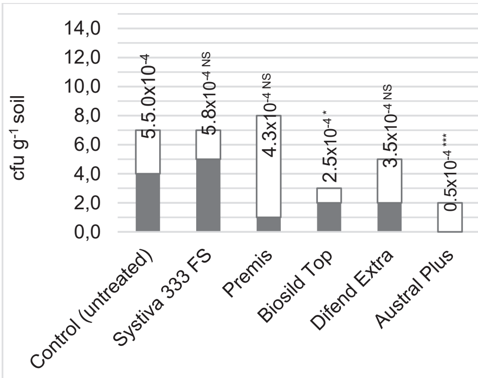


Figure 3. Numerical evolution of bacteria of the genus *Clostridium* spp. during the vegetation period of winter wheat crop under the influence of seed-protectant pesticides (DAS = days after sowing)

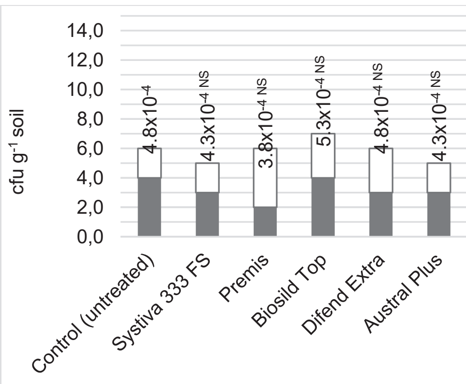
When wheat seed was treated with Systiva 333 FS and Difend Extra the values recorded were higher than the control variant. At the second observation, at 41 days after sowing, the largest differences between studied variants and control were registered. In the second part of the vegetation period, all the variants recorded a numerical density close to the control.



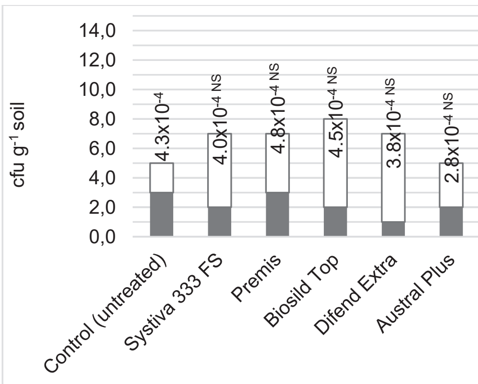
20 DAS (11.XI.2019)



41 DAS (02.XII.2019)



170 DAS (08.IV.2020)



184 DAS (24.IV.2020)

Figure 4. Effect of seed-protectant pesticides on *Clostridium* spp. during winter wheat growing season (DAS = days after sowing) [NS = not significant; *not significant; **significant; ***distinctly significant]

CONCLUSIONS

As a result of the study, it was observed that seed-protectant pesticides influence the numerical density of *Azotobacter* spp. and *Clostridium* spp. nitrogen-fixing bacteria in the soil.

Of the two genera, the genus *Azotobacter* is the one that is present with higher numerical values, being also the genus that is most strongly influenced by the action of pesticides.

It was observed that the numerical density of bacteria decreases in the first part of the vegetation period of the winter wheat crop, while in the second part of the vegetation period the numerical density of bacteria gradually increases reaching normal values, close to the control (no seed treatment).

It is well known that the use of pesticides for plant protection is an activity that cannot be replaced, but the application of the pesticides with the dose recommended by the manufacturer can avoid the occurrence of undesirable situations, like harmful effects on soil microbial growth, survival, and activity.

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OXIDIZED AND REDUCED FORMS OF IRON IN ALLUVIAL SOILS OF FLOODPLAINS OF RIVERS OF THE SIVERSKY DONETS BASIN

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Abstract

Alluvial soils form within the river floodplains. The conditions of soil formation for alluvial soils determines by the specifics of the ratio of biological and geological circulation of substances, rapid development of vegetation, and high biogenicity. They differ significantly from the soils of watersheds in their properties, genesis and use. Iron is requires careful study as one of the chemical elements that are necessary for the life of the biota and as one of the elements that determine the properties of soil. Iron, as a chemical element with variable valence, is in oxidized and reduced forms in soil. Their determination carries out in an acidic extract using α-α-dipyridyl by the colorimetric method. The results indicate that there is a connection with the iron content of the soil located on the floodplain, with the depth.

***Key words**: floodplain, alluvial soil, oxidized and reduced forms of iron.*

INTRODUCTION

Among the diversity of the world's landscapes, a special place occupies by floodplains, which distinguishes by their youth and vulnerability among parts of the river valley. In this area in the modern period, there are complex processes of formation and development of many components of the landscape, including soils (Cipriano-Silva, et al., 2020). Floodplain alluvial soils differ significantly from watershed soils both in their genesis and properties, ecological significance, and in use. The main factors of alluvial soil formation are the influence of floods, shallow groundwater, features of sedimentogenesis and constant introduction of material from the above areas. These soils characterize by dynamic of soil processes and complex biocoenotic structure (Bullinger-Weber G. & Gobat J.-M., 2006).

Alluvial soils (fluvisols) in the world occupy a relatively small area – 320 million hectares (Arnold R.W., Szabolcs I., & Targulian V.O., 1990). In Ukraine, their area is 1.4 million hectares, which is insignificant compared to the area of prevalent soils, especially chernozems. These soils make up the main fund of pastures and hayfields, which is due to the presence in this area of highly productive mesohygrophytic and hygrophytic cereal – motley grass meadows (Hikmatullah & Al-Jabri M., 2007). To support the functioning of the soil-plant system, it is necessary to study the ecological condition of floodplain soils. One of the indicators for assessing the ecological condition of soils is data on the content and dynamics of chemical elements. Because they are available for root uptake by plants and are actively involved in the biochemical cycle (Breemen N., 1988). In addition, studies in this area are great scientific value for studying the genesis and evolution of alluvial soils.

Iron, as one of the chemical elements, plays an important role both in soil genesis and in the processes of plant life (Breemen N., 1988).

Iron is one of the most abundant elements in nature. Clarke Fe in Earth's crust, according to Taylor S. R. (Taylor S.R., 1964), is 5.6%. Clarke Fe in soil is 3.8% (Chertko N.K. & Chertko E.N., 2008). Iron in the soil forms its own minerals, and is a part of silicates, sulfides, phosphates, arsenates and iron-organic complexes (Irmak S., Surucu A.K., & Aydin S., 2008).

Iron-containing minerals determine the color of soils, and the patterns of their transformation and content are used in the description of soil processes and soil classification to a large extent. Iron is involved in almost all elementary soil-forming processes: metamorphism of the

mineral and organic matter of the soil, migration, segregation and cementation of substances in the soil, podzolization, gleying, etc. (Villalobos & Tebo, 2005).
The character of its distribution along the profile of alluvial soils, which forme in a complex biogeochemical setting, is also of interest in addition to the iron content. The specificity of the distribution of this chemical element along the soil profile is associated with the unequal duration of the processes of saturation of the horizons with water both by the river water and by the soil water itself, the peculiarity of the species composition of plants, physic-chemical properties, and different granulometric and mineralogical compositions of alluvium (Barron & Torrent, 2013).
The issue of the content and distribution of iron in soils for floodplain alluvial soils of the Siversky Donets river basin insufficiently studied by our point of view.
The aim of our study was to study the content and profile distribution of Fe (II) and Fe (III) in the floodplain alluvial soils (fluvisols) of the Siversky Donets river basin in different parts of the floodplain territory.

MATERIALS AND METHODS

The studies were carried out within the following territories: the riverbed part of the Olkhovatka river floodplain, Kharkov district, Kharkov region (Figure 1), the central part of the Merla river floodplain, Bogodukhovsky district, Kharkov region (Figure 2), riverbed, central and near-terrace parts of the Siversky Donets river floodplain, Chuguevsky district, Kharkov region (Figure 3).
The soil cover of the floodplains of the rivers of the Siversky Donets basin on the territory of the Kharkov region of Ukraine is diverse. On the territory of the riverbed part of the Olkhovatka river floodplain, Kharkiv district, Kharkiv region, the following alluvial soils studied: Gleyic Fluvisol (N 49.775989°, E 35.936633°), Humi-Gleyic Fluvisol (N 49.771711°, E 35.934696°), Gleyic-Histic Fluvisol (N 49.771267082°, E 35.933323°).
On the territory of the central part of the floodplain of the Merla river, Bogodukhovsky district, Kharkov region, the following alluvial soils were studied: Gleyic Fluvisol (N 50.02082°, E 35.08576°), Gleyic Fluvisol (N 50.01830°, E 35.08676°), Calcari-Gleyic Fluvisol (N 50.03927°, E 35.14388°).

Figure 1. General view of Olkhovatka river floodplain

Figure 2. General view of Merla river floodplain

Figure 3. General view of Siversky Donets river floodplain

On the territory of the near-channel part of the floodplain of the Siversky Donets River Gleyic Fluvisol (N 49.750826°, E 36.545599°) studied.
On the territory of the central part of the floodplain of the Siversky Donets river Calcari-Gleyic Fluvisol (N 49.752337°, E 36.554064°) studied.

On the territory of the near-terrace part of the floodplain of the Siversky Donets River Humi-Gleyic Fluvisol (N 49.751103°, E 36.551698°) studied.

Mesophilic and hygrophilic herbaceous vegetation grew on the territory of the floodplains. The territories used as natural hayfields and pastures.

Soil samples take from each horizon in the summer. Soil samples of natural moisture poured in field conditions with 0.1 N H_2SO_4 solution in the ratio of 1 part of soil to 10 parts of acid.

Method for determining chemical parameters. Determination of iron oxides in an acidic extract carried out using α-α-dipyridyl by the colorimetric method. Fe (II) and Fe (III) were determined simultaneously in two portions from the same acid extract. In the first portion, Fe (II) was determined, in the second, the sum of Fe (II) and Fe (III), having previously converted Fe (III) into Fe (II) with hydroxylamine. The Fe (III) content is determined as the difference between the sum of Fe (II) and Fe (III) and the value of Fe (II), multiplying the resulting difference by a factor of 1.11.

RESULTS AND DISCUSSIONS

Three research sites were established within the near-channel part of the Olkhovatka River floodplain. The following soils were described on them: Gleyic Fluvisol, Humi-Gleyic Fluvisol, Gleyic-Histic Fluvisol.

The amount of reduced iron forms decreases with increasing depth in the Gleyic Fluvisol of the near-channel part of the Olkhovatka River floodplain. The soil profile for this indicator can be divided into two parts. The first is to a depth of 57 cm, which includes layers of 0-35 and 35-57 cm, where the content of the described forms of iron is slightly more than 2 mg/kg of soil. The second part – from a depth of 57 cm to 105 cm, which also includes two layers -57-70 and 70-105 cm. Here the amount of reduced forms of iron is less than 2 mg/kg of soil (Table 1).

The amount of oxidized forms of iron prevails over the reduced ones by 3-18 times, and is equal to 35.00-5.35 mg/kg of soil. Their number decreases with depth along the profile. The amount of oxidized forms of iron at a depth of 35-57 cm is greater than in the upper layer of 0-35 cm by 0.9 mg/kg of soil. The amount of this form of iron decreases deeper than 57 cm, especially in the layer 75-105 cm to 5.35 mg/kg of soil.

Table 1 Content and profile distribution of oxidized and reduced forms of iron in alluvial soils of the Olkhovatka river floodplain

Soil name	Depth, cm	Content of oxidized and reduced forms of iron, mg/kg soils	
		Fe (II)	Fe (III)
Gleyic Fluvisol	0-35	2.34	35.00
	35-57	2.06	35.90
	57-70	1.79	23.81
	70-105	1.60	5.35
Humi-Gleyic Fluvisol	0-35	2.23	36.97
	35-43	1.30	13.20
	43-70	3.50	58.50
	70-90	1.98	25.62
Gleyic-Histic Fluvisol	0-10	3.38	34.02
	10-25	3.63	24.20
	25-40	4.07	21.03
	40-105	9.91	33.30
	105-125	12.10	8.00

The content of reduced forms of iron in Humi-Gleyic Fluvisol unevenly distributed with increasing depth. Its smallest is in the 35-43 cm layer - 1.30 mg/kg of soil. The maximum value was recorded in the next layer of soil - 43-70 cm - 3.50 mg/kg of soil. The amount of this form of iron is 36% less from the maximum value in the upper layer to a depth of 35 cm. The amount of Fe (II) compounds is 1.5 times greater than the minimum value and amounts to 1.98 mg/kg of soil in the deepest layer, which was studied.

The amount of oxidized forms of iron prevails over the reduced forms, as in the previous soil. The tendency of distribution over layers with depth remains. Fluctuations in the content of oxidized forms of iron has varied between 58.50-13.20 mg/kg of soil. Their maximum founded at a depth of 43-70 cm, and a minimum - at a depth of 35-43 cm. The greatest difference between the depths in the content of oxidized forms of iron is 45.3 mg/kg of soil.

There is a clear tendency towards an increase in the content of reduced forms of iron with depth at the Gleyic-Histic Fluvisol of the riverbed floodplain of the Olkhovatka River. Their number increases evenly by an average of 0.3-0.4 mg/kg of soil to a depth of 40 cm. The content of these forms of iron increases sharply deeper than 40 cm. The difference in content between the layers is 3-5 mg/kg of soil.

The content of oxidized forms of iron is much higher than that of reduced iron. The amount of oxidized iron tends to decrease with increasing depth. The maximum values of the content of these forms are confined to the depths of 0-10 and 40-105 cm is 34.02 and 33.30 mg/kg of soil. This form of iron is approximately 10 mg/kg of soil less in the layers of 10-25 and 25-40 cm. The amount of iron drops sharply to 8.00 mg/kg of soil in the lower soil layer with a depth of 105-125 cm.

The amount of reduced forms of iron with depth varies between 1.58-0.88 mg/kg of soil in the Gleyc Fluvisol of the central floodplain of the Merla River and decreases with depth. The amount of reducing forms of iron in the upper horizon 0-20 cm compare to its content in the lower layer. The amount of Fe (II) compounds decreases significantly deeper than 50 cm to 0.88 mg/kg of soil in the layer of 120-130 cm. Almost the same amount of these compounds in the soil layers of 50-70 cm and 70-100 cm is observed (Table 2).

Table 2 Content and profile distribution of oxidized and reduced forms of iron in alluvial soils of the Merla river floodplain

Soil name	Depth, cm	Content of oxidized and reduced forms of iron, mg/kg soils	
		Fe (II)	Fe (III)
Gleyic Fluvisol	0-20	1.44	20.45
	20-50	1.58	30.17
	50-70	0.98	14.34
	70-100	1.00	12.28
	120-130	0.88	10.90
Gleyic Fluvisol	0-22	1.41	23.85
	22-55	2.51	21.29
	55-70	2.39	24.55
Calcari-Gleyic Fluvisol	0-22	2.55	18.97
	22-52	1.59	18.08
	52-65	1.76	13.57
	65-110	0.74	6.47
	110-130	0.14	0.75

The number of oxidized forms of iron significantly exceeds the number of reduced forms at all depths. The maximum content of oxidized forms at a depth of 20-50 cm is 30.17 mg/kg of soil. The number of these forms of iron is one and a half times less in the upper sphere of 0-20 cm is 20.45 mg/kg of soil. The amount of this form of iron from 50 cm and deeper gradually decreases and reaches a minimum value, which is three times less than the maximum, - 10.90 mg/kg of soil.

The trend towards a decrease the amount of reduced iron forms in the Gleyic Fluvisol of the central floodplain of the Merla River does not persist with depth. The maximum amount of these forms of iron is at the middle part of the profile at a depth of 22-55 cm is 2.51 mg/kg of soil. The amount of the reduced form of iron decreases by 1.1 mg/kg of soil compared to the maximum value for a horizon with a depth of 0-22 cm. Their amount decreases only by 0.14 mg/kg of soil in a horizon with a depth of 55-70 cm.

The oxidized forms of iron in this soil are, on average, 10 times more than the reduced ones. The distribution of their number with depth has the form of a curve with minimum values in the middle part of the profile and maximum values in the upper and lower horizons.

The maximum content of reduced iron forms in the upper part of the profile of the Calcari-Gleyic Fluvisol of the central floodplain of the Merla River exists up to a depth of 65 cm. The fluctuation of their amount occurs within the range from 1.76 mg/kg of soil to 2.55 mg/kg of soil with the highest value in the soil layer 0-22 cm. The content of reduced forms of iron from 65 cm and deeper sharply decreases to 0.74-0.14 mg/kg of soil.

The content of oxidized forms of iron in comparison with the reduced forms in the Calcari-Gleyic Fluvisol increases by 7-11 times with increasing depth. With increasing depth, the amount of Fe (III) decreases. The difference in the content of this form of iron between the upper layer and the lower one is more than 25 times (layer 0-22 cm - 18.97 mg/kg of soil, layer 110-130 cm - 0.75 mg/kg of soil). The amount of oxidized forms of iron changes little from the surface to a depth of 52 cm. Deeper changes in the content are more pronounced. In a layer with a depth of 52-65 cm, their number decreases by about 5 mg/kg of soil. In the 65-110 cm layer - more than twice, and in the 110-130 cm layer - more than eight times.

The amount of reduced forms of iron in the Gleyic Fluvisol of the near-channel part of the floodplain of the Siversky Donets River range from 1.70 mg/kg soil to 0.26 mg/kg soil. The peak in the content of these forms of iron at a depth of 27-50 cm is 1.70 mg/kg of soil. Deeper, their number decreases by more than four times and corresponds to the value of 0.30-0.26 mg/kg of soil. The number of reduced forms of iron above 27 cm also decreases in comparison with the maximum value is not so critical. The decrease is only 0.39-0.41 mg/kg soil (Table 3).

Table 3 Content and profile distribution of oxidized and reduced forms of iron in alluvial soils of the Siversky Donets river floodplain

Soil name	Depth, cm	Content of oxidized and reduced forms of iron, mg/kg soils	
		Fe (II)	Fe (III)
Gleyic Fluvisol	0-10	1.29	2.26
	10-27	1.31	1.37
	27-50	1.70	1.77
	50-77	0.30	0.81
	77-95	0.26	0.44
Calcari-Gleyic Fluvisol	0-8	1.75	3.25
	8-55	1.13	1.88
	55-65	1.04	1.26
	65-83	0.48	0.55
	83-105	0.24	0.35
Humi-Gleyic Fluvisol	0-8	1.32	3.65
	8-22	0.64	2.31
	22-42	0.86	2.27
	42-55	0.41	0.57
	55-76	0.28	0.34
	76-114	0.19	0.23

The amount of oxidized iron decreases with depth. The range in which the content of these forms of iron fluctuates is 2.26-0.44 mg/kg of soil. The content of Fe (III) compounds slightly increases at a depth of 27-50 cm in comparison with the topsoil.

The content of Fe (II) compounds in the Calcari-Gleyic Fluvisol of the central part of the floodplain of the Siversky Donets River averages 0.93 mg/kg of soil. The maximum amount of this form of iron exists in the upper soil layer 0-8 cm - 1.75 mg/kg of soil. The minimum - in the layer 83-105 cm - 0.24 mg/kg of soil. The decrease in the amount of reduced forms of iron occurs gradually to a depth of 65 cm, and deeper, the decrease has a sharp character, where the amount of iron forms decreases by more than 50%.

There are slightly more oxidized forms of iron in this soil. Their number does not exceed 3.25 mg/kg of soil and does not go below 0.35 mg/kg of soil. There are two sharp decreases in the content of these forms of iron along the soil profile. The first one is at a depth of 8-55 cm, where the indicator decreases by 1.37 mg/kg of soil. The second is at a depth of 65-83 cm is a decrease of 0.71 mg/kg of soil.

The amount of reduced forms of iron in the Humi-Gleyic Fluvisol of the near-terrace depression decreases with depth. The difference in terms of the content of reduced forms of iron between the layers is approximately 55%. An exception to this trend is the soil layer 22-42 cm, where the content of Fe (II) forms, on the contrary, increases to 0.86 mg/kg. Deeper, the downward trend in iron content persists. The total range of values for the content of these forms of iron is 1.32-0.19 mg/kg soil.

The number of oxidized forms of iron is 1.5-2 times higher than that of reduced iron, which is 3.65-0.23 mg/kg of soil. The amount of this form of iron with depth reduce by more than ten times. It is possible to distinguish two soil layers with a depth of 8-22 and 22-42 cm, where the amount of oxidized forms of iron varies little with depth.

CONCLUSIONS

The content of oxidized forms of iron in all studied alluvial soils is higher than the content of reduced forms of iron and is independent of the granulometric composition of the soil and its occurrence in parts of the floodplain.

According to the decrease in the content of iron forms, one can make the following series: alluvial soils of the floodplain of the Olkhovatka River - alluvial soils of the floodplain of the Merla River - alluvial soils of the floodplain of the Siversky Donets River.

According to the averaged data, the largest amount of iron founds in the soils of the riverbed part of the floodplains.

The smallest amount of iron is in Calcari-Gleyic Fluvisol.

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MICROBIAL AND ENZYME DECOMPOSITION OF PROTEINS TO RECLAIMED SUBSTRATES - VEGETATION EXPERIMENT WITH *Lolium perenne*

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Abstract

Vegetation experiment with Lolium perenne L. was carried out on reclaimed substrates from humus depot, tailings pond and mine, as applied vermiculite, mineral fertilization and liming. Microbial biomass nitrogen shows the highest values after the addition of 10% vermiculite, simultaneous application of fertilization and liming and self-fertilization, compared to controls, without ameliorants. Activities of the studied enzymes urease and protease follow the increase of the concentration of vermiculite, they are also higher in the samples with self-fertilization and the combined application of fertilization and liming. The values of microbial biomass nitrogen and enzymatic activities are highest in the variants with substrates from the mine. Microbial nitrogen biomass strongly correlates with urease activity and moderately with protease activity.

Key words*: reclamation, vermiculite, microbial biomass nitrogen, urease, protease.*

INTRODUCTION

Changes in soil microbiological properties precede chemical and physical changes, which take place more slowly over time. Microbial biomass and enzymatic activities can serve as sensitive early indicators of the dynamics of organic matter transformation, nutrient cycles, pollution stress and soil regeneration processes (Burns et al., 2013; Perez-de-Mora et al., 2012; Kennedy et al., 1993; Islam et al., 2000; Avidano et al., 2005). The creation of a sustainable ecosystem through biological reclamation of mine embankments and their inclusion in the surrounding landscape is essential (Petrov, 2019). Conducting liming, mineral and organic fertilization, addition of vermiculite and other activities in growth substrates for biological reclamation affect the quantity and composition of microorganisms in them, and respectively the values of microbial biomass and enzymatic activities of microbial origin. In previous studies of the same (Malcheva, 2021) and similar (Stefanova and Petrov, 2019) variants was established that non-spore-forming bacteria occupy a major share in the total microflora of variants of humus depot, tailings and mine , increasing microbial biomass carbon and the activity of enzymes catalase, cellulase, amylase and invertase (Malcheva, 2020).

In recent years, vermiculite has been widely used for the adsorption of metal ions, including heavy metals (Covelo et al., 2007; Abollino et al., 2008; Panuccio et al., 2009; Malandrino et al., 2006, 2011; Sis and Uysal, 2014; Kebabi et al., 2017; De Freitas et al., 2017). In addition, vermiculite significantly improves the drainage properties of substrates, increases porosity and crumbliness, prevents soil compaction and protects roots from sudden temperature fluctuations (Zheleva et al., 2019; Malcheva, 2020). Narendrula-Kotha and Nkongolo (2017) report that total microbial biomass has increased significantly 35 years after liming of regenerated mining deposits, but species microbial diversity remains statistically unchanged. Fuentes et al. (2006) also found that microbiological activity and nitrification increase in soils after liming due to the correction of soil acidity.

The addition alone or in combination of post-flotation lime, mineral fertilisation, sewage sludge, and mineral wool to the reclaimed soil-less substrate stimulates the activity of the enzymes catalase, urease and protease (Joniec, 2018). The increase in urea-N losses, possibly as a consequence of a higher urease activity, was compensated for by the increase in N immobilized in the biomass (Roscoe et al., 2000). The last cited authors found that the microbial biomass nitrogen content explained 97% and 69% of the variation in urease activity in the upper and deeper soil layer, respectively.

Purpose of the present study was to determine the effect of the application of vermiculite, mineral fertilization and liming on the accumulation of microbial biomass nitrogen and the degree of enzymatic degradation of proteins in reclaimed substrates with *Lolium perenne* L.

MATERIALS AND METHODS

The vegetation experiment began in March 2019 in greenhouse conditions. In plastic containers with a capacity of 1.5 l was poured 1 kg of substrate with the appropriate amount of vermiculite (5%, 10% and 20%). Mineral fertilization and liming are applied. Liming was used to neutralize the substrates. The experiment lasted 6.5 months. The following substrates were used: soil from the humus depot near tailings, tail from tailings (tail) and substrate from a copper mine (mine). Ryegrass seeds are sown with a higher density, and after the germination, the plants, are thinned to a density of 50 plants in a container. On the 58th day after the beginning of the vegetation experiment, liming was done in the mine substrates, and seven days later (64 days from the beginning of the vegetation experiment) fertilization was carried out (rates: N150P150K150 kg/ha) of all variants, without controls. The substrates were maintained at constant humidity. Two replicates were made for each variant of the study: Microbiological and enzymatic analyzes were performed on the 30th and 95th day of the experiment, before and after liming and fertilization. Microbiological and enzymatic analyzes were performed on the 30th and 95th day of the experiment, before and after liming and fertilization. The general scheme of the vegetation experience is presented in Table 1.

Microbial biomass nitrogen (MBN), urease and protease activity were analyzed using the following methods: Biomass N was calculated (Brookes et al., 1985) using the equation: biomass N = 1.85 EN, where EN = (total N from fumigated soil) - (total N from unfumigated soil). Total N in the extracts was measured by Kjeldahl digestion (Brookes et al., 1985); Spectrophotometric method was used for determination of urease activity of substrates (Kandeler and Gerber, 1988); protease activity of substrates was determined by the method Khaziev (1976): in the substrates are placed photoplates with gelatin coating with dimensions of 10/50 mm and every 15 days the percentage of the degraded area is reported with a reference net.

Table 1. Variants of the vegetation experience

Embankments from mine (M):	**Soil from humus depot (SD):**	**Tail from tailing (T):**
M-0 - Without vermiculite and without fertilizing; M-I - Without liming (L), without fertilizing (F) (5%, 10%, 20%); M-II - Without liming, with fertilizing (5%, 10%, 20%); M-III - With liming, without fertilizing (5%, 10%, 20%); M-IV - With liming, with fertilizing.	SD-0 - Without vermiculite and without fertilizing; SD-0F - Without vermiculite and with fertilizing; SD-I - Without fertilizing (5%, 10%, 20%); SD-II - With fertilizing (5%, 10%, 20%).	T-I - Without fertilizing (5%, 10%, 20%); T-II - With fertilizing (5%, 10%, 20%).

Correlation analysis was applied to determine the relationship between microbial biomass nitrogen and enzymatic activities using the software product MS Excel 2010.

RESULTS AND DISCUSSIONS

On 30th day of the study, microbial nitrogen biomass had higher values in the samples with mining substrates, followed by samples with tail and those from the soil depot, which is located near tailings (Figure 1).

The addition of vermiculite increases the values of biomass nitrogen of microbial origin. The best results are establish when adding 10% vermiculite. Similar results were obtained on day 95 of the study (Figure 2).

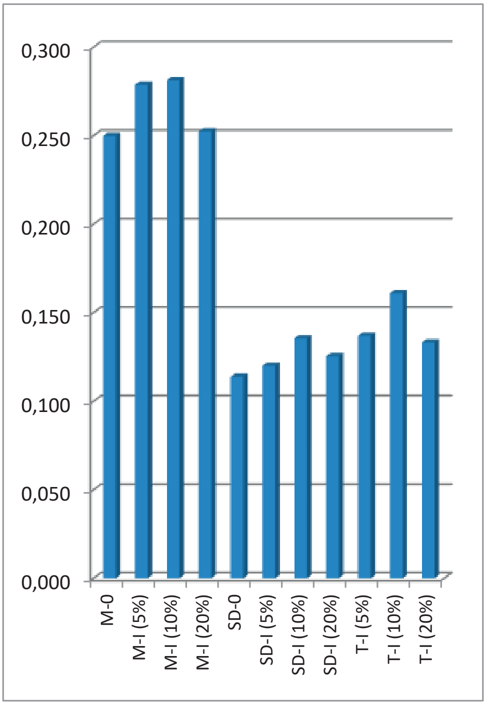

Figure 1. Microbial biomass nitrogen, 30th day (mg N/g soil)

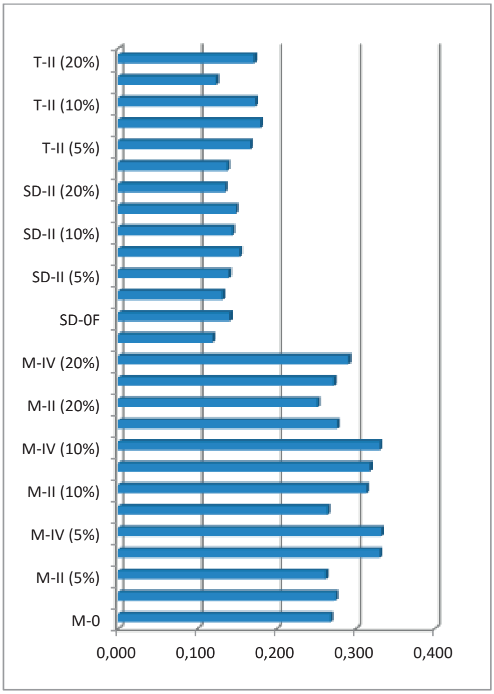

Figure 2. Microbial biomass nitrogen, 95th day (mg N/g soil)

Combined application of liming and fertilization in the mine variants as well as the fertilization in the "SD" and "T" variants increase biomass nitrogen of microbial origin to a higher degree, with the exception of: SD-II (10%), SD-II (20%) and T-II (10%). Variants "with liming, without fertilizing" (M-III) give better results than the "without liming, with fertilizing" (M-II), regardless of the vermiculite concentration.

Microbial nitrogen biomass and enzymatic activities depend on the development of microorganisms in the substrates and their activity. In a previous study on the microbiological activity of the same variants (Malcheva, 2021) it was found that the biogenicity of the tested substrates increases with the application of vermiculite, liming and fertilization. The main share in the composition of the total microflora for the two periods of research is occupied by the non-spore-forming bacteria, followed by the bacilli. Actinomycetes and micromycetes are the least represented. On the 30th day of testing, the total amount of microorganisms in the mine variants was lower than in the control, in contrast to the soil depot and tailings variants. With increasing time of exposure to vermiculite (95th day of the experiment) and after individual and combined application of liming and fertilization, the biogenicity was higher in the mine variants compared to those in the soil depot and those with tailings. This trend shows the importance of vermiculite for the adsorption of heavy metals, especially depending on the duration of action, as well as for improving the physico-chemical properties of substrates. The pH values increase after liming in the mine variants, which, together with the application of fertilization, also contributes to increasing the development of the microflora in the substrates and their activity. Increase in the values of microbial biomass and microbiological activity after liming of regenerated mining deposits and soils was established by other authors (Fuentes et al., 2006; Narendrula-Kotha and Nkongolo, 2017). The introduction of mineral fertilizers increases the amount and activity of microorganisms in the soil (Hart and Stark, 1997; Forge and Simard, 2001; Meena et al., 2014; Bogdanov et al., 2015; Plamenov et al., 2016). Other authors find out that this increase is in short term, and is often followed by reduction of the microbial biomass and activity (Ohtonen, 1992; Smolander et al., 1994; Périé and Munson, 2000). Vegetation type and litter quality seem to be more important for soil microbial activity than the substrate quality on the reclaimed sites (Stefanova and Petrov, 2019).

In general, the total amount of microorganisms is higher with the addition of 10% and 20% vermiculite. The highest values of trace elements, especially Cu and As, were found in the mine variants (Zheleva et al., 2019). This determines the lower total amount of microorganisms on the 30th day of the study (before liming and fertilization) in these variants, but not their lower activity (Malcheva, 2021). In general, the increase in biogenicity, MBN values and enzymatic activities in the addition of vermiculite in all variants proves the effect of the mineral for purification of heavy metal substrates.

In general, on the 30th day from the start of the experiment (before liming and fertilization) a decrease in urease values was established with an increase in the amount of vermiculite, except for M-20% (Figure 3).

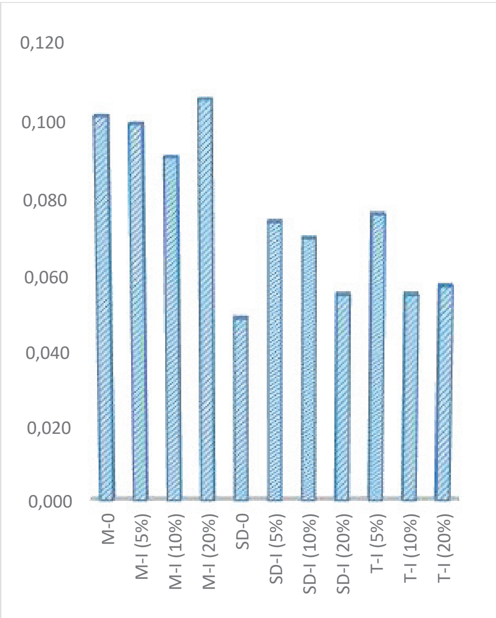

Figure 3. Urease activity of soil microorganisms, 30th day, mg N/g soil

The activity of the enzyme remains lower than that of the control. Probably initially the increasing amount of vermiculite stresses the microorganisms and suppresses their enzymatic activity. This conclusion is confirmed by the results of the 95th day (after liming, fertilizing and mowing) - increasing the activity of the enzyme with increasing the amount of vermiculite (Figure 4). A similar trend was found in the study of the enzymes cellulase, amylase and invertase, while the catalase activity increased with increasing amount of vermiculite for both study periods (Malcheva, 2020).

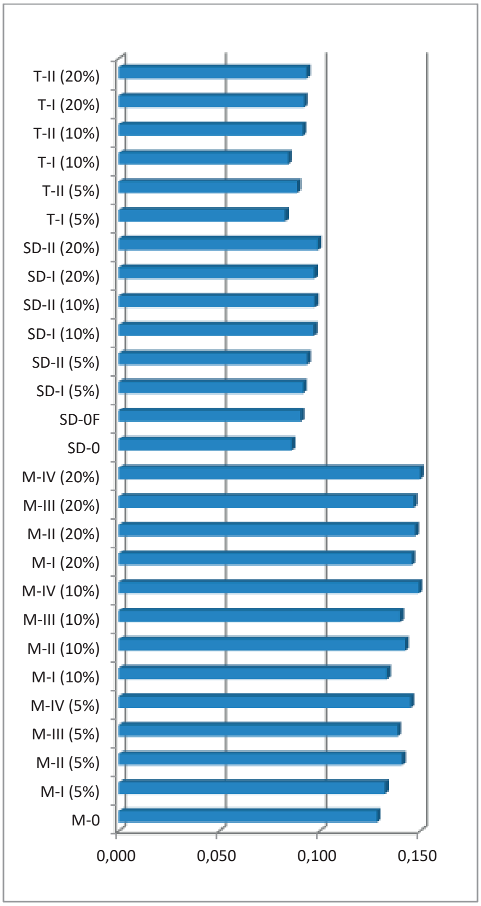

Figure 4. Urease activity of soil microorganisms, 95th day, mg N/g soil

Urease activity is highest in the mine variants, followed by the SD and T variants. The combined application of fertilization and liming at the mine increases of the enzyme activity to a higher degree. The "without liming, with fertilizing" (M-II) variants showed better results than with "liming, without fertilizing" (M-III), regardless of the vermiculite concentration. In the other variants - "SD" and "T" fertilization increases the urease activity, as the enzyme values increase with increasing concentration of vermiculite. The lowest and closer to those of the control samples are the values of urease in the substrates without liming and fertilization.

The protease activity of the studied variants follows the trends in urease - a higher percentage of degraded area on the 95th day of the study compared to the 30th day, as well as higher values of the enzyme with increasing vermiculite concentration, mainly after liming and fertilizing (Figures 5 and 6).

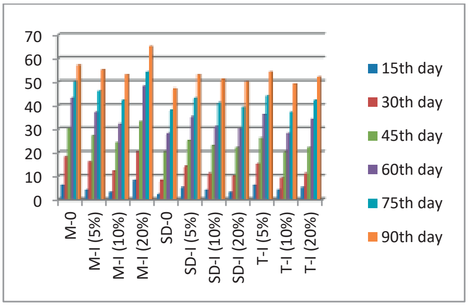

Figure 5. Protease activity of soil microorganisms, 30th day (% degraded area)

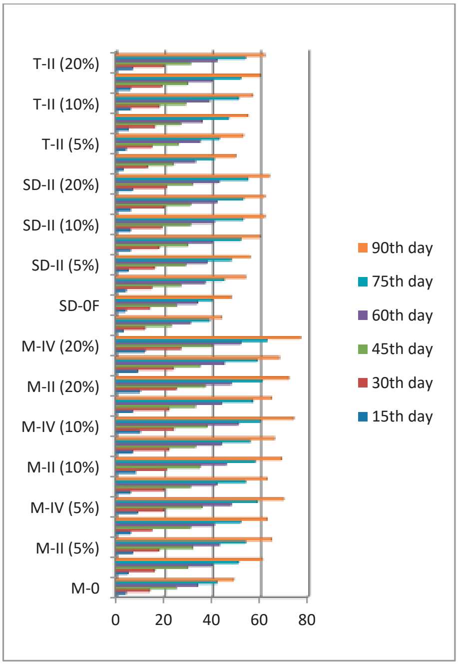

Figure 6. Protease activity of soil microorganisms, 95th day (% degraded area)

Protease values are highest with the addition of 20% vermiculite. The order of magnitude of the protease activity is: M>SD>T. The "without liming, with fertilizing" (M-II) variants showed better results than with "liming, without fertilizing" (M-III), regardless of the vermiculite concentration.

Some researchers have studied the influence of fertilization on the fertility of soil (Jia et al., 2001; Liu, 2004; Malcheva et al., 2016) and growth substrates for reclamation (Malcheva, 2020) by exploring soil enzymatic activity. The addition alone or in combination of post-flotation lime, mineral fertilisation, sewage sludge, and mineral wool to the reclaimed soil-less substrate stimulates the activity of the enzymes catalase, urease and protease (Joniec, 2018).

The correlation analysis is presented in the following Figures 7 and 8.

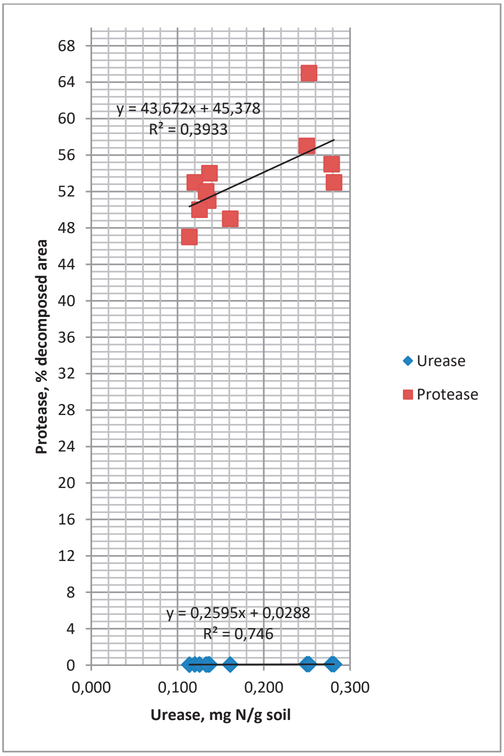

Figure 7. Dependence between MBN and enzyme activities, 30th day

Two times as strong a correlation was found between MBN and urease activity as compared to MBN and the protease activity of soil microorganisms. These dependencies were higher on day 95 of the study.

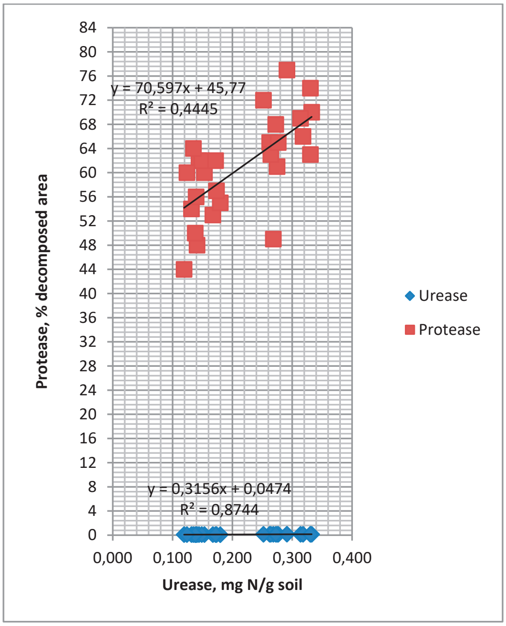

Figure 8. Dependence between MBN and enzyme activities, 95th day

CONCLUSIONS

Microbial biomass nitrogen values increased from the 30th to the 95th day of the study. For both study periods, MBN values were highest in the samples with mining substrates, followed by the samples with tail and those from the soil depot. Biomass nitrogen of microbial origin increases the most with the addition of 10% vermiculite. The best results for this indicator are obtained with the combined application of liming and fertilization in the variants of the mine, as well as fertilization in the variants from the soil depot and those with tail. Variants "with liming, without fertilizing" show better results than "without liming, with fertilizing", regardless of the vermiculite concentration.
Urease activity is highest in the variants from the mine, followed by the variants from the soil depot and those with tail. Before liming and fertilizing (30th day), a decrease in the enzyme activity with an increase in the amount of vermiculite was found. However, after liming, fertilizing and mowing (95th day), urease activity increases with increasing amount of vermiculite in all variants. The best results are obtained by adding 20% vermiculite. Combined application of fertilization and liming in the mine, as well as fertilization in the other variants, increases the activity of the enzyme to a higher degree. "Without liming, with fertilizing" options show better results than "with liming, without fertilizing", regardless of the concentration of vermiculite.
Protease activity increases to a higher degree also after liming and fertilization, as well as with increasing the concentration of vermiculite - the best results are obtained by adding 20% vermiculite. The values of the enzyme are the highest in the variants from the mine, followed by the variants from the soil depot and those with tail. Variants "Without liming, with fertilizing" show better results than "with liming, without fertilizing", regardless of the concentration of vermiculite.
In general, the applied activities - liming, mineral fertilization and addition of vermiculite increase the values of biomass nitrogen of microbial origin and the studied enzyme activities compared to control samples. A strong correlation is establish between microbial biomass nitrogen and urease activity. While between MBN and protease activity the dependence is moderate. These dependencies increase with the duration of the study.

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CHANGES OF PHYSICAL PROPERTIES IN SOILS UNDER TRADITIONAL SOIL MANAGEMENT IN ARABLE CROPS, IN THE SOUTHERN PART OF ROMANIA

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Abstract

The last decades made obvious that the life on Earth depends on the health status of our soils. The EU is committed to ensure that land is managed sustainably, and soils are protected or remediated according to their needs. The recently launched EU Green Deal puts soil in the heart of the Farm to Fork and Biodiversity strategies, with important targets to be reached by 2030. Therefore, research on how soil management practices affect the soil quality is required by relevant actors, sectors, and policy fields. The present work presents a comparative study on the changes of physical properties of soils in the southern part of Romania, under two different soil tillage practices: scarification (9 years, at 45 cm depth) and shallow plowing (25 cm) followed by disking. The two soil profiles from Calinesti, Teleorman County and Bucu, Ialomita County, revealed a chromic luvisols (luvisols class) in the Calinesti site and a calcaric fluvisols (protisols class) in the Bucu site. The formation conditions of the two types of soil are quite different in the terms of parental material, climate, vegetation, relief, anthropogenic influence, etc. Following the analysis of the physical properties (bulk density, total porosity, and degree of compaction) of the two soil types, evident differences were found, with implications in the crop yields.

Key words*: soils physical properties, chromic luvisols, calcaric fluvisols, soil management practices, economic efficiency.*

INTRODUCTION

According to the New EU soil strategy, healthy soils are one essential aspect to meet climate and biodiversity goals under the New European Green Deal. Included in the EU biodiversity strategy for 2030, this initiative has as main goals to protect soil fertility, reduce erosion and sealing, increase organic matter content, identify contaminated sites, restore degraded soils, define what constitutes ‘good ecological status’ for soils (EC Law, 2021).

The Soil Thematic Strategy for Soil Protection identified two decades ago the key soil threats in the EU as erosion, floods and landslides, loss of soil organic matter, salinization, contamination, compaction, sealing, and loss of soil biodiversity (EC Environment, 2021).

The main physical characteristics of the soil (structure, total porosity and aeration, bulk density, resistance to penetration, permeability) can be influenced by soil mechanical works, which in turn can influence the physical and mechanical characteristics of the soil (consistency, adhesiveness, plasticity, resistance to plowing, etc.) (Dumitru et al., 2011).

Currently, conservative (unconventional) soil works define extremely varied processes, from direct sowing (no-tillage, direct drill) in unprocessed soil to deep tillage without turning the furrow (scarification). Between these two extremes there are different methods, such as: reduced works (classic rationalized), minimum works (with coverage under 30%, minimum tillage), minimum works with vegetable mulch (with coverage over 30%, mulch tillage), sowing on billets (ridge tillage), partial or strip works (strip till, zone till), works with protective layer (cover crops) etc.

By ploughing, the upper horizon is covered, the restored soil layer is brought to the surface and sometimes enriched with nutrients, achieving favorable conditions for germination and growth of plants. At the same time, a loosening of the soil is carried out, its volume is increased by a minimum of 25%, hydrostable aggregates are created especially at bulk density values of 1.1-1.3 g/cm^3.

This volume disappears over time, by natural settlement, when preparing the seedbed or during the vegetation period (Penescu and Ciontu, 2001).

The apparent density or volumetric weight, correlates very well with the total porosity and the degree of subsidence of the soil being conditioned by the texture, content in organic matter and the agrotechnical works executed (Canarache, 1990).

The root system of crop plants develops optimally at specific apparent density values, but these values change over time, either by the natural laying of the soil or by the need for agrotechnical works carried out according to the requirements of each crop.

Even more, the use of nitrogen from fertilizers increases at optimum values of bulk density, while, at low bulk density values (as 1.0 g/cm^3) only 38% nitrogen is used and at higher bulk densities (as 1.4 g/cm^3) only 44% of the total administered quantity is used by plants (Cârciu et al., 2019).

Regardless of the applied technology, by cultivating the land, influences are exerted on the soil that modify the natural balance of the physical, chemical or biological state with repercussions on its fertility.

The impact of agricultural technologies on the soil has become a pressing and topical issue, as it can lead to the degradation of soil properties due to the need to conserve and improve it, including water conservation in the soil.

Through agricultural works performed within conventional technologies, various changes take place, such as: the appearance and formation of crust, reduction of mesofauna, mineralization of organic matter, compaction, which lead to negative phenomena (acceleration of erosion, reduction of humus content, etc.).

Adopting good agricultural practices, especially on the soil management, is a key factor in granting food, clean water, feed, energy, safe climate, diverse ecosystem services and biodiversity, etc. for future generations.

The present work presents a comparative study of physical and hydrophysical properties of soils in two locations in Romania, under two different soil tillage practices and the implications on the crop yields.

MATERIALS AND METHODS

Physico-geographical conditions

The experiment was conducted in two different locations, in Southeastern part of Romania, in Călinești area Teleorman County on a chromic luvisols (Figure 1) and in Bucu area, Ialomița County, on a calcaric fluvisols, as illustrated in Figure 2. The technology applied in the two reference areas was different, in terms of soil mobilization and seedbed preparation but also the imputations used.

Figure 1. Călinești area

Figure 2. Bucu area

Soil analysis

The samples were analysed in INCDPA Bucharest laboratories. Soil samples were dried at room temperature; soil subsamples were homogenized, milled, and sieved through a 250 μm sieve.

The following analytical methods were used to determine the chemical properties:

- organic matter (humus): volumetric determination (Walkley-Black humidification method, STAS 7184/21-82);
- $CaCO_3$ (carbonates): gasometrical method (Scheibler calcimeter, SR ISO 10693: 1998, %);

- the nitrogen content, by calculation, based on the humus content and the degree of saturation with bases (IN = humus x V/100);
- mobile phosphorus content (Egner-Riehm-Domingo method and colorimetric molybdenum blue, Murphy-Riley method ascorbic acid reduction);
- mobile potassium content (Egner-Riehm-Domingo extraction and flame photometry);
- pH (potentiometric method in aqueous suspension at soil/water ratio of 1/2.5 - SR 7184 /13-2001);
- hydrolytic acidity, extraction with sodium acetate at pH 8.2;
- degree of bases saturation V% (Kappen Schoffield method).

The following physical characteristics were determined:
- determination of granulometric fractions;
- pipette method, for fractions ≤ 0.002 mm;
- wet grinding method for fractions of 0.002-0.2 mm and dry grinding method for fractions > 0.2 mm; the results are expressed as a percentage of the material remaining after pretreatment;
- bulk density (BD): The known volume of metal cylinders (100 cm^3) at the instant soil moisture (g/cm^3) - total porosity (PT): by calculation (% by volume -% v/v);
- aeration porosity (PA): by calculation (% volume -% v/v);
- compaction degree (GT): by calculation (% by volume -% v/v), where: PMN - minimum required porosity, clay of the sample is calculated with the formula PMN = 45 + 0.163 A (% by volume -% v/v); PT = total porosity (% v/v); A - clay content (% w/w);
- hygroscopicity coefficient (HC): drying at 105°C of a pre-moistened soil sample at equilibrium with a saturated atmosphere with water vapor (in the presence of 10% H_2SO_4 solution) - % by weight (% w/g);
- wilting coefficient (WC, %, g/g), calculated based on hygroscopicity coefficient;
- field water capacity (FWC, % w/w), calculated based on Dumitru et al. (2009) formula, considering clay content (%), silt content (%), bulk density (g/cm^3), and layer depth (cm);
- useful water capacity (UWC, % w/w) is calculated as the difference between field capacity (% w/w) and wilting coefficient (% w/w);
- total water capacity (TC, % w/w) is determined as the report between total porosity (% v/v) and bulk density (g/cm^3).

For the complete soil characterization, in terms of both the physico-chemical properties of the soil and physico-geographic conditions in which the soil was formed, soil properties are represented as symbols grouped in ecopedological indicators, according to the methodology in force (ICPA, 1987; Munteanu and Florea, 2009).

According to the available data, the main physical-geographical conditions of the two testing sites are illustrated in Table 1.

Table 1. Main physical-geographical conditions

Location	Geology and lithology	Climatic factors	Groundwater	Soil types
Calinesti (altitude: 75-80 m)	N-V Burnaz plain, on the Moesic Platform, sedimentary deposits -fluvio-lacustrine deposits over which loess and löessoid deposits	*min. t.*>-30°C *January av.* -2- -3°C *max. t* >40°C *July av.* > 23°C *aagr* - 125-127 kcal/cm^2 *aaat* - 11 °C - 12.0°C *dwf* - 190-210 d *aar* - 500-550 mm	3-5 m in river meadows, 5-15 m in most interfluves	typical luvosols (and mollic)
Bucu (altitude: 15-20 m)	The Ialomița meadow; covered with fluvial and swampy deposits, that consist predominantly of clays (sandy or loessoid), fine and coarse sand, homogenized with gravel	*min. t.*>-30°C *January av.* -2- -4°C *max. t* >38°C *July av.* 22 - 23°C *aagr* - 125-127 kcal/cm^2 *aaat* - 10.8 °C - 11.0°C *dwf* - 190-210 d *aar* - 400-500 mm	1-2 m in the spring 2-3 m during summer and autumn	fluvisols and gleysols

min. t. - minimum temperature; January av. - average temperature in January; max. t - maximum temperature; July av. - average temperature in July; aagr - annual average global solar radiation; aaat - average annual air temperature; dwf - days without frost/year; aar - average annual rainfall

RESULTS AND DISCUSSIONS

Soil characterization

A. Călinești location

- Soil type: Chromic luvisols
- Landscape: plain
- Use: arable, sunflower
- Parent material: loessoid deposits
- Groundwater: > 10 m

Figure 3. Călinești soil profile

Morphological characterization of the Călinești soil profile

The analytical data for chromic luvisols from the studied area are presented in the Table 2.

A_0 (0-32) cm, medium clay, color 7.5 YR 2/2 to the wet material and 7.5 YR 3/3 to the dry material, well developed grainy structure, aggregates crumble easily, unassembled when moist, friable in the dry state, poorly plastic and adherent, gradual passage.

AB (32-56 cm), dusty clay, color 7.5 YR 3/2 at the wet state material and 7.5 YR 4/3 at the dry state material, well developed polyhedral structure at the top of the horizon and medium polyhedral developed at its base, the aggregates crumble easily, not smooth when moist, friable in the dry state, weak plastic and adherent, weak compaction.

Bt_1 (56-135 cm), uniform color in shades of 7.5 YR 3/2 at the wet state material and 7.5 YR 3/3 at the dry state material, the structure is medium and large prismatic, clay-clay texture; the material is hard in the wet state and hard in the dry state, moderately plastic and adherent, weakly compact and weakly cemented.

Bt_2 (135-180 cm), medium clay, uniform color in shades of 7.5 YR 3/3 at the wet material and 7.5 YR 4/4 at the dry material, the structure is medium and large prismatic, frequent fine cracks, the material is hard when moist and hard when dry, residual neoformation is observed in the form of clay films on the faces of the structural aggregates.

C_k (>180 cm), medium sandy clay, uniform color in shades of 7.5 YR 4/4 in wet and 7.5 YR 5/4 in dry, unstructured, friable in wet, moderately cohesive in dry, has rare grains of sand, rare spots of $CaCO_3$, strong effervescence.

Table 2. Characteristics of the chromic luvisols, Calinesti area

Horizon	Ao	AB	Bt_1	Bt_2	C
Depth (cm)	0-32	32-56	56-135	135-180	> 180
Coarse sand (2-0.2 mm)	14,8	5.1	6.4	5.3	26.5
Fine sand (0.2-0.02 mm)	26.6	21.8	20.8	11.5	19.5
Dust (0.02-0.002 mm)	28.6	41.7	28.6	38.5	18
Clay (< 0.002 mm)	30	31.4	44.2	44.7	36
Soil texture	silt	silt loam	silty clay loam	silty clay loam	sandy loam
Soil reaction (pH)	5.7	5.9	6.2	6.5	7.2
Humus content (%)	3.2	2.8	1.7	0.8	0.8
Apparent density (g/cm^3)	1.26	1.31	1.46	1.47	1.39
Total porosity (%)	52.5	50.6	46.5	43.0	46.7
Degree of compression GT (%)	-6	1	15.3	17.7	4
Carbonates (%)	0	0	0	0	4.7
Degree of saturation with bases V (%)	72	74	78	80	-
Total content of nitrogen IN	2.3	2.07	1.32	0.64	-
Mobile P (ppm)	34	31	26	24	-
mobile K (ppm)	184	165	132	117	-
Wilting coefficient (%)	10.2	10.8	11.7	11.8	-
Field capacity (%)	18.5	19.6	21.2	21.5	-
Useful water capacity (%)	8.4	8.8	9.6	9.8	-
Total water capacity (%)	41.6	38	32	29	-
Humus reserve (t/ha)	129	88	196	53	-

B. Bucu location
- Soil type: calcaric fluvisols
- Landscape: meadow
- Use: arable, sunflower
- Parent material: alluvial deposits
- Groundwater: > 2.5 m

Figure 4. Bucu soil profile

Morphological characterization of the soil profile from Bucu area
The analytical data for the clacaric fluvisols from the Bucu area are presented in the Table 3.

Ap (0-16 cm), dusty sandy clay, moderately developed grayish structure, brown with shades of 10 YR 3/2 to wet and dark brown 10 YR 5/4 to dry, reawakening, weak biological activity, non-plastic, non-adhesive, frequent fine pores, very frequent thin roots from cultivated vegetation, gradual passage, hardpan 18-26 cm;
Ao (16-28 cm), medium sandy clay, poorly developed grainy structure, strongly tanned, yellowish brown with shades of 2.5 Y 4/3 at wet and 2.5 Y 5/4 at dry, frequent fine roots, strongly tanned, clear wavy passage.
AC (28-46 cm), sand-fine clay, light brown with shades of 2.5 y 4/4 to wet and yellowish brown 2.5 y 6/4 to dry, very friable, unstructured, non-plastic, non-adhesive, frequent coarse pores, frequent fine roots, weak effervescence in the lower half of the horizon, gradual passage.
C_1 (46-82 cm), sand-clay coarse, light yellowish with shades of 2.5 Y 5/3 at wet and 2.5 Y 6/6 at dry, unstructured, reaven, very friable, frequent CaCO3 pseudomyceles, strong effervescence, clear straight passage.
C_2 (82-115 cm), sand-clay coarse, light yellowish with shades of 2.5 Y 5/3 at wet and 2.5 Y 6/6 at dry, unstructured, reaven, very friable, frequent $CaCO_3$ pseudomyceles, strong effervescence, clear straight passage.

Table 3. Characteristics of the calcaric fluvisols, Bucu area

Horizon	Ap	Ao	AC	C_1	C_2
Depth (cm)	0-16	16-28	28-46	46-82	82-115
Coarse sand (2-0.2 mm)	9.8	10.2	14.1	14.5	18.4
Fine sand (0.2-0.02 mm)	40.9	36.7	45.9	50.3	48.7
Dust (0.02-0.002 mm)	30.8	34.5	28.4	24.9	20.4
Clay (< 0.002 mm)	18.5	18.6	11.6	10.3	12.5
Soil texture	sandy loam	sandy loam	loamy sand	sand	sand
Soil reaction (pH)	7.2	7.2	7.4	8.1	8.4
Humus content (%)	2.16	2.07	1,21	0.37	-
Bulk density (g/cm^3)	1.52	1.53	1.63	1.64	1.44
Total porosity (%)	42	42	38	36	43
Degree of compression (%)	12	13	19	22	7
Degree of saturation with bases	94	96	98	100	100
Total content of nitrogen IN	2.03	1.98	1.18	0.37	-
Mobile P (ppm)	14	13	9	7	-
Mobile K (ppm)	112	87	67	45	-
Wilting coefficient (%)	3.9	4,2	4.6	4.5	-
Field capacity (%)	7.1	7.7	8.5	8.2	-
Useful water capacity (%)	3.2	3.44	3.9	3.7	-
Total water capacity (%)	42	29	45	49	-
Humus reserve (t/ha)	34	97	65	-	-

Aspects regarding the soil physical and hydrophysical properties

In Călinești experimental plot, the chromic luvisols was cultivated mainly with sunflower, and the soil works were represented by scarify-cation, each year in the last 9 years. At 45 cm the hardpan layer was found. The soil activity was more intense, with lumbricides (*Lumbricus terrestris*) present until 60 cm. The crop residues were found in the top 40 cm of the surface, fact which improved the soil physical indicators, as bulk density, total porosity, and compaction degree, as illustrated by Figure 5.

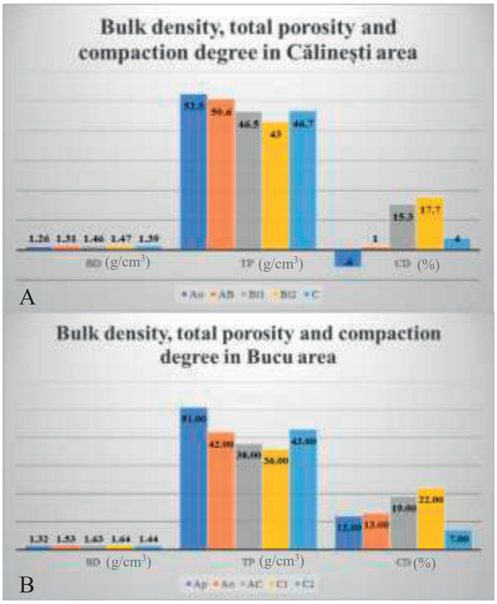

Figure 5. The soil physical properties in Călinești (A) and Bucu (B) experimental fields

The better water infiltration capacity, proved by the four hydrophysical indicators, as wilting coefficient, field water capacity, useful water capacity and total water capacity (Figure 6), is confirmed by the absence of puddles on the surface of the soil, even short time after rains. All these factors contribute to an increased sunflower production, higher than the average production, usually around 3600 kg/ha.

At Bucu experimental plot, also cultivated with sunflower, the soil works on calcaric fluvisols were represented only by superficial ploughing (max.15 cm). Due mainly to the lack of water, the works of soil mobilization were of poor quality and had as result the formation of hardpan below 13-15 cm. Due to the water weak infiltration and unfavorable soil physical characteristics, the root system was poor, and was formed above the hardpan.

In addition, puddles could be observed at the surface of the soil.

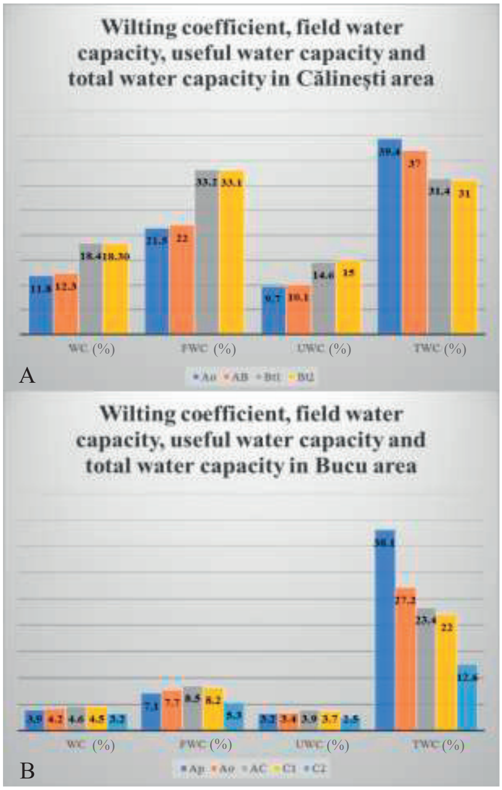

Figure 6. The soil hydrophysical properties in Călinești (A) and Bucu (B) experimental fields

Being a soil with a generally coarse texture, poorly supplied with nutrients, with low activity of the soil fauna, the sunflower yields were low, below 1000 kg/ha.

Previous studies showed that the root system develops optimally at bulk density values ranging from 1.0-1.4 g/cm^3. In Bucu area, the values higher than 1.53 g/cm^3 proved the difficulty of root system to develop. The values of the compaction degree (CD) change over time, either by natural laying of the soil or due the agrotechnical works carried out according to the requirements of the crop, but the obtained values proved that Bucu soil has a higher compaction degree, which is unfavorable to sunflower plants.

CONCLUSIONS

Two locations with different physical and geographical conditions were studied, which resulted in two types of soil, classified in different soil classes, namely: protisols (calcaric fluvisols) and luvisols (chromic luvisols).

In the conditions of the chromic luvisols from Călinești, mobilized by scarification for 9 years, without plowing, it was found that the physical properties of the soil were much improved, without puddles, a very favorable aerohydric regime resulted, the activity of the soil fauna intensified, which led to an increase in production per unit area.

In the conditions of the calcaric fluvisols from Bucu, worked superficially up to 15-17 cm, due to the accentuated compaction, the root system of the plants was restricted, weak infiltration of water, the appearance of the crust on the surface, which lead to low yields, under 1000 kg/ha.

The hydrophysical properties of the two soil types are very different, considering the values of total water capacity (TWC) and useful water capacity (UWC), hence the resistance of plants to drought in certain vegetation periods was much low in Bucu area.

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INFLUENCE OF YARA MINERAL FERTILIZER PRODUCTS ON GROCHEMICAL INDICATORS AND MICROBIOLOGICAL ACTIVITY IN SOILS AT COMMON WINTER WHEAT

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Abstract

Experiment was carried out on a test field in the village of Gurkovo, on an area of 9 ha, in three variants. Fertilization was performed with fertilizer products: Yara Mila Triple (16% N, 16% P_2O_5, 16% K_2O), Yara Vera Amidas (40% N, 14% SO_3) and Yara Bela Sulfan (24% N, 15% SO_3, 11 % CaO). A variety Avenue of common winter wheat was used. Macronutrients (digestible forms of N, P, K) in the soil during the tillering and maturity phenological phases of wheat shows quantitative dynamics, but between the second phase and the harvesting phase the values of macronutrients in all fertilization variants decrease, which is related to their good absorption. The used fertilizer products increase the biogenicity of soils, as established and regrouping of the separate groups of microorganisms in the composition of total microflora - reduces the amount of non-spore-forming bacteria at the expense of development of bacilli, actinomycetes and micromycetes. The increase of mineral nitrogen compounds in soil during the maturity phase to some extent inhibits the development of microorganisms, but not their mineralization activity.

Key words: *mineral fertilization, microorganisms, macronutrients*.

INTRODUCTION

The usage of easily assimilable and quickly operating mineral fertilizers, in effective combinations and fertilizer norms, appropriate weather and duration of fertilization improve the soil fertility, the quality and yield of the agricultural production. The soil fertilization increases the reserves of nutrient substances in the upper soil layer, as well as the quantity of the organic carbon (Karcauskiene and Repsiene, 2009), which leads to changes in the quantity and activity of the soil microflora (Giacometti et al., 2013). The changes in the microbial communities may be used for prognostication the effects of the ecosystemic disturbances by organic and conventional management practices (Bending et al., 2000; Bruggen-Van and Semenov, 2000; Poudel et al., 2002), since the microbial community possesses potential for faster growth and exchange, because of which is a more reactive component of the soil ecosystem for external stress, than the plants and animals (Panikov, 1999). The soil microorganisms are sensitive to changes in the surrounding environment (Schinner and Sonnletner, 1996) and are indicators that the microbial population changes after the fertilization (Hyman et al., 1990). The bringing in of mineral fertilizers as a whole increases the total quantity of the microorganisms in the soil (Milanov and Yorova, 1980; Donov et al., 1992; Prescott et al., 1992; Hart and Stark, 1997; Forge and Simard, 2001; Meena et al., 2014), as main share in the content of the general microflora after fertilization occupy the non-spore forming bacteria and bacilli, and less presented are the actynomycetes and micromycetes (Bogdanov et al., 2015; Naskova et al., 2015; Plamenov et al., 2016; Malcheva et al., 2018a; Malcheva et al., 2019a). The accumulation of microbial biomass in the initial soil-forming process in recultivating objects, especially after fertilization, is due mainly to the active development of the non-spore forming bacteria (Stefanova and Petrov, 2019). The non-spore forming aerobic and anaerobic bacteria play basic role for decomposition of green wastes in the separate phases of their composting (Malcheva et al., 2018b; Kostadinova and Dyakov, 2019), as well as after application of compost and biochar in the soil (Malcheva et al., 2019b; Malcheva et al., 2020). Other authors determine that the increase of the total microflora after fertilization is within short-term plan and is frequently followed by decrease of the microbial biomass and activity (Ohtonen, 1992; Smolander et al., 1994; Périé and Munson, 2000).

Yara Mila Triple is a balanced NPK 16/16/16 formula, appropriate for all crops, which are not sensitive to chlorine. The Yara Mila Triple granules are elaborated in such way, so there are not losses of nutrient substances flying off with storage, transportation and fertilizer bringing in. Yara Vera Amidas is based on covering of hot granulated carbamide with molten sulphur. It contains 40% total nitrogen, out of which 35% is in amidic form, which has to transform into an assimilable form for the plants - from ammonium to nitric, and 14% sulphur. The granulated form of Yara Vera Amidas allows even distribution on the soil surface and thus the uneven assimilation by the crops is avoided. Yara Bela Sulfan is a complex nitrogen fertilizer for simultaneous application of three important nutrient elements - nitrogen, sulphur and calcium. The ammonium nitrate is combined with calcium sulphate and thus is reached to the content of 24% nitrogen (out of which 12% nitrate and 12% ammonium), 15% sulphur and 11% calcium. The granules are with size, which allows thick and even covering of the area. The nutrient substances in Yara Bela Sulfan are completely soluble and provide the optimum dynamics of the absorption.

Efficiency improvement of the nitrogen fertilization is a prime issue because of the low efficiency of nitrogen. The sharing in portions of the fertilization with nitrogen has for purpose to provide the wheat quantitatively with this macroelement according to its necessities in each phase of development, to synchronize the availability of soluble nitrogen in the soil and the plant necessities (Sticksel et al., 2000; Golba et al., 2013), and also to increase the nitrogen usage efficiency. This production experiment is used on great number of farms notwithstanding the technique and purpose of production. During the recent years the purpose is to be changed the physical properties of conventional water-soluble phosphorous fertilizers, to be decreased the fixation of phosphate anions in the soil and to be increased the efficiency of the fertilizer phosphorus with assimilation by the plants.

The purpose of the study is to be determined the impact of Yara mineral fertilizer products on agrochemical indicators and microbiological activity in soils with common winter wheat.

MATERIALS AND METHODS

The experiment is carried out at an experimental field in the territory of village of Gurkovo, on area of 90 decare, in three variants. The fertilization is carried out with the fertilizer products: Yara Mila Triple (16% N, 16% P_2O_5, 16% K_2O), Yara Vera Amidas (40% N, 14% SO_3) and Yara Bela Sulfan (24% N, 15% SO_3, 11% CaO). The used variety is common winter wheat Avenue (selection of company Limagrain). The sowing is carried out on 11 October 2018 with sowing norm 600 germinating seeds/m^2.

Within the frameworks of the experimental area is carried out pro-sowing bringing in of Yara Mila Triple (16% N, 16% P_2O_5, 16% K_2O) - 25 kg/decare (4 kg/decare N active substance, a.s.). The distribution of the experimental variants, each of which with area of 30 decare is as follows:

Variant 1 (Field 1):

First fertilization 09 March 2019: Yara Vera Amidas (40% N) - 20 kg/decare (8 kg/decare N a.s.);

Second fertilization 18 April 2019: Yara Bela Sulfan (24% N) - 25 kg/decare (6 kg/decare N a.s.).

Total active substance nitrogen for the whole period of vegetation: 18 kg/decare N.

Variant 2 (Field 2):

First fertilization 09 March 2019: Yara Vera Amidas - 15 kg/decare (6 kg/decare N a.s.);

Second fertilization 18 April 2019: Yara Bela Sulfan - 25 kg/decare (6 kg/decare N a.s.).

Total active substance nitrogen for the whole period of vegetation: 16 kg/decare N.

Variant 3 (Field 3):

First fertilization 09 March 2019: Yara Vera Amidas - 14 kg/decare (5.6 kg/decare N a.s.);

Second fertilization 18 April 2019: Yara Bela Sulfan - 20 kg/decare (4.8 kg/decare N a.s.).

Total active substance nitrogen for the whole period of vegetation: 14.4 kg/decare N.

Before setting of the experiment, during the vegetation and after harvesting of the crop are taken soil samples for each of the variants for determining the content of macronutrient elements in the soil - content of ammonium and nitrate nitrogen, mobile phosphates and assimilable potassium.

The content of ammonium nitrogen (NH_4-N) is determined photometrically with indophenol blue as a result of extraction with calcium dichloride ($CaCl_2$) solution. The nitrate nitrogen (NO_3-N) is determined photometrically with Nitrospectral as a result of extraction with calcium dichloride ($CaCl_2$) solution.

The content of phosphorus and potassium is determined by a double lactate method of Egner-Rheem. The method is based on extraction of the mobile compounds of phosphorus and potassium by a solution of calcium lactate $(CH_3CH.OH.COO)_2Ca$, which is buffered with hydrochloric acid to pH 3.5-3.7, with proportion soil-solvent 1:50 and time of interaction 90 min.

The values of the soil reaction are measured potentiometrically by a pH-meter in compliance with the requirements of the methods for measuring soil pH as per the international standard ISO 10390.

The microbiological activity of the soil includes determining of non-spore forming bacteria, actinomycetes, micromycetes, bacillary microflora, bacteria, which are assimilating mineral nitrogen. They are determined by the method of dilution and culture on solid nutrient media (МПА and САА), cultivation in thermostats and next reporting of colony forming units (CFU), recalculated per 1g absolutely dry soil.

The statistical analysis includes calculation of average value out of three repetitions, standard diversion and variation coefficient (C.V.).

RESULTS AND DISCUSSIONS

The results of the carried out agrochemical analyses of soil samples with wheat are presented in Table 1.

The obtained data of the carried out agrochemical analyses before setting the experiment with common winter wheat prove poor reserve of the soil with macroelements and slightly acid reaction of the soil. The values are close in the three fields, which is important for the scientific reliability of the obtained results.

Table 1. Content of macroelements in the soil in different phases of development with wheat

№	Crop	Variant	pH	Mineral N		Assimilable P and K	
				NO_3-N	NH_4-N	P_2O_5	K_2O
Pre-sowing							
1	Wheat	Field 1	5.07	10.2	7.05	8.65	28
2		Field 2	4.9	10.3	6.98	9.03	28.5
3		Field 3	5.01	11.2	7.11	8.72	28.1
Tillering phase							
1	Wheat	Field 1	4.94	15.6	7.1	10.7	30.9
2		Field 2	5.2	10.9	6.48	14.9	34.4
3		Field 3	5.04	15.4	16.7	8.92	31.6
Maturity phase							
1	Wheat	Field 1	5.24	10.3	29.2	8.7	29
2		Field 2	5.01	14.9	31.3	12.5	28.5
3		Field 3	5.07	32.2	45.1	6.18	28.3
Harvesting phase							
1	Wheat	Field 1	5.21	8.9	25.4	5.2	26.5
2		Field 2	5.07	11.2	25.6	10.1	25.4
3		Field 3	5.1	28.3	37.3	4.3	26.2

It is determined by the soil analysis that the content of ammonium nitrogen, in all studied phases with the wheat is with relatively close values (4.94-5.24 mg/kg). In phase tillerering, field 2 distinguishes with a slightly higher value than the other two fertilizer variants. Field 1 has superiority in the reserve with ammonium nitrogen during the next two phases. The highest values of nitrate nitrogen in phase tillerering is determined in field 1, and in phase ear formation and after harvesting at field 3, where field 1 is with the lowest results. The comparatively close values of the nitrate nitrogen in phase tillering in the three fields (10.9-15.6 mg/kg) must be reported and its categorical increase in the next two phases in field 3 (around three times more in comparison with the other fertilizer variants). The obtained results in all phases of development and after harvesting are categorical regarding the values of assimilable phosphorus and potassium. The quantity of the studied macroelements is with the highest values at field 2. An exception is the results of the agrochemical analyses after harvesting, where the highest reserve of assimilable phosphorus is field 1.

The not big difference in the values of the studied soil macroelements pro-sowing and after harvesting, as well as their dynamics among the separate wheat phases of development, show the exceptionally good assimilability of the brought in fertilizer products.

On the grounds of the obtained results we may affirm that the quantity of the macroelements in the soil during the first two phases have their quantitative dynamics, but between the second phase and phase harvesting the values of all macroelements in all fertilization variants decrease, which is connected with their good assimilation.

The results of the microbiological analysis are presented in Table 2. The study is in seasonal dynamics, during the three phenological phases of the wheat development - tillering, ear formation, harvesting. An analysis of a control sample is carried out in the autumn before setting of the experiment.

Table 2. Quantity and qualitative composition of the soil microflora (x 10^3 cfu/abs. dry soil)

Crop	Phase	Variant	Total microflora	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Bacteria absorb mineral nitrogen
Wheat	Control	Field 1	2916	2142	396	234	144	3024
		Field 2	2520	1836	378	198	108	2700
		Field 3	2286	1584	360	216	126	2718
	Tillering	Field 1	6300	3192	588	2352	168	2604
		Field 2	5756.4	2902.8	656	2000.8	196.8	2328.8
		Field 3	7155	2130	930	1695	2400	1800
	Maturity	Field 1	3547.8	2284.2	712.8	437.4	113.4	1539
		Field 2	2608	1696	368	352	192	1936
		Field 3	2316.6	1620	372.8	210.6	113.4	1296
	Harvesting	Field 1	3998.4	2570.4	621.6	672	134.4	1747.2
		Field 2	3132.4	1968	442.8	524.8	196.8	2050
		Field 3	2851.2	1814.4	486	307.8	243	1490.4

Fertilization increases the biogenicity of soils in comparison with the studied controls (unfertilized samples) in different degree during the separate phases of the wheat development. The total quantity of microorganisms is the highest with the fertilization during phase tillering - around 2 times with variants 1 and 2, around 3 times with variant 3. In the same order decreases the total microflora during phase ear formation, but the quantity of the microorganisms remains higher in comparison with the controls. A slight increase of the total quantity of microorganisms is observed during phase harvesting in comparison with phase ear formation. Consequently regarding the seasonal dynamics the development of the microorganisms is higher during the spring in comparison with the summer. It is drawn attention as a tendency that with the fertilization, applied in variant 1, the total quantity of microorganisms is preserved higher during the three phases, with variant 2 intermediate in comparison with the other two variants, and with variant 3 - the highest during phase tillering and the lowest with the other phases. Generally this tendency correlates with the quantity of the nitrogen and sulphate compounds brought in with the fertilizers - the highest quantity with variant 1, follows variant 2 and the lowest with variant 3. The chemical analysis shows that the content of assimilable nitrogen compounds is the highest with variant 3, follow variant 2 and variant 1. Consequently the assimilation of the nitrogen compounds by the wheat plants follows the ascending order: variant 1 > variant 2 > variant 3, which presents and the development of the microorganisms as a whole. Thanks to the ammonification the soil is enriched additionally with ammonium and ammonium salts available for the plants. Respectively the yields of the fields are relatively in the same ascending order. The development of the microorganisms also decreases by depletion of the reserve of assimilable phosphorus and potassium - it is higher with phase tillering, and lower with the other two phases.

Highest percent in the content of the total microflora with the fertilized variants occupy the non-spore forming bacteria - 50-65%, with exception of variant 3, phase tillerering - 30%. The share of the non-spore forming bacteria is 69-74% with the control samples, and next in decreasing order are the bacilli, actynomycetes and micromycetes. While with the fertilized variants is observed regrouping of the micro-organisms - the actynomycetes occupy higher percent share than the bacilli during phases tillerering and harvesting. The micromycetes (the moulded fungi) also increase their quantity with fertilization, but their percent share remains the lowest in the content of the total microflora, with exception of variant 3, phase tillerering, where their quantity is the highest of all studied groups of microorganisms. The highest moisture of the soil is determined with this variant, which is a factor contributing for the better development of the moulded fungi. This regrouping of the microorganisms with phases tillerering and harvesting has impact on the decomposition of the organic substances in the soils. On the one hand the higher quantity of the non-spore forming bacteria and baccilli (ammonifiers) increases the initial stages of the organic matter destruction. Bacilli are also spore-forming species, which increases their resistance to changes (sometimes unfavourable) conditions of the environment. On the other hand the increase of the actinomycetes and micromycetes quantity shows that they also include actively in the decomposition of the organic matter, as with the assimilation of plainer substrata (carbohydrates, proteins, lipids, starch), as well as with the accumulation of more complex and difficult for assimilation substrata - mainly cellulose, hemicellulose, polysaccharides, when the speed of decomposition is also slowed down (final states of deconstruction). Some strains non-spore forming bacteria are less resistible to the direct impact of the nutrient substances, brought in with the fertilizers under the form of nitrogen and sulphur compounds, even more so they do not form spores surviving extreme conditions, “they stress” and die or they decrease their activity - not always higher quantity means higher activity. The reproduction and activity of the microorganisms are influenced by variety of factors - moisture, temperature, pH, reserve of nutrient substances, soil pollution, vegetation and etc. It is determined that the increase of the mineral nitrogen compounds in the soil during phase ear formation suppresses the development of the microorganisms, but not their activity - with the lowest total quantity of microorganisms and bacteria, assimilating mineral nitrogen with variant 2 and 3 during phase ear formation are determined the highest values of the mineralization coefficient in comparison with the same variants with the other phases. The quantity of bacteria, assimilating mineral nitrogen, however, is higher with the controls, than with the fertilized samples, which means that in some degree the speed of decomposition is impeded after fertilization - the values of the mineralization coefficient with the samples are higher (Figure 1).

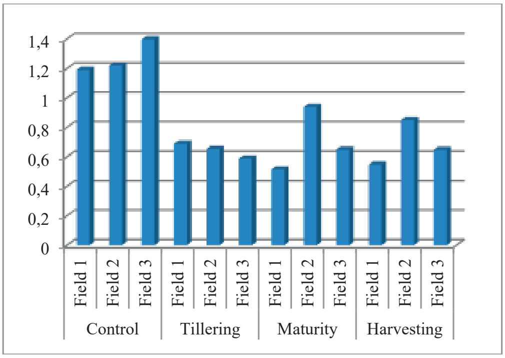

Figure 1. Mineralization coefficients

The statistical analysis showed that the extract is homogeneous - low values of standard deviation and coefficient of variation under 10% with all groups of microorganisms, for all variants and phases (Table 3).

The usage of Yara mineral fertilizers leads to increase of the content of macroelements in the soil in comparison with the control sample before setting of the experiment. The trend of increase continues until phase ear formation as in phase after harvesting is observed decrease of the reserve, which is an adequate process on basis the botanical characteristics of the wheat and the phases of vegetation. The best results are obtained with the variant fertilization of field 1 regarding ammonium nitrogen, field 3 - nitrate nitrogen, assimilable phosphorus and potassium - field 2.

On the grounds of the obtained results may be affirmed that the fertilizer variants suggested for testing have exceptionally favourable impact on the soil reaction and the content of macroelements in the soil. Their quantities after phase harvesting are sufficient to provide good initial development of a next crop on the same area in the plan of crop rotation.

Table 3. Statistical indicators: Standard deviation (STDEV), coefficient of variation (C.V., %)

Crop	Phase	Variant	Total microflora		Non-spore-forming bacteria		Bacilli		Actinomycetes		Micromycetes	
Statistical indicators			STDEV	C.V.	STDEV	C.V.	STDEV	C.V.	STDEV	C.V.	STDEV	C.V.
Wheat	Control	Field 1	4.546	3.820	0.816	3.711	0.471	3.722	0.000	0.000	5.888	3.505
		Field 2	3.559	3.489	0.000	0.000	0.816	7.423	0.471	7.443	4.320	2.880
		Field 3	6.532	7.423	1.700	8.359	0.000	0.000	0.471	6.428	5.099	3.377
	Tillering	Field 1	4.320	2.274	0.816	2.333	3.742	2.673	0.816	8.165	4.082	2.634
		Field 2	2.449	1.384	0.816	2.041	3.266	2.677	0.943	8.081	7.483	5.345
		Field 3	3.266	2.300	1.414	2.281	2.944	2.605	4.899	3.062	2.449	2.041
	Maturity	Field 1	3.742	2.654	1.247	3.530	1.247	3.024	0.471	4.041	4.967	5.228
		Field 2	3.266	3.081	0.816	3.550	0.816	3.711	0.943	8.081	6.164	5.095
		Field 3	3.266	3.266	1.414	6.149	0.943	7.071	0.000	0.000	1.633	2.041
	Harvesting	Field 1	2.160	1.412	0.471	1.263	1.633	4.082	0.471	5.657	7.071	4.562
		Field 2	1.414	1.179	0.816	2.722	0.471	1.458	0.816	6.804	4.320	3.086
		Field 3	1.633	1.458	1.247	4.204	0.816	4.297	1.247	8.134	2.828	3.074

The usage of the fertilizer products increases the biogenicity of the soils in comparison with the studied controls (unfertilized samples) in different degree during the separate phases of the wheat development. The total quantity of microorganisms is the highest with the fertilization during phase tillering - around 2 times with variants 1 and 2, around 3 times with variant 3. The biogenicity decreases during phase ear formation and harvesting, as the total microflora is the highest with variant 1, variant 2 follows, and variant 3 is with the lowest quantity. As a tendency is drawn attention that with the fertilization with variant 1 the total quantity microorganisms is preserved higher during the three phases, with variant 2 intermediate in comparison with the other two variants, and with variant 3 - the highest during phase tillering and the lowest with the other phases.

The highest percent share in the content of the total microflora with the fertilized variants occupy the non-spore forming bacteria - 50-65%, with exception of variant 3, phase tillerering - 30%. Regrouping of the rest groups of microorganisms is determined in comparison with the controls - the percent participation of the actinomycetes is increased significantly, which take part in the final stages of the organic matter destruction. They are in higher quantity than the bacilli with phase tillering for all variants and phase harvesting for variants 1 and 2. The micromycetes are less presented in the content of the total microflora with exception with variant 3, phase tillering, where their quantity is the highest. The quantity of bacteria, assimilating mineral nitrogen is higher with the controls, than with the fertilized samples, which means that in certain degree the speed of decomposition is impeded after fertilization - the values of the mineralization coefficient with the controls are higher.

Similar results for distribution of the separate groups of microorganisms in the total microflora content, after mineral and organic fertilization and in the phases of composting, are determined also in other own and somebody else's researches (Naskova et al., 2015;

Plamenov et al., 2016; Malcheva et al., 2018a; 2018b; Malcheva et al., 2019a; 2019b; Stefanova and Petrov, 2019; Kostadinova and Dyakov, 2019; Malcheva et al., 2020).

The researches regarding the effects of the nitrogen fertilization on the microbial biomass remain contradictory. For example, Zhang et al. (2005) have observed significant increase of the microbial biomass up to two years with usage of nitrogen fertilizers at pastures in China, but Sarathchandra et al. (2001) announce for significant decrease of the microbial biomass at perennial pasture of New Zealand because of a two-year nitrogen fertilization. Meanwhile, Johnson et al. (2005) have determined that two year of application of N has not influenced the microbial biomass in soils in Scotland. These tendencies depend on the differences regarding the soil moisture, content of organic substances in the soil, total content of N, pH, duration of bringing in of N (Williams et al., 2007), but the specific basic factors are still not completely identified (Arnebrant, 1990; Zhang et al., 1998). The degree of the fertilization effect on the microbial biomass depends on pH according to researches of Geisseler and Scow (2014) - the fertilization decreases the microbial biomass with soils with pH under 5, but has a significantly positive effect with higher values of pH of the soil.

CONCLUSIONS

The usage of Yara mineral fertilizers leads to increase in the content of macroelements in the soil in comparison with the control sample before setting of the experiment. The trend of increase continues until phase ear formation as in phase after harvesting is observed decrease of the reserve, which is an adequate process on base the botanical characteristics of the wheat and the phases of vegetation. The best results are obtained with the variant fertilization of field 1 regarding ammonium nitrogen, field 3 - nitrate nitrogen, assimilable phosphorus and potassium - field 2. The quantities of the macroelements after phase harvesting are enough to provide good initial development of a next crop on the same area in the plan of crop rotation.

The usage of the fertilizer products increases the biogenicity of the soils in comparison with the studied controls (unfertilized samples) in different degree during the separate phases of the wheat development. The total quantity of microorganisms is the highest with the fertilization during phase tillering - around 2 times with variants 1 and 2, around 3 times with variant 3. The biogenicity decreases during phase ear formation and harvesting, as the total microflora is the highest with variant 1, follows variant 2, and variant 3 is with the lowest quantity. As a tendency is drawn attention that with the fertilization with variant 1 the total quantity of microorganisms is preserved higher during the three phases, with variant 2 intermediate in comparison with the other two variants, and with variant 3 - the highest during phase tillering and the lowest with the other two phases.

The highest percent share in the content of the total microflora with the fertilized variants occupy the non-spore forming bacteria - 50-65%, with exception of variant 3, phase tillering - 30%. Regrouping with the rest groups microorganisms is determined in comparison with the controls - the percent participation of the actinomycetes is increased significantly, which participate in the final stages of the organic matter destruction. They have higher quantity than the bacilli with phase tillering for all variants and phase harvesting for variant 1 and 2. The micromycetes are the less presented in the content of the total microflora, with exception of variant 3, phase tillering, where their quantity is the highest. The quantity of the bacteria, assimilating mineral nitrogen is higher with the controls, than with the fertilized samples, which means that in some degree the speed of decomposition is impeded after fertilization - the values of the mineralization coefficient with the controls are higher.

The soil microbiological activity depends on and is in correlation with complex of factors - moisture, temperature, pH, mechanical composition, nutrient reserve, choice of a fertilizer and variants of fertilization, type of vegetation, processing of the soil and etc. Since they are sensitive indicators, their study in dynamics with fertilization with different types of fertilizers in appropriate combinations and norms, with different vegetation is necessary with purpose evaluation of the fertilization impact on the microbiological and enzyme activity of the soil, and from there on the soil fertility.

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THE USE IN AGRICULTURE OF CALCAREOUS AMENDMENTS AND THEIR INFLUENCE ON THE CHEMICAL PROPERTIES OF THE REDDISH LUVOSOIL FROM THE MOARA DOMNEASCA STATION, SOUTHEASTERN ROMANIA

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Abstract

The increasing volume of waste generated by the steel industry, with repercussions given by environmental pollution and the removal from the agricultural circuit of significant areas of land that is being used for waste storage, requires the identification of efficient solutions for recycling this waste, while also requiring the use of elements with beneficial effects on the soil and on the cultivated plants. The metallurgical industry produces various residues, and some of them, such as steel slag, can be used successfully in the agricultural activity. Steel slag can have a beneficial influence on the physico-chemical properties of the soil and on the agricultural production. In order to mitigate the environmental consequences caused by the disposal of large quantities of slag in landfills and in order to reduce transport costs for its disposal, the steel industry encourages the sustainable use of slag in various fields of application, such as agriculture. Research conducted in 2020, on the experimental field at the "Belciugatele" research station/"Moara Domneasca" development farm, followed the influence of various calcareous amendments on the chemical properties of reddish luvosoil from this area. During the experiment, different doses of dolomite, calcium carbonate and two types of slag from the steel industry were applied and changes in the reaction of the soil and the content of macroelements and microelements in the soil were monitored.

Key words*: soil, amendments, slag, chemical properties, pollution.*

INTRODUCTION

The use, in agriculture, of solid metallurgical waste such as steel slag, has become very important for its recycling and recovery, which would lead to a reduction of overall environmental pollution, while also contributing to soil improvement and higher yields.
Every year, in Europe, over 40 million tonnes of iron and steel slag are produced as a result of industrial processes (Branca & Colla, 2012; Yildirim & Prezzi, 2011).
Based on its properties, slag is classified as a non-hazardous waste and can be stored, but this procedure requires a large area of land for storage. There is always the risk that various components of the slag may be leached into the soil's profile and contaminate the water table, and implicitly, groundwater.
Therefore, it is necessary to research these types of wastes that could be used as valuable secondary raw materials in other areas of the economy, such as agriculture.
The main chemical components that slags contain in their composition and which are important for their use in agriculture are CaO, MgO, SiO_2, P_2O_5, Fe and MnO. Concentrations of elements in slag vary greatly depending on the raw materials used, the type of steel manufactured, furnace conditions and other aspects (Yi et al., 2011; Shi, 2004).
It was shown that, due to the high calcium content, slag can help increase the soil's pH and can help mobilize nutrients, which leads to an increased agricultural production (Branca & Colla, 2012). Balanced plant nutrition is one of the main factors affecting their growth and yield. The application of industrial waste as a fertilizer or as an amendment to correct the soil's pH reaction has become a common practice in agriculture (Liu et al., 2002; Yang & Zhang, 2005).

The application of steel slag was studied on acidic soils, cultivated in the no-tillage system, with excess of Al^{3+}, and after 27 months from the application of slag in doses of 2 t/ha, 4 t/ha and 8 t/ha (Fernandes et al., 2018), without incorporation, an increase in the degree of saturation in bases was recorded, with V% = 70%, at a depth of 20-40 cm, when the dose of 4 t/ha of slag was applyed, while an increase in the pH value was also recorded, from a pH of 4.6 in the non-fertilized variant to a pH of 5.6 in the variant where 4 tonnes of slag/ha were applied. During the same period, the effects of $CaCO_3$ on the soil were observed only at a depth of 0-20 cm.

Due to the high level of CaO and MgO and also due to the high pH level, that can reach up to 12.5, the repeated application of steel slag can make the soil excessively alkaline, which can reduce the bioavailability and the plant's uptake/absorption of macroelements such as P and microelements, such as Fe, Cu and Zn and can impede their growth and overall yields (Chand et al., 2015).

MATERIALS AND METHODS

Because slag is rich in Ca and other fertilizer components such as Mg, Fe, N, P, K, in our research we aimed to evaluate the impact of the application of two types of steel slag (furnace slag - LF and converter slag - CV) (Tables 1 and 2) on the soil's quality while also aiming to compare their influence on the soil's chemical properties, with the influence of amendments used traditionally, respectively calcium carbonate and dolomite.

The steel slag used in this study, as a potential source of certain nutrients beneficial to both soil and plants, is a by-product of the steel industry and comes from the ArcelorMittal Galati Steel.

Table 1. Composition of different types of slag from Romania, ArcelorMittal Galati

	Fe	SiO_2	CaO	MnO	MgO	Al_2O_3	P_2O_5
	%	%	%	%	%	%	%
A-LF	2.38	15.55	48.52	6.12	3.50	16.63	0.50
B-LF	0.98	11.89	52.56	0.56	4.15	22.28	0.29
C-LF	11.28	7.88	40.46	5.93	5.11	22.54	0.60
D-CV	19.09	13.25	50.80	3.05	1.48	1.85	1.71
E-CV	1.17	12.09	49.65	0.98	5.20	24.04	0.31
F-CV	5.55	7.54	46.71	2.25	6.75	24.07	0.50

The most significant oxide components of slag are: CaO (usually 46-52.5%), Al_2O_3 (2-22.5%), SiO_2 (7.5-15.5%) and MgO (3.5-6.8%). The slag generated by the furnace has a Fe content between 1% and 19%, while the MnO content is between 0.5% and 6.2% and less than 2% of P_2O_5 (Table 1).

LF slag (ladle furnace slag) is a secondary metallurgy slag resulted from the process of refining steel slag generated in the different primary unit (electric arc furnace).

The most usual applications of dusty LF slag are focused, at present, on the following areas (Popescu et al., 2016):

- The reintroduction into the steelmaking units: electric furnace slag frothing, converter with oxygen blast-furnace desulfurization or ladle (Zhao et al., 2015);
- The use as an additive of clinker in the cement industry (Setien et al., 2009; Shi, 2004);
- Used for acid mine water treatment (Mark & Gutta, 2009);
- Used as a fertilizer in agriculture, because of trace elements found in slag, which may act as micronutrients (Bonenfant et al., 2008), or a neutralizer for acidic soils (Smallfield, 2000).

The current steelmaking process is based on the Basic Oxygen Steelmaking process, where a basic slag is produced in the Linz-Donawitz converter. The LD slag contains about 1-3 wt% of P_2O_5, which is too low to be used as a phosphate fertilizer, but, at the same time, it is too high to be used in the BF or recycled in the sinter plants.

Because blast furnace slag contains the fertilizer components CaO, SiO_2, and MgO, it is used as calcium silicate fertilizer. In addition to these three components, steelmaking slag also contains components such as FeO, MnO, and P_2O_5, and is used for a broader range of agricultural purposes, including dry field farming and pastures. Its alkaline property can also correct soil acidity (Humaria, 2014).

Table 2. Chemical composition of slag used in the experiment

Materials	Na	Ca	Mg	Cd	Cr	Cu	Ni	Mn	Pb	Zn	Fe
	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%
LF	0.28	11.7	5.43	U/nd*	7.61	2.39	4.88	9084	0.3	6.3	0.08
CV	0.023	18.5	1.62	U/nd*	725	20.1	3.68	28164	3.75	40.8	12.2

*U/nd - undetectable by the test method used

The concentrations of heavy metals in the slag were analyzed by atomic absorption spectrophotometry (AAS), from extracts with wet mineralization, ICPA Methodology (1983), Chapter 14 pt 134.

The study was conducted at the "Belciugatele" research station/"Moara Domnească" development farm belonging to the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV Bucharest), where an experimental field which included 9 variants in 3 repetitions V1 (control), V2 (carbonate calcium - 3 tonnes/ha), V3 (dolomite - 3 tonnes/ha), V4 (LF-slag 1 tonne/ha), V5 (LF-slag 3 tonnes/ha) , V6 (LF-slag 5 tonnes/ha), V7 (CV-slag 1 tonne/ha), V8 (CV-slag 3 tonnes/ha), V9 (CV-slag 5 tonnes/ha) where the slag was incorporated immediately after application.

The aspect of the slag used in this study is presented in Figures 1 and 2.

Figure 1. LF slag (foto. 2019)

Figure 2. CV slag (foto. 2019)

RESULTS AND DISCUSSIONS

The type of soil found in the area of research is the reddish luvosoil, that had, before the application of the slag, the following chemical characteristics (Tables 3 and 4), where we can observe in the surface horizon a pH of 5.98, a humus content of 1.95, Nt of 0.110 %, P_{AL} 41 mg/kg, K_{AL} 200 mg/kg, Ca^{2+} 2.98 me/100 g and a heavy metal content below the maximum permissible limits.

Table 3. The main chemical properties of the reddish luvosoil in the reseach area

	Tests performed						
Depth	pH	Humus	Nt	P_{AL}	P_{AL}[1]	K_{AL}	Ca^{2+}
cm	pH units	%	%	mg/kg			me/100 g
0-20	5.98	1.95	0.110	41	41	200	2.98
20-40	5.70	1.78	0.102	29	29	146	11.83

[1]recalculated values in accordance with the soil´s pH

Table 4. The main chemical properties of the reddish luvosoil in the reseach area

	Tests performed								
Depth	Cd	Cu	Co	Cr	Mn	Ni	Pb	Zn	Fe
cm	mg/kg								%
0-20	0.21	22.6	12.4	21.5	863	30.6	19.1	64.5	2.839
20-40	0.15	21.9	11.6	21.7	785	33.2	17.1	67.0	3.106

The analysis of the soil's pH reaction at a depth of 0-20 cm shows a very significant increase in the pH value when applying a dose of 3 tonnes of LF slag per hectare, respectively 6.03, compared to 5.96 in the control variant, as shown in Table 5.

Table 5. The influence of the applied amendments on the soil's pH reaction at a depth of 0-20 cm

Variant	Soil's pH		Difference		Significance
		%		%	
V1-control	5.96	100	Mt	-	
V2-$CaCO_3$	6.03	101.11	0.06	1.11	***
V3-$CaMg(CO_3)_2$	5.96	100.05	0.00	0.05	-
V4-LF 1 t/ha	5.85	98.21	-0.10	-1.78	ooo
V5-LF 3 t/ha	6.03	101.11	0.06	1.11	***
V6-LF 5 t/ha	5.88	98.71	-0.07	-1.28	ooo
V7-CV 1 t/ha	6.10	102.29	0.13	2.29	***
V8 CV 3 t/ha	6.09	102.18	0.13	2.17	***
V9-CV 5 t/ha	6.51	109.16	0.54	9.16	***
LSD 5% = 0.02 *LSD 1% = 0.03* *LSD 0.1% = 0.04*					

At a depth of 20-40 cm, the increase in the value of the soil's pH reaction is very significant in all experimental variants, when compared to the control variant. The highest increase was recorded in the variant where dolomite was applied, with a pH of 6.11, compared to a pH value of 5.71 in the control variant. For the variants where LF slag was applied, the soil's pH reaction was of 5.74 for the variant with 1 tonne of slag per hectare, 5.97 for the variant where 3 tonnes of slag per hectare were applied and 5.92 for the variant where 5 tonnes of LF slag were applied per hectare, as detailed in Table 6.

Table 6. The influence of the applied amendments on the soil's pH reaction at a depth of 20-40 cm

Variant	Soil's pH		Difference		Significance
		%		%	
V1-control	5.71	100	Mt	-	
V2-$CaCO_3$	6.02	105.36	0.30	5.36	***
V3-$CaMg(CO_3)_2$	6.11	107.05	0.40	7.05	***
V4-LF 1 t/ha	5.74	100.58	0.03	0.58	***
V5-LF 3 t/ha	5.97	104.55	0.26	4.55	***
V6-LF 5 t/ha	5.92	103.61	0.20	3.61	***
V7-CV 1 t/ha	6.17	107.99	0.45	7.99	***
V8-CV 3 t/ha	6.19	108.45	0.48	8.45	***
V9-CV 5 t/ha	6.24	109.27	0.53	9.27	***
LSD 5% = 0.03 *LSD 1% = 0.05* *LSD 0.1% = 0.07*					

The application of CV slag determined an increase of the soil's pH reaction at both the depth of 0-20 cm and the depth of 20-40 cm in all variants where CV slag was applied. The most significant increase was recorded at a depth of 0-20 cm, when a dose of 5 tonnes per hectare was applied, where the pH value increased to 6.5, as compared to the value of 5.96 in the control variant. At a depth of 20-40 cm, also when applying a dose of 5 tonnes per hectare, the highest increase of the soil's pH reaction was registered, respectively a value of 6.24, compared to 5.71 for the control variant.

The application of steel slag in the experiments undertaken on the reddish luvosoil from Moara Domnească determined an increase of the soil's pH reaction and of the main chemical properties of the soil, in the first 3 months from the application, as shown in Tables 7 and 8.

Table 7. The influence of the applied amendments on the chemical properties of the reddish luvosoil from Moara Domneasca at a depth of 0-20 cm

Treatment	Humus	Nt	P_{AL}	K_{AL}	Ca^{2+}	Mg
	%	%	mg/kg	mg/kg	me/100 g	me/100 g
V1-Control	2.01	0.106	35	159	2.98	17.1
V2- $CaCO_3$	1.78	0.107	35	174	12.75	18.2
V3- $CaMg(CO_3)_2$	1.95	1.110	41	200	3.27	18.4
V4-LF 1 t/ha	2.07	0.106	41	123	10.05	18.2
V5-LF 3 t/ha	2.13	0.227	51	204	15.58	20.0
V6-LF 5 t/ha	2.01	0.115	70	166	11.55	18.2
V7-CV 1 t/ha	1.78	0.110	46	182	16.84	18.9
V8-CV 3 t/ha	2.07	0.117	43	160	12.68	19.8
V9-CV 5 t/ha	1.84	0.109	85	130	17.64	20.1

The application of the amendments did not lead to an increase of the humus content in the soil, the highest value was registered when applying a dose of 3 tonnes of LF slag per hectare, respectively 2.13% compared to 2.01% in the control variant.

The total nitrogen content of the soil registered increases in most experimental variants, the highest value being registered in the variant where 3 tonnes of LF slag were applied per hectare, respectively 0.227%.

The increase of the phosphorus content in the soil was recorded in all experimental variants. The highest values were recorded when applying the dose of 5 tonnes per hectare, both in the case of LF slag (70 me/kg) and in the case of CV slag (85 me/kg).

Significant increases in the soil's potassium content were recorded in most experimental variants compared to the variant where no calcareous amendments were applied, the highest value being recorded in the variant where the dose of 3 tonnes of LF slag were applied per hectare.

In the case of calcium, there were increases in all experimental variants, the highest value being recorded where converter (CV) slag was applied on the soil in a dose of 1 tonne per hectare.

As for the case of calcium and magnesium, there were increases in all experimental variants compared to the control variant, where the value was of 17.1 me/100 g.

The largest increases were recorded when applying LF slag in a dose of 3 tonnes/ha (20 me/100 g) and when applying CV slag at a dose of 5 tonnes/ha (20.1 me/100 g).

Table 8. The influence of the applied amendments on the chemical properties of the reddish luvosoil from Moara Domneasca at a depth of 20-40 cm

Treatment	Humus	Nt	P_{AL}	K_{AL}	Ca^{2+}	Mg
	%	%	mg/kg	mg/kg	me/100 g	me/100 g
V1-Control	1.60	0.092	37	160	3.21	19.4
V2- $CaCO_3$	1.78	0.102	29	146	11.83	20.6
V3-$CaMg(CO_3)_2$	1.78	0.109	53	144	4.11	20.4
V4-LF 1 t/ha	1.95	0.115	39	132	2.98	20.2
V5-LF 3 t/ha	2.01	0.121	37	186	5.43	19.1
V6-LF 5 t/ha	1.95	0.107	44	144	19.75	20.4
V7-CV 1 t/ha	1.30	0.085	12	148	24.79	20.0
V8-CV 3 t/ha	1.66	0.095	19	148	1.49	20.5
V9-CV 5 t/ha	1.54	0.092	30	138	3.47	21.8

After 3 months from application, the influence of the amendments on the depth of 20-40 cm, as compared to the depth of 0-20 cm, is not significant, with the only exception being in the case of calcium, where an increas can be observed in most experimental variants, when compared to the control variant. The highest increase, of 19.75 me/100 g, was registered when applying a dose of 5 tonnes of furnace slag (LF) per hectare and of 24.79 me/100 g, when applying converter slag (CV) in a dose of 1 tonne per hectare.

The analyzes performed, in the experimental field, on the reddish luvosoil, in order to determine the soil's content in heavy metals, show that all the heavy metals analyzed had a content below the maximum allowable limits (Table 9).

Table 9. The heavy metal content of the soil at Moara Domneasca at a depth of 0-20 cm

Heavy metals	Cd	Cu	Co	Cr	Mn	Ni	Pb	Zn	Fe
				mg/kg					
V1-Control	0.19	22.6	12.2	21.8	788	32.40	17.0	64.3	2.767
V2- $CaCO_3$	0.18	22.5	11.5	21.4	821	31.57	21.8	68.6	2.846
V3- $CaMg(CO_3)_2$	0.19	21.2	13.0	25.8	849	41.20	19.6	69.7	2.922
V4-LF 1 t/ha	0.13	20.6	10.5	22.3	77	32.65	20.9	61.9	2.700
V5-LF 3 t/ha	0.10	23.6	10.8	20.2	802	38.39	21.1	66.9	2.783
V6-LF 5 t/ha	0.11	22.7	10.5	22.8	842	34.93	20.9	67.8	2.812
V7-CV 1 t/ha	0.19	20.4	14.3	24.7	812	37.70	19.7	65.8	2.873
V8-CV 3 t/ha	0.18	22.6	13.9	30.6	848	48.00	17.3	64.5	3.062
V9-CV 5 t/ha	0.32	21.3	13.6	28.2	852	44.70	19.4	68.9	2.977
Maximum heavy metal allowable limit for soil	3	100	-	100	-	50	50	300	-

CONCLUSIONS

Our research highlighted that slag from steel processing plants can be used as an amendment to correct the soil's pH reaction.

A high CaO content, of 50%, may recommend the use of these by-products of the steel industry for soil improvement.

In addition to the calcium intake, this waste also brings an important contribution of microelements in the soil, necessary for the growth and development of crops.

There were significant increases in the value of the soil's pH reaction both at a depth of 0-20 cm and at a depth of 20-40 cm when applying the converter slag (CV) in a dose of 5 tonnes/ha.

After 3 months from the application of these residues, no accumulations of heavy metals was found in the soil, even for the maximum applied doses.

The results of research on the use of slag from the steel industry in agriculture have shown that the correct application of these residues brings benefits to the soil's chemical properties, by increasing the pH value of acidic soils, by increasing the nutrient contents of phosphorus, calcium and magnesium, and all these aspects have a contribution in achieving higher crop yields.

Environmental risk assessment based on the application of slag requires further investigation, which is an opportunity for both the steel and fertilizer industries to promote the economy/preservation of natural resources, in order to reduce CO_2 emissions, to prevent the accumulation of large amounts of waste, and to bring social awareness in regards to the sustainability of some industrial activities.

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THE IMPACT ON SOME PHYSICO-MECHANICAL PROPERTIES OF SOIL PROCESSING USING THE VIBROCOMBINATOR IN FORESTRY NURSERY

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Abstract

The mechanization technologies of soil works have a major impact on physical state of soil. This situation is generated by the mechanical action of working parts which are involved in soil works and by the traffic of running systems of tractors and agricultural machines. In order to carry out the research, we settled in six forestry nursery of the West of Romania so that we could have six different types of soils which are representative for that specific area. From each profile was collected soil samples in three steps of 6, 12 and 18 cm. For each sample were performed six repetitions (N = 6). We started by measuring the particle size distribution (granulometric composition) and the main physical properties of the soil (moisture, bulk density, total porosity and soil compression degree). Advanced methods of statistical analysis (univariate three-way ANOVA and multivariate analysis, PCA, Manova and HCA) began to be successfully used in recent years for the study of soil behavior at the interaction with the working bodies. Vibro-cultivators are machines for seedbed preparation. They are equipped with tools sustained by elastic suspension. The elasticity of supports facilitates the oscillations of working tool - elastic support assembly. This set has a natural mode shapes which corresponds to a natural frequency of vibration. Seedbed preparation for crop establishment (sowing) is one of the most important works in forestry nursery, as is done with high energy consumption and high costs.

Key words: *vibro-combinator, soil tillage, bulk density, total porosity, compression degree.*

INTRODUCTION

Compaction causes a rearrangement of the soil particles and many properties of the soil are influenced as a result. Pore size distribution is altered, total porosity is decreased, and there are changes in the movement and content of heat, air, water and nutrients in the soil. The restricted growth of roots commonly observed in compacted soil has been variously attributed to all of these properties, and to the high mechanical resistance which compacted soil presents to plant roots (Shierlaw J. et al., 1984; Boja et al., 2013).

Soil compaction, as a consequence of frequent cultivation with heavy machinery, is one of the most important problems that modern mechanized agriculture is facing. Although the negative effects of heavy farm machinery on the physical characteristics of soil fertility, e.g. decreased aggregate stability, soil crusting, and formation of traffic pans and plough-pans, is well documented, much less is known about how soil compaction affects biological soil fertility (Neve et al., 2000).

These mechanization technologies have been tested to determine which of them correspond to the highest degree of sustainable agriculture concept and ensure protection, preservation and improvement of agricultural lands. The testing results of mechanization technologies for soil works variants which include a wide spectrum of conservative and unconventional works, performed with appropriate equipment, were compared both between them and also with witness variant which involved the classical

and conventional technologies for soil processing (Țenu et al., 2009).
The structure is a distinctive characteristic, appropriate to soil, being of great importance for physical, chemical and biological processes which are developed in soil and in the soil-plant-atmosphere system. Many authors consider the structure as a basic characteristic, on which depends the soil fertility (mainly water and air regime, thermal and nutrient regime) (Boja et al., 2018a; Boja et al., 2018b).
The degradation of the structure is determined by two groups of causes: changing the chemistry of the soil by decreasing soil humus content, and sometimes, especially as a result of unbalanced fertilization or irrigation with poor quality water by alkalization or acidification of soil; the direct destruction actions of structural elements, including soil dusting due to excessive work, or inadequate humidity, compaction due to exaggerated traffic especially when it is performed on wet ground, formation of crust under rain drops action or sprinkling-irrigations, etc. (Țenu et al., 2009).
The reduction of soil volume (a simple reduction in pore space) due to external factors is called soil compaction. Soil compaction is defined as increase in soil bulk density or decrease in soil volume and porosity (Figure. 1) due to mechanical stress on soil (e.g., from traffic of farm machinery). It can also occur due to natural reconsolidation of soil. There are two types of compaction, namely, surface compaction and subsoil compaction. The compaction that occurs in the surface "plow layer" is called surface compaction, while the compaction that occurs as a result of a surface load below the plow layer is called subsoil compaction.

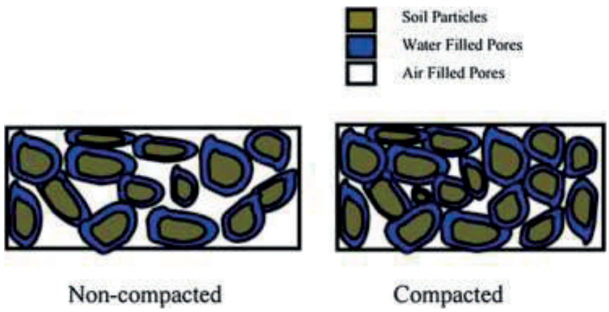


Figure 1. Effect of soil compaction on pore space (Neve et al., 2000; Boja et al., 2018b)

Nowadays, humanity is facing a major controversy over the choice of appropriate technology of soil tillage. It is the time that is required an intelligence choice between conventional technologies (classical) for seedbed preparation, assuming an intense mechanical processing of soil, which affects soil structure and soil organic matter, and the conservative tillage technologies for seedbed preparation, which removes these disadvantages in terms of an accepted decrease of the production (Benites et al., 2000).
At present, increase in the size of farm equipment used to carry out various farm operations increases the risk of soil compaction. The agricultural soil compaction can take place due to frequent movement of farm machinery. Factors responsible for compaction due to vehicular traffic include weak soil (soil density and moisture content effect) and excessive loads (size of vehicles, tire size, and number of passes are directly related to compaction). Soil tillage operations are also responsible for soil compaction (Pisante et al., 2010).
The advantages of using vibro-combinators are: required preparation of seedbed in difficult working conditions and preservation of moisture and total porosity and reducing of soil compression degree. Such important factors can ensure fast, uniform and early germination of seeds, these requirements standing at the basis of abundant harvests (Boja et al., 2018a).
The paper presents a study on the optimization of working regime of vibro-cultivators based on environmental impact assessment for use in seedbed processing. Study presents a method to determinate some phisical and mechanical

proprieties before and after soil tillage works of aggregates consisting of tractor and vibro-cultivators, in six parcels in the plains of the West of Romania.

Vibro-cultivators are machines for seedbed preparation. They are equipped with tools sustained by elastic suspension. The elasticity of supports facilitates the oscillations of working tool - elastic support assembly. This set has a natural mode shapes which corresponds to a natural frequency of vibratio (Cardei et al., 2015).

Modern agricultural operations now demand the utilization of a wide variety of equipment and specialist machinery systems, with many having rotary elements such as axles, gears, pulleys etc. With these agricultural machinery systems which have rotary elements, uncontrolled vibrations may become an important problem to consider. When the initial 'switch-on' frequency meets with the natural frequency of a machine element in the system, undesired noise, high levels of vibration and mechanical failures may occur during operation (Celik et al., 2010).

Generally, combinators consist of a vibro-cultivator A (cultivator for total processing of soil), composed of: frame 1, coupling device at the power source 2, wheels for limiting of working depth 3, soil loosening bodies 4, and a helix harrow B, which consists of frame 5, two rodrotors 6, and horizontality adjustment system 7 (Figure. 2).

Worldwide, more and more prestigious companies have incorporated into the range of products such vibro-combinators.

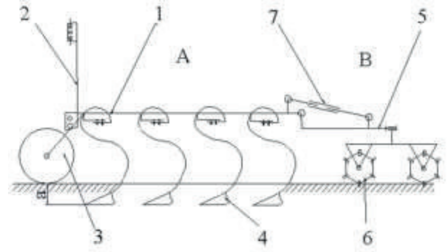


Figure 2. General scheme of a vibro-combinator (Căproiu et al., 1982; Biris et al., 2015; Boja et al., 2018b)

Deep tillage tools are one of the primary components of agricultural equipment which experience high level soil reaction forces during tillage operations. These forces may cause plastic deformation or failure which is undesirable for tillage machines/tools. The active tillage elements of agricultural machineries require extensive studies in order to obtain a proper soil fragmentation and displacement. (Petrescu et al., 2015; Boja et al., 2018a)

MATERIALS AND METHODS

In order to obtain a global image on the impact of the new vibro-combinator (the prototype SANDOKAN 2) (Table 1) in terms of the physical-mechanical properties of the soil, it was necessary to determine its properties before the passage of the equipment (in the state of the soil), and after its passage on all the six parcels and trials. These parcels will be suggestively named: soil 1 - soil 6; and the three types of active elements (Gamma, Delta1 and Delta2) (Figures 3 and 4).

Table 1 Main characteristics of the prototype vibro-combinator SANDOKAN 2

No. crt.	Characteristics	MU	Values
1	Mass	kg	5670
2	Length in transport	m	6.6
3	Height in transport	m	3.95
4	Width in transport	m	2.93
5	Width of the gamma active parts, reversible chipper type	mm	35
6	Width of the delta 1 active parts, arrow type	mm	150
7	Width of the delta 2 active parts, arrow type	mm	250

Figure 3. The prototype vibro-combinator SANDOKAN 2 equipped with the three types of active elements (GAMMA, DELTA 1, DELTA 2) (Boja et al., 2018a; Boja et al., 2018b)

Figure 4. Geometrical models for the three active elements (Petrescu et al., 2015)

The physical properties were determined by using the method of the cylinders with a constant volume of 100 cm^3, carrying out six repetitions at different depth, from 6, 12 and 18 cm. The methods of analysis and interpretation of the results as well as the work procedure for the determination of the physical – mechanical properties are those indicated in the specialized literature (Boja et al., 2012; Biris et al., 2015).

Statistical analysis. All data were subjected to univariate three-way analysis of variance (ANOVA, P = 0.05) and done with KyPlot (KyPlot Version 5.0.2, http://www.kyplot.software.informer.com) (Boja et al., 2018c; Boja et al., 2020). The ANOVA factors were: Soil (soil type), h (depth), Device (active element) and their six order interaction. The means pairwise comparisons were investigated by Tukey's post-hoc test (P = 0.05). Multivariate analysis: principal component analysis (PCA) was performed with P.A.S.T. version 3.04 statistical software, http://folk.uio.no/ohammer/past/) (Hammer et al., 2001; Boja et al., 2018c; Boja et al., 2020).

RESULTS AND DISCUSSIONS

When analysing the granulometric curves presented in Figure 5 and Table 2, one can notice the fact that there was a sandy-clay-dusty texture in soil 2, 4 and 3, 5 encompassed in the experiment at a participation quota that scarcely varies, with the exception of the 1st soil where the particle size distribution is different: clay-dusty-sandy texture.

Table 2. Average values of the granulometric analysis at different depths of prelevation

Type of soil	Depth of prelevation, (cm)	Values of the granulometric analysis		
		Sand, %	Dust, %	Clay, %
SOIL 1 (S1)	6	26.2	28.6	44.8
	12	26.8	28.7	44.3
	18	27.4	28.7	44.4
SOIL 2 (S2)	6	35.7	30.2	34.5
	12	35.1	30.2	34.8
	18	35.1	30.1	34.7
SOIL 3 (S3)	6	43.4	27.8	28.5
	12	43.2	28.1	28.7
	18	43.2	28.5	25.9
SOIL 4 (S4)	6	33.9	33.5	32.1
	12	33.1	33.1	34.5
	18	32.8	31.4	33.3
SOIL 5 (S5)	6	33.4	35.2	31.5
	12	32.9	34.8	32.8
	18	29.1	32.2	30.1
SOIL 6 (S6)	6	42.4	31.9	26.4
	12	42	31.1	26.2
	18	38.5	26.5	24.1

From the analysis of the values gathered for the participation quotas of the granulometric fractions, we could infer some interesting differentiations among the six types of soil in which we tried the vibro-combinator, as follows: All the six types of soil that we tried on the vibro-combinator are a relatively close mix, but in different proportions among the three granulometric fractions; The sand fraction (gravel + fine) is predominant in the soil 3 and soil 6 (43.2%); For the dust fraction (I + II), the differences among the three types range only for 2%, the highest value being registered on the soil 2 and soil 4 (30.1%); The participation quotas of the clay granulometric fraction are among the biggest, varying between 28.7% (soil 3) and 34.7% (soil 2), and reaching 44.5% for soil 1; The dust granulometric fraction is almost constant for all the six types of soil.

To synthesise more efficiently the data taken and to be able to describe completely the intrinsic characteristics of the sample, it was chosen a statistic processing with the aid of the program KyPlot. The results obtained are given in Table 3, having as a purpose to underline the variance of apparent density, soil moisture, total porosity and soil compression degree, and comparative with each types of soils and three active elements (Gama, Delta 1, Delta 2). Thus, for each types of soils included in the experiment resulted in eight statistical indicators for each technical work use a new vibro-combinator, but also witness sample. The mechanical processing of the soil through traditional and modern methods is currently put under question due to the high energy consumption and the continuous degradation of the arable horizon through erosion and excessive compaction.

It is known that the bulk density varies between 1 and 2 g/cm^3, according to the type soil and horizon, being generally lower in the case of the soils rich in humus and in the structured soils as compared to the unstructured soils. The values of the bulk density are in tight correlation with the degree of settlement of the soil. The high bulk density means a decrease of the capacity to retain water, of the permeability, of aeration and an increase of the mechanical resistance opposed by the soil during its sampling. On the contrary, low bulk densities can reduce the bearing of the soil, making difficult the mechanized execution of the works, even the driving of the operation machinery (Spoljar et al., 2009; Spoljar et al., 2011; Boyraz et al., 2014; Boja et al., 2016; Calistru et al., 2016; Vidrean et al., 2018).

By analyzing the values of total porosity, we can say that for the 1st type of soil we noticed an increase of the total porosity from 40.19%, which represents the initial state of the soil, to 44.36% (value obtained after the working of the soil with the vibro-combinator equipped with Gamma elements), 45.64% (with Delta 1 elements) and 45.71% (with Delta 2 elements).

The degree of settlement for the 1st type of soil presents values > 18%, which means that the soil is strongly settled for all levels of depth and after the passage with the three types of active elements of the cultivator.

The values gathered for the 2nd type of soil varies from weakly settled (1-10%) to moderately settled (11-18%). However, it is important to specify the fact that the lowest values of the degree of settlement appeared after preparing the germination bed with the aid of the active elements Delta 2.

In the case of the 3rd type of soil, we had negative values for this mechanical index of the soil at all depth, especially for the types of active elements, which means there is a soil moderately loose (-17…-10%) - fact that can be explained by the fact that this parcel has been annually worked.

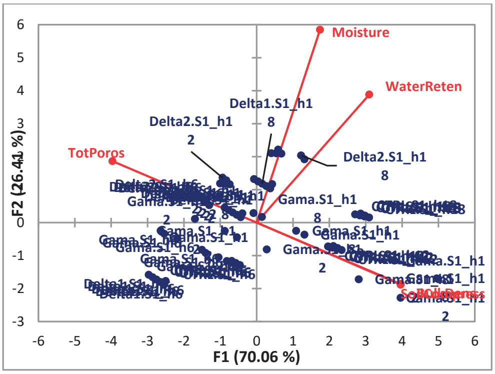
Analyzing the influence of the active elements on the different types of soils, some conclusions can be made (Table 3 and Figures 5-10): in terms of apparent density values (Da), the lowest value is found on all soil types (S1-S6) when working with the active elements Delta 2; the total soil porosity has maximum values when the vibro-combinator is equipped with the Delta 2 active elements, logical situation due to the existing relation to density and porosity; soil moisture values reach peak values after processing with Delta 2 to S1 and S2, and in S3 the maximum value of soil moisture is reached after processing with Delta1; the soil compaction degree has a similar humidity variation, namely: minimum values for S1 and S2 using Delta 2 and in S3 following the use of Delta 1.

Analyzing the impact of active organisms on soil depth, some conclusions can be drawn

(Table 3 and Figures 5-10): apparent density (Da) records minimum values when using Delta 2 for all three depths (6 cm, 12 cm, 18 cm); total porosity has an inverse variation such as that of apparent density: the highest values are found for all three depths when working with Delta 2; and soil moisture values respect the same law that: for all three depths the maximum value occurs after processing with Delta 2; the soil compaction degree has a similar variation, that is, the smallest values are recorded at all depths when working with Delta 2; when working with Delta 2 active elements, all physico-mechanical soil indicators have optimal values regardless of working depth; the same legality is preserved (with few exceptions) and when analyzing the impacts of the active organ of the vibro-combinator on the soil types contained in the experimental field.

Table 3. Results for the soil physical and mechanical properties (values are expressed as mean ± standard deviation) for the interaction factor Device*h*Soil (CTRL, Gamma, Delta 1, Delta 2)

Device*h*Soil	Soil moisture (%)	Bulk Density (g/cm^3)	Total Porosity (%)	Soil compression (%)	Water retention (m^3/ha)
CTRL.06.S1	16.18j±0.09	1.50cde±0.02	42.18hij±0.63	19.23cdef±1.20	361.69ef±4.29
CTRL.12.S1	20.25r±0.09	1.56ab±0.01	40.19kl±0.40	23.16a±0.77	150.91k±1.61
CTRL.18.S1	22.25t±0.09	1.41bc±0.03	45.71jk±1.32	12.62bc±2.52	186.58m±6.33
CTRL.06.S2	22.36s±0.16	1.46ab±0.01	43.91kl±0.29	15.92defg±0.55	404.69h±5.18
CTRL.12.S2	29.86r±0.16	1.45def±0.06	44.36ghi±2.22	15.19ab±4.25	141.00lm±4.31
CTRL.18.S2	33.93mn±0.15	1.74a±0.01	33.27l±0.40	36.29cd±0.77	421.44f±3.84
CTRL.06.S3	20.93u±0.28	1.75cd±0.01	32.89ij±0.40	37.05defgh±0.77	698.62op±6.12
CTRL.12.S3	28.23l±0.28	1.41defgh±0.02	45.64efghi±0.63	12.74ab±1.20	86.92k±1.59
CTRL.18.S3	35.03mn±0.28	1.63cdef±0.19	37.31ghij±7.27	28.56cde ±13.92	343.31g ±40.44
Delta 1.06.S1	10.25f±0.19	1.31defgh±0.06	49.81efghi±2.16	1.61fghi±4.27	186.63hi±10.22
Delta 1.12.S1	20.05v±0.19	1.69fghi±0.17	34.87defg±6.51	33.24defgh±12.47	568.50q±57.85
Delta 1.18.S1	22.15g±0.19	1.44defg±0.02	44.71fghi±0.80	11.68efgh±1.59	192.88ij±2.54
Delta 1.06.S2	21.75e±0.19	1.48defg±0.02	43.17fghi±0.80	14.80fghi±1.59	529.47b±6.80
Delta 1.12.S2	28.75mno±0.19	1.52fghi±0.02	41.41defg±0.63	20.73defgh±1.20	607.34no±6.89
Delta 1.18.S2	31.25h±0.19	1.39defgh±0.01	46.54efghi±0.34	8.07fgh±0.68	179.45j±1.41
Delta 1.06.S3	21.03f±0.28	1.46defgh±0.03	44.04fghi±1.11	13.09ghi±2.19	548.55c±11.79
Delta 1.12.S3	28.43u±0.28	1.54defgh±0.03	40.83efghi±1.12	21.83efgh±2.16	717.94p±19.57
Delta 1.18.S3	35.33g±0.28	1.35efgh±0.03	48.17efgh±1.24	4.84fghi±2.46	175.88j±5.73
Delta 2.06.S1	22.03i±0.28	1.19kl±0.01	54.17ab±0.38	-9.11kl±0.76	149.62k±2.42
Delta 2.12.S1	23.13de±0.28	1.45defgh±0.04	44.42efghi±1.47	12.33ghi±2.90	498.59b±16.30
Delta 2.18.S1	25.93no±0.28	1.48hij±0.02	43.27cde±0.72	14.58hij±1.42	961.56nop±13.47
Delta 2.06.S2	23.83hi±0.28	1.18l±0.01	54.68a±0.40	-10.14l±0.80	144.60k±2.36
Delta 2.12.S2	29.43a±0.28	1.44defg±0.06	44.81fghi±2.16	11.58ghi±4.26	506.86a±24.65
Delta 2.18.S2	34.33k±0.28	1.46ijk±0.04	43.85bcd±1.36	13.44ij±2.68	821.35no±24.74
Delta 2.06.S3	20.83i±0.28	1.16l±0.01	55.42a±0.37	-11.62l±0.75	146.23kl±2.60
Delta 2.12.S3	28.03m±0.28	1.49efgh±0.02	42.79efgh±0.81	15.53ghi±1.59	908.47n±11.63
Delta 2.18.S3	34.73op±0.28	1.45ghi±0.06	44.42def±2.16	12.31jk±4.26	893.03nop±41.97
Gamma.06.S1	16.25pq±0.19	1.22l±0.02	53.05a±0.70	-7.77l±1.43	755.55p±13.88
Gamma.12.S1	17.55i±0.19	1.15kl±0.01	56.00ab±0.41	-12.79l±0.83	142.96kl±2.15
Gamma.18.S1	18.65cd±0.19	1.19kl±0.02	54.33a±0.65	-9.36l±1.31	405.10de±9.42
Gamma.06.S2	21.52q±0.15	1.21l±0.02	53.62a±0.70	-8.94l±1.43	766.76p±15.14
Gamma.12.S2	31.42bc±0.15	1.22jkl±0.02	53.08abc±0.60	-6.84l±1.20	413.25d±8.82
Gamma.18.S2	36.22bc±0.15	1.18kl±0.01	54.90ab±0.55	-10.52l±1.12	394.34de±8.03
Gamma.06.S3	20.93q±0.28	1.19l±0.02	54.20a±0.64	-10.11l±1.30	744.34p±12.74
Gamma.12.S3	28.23b±0.28	1.21kl±0.02	53.75ab±0.65	-8.20l±1.31	399.72d±8.70
Gamma.18.S3	35.03q±0.28	1.24l±0.02	52.37a±0.66	-6.40l±1.34	780.73op±14.00



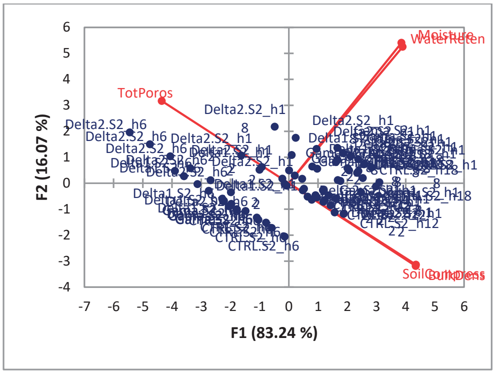
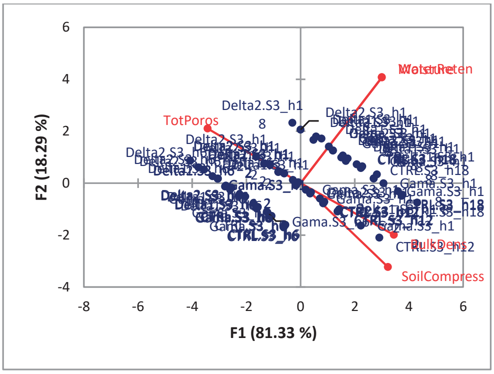


Figure 5. Interval plot for Soil 1 (from three-way ANOVA) for soil types (factor Soil), depth (factor h) and active elements (factor Device)

Figure 6. Interval plot for Soil 2 (from three-way ANOVA) for soil types (factor Soil), depth (factor h) and active elements (factor Device)



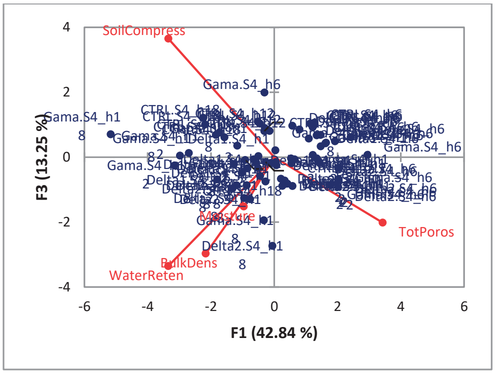
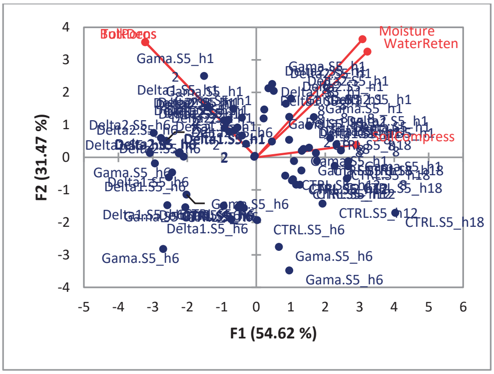


Figure 7. Interval plot for Soil 3 (from three-way ANOVA) for soil types (factor Soil), depth (factor h) and active elements (factor Device)

Figure 8. Interval plot for Soil 4 (from three-way ANOVA) for soil types (factor Soil), depth (factor h) and active elements (factor Device)



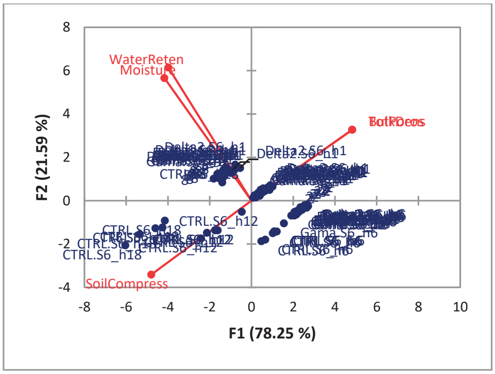


Figure 9. Interval plot for Soil 5 (from three-way ANOVA) for soil types (factor Soil), depth (factor h) and active elements (factor Device)

Figure 10. Interval plot for Soil 6 (from three-way ANOVA) for soil types (factor Soil), depth (factor h) and active elements (factor Device)

Multivariate analysis

To evaluate the vibro-combinators soil tillage performances were studied the variables: apparent density (g/cm^3), total porosity (%) and soil compression (%). To evaluate the soil environmental impact of the vibro-combinators were considered the variables: soil moisture (%) and water retention (m^3/ha). In order to assess simultaneously the vibro-combinators soil tillage performances and environmental impact, was involved the multivariate analysis: principal component analysis (PCA) and multivariate analysis of variance (MANOVA, P = 0.05). The PCA and MANOVA were done separately for each soil types S1 - S6. The PCA method involved as input data the variables correlation matrix and between sample groups algorithm. The MANOVA algorithm used as input data the first two principal components (PCs) coordinates of the group samples. The group samples were described by the interaction factor Device*h (i.e. active elements*depth).

For all soil types the first two PCs present eigenvalues greater than unity and a cumulative percentage of explained variance greater than 95.0%. Due to this reason these PCs are sufficient to describe the experiment with statistically significance.

The PCAs biplots gathers in the same graphical representation the samples scores and variable loadings (Figure 11). The sample groups are marked by points inside a convex hull and the variables are represented by vectors with the staring points in the coordinate system origin. The variable vectors end points shows the direction that describes the highest abundance (or levels) of the corresponding variables. This means that the group samples placed in the one vector direction (marked by its end point), have high abundance/level of that variable. When the sample groups are placed in the opposite direction, they have lowest abundance/levels for that variable. Analysing Figure 11, for the soil type S1, the PCA biplot prescribe (Tables 4-6).

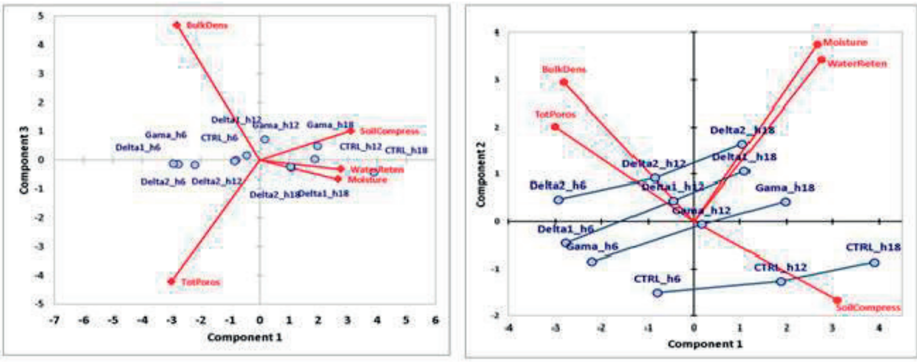


Figure 11. Principal component analysis (PCA) biplot for different depths (factor h) and for the three active elements (factor Device) for soil type S1-S6

Table 4. Statistical significance values of multivariate analysis of variance (MANOVA, P = 0.05) for the soil type S1-S2

MANOVA	CTRL.6.S1	Delta1.6.S1	Delta2.6.S1	Gama.6.S1	CTRL.12.S1	Delta1.12.S1	Delta2.12.S1	Gama.12.S1	CTRL.18.S1	Delta1.18.S1	Delta2.18.S1	Gama.18.S1
CTRL.6.S1		0.000	0.000	2.239	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Delta1.6.S1	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Delta2.6.S1	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gama.6.S1	2.239	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CTRL.12.S1	0.000	0.000	0.000	0.000		3.940	0.001	0.002	0.000	0.000	0.000	0.000
Delta1.12.S1	0.000	0.000	0.000	0.000	3.940		0.001	0.003	0.000	0.000	0.000	0.000
Delta2.12.S1	0.000	0.000	0.000	0.000	0.001	0.001		0.000	0.000	0.000	0.000	0.000
Gama.12.S1	0.000	0.000	0.000	0.000	0.002	0.003	0.000		0.000	0.000	0.000	0.000
CTRL.18.S1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.241	0.000	0.000
Delta1.18.S1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.241		0.000	0.000
Delta2.18.S1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000
Gama.18.S1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Table 5. Statistical significance values of multivariate analysis of variance (MANOVA, P = 0.05) for the soil type S3-S4

MANOVA	CTRL.6.S2	Delta1.6.S2	Delta2.6.S2	Gama.6.S2	CTRL.12.S2	Delta1.12.S2	Delta2.12.S2	Gama.12.S2	CTRL.18.S2	Delta1.18.S2	Delta2.18.S2	Gama.18.S2
CTRL.6.S2		0.793	0.001	0.352	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Delta1.6.S2	0.793		0.001	2.592	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Delta2.6.S2	0.001	0.001		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gama.6.S2	0.352	2.592	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CTRL.12.S2	0.000	0.000	0.000	0.000		0.069	13.997	0.004	0.000	0.000	0.000	0.000
Delta1.12.S2	0.000	0.000	0.000	0.000	0.069		0.327	0.000	0.000	0.000	0.000	0.000
Delta2.12.S2	0.000	0.000	0.000	0.000	13.997	0.327		0.002	0.000	0.000	0.000	0.000
Gama.12.S2	0.000	0.000	0.000	0.000	0.004	0.000	0.002		0.000	0.000	0.000	0.000
CTRL.18.S2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.938	0.000
Delta1.18.S2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000
Delta2.18.S2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.938	0.000		0.002
Gama.18.S2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	

Table 6. Statistical significance values of multivariate analysis of variance (MANOVA, P = 0.05) for the soil type S5-S6

MANOVA	CTRL.6.S3	Delta1.6.S3	Delta2.6.S3	Gama.6.S3	CTRL.12.S3	Delta1.12.S3	Delta2.12.S3	Gama.12.S3	CTRL.18.S3	Delta1.18.S3	Delta2.18.S3	Gama.18.S3
CTRL.6.S3		0.957	0.210	42.092	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Delta1.6.S3	0.957		21.324	3.674	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Delta2.6.S3	0.210	21.324		0.564	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gama.6.S3	42.092	3.674	0.564		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CTRL.12.S3	0.000	0.000	0.000	0.000		1.358	1.110	2.199	0.000	0.000	0.000	0.000
Delta1.12.S3	0.000	0.000	0.000	0.000	1.358		36.244	3.884	0.000	0.000	0.000	0.000
Delta2.12.S3	0.000	0.000	0.000	0.000	1.110	36.244		2.363	0.000	0.000	0.000	0.000
Gama.12.S3	0.000	0.000	0.000	0.000	2.199	3.884	2.363		0.000	0.000	0.000	0.000
CTRL.18.S3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		2.540	1.089	0.278
Delta1.18.S3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.540		4.215	0.228
Delta2.18.S3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.089	4.215		1.277
Gama.18.S3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.278	0.228	1.277	

CONCLUSIONS

The advantages of using vibro-combinators are: perfect preparation of seedbed in difficult working conditions and preservation of soil moisture. Such important factors can ensure fast, uniform and early germination of seeds, these requirements standing at the basis of abundant harvests. The research investigated the soil tillage performances and the environmental impact of several active elements of the vibro-combinators, at certain soil depths and soil types.

The multivariate analysis allowed to assess for each soil type which active elements performs both best soil tillage and environmental protection of the soils. From the technical point of view, the 6 cm depth is the most important to soil tillage for crop production. For this depth the active elements of the vibro-combinator: Delta 2 and Delta 1 are those that performs both best soil tillage and environmental protection of the studied soils.

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THE DIAGNOSTICS AND CARTOGRAPHIC-ANALYTIC ASSESSMENT OF THE ARABLE SOILS DEGRADATION IN UKRAINE BY SPACE SCANNING DATA

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Abstract

The using of multispectral space scanning data of high spatial resolution is justified for definition and description of degraded soils on arable lands. It is established main criteries of degradation processes in soils, which can be reliably determined by the decoding results of space images. Methodological principles for multispectral satellite imagery data decoding are developed for mapping of soil cover, as well as for quantitative estimation of its complexity and lateral heterogeneity of degraded soils. A coherent analysis of satellite images decoding results and data obtained from field surveys in different regions proves the soil decoding technology for multispectral satellite imagery data in the optical range, developed herein, is highly effective for determining the elements of local soil heterogeneity, which have soil degradation. The results of verification of cartographic-analytical assessments by space survey data are presented, which will allow introducing of space-differentiated soil quality management system to their rationally use, protect and prevent the development of soil degradation.

Key words: *arable soil, diagnostics, degradation, multispectral space scanning.*

INTRODUCTION

The United Nations (UN) Report on Status of the World's Soil Resources highlights that most of the world's soil resources are in poor conditions and identifies that soil degradation continues to be one of the major environmental and agricultural threats worldwide (FAO, 2015) which affects food security and the environment.

Modern soil studies (Medvedev et al., 2020; Vozhehova et al., 2019; Shein et al., 2007; Li et al., 2020; Lema et al., 2019; Sanchez, 2019; Zhang, 2019) confirm that the condition of the soil cover has deteriorated in recent decades and has become close to catastrophic. In particular, according to the international project "The Global Assessment of Soil Degradation", it is determined that the processes of soil degradation are spread over an area of about 1.7 billion hectares (Dobrovolsky, 2002). At the same time, physical degradation of soils, defined as a set of processes that cause destruction, movement and deposition of soil particles and mass, simplification of soil structure and microstructure and negative changes in its regimes (water, air and temperature), is the greatest threat (Medvedev, 2013). Usually, physical degradation is an inevitable companion of unbalanced and excessively intensive soil use (Baliuk et al., 2012). Thus, according to the National Report on the State of Soil Fertility of Ukraine, the risk of over compaction exists on almost 22 million hectares of arable land, which causes losses of $ 160-500 million annually. In particular, the content of agronomically valuable aggregates is lower than the permissible level by almost 30% of the area of arable soils, while the probability of formation of lumps during tillage reaches 12%, which is about 3.5 million hectares (National Report on the State of Soil Fertility of Ukraine, 2010).

For Ukraine, which is characterized by a large area of arable land (32 million hectares), for which the risk of physical soil degradation is too high, an unalterable approach to monitoring adverse events on agricultural land is to use multispectral space scan data as primary digital source of information about state of soil resources. The use of space images with high-quality and spatial resolution makes it possible to do quantitative description of the state of the soil surface at the level of detailed or large-scale surveys and compared to ground research and aerial photography is much less labor-intensive and costly (Ge, Thomasson, 2011). In this regard, in Ukraine there is a usage of space survey data to create a national system of geographic information support and environmental monitoring as part of the European program "Copernicus" in the global system "GEOSS".

The logic and expediency of using space scanning data for a comprehensive study of degradation phenomena in the soil cover is confirmed by world experience (Zhang et al., 2018; Filep et al., 2016; Mirzaee et al., 2016). For example, Cavalli et al. (2020) used advanced high-resolution radiometer`s (AVHRR) data to assess soil erosion in the southeastern region of São Paulo, Brazil. X.H. Wang et al. (2011) studied the problem of soil and water loss using TM Landsat on the Loess Plateau (China). AVHRR images from the NOAA meteorological satellite, which are rapidly updated and cover a large area, are often used to monitor vegetation status by calculating a set of vegetation indices that indirectly characterize the degree of soil degradation (Thiam, 2003; Symeonakis et al., 2004).

The experience of scientists from the University of São Paulo (Brazil) (Nascimento et al., 2021) is also valuable. They proposed the use of a soil degradation index (SDI) by temporal satellite images. The results of their research indicate that soils with a higher content of physical clay have a lower risk of degradation.

In the scientific works by Omuto C.T. it is emphasized that the control of the manifestation of physical soil degradation remains a challenge for many scientists due to the lack of proper assessment protocols. The author investigated soil degradation in Eastern Kenya and consistently applied a soil testing model to determine the phases of physical degradation. Visual assessment of signs of degradation, RUSLE model and diffuse infrared spectral reflection were used in the model of soil testing as predictors (prognostic parameters) of physical degradation. Visual assessment proved to be a cheap and fast method of determining the final stages of physical degradation with an accuracy of 60%. Visual evaluation in combination with the RUSLE model increased the evaluation accuracy to 80%. Infrared spectral reflection, which is sensitive to slight changes in soil physical conditions, was also identified as a potential predictor of early signs of physical soil degradation (Omuto, 2008).

Belarusian scientists (Olshevsky et al., 2018) emphasize that the development of technologies for collecting, processing, storing and using space surface scanning data, reducing their cost, the ability to select channels for spectral scanning of the Earth's surface and observation period, as well as obtaining relevant data and applications of automation software allows us to consider these methods as the most acceptable for detecting and mapping the processes of degradation and degraded lands.

The above highlights the need to develop a system for using multispectral high-resolution space scanning data to identify and quantify areas of soil cover within agricultural lands that are prone to degradation processes, in particular, physical degradation.

MATERIALS AND METHODS

A practicing of a creation of system for using high-resolution multispectral space scanning data to identify and qualitative assessment of soil cover areas within agricultural lands, based on space scanning data was carried out on the example of the Romaniv polygon. This polygon occupies 60 ha in Volyn Region.

The test polygon is located in one of the northern physical and geographical areas of the Volyn upland region (Volyn Opillya) of the Western Ukrainian region of the zone of deciduous forests of Ukraine (National Atlas of Ukraine, 2007), which is a flat and hilly areas that emerged among forests in the process of their ancient agricultural development. The main features of this area are due to the spread of forest rocks and the increase of Upper Cretaceous sediments, which are soil-forming rocks together with sands, sandy clays and Lower Sarmatian limestones (Marinich, Shishchenko, 2005). The most common are dismembered hills with dark gray and gray forest soils, forming a wavy plain, which is divided by beams with slopes, which have wide waterlogged bottoms. The sandy loam composition of most soils contributes to the development of erosion processes, which necessitates the implementation of soil-protective agriculture.

We have tested developed technologies for determination of degraded soils using Sentinel-2 satellite data that provides digital images of the Earth's surface in the multispectral bands with a resolution of 10 m. These data is a multispectral operational imaging mission within the GMES (Global Monitoring for Environment and Security) program, jointly implemented by the EC (European Commission) and ESA (European Space Agency) for global land observation at high resolution with high revisit capability (Sharing Earth Observation Resources…). Imagery was acquired on 29 April 2018 from the bare, dry soil surface.

Research included: statistical analysis of the image, creation of a provisional soil map and system of soil sampling, field investigation of the soil pattern and laboratory analysis of soil samples, expert assessment of image complexity and analytical results as the basis for image classification and soil-cover models, parameterization and geo-statistical analysis of the spatial variation of soil indicators, and extrapolation procedures based on interpretation of spectral signatures.

With the aid of a GPS, a regular grid of elementary sites was established (one per 2 ha) for 35 soil sampling were collected from the 0-10 and 10-20 cm layer, and 6 soil pits were dug to characterize the soils (morphological structure of the soil profile, depth of humus profile, spatial configuration of plow layer) in the field. Samples were collected according to Soil Survey Standards of Ukraine (ISO 10694-1995, DSTU 4287:2004, DSTU 4728-2007, DSTU 4730-2007). Also in the field it was investigated the physical soil properties (bulk density, soil penetration resistance - according to DSTU 5096: 2008). At the laboratory-analytical stage of the research, it was determined: total humus content (DSTU 4289: 2004); pH (DSTU ISO 10390: 2007).

Statistical and data processing methods used SNAP ESA and TNT programs for pre-processing of space images, NDVI calculation, primary image processing, transformation, general statistical analysis and image classification; and STATISTICA 10 for variance, correlation and regression analysis.

RESULTS AND DISCUSSIONS

According to basic research on the description of anthropogenic soil degradation by individual elementary soil processes and their combinations, 13 main types were identified (Krupenikov, 2005), which, in our opinion, can be used for a large list of soil types. The analysis of these types of degradation showed that most of them can be directly or indirectly determined from high-resolution space survey data, in particular: humus degradation and dehumification; physical and hydrological degradation; deflationary degradation or wind erosion of soils; irrigation degradation and deterioration of soil material composition and significant reduction of soil bioproductivity and quality, crop yield. The aggregate manifestation of various degradation processes causes geographical or spatial degradation of the soil, which is manifested in the complication of the structure of the soil cover within agricultural lands.

Due to the fact that aerospace methods in soil science are based on the study of the spectral reflectivity of the soil surface layer (Gebrin-Baidi, 2016; Orlov, 2001; Schmid et al., 2016; Žížala et al., 2017) it is possible to determine and monitor degraded soils based on the results of contour decoding of space scanning data with high and ultrahigh spatial resolution. In the development of this direction, as a result of the conducted research the system of cartographic-analytical definition of degraded soils on the basis of decoding of space survey data is developed (Figure 1).

The information block of this system is a meaningful basis, which is stored in the form of databases for further analysis, processing, assessment, multi-purpose search and replenishment. Data are collected both from surveillance networks for environmental monitoring, primarily from those that use space scanning data, and from third-party sources (administrative bodies, design and production organizations, foundations, scientific libraries, archives, etc.).

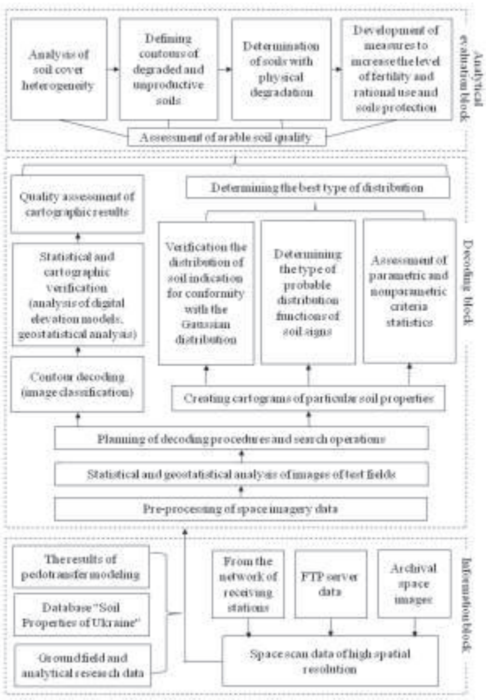

Figure 1. System of cartographic-analytical determination of degraded soils on the basis of space scanning data

The information that is received and stored is usually unified, i.e reduced to a form that is convenient for further use in a database for decoding space scanning data.

The decryption (decoding) unit is a set of basic procedures aimed at transforming digital information of multispectral high-resolution space images into useful information for soil scientists on the patterns of distribution of soil differences and their quantitative description. It implies the use of various methods and technologies, including: technology for collecting and transmitting information, collecting and maintaining long-term archives of information, pre-processing of space scanning data and their classification, conducting ground research and analysis of soil samples, and methods of coherent data analysis ground and remote sensing of agrocenoses, dynamic mapping and operative presentation of the results of cartographic modeling of the soil cover on the basis of space images.

The block of analytical estimation provides the complex analysis of results of decoding by use both exact methods of data processing (mathematical modeling), and their expert estimation directed on definition of degree of degradation of arable soils, and first of all their physical degradation, and on development of measures for increasing their fertility, rational use and protection.

Elaboration of the developed methodical approaches is carried out on the example of the polygon "Romaniv".

Preliminary data analysis included collecting thematic information about the study area and schematic linkage of thematic data with major interpretive features of the satellite imagery: brightness and structure of the image (the size and shape of features of the land surface and the nature of brightness distribution within them). The first stage in decoding the imagery was definition of common statistical indicators and analysis of distribution curves of its optical brightness, which showed that the variation has several modal values and, thus, the image of the bare soil surface encompasses diverse objects. Analysis of plots of the optical brightness of the image suggested that the soil cover of polygon could best be depicted by three distinct mapping units. The provisional soil map (Figure 2) was created in the final stage of image processing by the K-means method of cluster analysis.

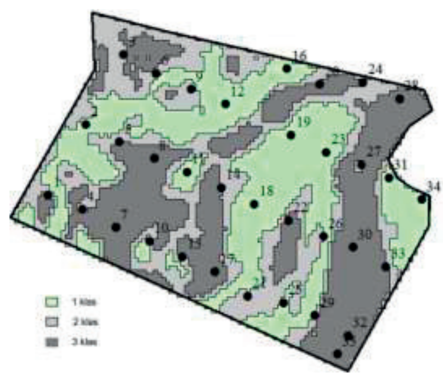

Figure 2. Soil map of the Romaniv polygon, with derived from classification of Sentinel-2 satellite data (the processing of a space image and the construction of a schematic map was carried out with the participation of leading engineer of Soil Erosion Control Laboratory and Remote Sensing Alexander Sherstyuk)

Further, we observed correspondence between soil delineations and individual elements of the micro-relief (micro-watershed, breaks in slope, bottom of a drainage hollow, etc.) so the map reflects a certain orderliness of the soil cover; its boundaries separate soil bodies with specific internal structure and variability characteristics. Joint analysis of the constructed contour map, the results of the field survey and the archival soil map confirmed the effectiveness of the decoding of multispectral imagery for identification of soils with different physical properties, which are the main criteria for physical degradation of soils. The taxonomic units represented by the mapping units in Figure 2 are listed below, according to the Ukrainian soil nomenclature and the World Reference Base (WRB), using the scheme for harmonisation drawn up by Medvedev and others (Medvedev et al., 2003; Soil heterogeneity…, 2009):

1. Conformed with the combination of Chernozem podzolized, Meadow-chernozemic and Meadow soils (Chernic chernozem, Phaeozems Haplic and Umbrisols Greyis in WRB);
2. Conformed with the combination of Light gray and Gray podzolized soils and Dark gray podzolized soil (Eutric Podzoluvisols, Gleyis Greyzems, Haplic Greyzems in WRB);
3. conformed with the combinations soddy medium podzolic soils loamy sand and Dark gray podzolized soil (Gleyic podzoluvisols and Haplic Greyzems in WRB).

Graphical representation of the variation of the main indicators of soils, determined according to ground sample surveys (training sample), showed a significant difference in classes, determined on the basis of the cosmic image by median value and interquartile distance (Figure 3). The most significant difference was between class 1 and two other classes - 2 and 3. The identified soil classes are well differentiated by the total humus content (Figure 3*a*) and pH (Figure 3*b*). There is also a significant difference for soil penetration resistance in layers of 0-20 and 20-30 cm (Figure 3*e*, 3*f*). In particular, the lowest values of soil penetration resistance are characteristic of the most humus class of soils – the combination of Chernozem podzolized, Meadow-chernozemic and Meadow soils (Chernic chernozem, Phaeozems Haplic and Umbrisols Greyis in WRB). At a time when for low humus soils there was an increase in soil penetration resistance from 18 to 24 kgf/cm^2 in the layer of 0-20 cm, from 24 to 36 kgf/cm^2 in the layer of 20-30 cm.

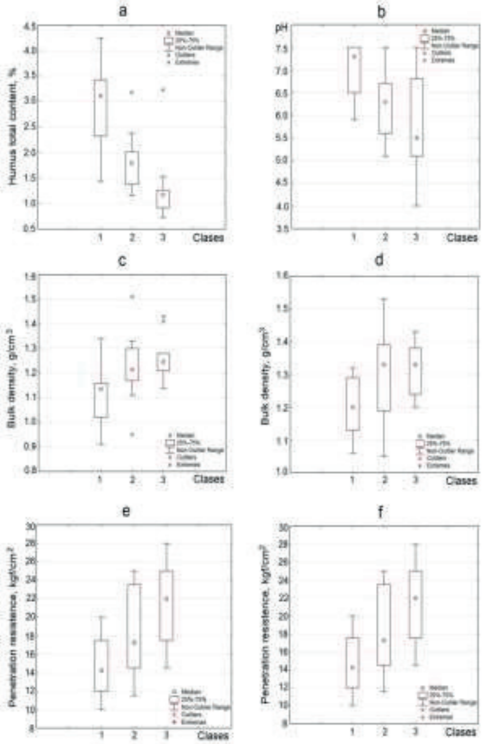

Figure 3. Graphical representation of the main statistics of soil indicators for soil classes, which determined by space survey data

For the density of the soil structure, the difference between 2 and 3 classes is less significant. The density of Light gray and Gray podzolized soils and Dark gray podzolized soil (Eutric Podzoluvisols, Gleyis Greyzems, Haplic Greyzems in WRB) and soddy medium podzolic soils loamy sand and Dark gray podzolized soil (Gleyic podzoluvisols and Haplic Greyzems in WRB) in the surface layer is about 1.2 g/cm^3, for a layer of 15-20 cm - more than 1.3g/cm^3 (Figure 3*c*, 3*d*). At a time when the more humus soils was characterized by a value of about 1.1 g/cm^3.

In general, with the spatial analysis of the distribution of experimental soil characteristics it is determined that this landfill is characterized by low supply of organic matter (50% of the area contains less than 2% of the total humus content), moderate (17% of the area) and high (1%) acidity. At the same time, approximately 70% of the landfill area has a structure density in the seed layer of less than 1.3 g/cm^3. Not more than 30% of the field is over compacted at the depth of the plow sole (structure density is more than 1.4 g/cm^3).

According to the known approaches to the assessment of physical properties of soils, the structure of the soil with values of equilibrium density from 1.2 to 1.4 g/cm^3 is considered to be insufficiently stable and potentially susceptible to physical degradation (Kuznetsova, 1979). And thus, we can conclude that according to this indicator, a third of the area of the landfill (distribution of class 3 according to the decoding) has signs of physical degradation, which requires the development of differentiated agricultural technologies.

At the next stage of data processing, two-dimensional models of soil penetration resistance distribution within the landfill were analyzed (Figure 4). It was found that the spatial distribution of this indicator does not coincide with the contour of the archival soil map (scale 1:25000), while it is interrelated with the contour of the allocations, which is determined by space survey data.

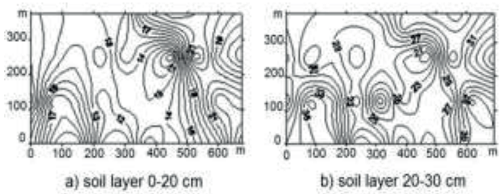

Figure 4. Two-dimensional models of soil penetration resistance distribution (Medvedev, 2009)

Comparison of the contour of the newly created map (Figure 2) with the archival soil map of the polygon allowed determining their significant difference, which is explained, first of all, by the difference in approaches to their creation.

Thus, the traditional approach in genetic soil science (thanks to which all existing soil maps in Ukraine were created in the period from 1961 to 1990 of the last century) is dominated by the use of soil-geographical principle in determining soil differences, which to some extent ignores the lateral heterogeneity of soil properties complex: morphological, physical, physico-mechanical, etc. Data from space scanning and decoding results make it possible to determine another type of soil contour, which provides, above all, accurate information about the regular variation of the complex of soil properties in the soil surface, which often allows an experienced specialist to determine its genetic affiliation.

In this regard, the analysis of the contours of soil maps, built on multi-temporal data from high-resolution space scanning, is appropriate and informative enough to determine the degradation processes in soils due to unbalanced agriculture. At the same time, at the initial stages of development of physical degradation there is an increase in the lateral heterogeneity of the whole complex of soil properties, which directly or indirectly form the optical characteristics of the soil surface. The constant increase in soil heterogeneity causes a very negative phenomenon for precision agriculture – "motley field". Thus, the increase in the total number and complexity of contours, which is established by the results of contour decoding of different time space information, may be evidence of the cumulative manifestation of various degradation processes within agrocenoses.

In general, according to the results of research on the test polygon, taking into account the ratio of areas characterized by different values of soil penetration resistance and density of the soil structure, the implementation of precision farming technologies is recommended. In particular, within the polygon it is advisable to practice no-till and minimum tillage instead of zonal pre-sowing and plow tillage for 1 class of soils, which is determined by space scanning data. It is also appropriate to use technologies of differentiated deep tillage to eliminate excessive density in the plow sole and eliminate acidity by liming for 2nd and 3rd classes.

CONCLUSIONS

The use of the results of decoding the data of multispectral space scanning of high spatial resolution to determine the contours of arable soils prone to degradation is updated.

A system of cartographic-analytical determination of degraded soils on the basis of space scanning data is proposed, which provides a comprehensive and expert analysis of the results of decoding space images by accurate data processing methods, aimed at determining the degree of degradation of arable soils by physical properties.

Approbation of the developed methodological approaches to assessing the condition of arable soils, carried out by Sentinel-2 satellite data, proves their effectiveness for spatial differentiation of soil cover, which determines soil areas that are significantly different in terms of soil density and soil penetration resistance.

It is substantiated the expediency of the analysis of the contour of soil maps, based on multi-temporal data of space scanning of high spatial resolution, for determination of degradation processes in soils due to unbalanced agriculture.

Directions for further research include the systematization of equations which explain the relationship of optical properties of the soil surface with fundamental physical and chemical properties, so as to make real and useful differentiation of soil quality from satellite imagery.

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THEORETICAL RESEARCH ON TECHNOLOGICAL PROCESS OF A STUD SEEDER WITH A FURROW-FORMING WORKING BODY

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Abstract

The maximum yield of grain crops with good technological qualities of grain at minimum costs is directly related to the traction resistance of the unit and the exact distribution of seeds in depth and sowing area. The use of cultivator-cultivators for subsoil-spread sowing is more effective than conventional seeders, as it allows evenly distributing seeds over the sowing area, eliminating gaps between individual technological operations, shortens sowing times, better use of the first spring maximum of soil moisture, and compaction of loose soil with wheels of tractors and machines. The working bodies of serially produced seed drills for subsoil-spread stubble sowing do not fully meet the agrotechnical requirements, since their use in production revealed a number of disadvantages that seriously worsen the quality of work, especially with high soil moisture (more than 18%), due to soil sticking to working bodies and its mixing, which leads to roll formation and removal of wet layers to the surface of the field. In this regard, increasing the yield of grain crops and improving the quality indicators of sowing by using a coulter for subsoil-spread sowing is an urgent scientific and technical problem. Based on the research materials, prototypes of seeders with experimental stubble coulters were developed and manufactured. Studies of the developed seeding machines have shown stable performance in sowing grain crops.

Key words: *opener, soil, seeder, ecology, furrow former.*

INTRODUCTION

The development of designs for grain seeders, seeding units and complexes is in the direction of creating units for subsoil-spread sowing, since it allows you to optimally use the area of plant nutrition, improve their growing conditions, reduce sowing time and reduce labor and energy costs. The effectiveness of the use of opener designs for subsoil-spread sowing of agricultural seeds is largely determined by a decrease in the traction resistance of the working bodies to the soil with a simultaneous increase in the quality of sowing (Kalabushev, 2018; Kalabushev, 2019). The existing designs of furrow formers do not fully ensure the quality of the seeders, often have an increased traction resistance, which significantly increases the cost of production and reduces the yield.

MATERIALS AND METHODS

In order to reduce the traction resistance of the units and obtain more uniform crops, domestic and foreign scientists have created a large number of openers of cultivator seeders. In turn, the analysis of the qualitative and quantitative indicators of their work allows us to assert that a wide variety of their designs is not an indicator of perfection, but, on the contrary, the result of insufficient completeness of their study. From the foregoing it follows that the work carried out in the direction of improving the quality indicators of sowing remains relevant (Shumaev, 2020; Kuhmazov, 2020).

To reduce the traction resistance of the seeder-cultivator and increase the uniformity of the distribution of seeds, we propose the design of the combined opener of the seeder-cultivator (Figure 1).

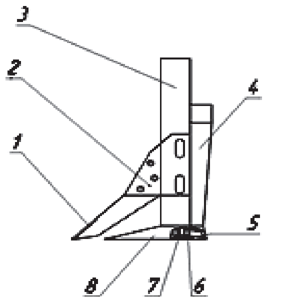

Figure 1. Combined opener of the seed drill-cultivator:
1 - loosening tooth; 2 - bracket; 3 - rack; 4 - seed tube;
5 - seed distributor; 6 - sole; 7 - fastening screw;
8 - lancet paw

The combined opener of the seeder-cultivator contains a stand 3 with a loosening tooth 1, a seed pipe 4 and a lancet paw 8. The loosening tooth 1 is fixed in front of the toe of the lancet paw 8 of the opener by means of a bracket 2 mounted on a stand 3, while the cutting plane of the loosening tooth 1 and a lancet paw 8 are the same. The arrow-shaped paw 8 of the opener is attached to the base of the pillar 3 of the opener by means of the fastening screw 7 and the sole 6, in the rear of which the seed distributor 5 is fixed, two side and one rear surfaces of the distributor 5 are made in the form of a fifth degree polynomial. The rib formed by the side surfaces of the seed distributor is made with a rounding radius to reduce the injury of seeds.

The technological process of the combined opener of the seeder-cultivator is as follows. When the combined coulter moves, loosening tooth 1 enters the stubble layer of the soil, cuts it, forming a gap and loosens the soil. During the movement, the process of constant formation of the soil compaction core on the toe of the loosening tooth 1 takes place, but the soil compaction core on the toe of the lancet paw 8 of the combined coulter does not receive its development and is constantly destroyed, which creates the best conditions for the stability of the movement of the combined coulter in depth, so how the formation of a soil compaction core on the toe of the lancet paw is excluded 8. The soil and plant residues passing along the front working face of the loosening tooth 1 rise and, continuing to slide along the surface of the loosening tooth 1, fall onto the field surface. Pointed share 8 cuts plant roots, loosening the soil and killing weeds, and the sole 6 levels the bottom of the furrow, forming an even, compacted seedbed. At the same time, the seeds from the seed distributor through the seed pipe 4 enter the lateral and rear surfaces of the seed distributor 5 and are laid at the bottom of the furrow, while the seeds that fall on the lateral surfaces of the seed distributor 5 are evenly distributed along the bottom of the furrow to the right and left of the longitudinal-vertical the planes of symmetry of the combined coulter over the entire working width of the lancet paw 8, and the seeds that fall on the rear surface of the seed distributor 5 are distributed in the rear part of the sub-plow space and partially on the sides, which ensures the best uniformity of seed distribution over the sowing area using the maximum capture width of the lancet paw 8 and the creation of better conditions for seed germination and plant development, which leads to an increase in crop yields. A layer of soil coming off the lancet paw covers the sown seeds.

Since the combined opener of a seeder-cultivator of this design was used for the first time during sowing, the task of theoretical research was to study the interaction of its design parameters and the soil.

RESULTS AND DISCUSSIONS

With the theoretical substantiation of the design parameters of the combined opener of the seeder-cultivator, let us consider its traction resistance, the thickness of the loosening tooth (Figure 2), the deformation zone of the loosening tooth and the distance from the nose of the loosening tooth to the toe of the lancet paw in the combined opener.

When cutting bonded and plastic soils, a compacted core of the material being processed is formed in front of the cutting profile, and further cutting is carried out not with a blade, but with this core.

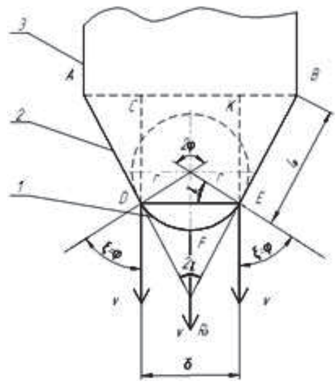

Figure 2. Elements of a loosening tooth: 1 - blade; 2 - chamfer; 3 - skeleton

The required blade width is determined by the chord dc = δ (Figure 2), which is equal to

$$\delta = 2\, r \sin \varphi \qquad (1)$$

where: r is the radius of curvature of the loosening tooth blade, m; φ - angle of friction of the soil against steel, deg.

Considering Figure 2, it can be argued that the width of the loosening tooth will be

$$b = 2(r \cdot \sin\varphi + l_{Л} \sin\gamma_1), \qquad (2)$$

where 2γ1 is the opening angle of the loosening tooth, deg.

When the loosening tooth moves at a certain depth, the soil chipping would occur in the direction of the action of the resultant force Rl, located at an angle $(\xi_1 + \varphi)$, that is, in the direction nm (Figure 3) of the deformation zone.

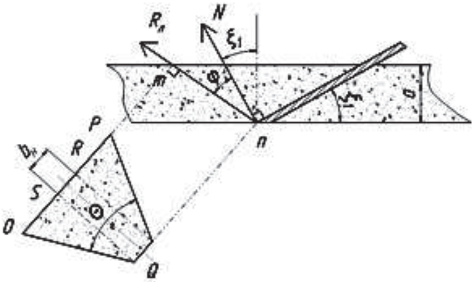

Figure 3. Scheme for determining the soil deformation zone with a loosening tooth: ζ1 - the angle of entry of the paw into the soil; ζ2 - the angle of entry of the paw into the soil; φ2 - angle of friction of the soil along the blade of the paw; a - tillage depth; N - normal lancet paw response; Rl - resultant force; Θ - angle of soil deformation by a loosening tooth, degrees

Dependence of the size of the deformation propagation zone on the size of the soil compaction core on the loosening tooth:

$$b_{Д.Н.} = \frac{2a \cdot tg\frac{\theta}{2}}{\cos(\xi_1 + \varphi)} + 2r \cdot \sin\varphi + 2l_{Л} \sin\gamma_1, \qquad (3)$$

where: Θ - angle of soil deformation by a loosening tooth, degrees; a - tillage depth, m; ζ1 - angle of entry of the paw into the soil, degrees; ll - blade length, m

In the process of operation of the combined opener of the seeder-cultivator, its paw quickly becomes dull, while the toe of the paw is closed with the width bn. When the paw moves at a depth a, the soil chipping will occur in the direction of action of the resultant paw, Rl which is located at an angle (ζ2 + φ2) (Figure 4), where ζ2 - the angle of entry of the paw into the soil, φ2 is the angle of friction of the soil along the edge of the paw. A loosening tooth is installed in front of the paw, therefore, in order to exclude the influence of soil deformation from the paw on the loosening tooth, the horizontal distance between them should correspond to the segment mp. Therefore, the horizontal distance lg between the toe of the paw and the loosening tooth must satisfy the condition

$$l_r \leq lb + mp, \qquad (4)$$

where lb is the overhang of the toe of the loosening tooth, m.

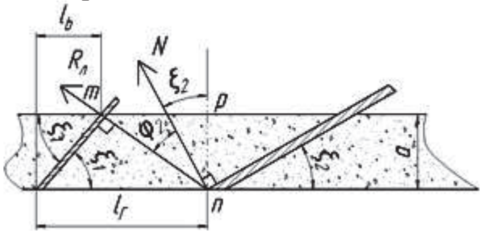

Figure 4. Scheme for determining the distance from the toe of the loosening tooth to the toe of the lancet paw: ζ1 - the angle of entry of the paw into the soil; ζ2 - the angle of entry of the paw into the soil; φ2 - angle of friction of the soil along the blade of the paw; lg - horizontal distance between the toe of the lancet paw and the loosening tooth; a - tillage depth; Rl - resultant force

The maximum horizontal distance between the toes of the lancet paw and the loosening tooth is determined from the expression:

$$l_r \leq a \cdot [ctg\zeta 1 + \tan(\zeta 2 + \varphi 2)] \qquad (5)$$

To determine the traction resistance of the combined opener of a seeder-cultivator, let us consider its operation, provided that the combined opener moves uniformly, at a constant depth and in a homogeneous environment, and the air resistance is neglected due to its small value.

The total value of the traction resistance of the combined opener of the seeder-cultivator can be represented as the sum of the traction resistance of the loosening tooth and the lancet, that is:

$$RXc = RXз + RXлс, \qquad (6)$$

where RXз, RXлс - traction resistance of the ripper tooth and lancet paw, N.

In this expression, the second component RHls is:

$$RKhls = RKhl - k\,RKhl = RKhl\,(1\text{-}k), \qquad (7)$$

where RXl –traction resistance of the lancet share, N; k– coefficient depending on the size of the deformed soil by the loosening tooth.

The coefficient k depends on the area of the soil deformation zone in the transverse-vertical plane with a loosening tooth and a lancet paw, which is determined by the expression:

$$k = \frac{S_З}{S_Л} = \frac{(b_{Д.Н.} + b) \cdot a}{2 \cdot b_Л \cdot a} = \frac{b_{Д.Н.} + b}{2 \cdot b_Л}, \quad (8)$$

where: Sz, Sl - area of the deformation zone of the loosening tooth and paw in the transverse-vertical plane, m^2; bl - capture width of the lancet paw, m.

Our task is to determine the traction resistance of the loosening tooth and lancet paw when interacting with the soil. It should be noted that each working surface of the loosening tooth and lancet paw is a triangular wedge with angles characterizing the installation of the working surface of the loosening tooth $\alpha_1, \xi_1, \gamma_1$ and lancet paws $\alpha_2, \xi_2, \gamma_2$... Therefore, in what follows we would carry out theoretical calculations, which are based on the theory of the trihedral wedge of V.P. Goryachkin and his followers.

The traction resistance RKhz of the loosening tooth of the combined opener of the seeder-cultivator would consist of the following components:

$$R_{Хз} = 2 \cdot (R_{Хз_G} + R_{Хз_F} + R_{Хз_Д} + R_{Хз_С}, \quad (9)$$

where: $R_{Хз_G} R_{Хз_F} R_{Хз_Д} R_{Хз_С}$ - traction resistance of the loosening tooth, depending on the weight of the seam, the dynamic pressure of the seam, the resistance of the soil to compression by the nape of a blunt blade, the resistance of the soil to deformation, N.

For analogy with expression (9), the traction resistance of the lancet paw would be equal to $R_{Хл}$:

$$R_{Хл} = 2 \cdot (R_{Хл_G} + R_{Хл_F} + R_{Хл_Д} + R_{Хл_С}), \quad (10)$$

where: $R_{Хл_G} R_{Хл_F} R_{Хл_Д} R_{Хл_С}$ - traction resistance of the duckfoot, depending on the weight of the soil layer, the dynamic pressure of the layer, the resistance of the soil to compression by the back of the head of a blunt blade, the resistance of the soil to deformation, N.

Consider the forces acting on the loosening tooth when the soil layer is located on the surface of the loosening tooth. As already noted, the working surface of the loosening tooth is a triangular wedge. Forces would act on the right working surface of the loosening tooth (Figure 5): Nz is the normal reaction to the loosening tooth from the soil, Fz is the friction force of the soil against the working surface of the loosening tooth.

Let us determine the projections of the forces Nz and Fz on the coordinate axes Ox, Oy, Oz. Then the projection of the force Nc on the axis would be:

- on the Ox axis:

$$N_{зx} = N_з \cdot \cos(90 - \zeta_1) \cdot \sin\gamma_1 = N_з \cdot \sin\zeta_1 \cdot \sin\gamma_1, \quad (11)$$

- on the Oy axis:

$$N_{зy} = N_з \cdot \cos(90 - \zeta_1) \cdot \cos\gamma_1 = N_з \cdot \sin\zeta_1 \cdot \cos\gamma_1, \quad (12)$$

- on the Oz axis:

$$N_{зz} = N_з \cdot \sin(90 - \zeta_1) = N_з \cdot \cos\zeta_1, \quad (13)$$

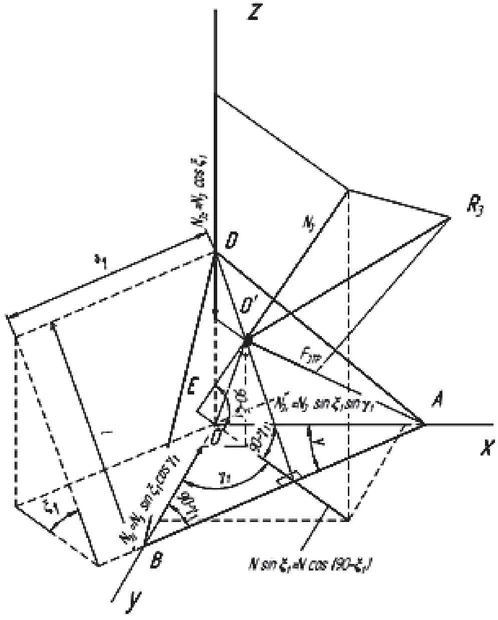

Figure 5. Scheme for the analysis of the work of a trihedral wedge

Since the working surface of the wedge OAB (Figure 6) penetrates into the soil, and the soil particle along this surface moves in the direction OA, it is difficult to determine the projection Fz, then to solve this problem we will decompose the force Fz into two components: Fz1 - parallel to the knife blade AB of the loosening tooth; Fz2 - along the directional perpendicular blade of the knife AB of the loosening tooth.

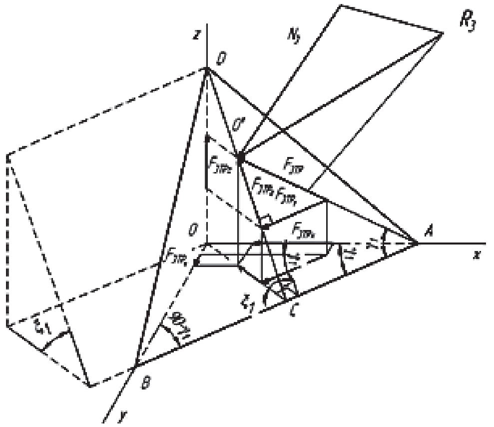

Figure 6. Determination of projections on the coordinate axis of the friction force $F_{3_{TP}}$

The resulting force on the x-axis would be:

$$F_{3_{TPX}} = F_{3_{TP1X}} + F_{3_{TP2X}} = \\ = F_{3_{TP1}} \cdot \cos\gamma_1 + F_{3_{TP2}} \cdot \cos\zeta_1 \cdot \sin\gamma_1 \quad (14)$$

In determining $F_{3_{TP1X}}$ and $F_{3_{TP2X}}$ it should be borne in mind that when the loosening tooth rises to the working surface, the base of the seam, initially occupying the AOC position, goes into AO'S. Therefore, the segment CO is equal to the segment CO ', and the angle OAC = O'AC $= \gamma_1$, hence the angle between the vectors $F_{3_{TP}}$ and $F_{3_{TP1}}$ in the ABD plane will also be equal to γ_1...

Then, respectively, the forces of friction against the tooth $F_{3_{TP1}}$ and $F_{3_{TP2}}$ would be equal:

$$F_{3_{TP1}} = F_{3_{TP}} \cdot cos\gamma_1 \quad (15)$$

$$F_{3_{TP2}} = F_{3_{TP}} \cdot \sin\gamma_1 \quad (16)$$

Substituting formulas (15) and (16) into expression (14), we obtain:

$$F_{3_{TPX}} = F_{3_{TP}} \cdot \cos^2\gamma_1 + F_{3_{TP}} \cdot \cos\zeta_1 \cdot \sin^2\gamma_1$$

or

$$F_{3_{TPX}} = F_{3_{TP}} \cdot (\cos^2\gamma_1 + \cos\zeta_1 \cdot \sin^2\gamma_1)$$

Oy axis: $F_{3_{TP1y}} = F_{3_{TP1}} \cdot \sin\gamma_1$

$$F_{3_{TP2y}} = F_{3_{TP2}} \cdot \cos\zeta_1 \cdot \cos\gamma_1$$

Resultant strength $F_{3_{TPy}}$ per axis Oy will be:

$$F_{3_{TPy}} = F_{3_{TP1y}} - F_{3_{TP2y}} = \\ = F_{3_{TP1}} \cdot \sin\gamma_1 - F_{3_{TP2}} \cdot \cos\zeta_1 \cdot \cos\gamma_1 \quad (17)$$

And taking into account equations (3.27) and (3.28) will take the form:

$$F_{3_{TPy}} = F_{3_{TP}} \cdot \cos\gamma_1 \cdot \sin\gamma_1 - \\ - F_{3_{TP}} \cdot \cos\zeta_1 \cdot \cos\gamma_1 \cdot \sin\gamma_1$$

or

$$F_{3_{TPy}} = F_{3_{TP}} \cdot \cos\gamma_1 \cdot \sin\gamma_1(1 - \cos\zeta_1) \quad (18)$$

Oz axis: $F_{3_{TP1z}} = 0 \qquad F_{3_{TP2z}} = -F_{3_{TP2}} \cdot \sin\zeta_1$

Resultant strength $F_{3_{TPz}}$ per axis Oz will be:

$$F_{3_{TPz}} = F_{3_{TP1z}} - F_{3_{TP2z}} = -F_{3_{TP2}} \cdot \sin\zeta_1 \quad (19)$$

Taking into account formula (15), the equation will take the form:

$$F_{3_{TPz}} = -F_{3_{TP2}} \cdot \sin\zeta_1 \cdot \sin\gamma_1 \quad (20)$$

After the projections of the forces N3 and $F_{3_{TP}}$, we determine the force of static pressure on the surface of the loosening tooth N3G, which is due to the weight of the layer located on the surface of the loosening tooth. In a state of equilibrium, the following forces act on the soil layer: the weight of the soil layer G3 on the loosening tooth; normal reaction of the working surface N3; friction force $F_{3_{TP}}$ soil on the working surface; backing force Q3G from the front of the wedge of untreated soil. The Q3G force will be located in a horizontal plane parallel to the xOy plane (Figure 7).

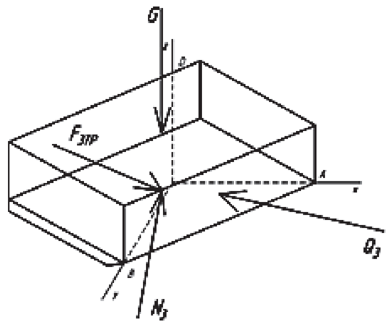

Figure 7. Scheme for determining force N_3

Let's project the forces acting on the soil layer onto the Oz axis:

$$\sum F_{3_z} = 0 \quad -G_3 + N_{3z} - F_{3_{TPz}} = 0 \quad (21)$$

Substituting formulas 3.23 and 3.33 into the resulting expression, we get:

$$-G_3+N_3 \cdot cos\zeta_1 - - F_{з_{ТР2}} \cdot \sin\zeta_1 \cdot \sin\gamma_1 = 0 \quad (22)$$

Then, the weight of the soil layer on the loosening tooth will be determined as: G_3

$$G_3 = N_3 \cdot cos\zeta_1 - F_{з_{ТР2}} \cdot \sin\zeta_1 \cdot \sin\gamma_1 \quad (23)$$

Considering that $F_{з_{ТР}} = N_3 \cdot f$ we get:

$$G_3 = N_3 \cdot cos\zeta_1 - N_з \cdot f \cdot \sin\zeta_1 \cdot \sin\gamma_1 = = N_3 \cdot (cos\zeta_1 - f \cdot \sin\zeta_1 \cdot \sin\gamma_1) \quad (24)$$

Where does the normal reaction come from? N_3:

$$N_{3G} = \frac{G_3}{cos\zeta_1 - f \cdot \sin\zeta_1 \cdot \sin\gamma_1} \quad (25)$$

Then the friction force of the soil on the working surface of the loosening tooth $F_{з_{ТР}}$ defined by the expression:

$$F_{з_{ТР}} = \frac{f \cdot G_3}{cos\zeta_1 - f \cdot \sin\zeta_1 \cdot \sin\gamma_1} \quad (26)$$

Respectively:

$$N_{3Gx} = \frac{G \cdot \sin\zeta_1 \cdot \sin\gamma_1}{cos\zeta_1 - f \cdot \sin\zeta_1 \cdot \sin\gamma_1} \quad (27)$$

$$F_{з_{ТРGx}} = \frac{f \cdot G \cdot (\cos^2\gamma_1 + \cos\zeta_1 \cdot \sin^2\gamma_1)}{cos\zeta_1 - f \cdot \sin\zeta_1 \cdot \sin\gamma_1} \quad (28)$$

Formation weight G is defined as:

$$G = V \cdot \gamma_{об}, \quad (29)$$

where V - the volume of the cut soil layer, m^3; $\gamma_{об}$ - volumetric weight of soil, N/m^3.

The volume of the soil layer V is found from its geometric parameters:

$$V = \frac{b \cdot a \cdot l_a}{2 \cdot \sin\gamma_1}, \quad (30)$$

where: b is the width of the loosening tooth, m; a - depth of tillage, m; l_a is the length of the blade of the working body at a given depth, m.

Then the weight of the bed G will be equal to:

$$G = \frac{b \cdot a \cdot l_a \cdot \gamma_{об}}{2 \cdot \sin\gamma_1}, \quad (31)$$

The value of the component of the traction resistance of the loosening tooth, depending on the weight of the formation:

$$R_{3Gx} = b \cdot a \cdot l_a \cdot \gamma_{об} \cdot \frac{\sin\xi_1 + f \cdot \sin\gamma_1 \cdot (ctg^2\gamma_1 + \cos\xi_1)}{2 \cdot \cos\xi_1 (1 - f \cdot tg\,\xi_1 \cdot \sin\gamma_1)}, \quad (32)$$

where is the length of the blade of the working body at a given depth, m; γobl_a–volumetric weight of soil, N/m^3; f is the coefficient of friction of the soil against steel.

The traction resistance of the loosening tooth, depending on the dynamic pressure, will be

$$R_{3Fx} = b \cdot a \cdot v^2 \, \frac{\gamma_{об}}{2 \cdot g} \cdot \sin^2\gamma_1 \cdot \frac{\sin\xi_1 + f \cdot \sin\gamma_1 \cdot (ctg^2\gamma_1 + \cos\xi_1)}{c\,tg\,\xi_1 - f \cdot \sin\gamma_1}, \quad (33)$$

where: v - speed of movement of the combined opener of the seeder-cultivator, m/s; b - bed width, m; g - free fall acceleration, m/s^2.

To determine the resistance of the soil to deformation R3D by a loosening tooth, let us assume that this force is proportional to the cross-sectional area of the formation:

$$R_{x3Д} = k_1 \cdot a \cdot (r \cdot sin\,\varphi + l_л \, sin\gamma_1), \quad (13)$$

where k –coefficient taking into account the properties of the soil and the geometric shape of the loosening tooth; a– bed height, m; φ– angle of friction of the soil on the loosening tooth, deg.

Resistance of a loosening tooth, depending on the resistance of the soil to compression by the back of the head of a blunt blade

$$R_{x3C} = \lambda_1 \cdot G_м \cdot \sin\gamma_1 \cdot \frac{\sin\mu_3 + f \cdot \sin\gamma_1 \cdot (ctg^2\gamma_1 + \cos\mu_3)}{\cos\mu_3 - f \cdot sin\,\gamma_1 \cdot sin\,\mu_3}, \quad (34)$$

where: λ_1 - coefficient taking into account the pressure of the seeder-cultivator on the loosening tooth; μ_3 - occipital angle, deg.

Thus, the traction resistance of the loosening tooth of the combined opener of the seeder-cultivator can be represented as:

$$R_{X3} = 2 \cdot (b \cdot a \cdot l_a \cdot \gamma_{об} \cdot \frac{sin\,\xi_1 + f \cdot sin\,\gamma_1 \cdot (ctg^2\gamma_1 + cos\xi_1)}{2 \cdot cos\,\xi_1 (1 - f \cdot tg\,\xi_1 \cdot sin\,\gamma_1)} + b \cdot a \cdot v^2 \, \frac{\gamma_{об}}{2 \cdot g} \times \times sin^2\gamma_1 \cdot \frac{sin\,\xi_1 + f \cdot sin\,\gamma_1 \cdot (ctg^2\gamma_1 + cos\xi_1)}{c\,tg\,\xi_1 - f \cdot sin\,\gamma_1} + k \cdot h \cdot (sin\,\varphi_1 \, r + l_л \, sin\gamma_1) + \quad + \lambda_1 \cdot G_м \times sin\,\gamma_1 \cdot \frac{sin\,\mu_3 + f \cdot sin\,\gamma_1 \cdot (ctg^2\gamma_1 + cos\,\mu_3)}{cos\,\mu_3 - f \cdot sin\,\gamma_1 \cdot sin\,\mu_3} \quad (35)$$

Making similar calculations with the parameters of the lancet paw, taking into account that the angle of friction of the soil along the loosening tooth φ is equal to the angle of friction of the soil along the lancet paw and their occipital angles are equal, it is possible to obtain a dependence that allows us to estimate the traction resistance of the lancet paw, which looks like: μ_3:

$$R_{Хл} = b_2 \cdot a \cdot l_{2а} \cdot \gamma_{об} \cdot \frac{sin\,\xi_2 + f\,\cdot sin\,\gamma_2 \cdot (ctg^2\,\gamma_2 + cos\xi_2)}{2 \cdot cos\,\xi_2 (1 - f \cdot tg\,\xi_2 \cdot sin\,\gamma_2)} + \; + b_2 \cdot a \cdot v^2\; \frac{\gamma_{об}}{2 \cdot g} \cdot sin^2\,\gamma_2 \cdot \frac{sin\,\xi_2 + f\,\cdot sin\,\gamma_2 \cdot (ctg^2\,\gamma_2 + cos\xi_2)}{c\,tg\,\xi_2 - f\,\cdot sin\,\gamma_2} + k_2 \cdot a \frac{b_2}{2} + + \lambda_2 \cdot G_м \times \sin\gamma_2 \cdot \frac{\sin\mu_3 + f \cdot \sin\gamma_2 \cdot (ctg^2\,\gamma_2 + \cos\mu_3)}{\cos\mu_3 - f \cdot \sin\gamma_2 \cdot \sin\mu_3}, \quad (36)$$

where: b_2 - bed width, m; $l_{2а}$ - length of the blade of the working body at a given depth, m; - angle of crumbling of the lancet paw, degrees; $\xi_2 \gamma_2$ - wing opening angle of the lancet paw, degrees; k_2 - coefficient taking into account the properties of the soil and the geometric shape of the duckfoot; λ_2 - coefficient taking into account the pressure of the seeder-cultivator on the duckfoot share; μ_3 - occipital angle, deg.

CONCLUSIONS

Calculations have established that the use of a combined opener with a loosening tooth 0.02 m wide, installed at a distance from the toe of the lancet paw to the toe of the loosening tooth 0.07 m and a working depth of 0.06 m, will reduce the traction resistance of the opener from 1.18 to 1 , 13 kN.

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DISPERSION AND REGRESSION ANALYSIS ON GRAIN YIELD AND NITROGEN FERTILIZATION OF TRITICALE VARIETIES I

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Abstract

The aim of the study is to analyze the responsiveness of two Bulgarian varieties of triticale to the rate of nitrogen fertilization in the formation of grain yield. The study area was Field Crops Institute - Bulgaria. The test period concluded 2015/2017. Were tested two varieties - 'Colorit' and 'Respect'. Were included in the experimental production tree rates of nitrogen fertilizer (kg/ha) - N_{60}, N_{120} and N_{180}. The phosphorus fertilizer was 60 kg/ha. Dispersion and regression analysis was applied to establish statistically significant influences of the studied factor and differences between the tested variants. Mineral fertilization at rates of 60, 120 and 180 kg N/ha had a confirmed statistical effect. The regression equations confirmed that the nitrogen rate has a strong influence on the grain yield. For both tested varieties, the largest increase in grain yield compared to the theoretical yield can be expected when fertilizing with 60 kg N/ha.

***Key words**: grain yield, nitrogen fertilization, statistical analysis, triticale.*

INTRODUCTION

Triticale is the first man-made culture. From the announcement of the first constant wheat-rye hybrids, triticale has come a long way to the formation of modern varieties. Today, culture is a key ingredient in the diet of farm animals. World grain production for 2018 amounts to 12,802,592 tons (FAOSTAT).
Interest in triticale is manifested in all research areas in the field of crop production. The main goal of scientists is to increase grain yield. In plant growing every human interventional invariably represents major and sometimes irrevocable change in the nature and properties of the original soil (Bijay-Singh, 2018). Fertilization is an important measure to increase soil nutrients and improve crop growth conditions (Hu et al., 2020). The impact of synthetic fertilizers on the environment can be negative and has been debated for decades by the scientific community. In this sense, it is necessary for specialists to forecast the yield depending on the application of fertilizers. One of the most reliable prediction methods is regression analysis. Linear regression analysis is probably the simplest and most popular way to measure the relationship between continuous predictor and response variables (Hope, 2020). Regression analysis answers question about the dependence of a response variable on one or more predictors, including prediction of future values of a response, discovering which predictors are important, and estimating the impact of changing a predictor or a treatment on the value of the response (Weisberg, 2005). This technology has been used for for nitrogen application strategies to obtain optimum yields and grain quality (Hansen, Jorgensen and Thomsen, 2002). The analysis of variance determines the influence of a given factor (factors) on a selected feature, and assesses the degree of this influence. An important task of the variance analysis is the estimation of the differences between the samples (gradations) in the dispersion complex, as the random variant serves as an error of the differences. In this respect, analysis of variance is a more efficient and economical method than others.
The aim of the study is to analyze the responsiveness of two Bulgarian varieties of triticale to the level of nitrogen fertilization in the formation of grain yield.

MATERIALS AND METHODS

The study area was Field Crops Institute-Chirpan, Bulgaria (42 ° 11'58 "N, 25 ° 19'27" E). The test period concluded 2015/2017. The experimental plot was 12 m^2 in four replications. Were tested two varieties triticale - 'Colorit' and 'Respect' with sunflower predecessor. Were included in the experimental production tree rates of nitrogen fertilizer (kg/ha) - N_{60}, N_{120} and N_{180}, incorporated at tillering phase. The phosphorus fertilizer was 60 kg/ha, incorporated in autumn. As a control option was adopted N_0P_0.

To determine the statistically significant effects of the studied factors and differences (LSD) between the tested variants, analysis of variance (ANOVA) was applied. The following model was applied:

$$Yi=\mu+ ai+ei$$

where: *Yi* are the meaning and number of the dependent and factor variables; μ are the average of the test results; *ai* is the effect of factors; *ei* is random error.

Data regression was determined with the software Statistica 13.0 (TIBCO, Software, 2018). The following model of regression dependence equation was used:

$$y=a+bx,$$

where: *y* are the values of the dependent variable or function (in our case grain yield); *x* are the values of the independent variable or argument (in our case fertilization rates); *a* is the parameter (coefficient) reflecting the distance from the zero point of the coordinate system to the beginning of the regression line; *b* is the angular coefficient characterizing the slope of the regression line.

RESULTS AND DISCUSSION

The data presented in Table 1 shows that the significant effect of fertilization on grain yield (GY) increased with increasing fertilizer rate for both varieties. It is important to note that the N_{120} and N_{180} variants have the same statistically significant effect (P=0.1%) for both the 'Colorit' variety and the 'Respect' variety. Application of 60 kg N/ha showed higher statistical reliability (P=1.0%) for 'Respect' variety compared to 'Colorit' variety (P=5.0%).

Table 1. GY variance analysis on average for the study period (2015-2017)

Fertilization rates	'Colorit'	'Respect'
	relative to the variant without fertilization	relative to the variant without fertilization
N_0	-	-
N_{60}	*	**
N_{120}	***	***
N_{180}	***	***

ns: no significant; *, **, *** significant at P=5%, P=1% and P=0.1%

The analysis of the total dispersion shows that the effect of fertilization for the variety 'Colorit' was proved with a degree of influence of 40.4% (Table 2), while the degree of influence of the Respect factor was 45.2% of the total variant. Gulmezoglu and Aytac (2010) reported that the effect of N fertilizer levels on grain yield were statistically significant (P<0.01). Janašauskaite (2013) reached the same results.

Table 2. Dispersion analysis of GY average for the study period (2015-2017)

Varieties	Source of variation	*SS*	*df*	*MS*	η
Colorit	A	243526	3	81175.34***	40.37436
	Error	359644	44	8173.727	59.62565
	Total	603170	47		
Respect	A	89293.5	3	29764.5***	45.01917
	Error	109052	44	2478.5	54.98083
	Total	198345.5	47		

ns: no significant; *, **, *** significant at P=5%, P=1% and P=0.1%

Table 3 presents the statistical significance of the variants compared to the previous (lower) nitrogen rate. In the case of 'Colorit' variety with a proven effect on the formation of GY was 60 kg N/ha at P=5%. With increasing fertilization rates, both norms remained outside the statistically significant effect. Similar results were observed for the 'Respect' variety. The application of N_{60} showed a higher degree of significance P=1% compared to the variety 'Colorit'. The results of our study are confirmed by the observation of Bielski et al. (2020). The authors reported that when applying a higher fertilization rate in the study, the GY was high, but the difference with the lower rate was not statistically significant.

Table 3. Statistical significance between fertilization variants in the formation of GY average for the test period (2015/2017)

Fertilization rates	'Colorit'	'Respect'
	relative to the lower fertilization rate	relative to the lower fertilization rate
N_0	-	-
N_{60}	*	**
N_{120}	ns	ns
N_{180}	ns	ns

ns: no significant; *, **, *** significant at P=5%, P=1% and P=0.1%

In order to differentiate the influence of mineral fertilization in the formation of grain yield, a regression analysis was performed for each variety, on average for the test period. When comparing 4 mathematical models for each variety, it was found that the grain yield changes under the influence of the increasing nitrogen rate in regression lines.

Figure 1 shows that for the variety 'Colorit' the values of the nitrogen norm showed a high, positive correlation with the values of the realized grain yield (r=0.979**). In this case, the dependence assumes proven positive values. The results showed that the nitrogen rate has a strong influence on the grain yield. Our results are confirmed by a number of studies, including those of Los Galetto et al. (2017) and Gulmezuglu and Aytac (2010). The statistical reliability of the coefficients in the equation and the mathematical model was confirmed. The four fertilization rates studied were within the confidence interval. The variant without fertilizer and 180 kg N/ha variants showed a lower-than-expected grain yield. Fertilization with N_{120} coincided with the predicted GY. However, the increase in the impact of N_{60} was 209.2 kg /ha more than the theoretical change.

For the 'Respect' variety, a strong and positive correlation was also observed between the values of the nitrogen rates and the realized grain yield (r=0.973**). The dependence also assumes proven positive values. The coefficients in the equation are statistically significant and the mathematical model was reliable. From Figure 2 it can be seen that the variant without fertilization and the maximum fertilizer rate realized a lower GY than the theoretical yield. Fertilization with 60 and 120 kg N/ha resulted in higher yield values than expected. However, for N_{60} the increase was higher (117.4 kg/ha) compared to N_{120} (78.1 kg/ha).

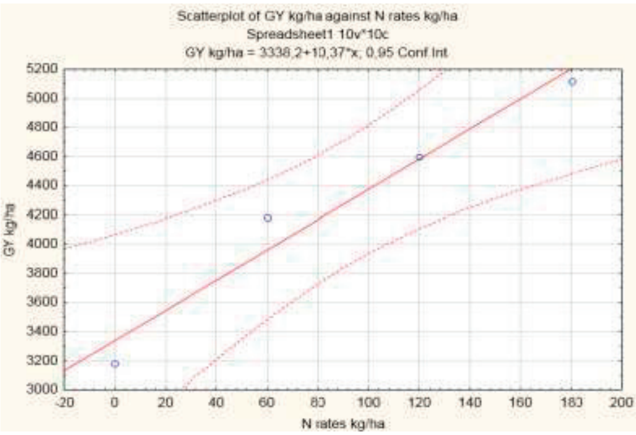

Figure 1. Theoretical change in grain yield under the influence of mineral fertilization on average for the test period (2015-2017) for the variety 'Colorit'

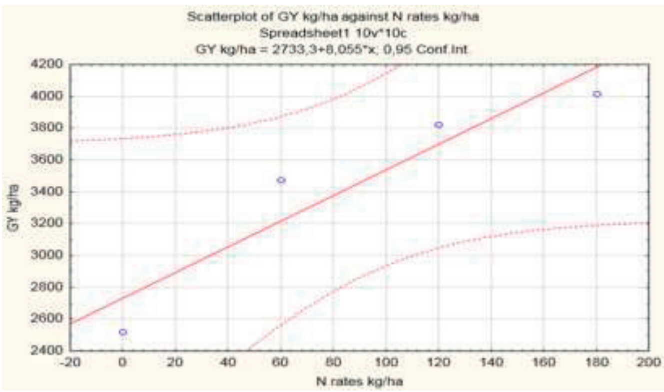

Figure 2. Theoretical change in grain yield under the influence of mineral fertilization on average for the test period (2015-2017) for the variety 'Respect'

CONCLUSIONS:

The variance analysis showed a significant effect of fertilization in both triticale varieties. Mineral fertilization at rates of 60, 120 and 180 kg N/ha had a confirmed statistical effect. However, the differences between N_{60}-N_{120} and N_{120}-N_{180} were unproven for both varieties. Only the difference between the control variant and N_{60} had a proven effect. The regression equations confirmed that the nitrogen rate has a strong influence on the grain yield. For both tested varieties, the largest addition to grain yield compared to the theoretical yield can be expected when fertilizing with 60 kg N/ha.

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COMPARISON RESULTS OF GRAIN CRIMPING PRODUCTS WITH ROLLER AND DISC WORKING BODIES

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Abstract

A description of the grain conditioner with disc working bodies and its differences from the industrially produced conditioners with disc working bodies is given. The results of a comparative analysis of the obtained product on both types of working bodies of grinders on barley, wheat, rye, oats, buckwheat and millet are presented. There is information both on the productivity of the used conditioners, and on the amount of power consumed for their drive and specific energy consumption. Unlike the grain roller conditioner, the disc conditioner has a lower capacity. However, energy consumption per kilogram of product is significantly lower.

Key words*: grains, crimping products, roller and disc working bodies.*

INTRODUCTION

In modern industry, a large amount of materials must be shredded. Grinding products are used both in mining to obtain materials for industrial processing (Liu et al., 2018; Yang et al., 2018), in the technology of obtaining construction components (Ostroukh et al., 2018), for chemical production and use (Gladkova et al., 2018; Slimani et al., 2018), for the production of food for humans and animals (Smejtková et al., 2016).

Various designs with different working bodies are used as grinders for lumpy materials (Kupchuk et al., 2019; Iskenderovet al., 2018). In some cases, vibration effects are used to increase the efficiency of the process (Rabat et al., 2018;Vaisberg et al., 2019). The main attention is paid, as a rule, to reducing energy consumption (Stepanenko et al., 2018; Norov et al., 2019) and obtaining a given particle size distribution (Lvov, 2018). For this purpose, theoretical studies are carried out to obtain the necessary equations and establish laws (Sysuev et al., 2017). The obtained mathematical expressions (Sysuev et al., 2016) allow to analyze the process by numerical methods, optimizing its parameters. An increase in the efficiency of the technological process and an increase in productivity are substantiated (Sysuev et al., 2015). In agriculture, the effect of the resulting grinding products on the vital activity of animals and changes in nutrition is also established (Inoue et al., 2015; Miyaji et al., 2017).

The purpose of the research was to establish the influence of the design features of the working bodies (cylindrical and conical shapes of smooth rollers) of the grain conditioner on the quality indicators of the product obtained from the kernels of a number of agricultural crops.

MATERIALS AND METHODS

The research methodology provided for comparative studies of the operation of roller conditioners with a smooth working surface, having a position in the form of cylindrical rollers (Figure 1) and conical disks (Figure 2). In the course of the experiment, for the recommended setting of the conditioners, the following indicators were measured by conventional methods: the average design thickness of the flakes (measured with a caliper 100 flakes); sieve analysis was the weighted average particle size - modulus of grinding; the fraction of the dusty fraction after sieving as the exit from the last sieve with holes of 0.25 mm); sorption coefficient as the ratio of the mass of retained moisture to the crushed product; the productivity of conditioners as the ratio of the mass of the product passed through to the time of its collection The drive power was measured with a KI-505 device. Energy consumption was defined as the ratio of the consumed power to the productivity. To determine the corrected energy consumption, the grinding modulus value was multiplied by the energy consumption.

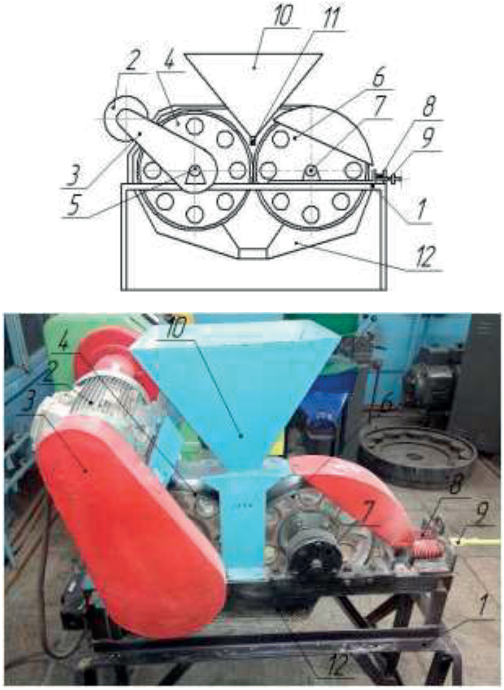

Figure 1 - Design and general view of the roller conditioner (Petent, 2008): 1 - frame; 2 - electric motor; 3 - V-belt transmission; 4 - drive roll; 5, 7 - bearing support; 6 - driven drum; 8 - safety spring; 9 - adjustment screw; 10 - loading hopper; 11 - damper; 12 - unloading tray

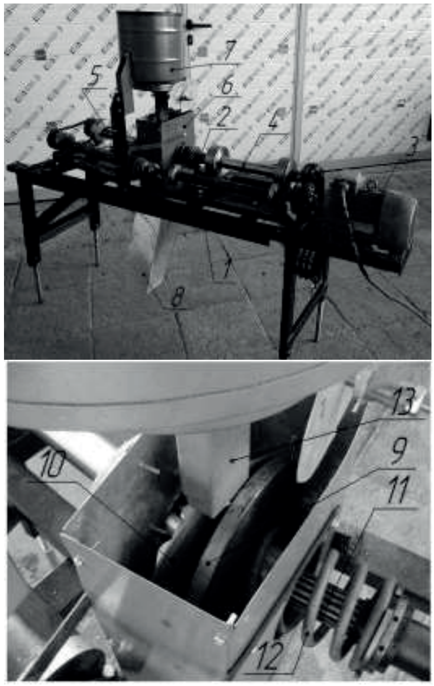

Figure 2 - General view and internal structure of a conical roller conditioner (Patent, 2015): 1 - frame; 2 - drive shaft; 3 - electric motor; 4, 5 - driven shafts; 6 - protective casing; 7 - loading hopper; 8 - unloading tray; 9, 10 - crimping discs; 11 - stock; 12 - spring; 13 - power supply

RESULTS AND DISCUSSIONS

The average values for three replicates of measuring the performance of the conditioner with smooth cylindrical rollers and the conditioner with disc working bodies are presented in Table 1. At the same time, information characterizing the quality of the product obtained is shown in Table 2.

Table 1. Performance indicators of conditioners

View grains	Roller conditioner			Disc conditioner working bodies		
	Q, kg/h	P, kW	Y, kWh/t	Q, kg/h	P, kW	Y, kWh/t
Barley	208	2.01	9.66	183	0.96	5.25
Wheat	321	2.13	5.27	216	0.98	4.54
Rye	340	1.8	5.29	206	0.96	4.66
Oats	83	1.6	19.2	76	0.88	11.5
Buckwheat	295	1.92	6.51	187	0.97	5.19
Millet	605	1.7	2.81	360	0.93	2.58
Crop average	*308.7*	*1.9*	*8.1*	*204.7*	*0.9*	*5.6*

Table 2. Indicators of crimped grain

Grain type	Roller conditioner				Disc conditioner working bodies			
	a, mm	G	M, mm	υ, %	a, mm	G	M, mm	υ, %
Barley	-	1.04	2.07	four	1.00	1.23	3.23	0.8
Wheat	1.02	0.9	2.43	3	0.98	0.96	2.91	0.6
Rye	0.98	1.02	2.90	2.5	1.01	1.08	2.65	1.4
Oats	0.83	1.24	2.55	2.9	0.89	1.29	3.3	0.6
Buckwheat	-	0.73	0.86	24	0.6	0.76	2.13	8
Millet	-	0.86	1., 8	18	-	0.89	1.59	11
Crop average	-	*0.97*	*2.02*	*9.1*	-	*1.04*	*2.64*	*3.7*

Table 3. Percentage of change in the performance of the disc conditioner relative to the cylindrical rollers

	Q, kg/h	W, kW	Y, kWh/t	Yk, kW*h* mm/t	M, mm	a, mm	G	υ, %
Barley	-12.02	-52.24	-45.65	-15.20	56.04	-	18.27	-80.00
Wheat	-32.71	-53.99	-13., 85	3.16	19.75	-3.92	6.67	-80.00
Rye	-39.41	-46.67	-11.91	-19.50	-8.62	3.06	5.88	-44.00
Oats	-8.43	-45.00	-40.10	-22.49	29.41	7.23	4.03	-79.31
Buckwheat	-36.61	-49.48	-20.28	97.45	147.67	-	4.11	-66.67
Millet	-40.50	-45.29	-8.19	14.05	24.22	-	3.49	-38.89
Crop average	*-33.69*	*-49.10*	*-30.82*	*-10.04*	*30.77*	-	*7.25*	*-58.82*

Comparing the numerical values of the indicators, the percentage of their change was revealed, shown in Table 3.

Considering that one conditioner had cylindrical, and the second conical rollers, and the presence of differences in their geometric parameters, then for an objective comparison, a comparison of the obtained indicators was used.

The performance of conditioners is determined by the length of the conditioning zone and the rotational speed of the rollers. The gap between the rollers, which is either forced or dependent on the performance of the device and associated with the condition of ease of gripping the material, also affects. So, due to the lower initial productivity of the disc conditioner, its average productivity on crops is 33-34% less (Figure 3). In this case, the influence of the properties of the material being flattened also affects. The smallest decrease in the performance of conditioners is observed on such a cohesive mass as oat grain - (-8.4%). Apparently, the improvement of conditions for the capture of oat material by the large diameter of the conditioner discs affects. At the same time,

the modulus of grinding increases by 29.4%, and the thickness of the flakes increases by 7.2%. At the same time, the greatest decrease in productivity on such a fluid material as millet seeds is (-40.5%). Similar, but slightly lower values are for rye, buckwheat and wheat seeds, which have less fluidity and larger sizes. In this case, the grinding module increases accordingly by (-8.62%; 147.67%; 19.75%), and the thickness of the flakes increases by 3% in rye, and in wheat it decreases by 3.9%. Buckwheat does not form flakes due to its high fragility. A significant difference in the quality properties of the product requires its separate consideration.

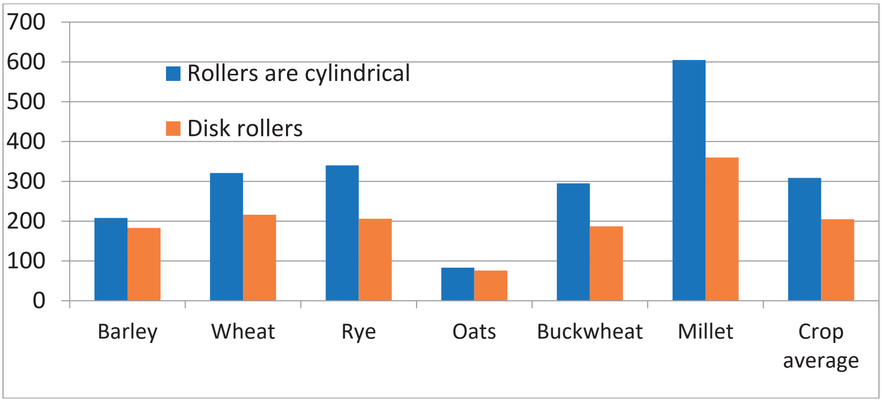

Figure 3 - Histogram of comparison of productivity values Q (kg/h) of disk and cylindrical roller conditioners

With a decrease in average productivity by 33.7%, power costs are reduced by 49% (45-54%). That is, the energy consumption of the process decreases. The average decline is 30.8%, and varies by crop from 8 to 46%. Taking into account the change in the particle grinding modulus, the energy consumption was considered taking into account the particle grinding modulus. In this case, the adjusted values of energy consumption are reduced by 10% (Figure 4). Oats have a decrease of 22.5%, rye of 19.5%, barley of 15.2%. At the same time, a number of crops require an increase in energy consumption: buckwheat by 97.5%, millet by 14%, wheat by 3%. Thus, in terms of energy performance, disc working bodies should be recommended for crushing oats, rye, barley, and wheat. For crushing millet and buckwheat grains, preference should be given to cylindrical rollers.

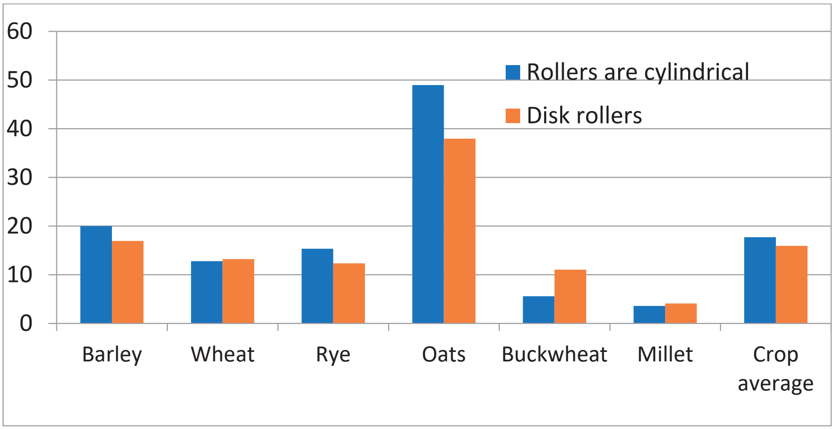

Figure 4 - Histogram of the comparison of the values of the corrected energy consumption Y (KWt mm/t) for disc and cylindrical roller conditioners

It should be noted that although there is an increase in the grinding modulus of almost all seeds of the studied crops (Table 2), as well as a slight increase in the thickness of the flakes in oats and rye, the quality properties of the resulting product improved in all crops: moisture adsorption by the grain product increased (Figure 4) by 7.3% on average. In barley, wheat and rye, the improvement was over 6%. No deterioration of these properties was observed. The growth of the dusty fraction contributes to an increase in the loss of material from dusting, and also contributes to the deterioration of animal respiration. The disc conditioner shows a decrease in dust fraction on all crops, and on average is 59%. The smallest decrease in dusting was observed in millet and rye.

This phenomenon is explained by the different rate of change in the size of the medial roller space of the conditioners when the rollers are turned. As a result, a relative shift of individual layers of caryopsis occurs between each other, leading to the formation of cracks and microcracks inside the caryopsis. The presence of cracks promotes the adsorption of liquids (for example, gastric juice, which improves the digestibility of the product, which is a positive reaction). The high shear value promotes the formation of deep cracks that destroy the flakes. Chipping off small particles contributes to the growth of the dust fraction. In barley, millet, and buckwheat, the flakes (at least half of the caryopsis) disintegrated into microflakes on cylindrical rollers, remaining on the disk set.

CONCLUSIONS

In contrast to the comparable cylindrical grain roller conditioner, the experienced disc conditioner has 33.7% lower capacity on average. The power consumption is, on average, 49% less. At the same time, the grinding modulus increases by 30.8%. However, adjusted for the modulus of grinding, the energy consumption per ton of product is significantly lower. Average values of corrected energy consumption are reduced by 10%. Oats have a decrease of 22.5%, rye of 19.5%, barley of 15.2%. At the same time, a number of crops require an increase in energy consumption: buckwheat by 97.5%, millet by 14%. Thus, in terms of energy performance, disc working bodies should be recommended for crushing oats, rye, barley, and wheat. For crushing millet and buckwheat grains, preference should be given to cylindrical rollers.

The quality properties of the product obtained on the disc conditioner improved in all crops: the moisture adsorption of the grain product increased by an average of 7.3%. In barley, wheat and rye, the improvement was over 6%. The deterioration of these properties was not observed in any culture. The disc conditioner shows a decrease in dust fraction on all crops compared to cylindrical rollers, and on average is 59%. The smallest reduction in dusting was observed in millet and rye. In barley, millet, and buckwheat, the flakes were broken into microflakes on cylindrical rollers, remaining on the disk set.

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EFFECT OF IRRIGATION ON THE EXCHANGEABLE CATIONS COMPOSITION IN CHERNOZEMS

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Abstract

The results of studies of the exchangeable cations composition in chernozems of the Forest-steppe and Steppe of Ukraine in irrigation are presented. The degree of change in the qualitative composition of the soil absorbing complex cations is determined by the irrigation water quality and irrigation norms. With irrigation by suitable water a slight decreasing of the content of absorbed calcium and increasing of the sodium content are noted. Changes in the cations composition of the absorbing complex are most significant on systematic irrigation by limited suitable and unsuitable waters. In the soil alkalinization in a weak and medium degree is developing. The part of absorbed sodium and potassium cations ranges from 3.0 to 6.6% of the total cations. Changes in the soil absorbing complex are due by increasing of the activity of sodium ions and decreasing of the activity of calcium ions in the soil solution. The regression model of the dependence between the absorbed sodium and potassium cations content in chernozem ordinary and the salinity of irrigation water and the Ca/Na ratio in the water extract was developed.

Key words*: alkalinisation, irrigation water, soil, soil absorbing complex.*

INTRODUCTION

Irrigated agriculture has a paramount importance for the food supply of the country, feeding the burgeoning global population, sustainable functioning of the agroindustrial complex, increasing crop yields and adapting to weather and climatic conditions (Singh, 2021; Climate, 2007; Vorotyntseva, 2020). Irrigation has contributed greatly to the improvements in global agricultural productivity and output in recent decades (The state.., 2011).

The importance of irrigation in the world's agriculture is rapidly increasing. The food need will increase by 70-100% to 2050. So the problem arises of how to simultaneously guarantee food security and preserve natural resources and soil fertility (Gomiero et al., 2011). Sustainable land use and soil protection play perhaps the most important role in food security and human security in general (Nkonya et al., 2016; Conception…, 2021). The value of this agricultural measure grows in terms of climatic transformations that are marked the last years worldwide (Climate..., 2007), especially in areas with insufficient moisture: insufficiently moist Forest-steppe and arid Steppe (40%) of Ukraine (Baliuk et al., 2018; Vorotyntseva, 2020). In addition, in modern conditions one of the factors affecting the properties and soils state in particular the composition of the soil absorbing complex is climate aridization. This can lead to the intensification of salinization soil processes, especially in hydromorphic conditions (Pankova, 2017). In such climatic conditions the likelihood of aridization processes increases.

Irrigated lands are anthropogenically transformed natural systems. Under the influence of irrigation, a complex transformation of the soil composition and properties, changes in the direction and intensity of the soil processes evolution were occurred (Baliuk et al., 2009; 2017; Vorotyntseva, 2016). Irrigation increases agricultural productivity and inevitably affects soil properties.

The sustainability of irrigated agriculture depends on the quality of irrigation water used (Rengasamy, 2018; Vorotyntseva, 2016; 2017). Climatic and hydrogeological conditions of the region, relief, initial soil properties, irrigation technology and agriculture also effect on the soil properties transformation. Irrigation enhances soil formation processes. On irrigated

areas, both fast-flowing and long-term processes of the formation of soil signs and properties are activated.
The evolution of soils can develop in the direction of both the preservation of properties and the development of degradation processes. This determines the ability of the soil to fulfill its biospheric, ecological, ecosystem and social functions (Kramer, 2020).
A recent FAO estimate reported that a large portion of total global soil resources are degraded and this problem is persistently expanding (The state.., 2011; Kramer, 2020; Baliuk et al., 2017).
The Sustainable Development Goals identify the need to restore degraded soils and improve soil health. Therefore, sustainable soil management is essential. It ensures balanced development, reduction of degradation and adaptation to arid conditions for the food security of the country (Vorotyntseva, 2020; Shah et al., 2019).
The analysis of international experience shows that improving the efficiency of irrigation, rational use of water resources, sustainable management of irrigated soils is an urgent and significant issue at the global level (Kachi, 2016; Lamaddalena, 2013, Voluntary.., 2017). The shortage of fresh water makes it necessary to use saline waters in areas of unstable and insufficient humidification (Baliuk et al., 2020; Li et al., 2020; Wei et al., 2019; Yuan et al., 2018).
The controlled management of the soil salinization is imperative for achieving most of the Sustainable Development Goals (SDGs) of the United Nations. It is necessary for achieving the "Zero Hunger" (SDG2) and "Life on Land" (SDG15) among other SDGs (Singh, 2021).
Soil salinity and alkalinization are the most prevalent and widespread problem limiting crop productivity in irrigated agriculture (Tomaz et al., 2020; Kramer et al., 2020; Baliuk et al., 2009; Handbook…, 2018).
The use of salt water for irrigation requires complex researches of the "irrigation water-soil-plant" system to study the dynamics and interconnection of soil processes and regimes during irrigation, monitoring and management of the fertility of irrigated lands. Studies of salt regimes and exchangeable cations composition in these soils are especially relevant to evaluate possibility and preventing the development of degradation processes, developing predictive models of soil fertility, and also well as measures to protect and improve their fertility (Baliuk, 2017, 2020; Vorotyntseva, 2016).
The aim of our researches was to study long-term effect of irrigation with sweet ("suitable") and saline waters ("unsuitable") on the salt regime, the composition of the soil absorbing complex of chernozems in the Forest-steppe and Steppe of Ukraine.

MATERIALS AND METHODS

The research was conducted in the agroclimatic zones with different humidification - Forest-steppe (Kharkiv region) and Steppe zones of Ukraine (Donetsk region). Hydrothermal coefficient of Selyaninov (HTC) characterizes the level of moisture in the zone. In the Forest-steppe it is 0.9-1.4, in the Northern steppe - 0.7-0.9. But due to the dryness of the climate, stable yields can only be obtained with irrigation.
The interconnected system "irrigation water - soil - crop" was studied. The researches were conducted on the key experimental stationars with sprinkler irrigation. They differ in natural and climatic conditions, soils, irrigation water quality.
The main subtypes of chernozems dominated in the composition of the irrigation were selected as a study objects. Irrigation waters of natural sources are essentially different on the chemical composition and quality. Therefore, they have a different effect on the intensity of soil processes and the transformation of the physicochemical properties of the soil.
As an object of comparison, we used non-irrigated soils similar in properties before irrigation on non-irrigated experimental stationars stationary plots (key-analog method).
Experimental stationar 1 - ***Merefa stationar*** - in Kharkiv District of Kharkiv Region (49.77 N, 36.03 E), is located in the southern part of the Left-Bank Forest-Steppe of Ukraine. This is stationar of Institute of Vegetable and Melon Growing. The soil is chernozem typical (Chernozems Chernic, WRB).
Monitoring site is with a longterm vegetable-fodder crop rotation (Figure 1). On the experimental site grown tomato, cucumber,

cabbage, onion, beet. Irrigation was carried out by DDA-100 M during 24 years. Groundwater was located at a depth more 8-10 m.

Figure 1. Growing vegetables in rotation (Merefa stationar)

Irrigation was carried out with water from the Mzha river. Duration of irrigation is 24 years. Irrigation norms in the experiment were 350-1350 m^3/ha, depending on the crops grown and the weather. The mineralization of the irrigation water is 0.6-0.7 g/dm^3; pH - 8.1; type of salt - principally calcium and magnesium bicarbonates and chlorides (Table 1). On national classification (State standard of Ukraine 2730:2015) the irrigation water is classified as "suitable" for irrigation.
The content of physical clay (<0.01 mm) in layer 0-25 cm of chernozem typical was 55%. The soil was characterized by a heavy loamy granulometric composition. Humus content was 3.3% (layer 0-25 cm).

Table 1. Chemical composition of irrigation waters

Values of indicators	Mineralization of water, g/dm^3	pH	Ion content, meq/dm^3						
			HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+
Mzha river (Experimental stationar 1)									
mean	0.68	8.1	6.9	1.1	0.9	4.1	2.7	2.0	0.10
min	0.60	7.5	6.2	0.6	0.3	3.6	2.2	1.2	0.09
max	0.70	8.3	8.8	1.5	2.7	4.6	3.0	2.5	0.16
Water of the pond (Experimental stationar 2)									
mean	2.50	8,0	6.0	9.0	22.9	7.5	12.2	17.4	0.80
min	2.36	7.8	5.5	8.1	21.2	6.8	11.7	16.5	0.65
max	2.60	8.0	6.7	9.8	23.0	8.2	12.8	17.4	0.82

Experimental stationar 1 - ***Pervomaysk stationar*** - in Yasinovatsky District of Donetsk Region (48.06 N 37.36 E) is located in the Northern Steppe of Ukraine. Soil is chernozem ordinary (Chernozems Chernic, WRB). The stationar was laid on the fields of vegetable-fodder crop rotation. The crop rotation included the alternation of crops: winter wheat, beetroot, cabbage, pepper, bow; barley; tomatoes, cucumbers; corn.
Irrigation was carried out by DDA-100 M with water from the local pond. Duration of irrigation was 50 years. Irrigation norms in the experiment were 1000-1500 m^3/ha depending on the crops grown and the weather.
Groundwater was located at a depth more 10 m. The content of physical clay (<0.01 mm) in layer 0-30 cm of chernozem ordinary was 69%. The soil was characterized by a light clay granulometric composition. Humus content was 3.9% (layer 0-25 cm).
The mineralization of the irrigation water is 2.4-2.6 g/dm^3; pH - 8.0; type of salt - magnesium and sodium chlorides and sulphates. On national classification the irrigation water was classified as "unsuitable" for irrigation on the dangers of soil alkalinization and partially suitable on the dangers of soil salinization and alkalization. The chemical composition of irrigation water is presented in Table 1. The water is contaminated with toxic metals such as lead, cadmium and cobalt. Thus the irrigation water is mineralized and contains toxic compounds. Such irrigation will affect the properties of the soil and the quality of vegetable products. It is used for irrigation due to the limited amount of fresh water.
On the experimental stationars the influence of irrigation on soil processes, properties and morphology of chernozems was studied. On each stationar profile pits were laid to a depth of 1.5-1.7 m and probing from genetic horizons. In addition, auger probes on experimental sites from boreholes were taken. The soluble salt content in water extracts and pH of water suspensions (with the soil-to-solution ratio of 1:5) were determined. The

composition of exchangeable bases was determined after extraction from the soil with 1 mol/dm^3 ammonium acetate solution (pH 7.0)

RESULTS AND DISCUSSIONS

Our long-term studies have established that irrigation leads to significant changes in the direction of natural soil processes and regimes, as well as indicators of the morphological structure of the profile, composition and properties of chernozem ordinary. The chemical composition of irrigation water is one of the main factors determined the degree of these changes.

With long-term irrigation by limited suitable and unsuitable waters the irrigation alkalinization in the morphological profile of the soil in the humus-eluvial (He) and humus-iluvial (Hi) horizons is visualized. There is glossiness on the edges of structural aggregates. Compaction of the surface horizon has occurred. The lumpy-granular soil structure has changed to lumpy-silty structure (in the plow horizon) and lumpy-prismatic (in subsurface horizon).

The irrigated soil profile in comparison with the non-irrigated analogue has a greater thickness of the humus-accumulative horizon. This is due to the improvement of the water regime and the creation of favorable conditions for the plants growth. The depth of the carbonate horizon (carbonates in the form of a white soft spot) increases with irrigation. This indicates the leaching of calcium by irrigation water.

Our research has established that gallochemical processes are increased during irrigation. This leads to the transformation of the cation-anionic composition of salts, a change of their chemical composition, type of salinity. The degree of these changes are mainly determined by the chemical composition of the irrigation waters and the initial properties of the soil.

Soil salinization is one of the most common processes on irrigated soils, especially when using saline waters. It restricts the sustainability of agriculture. Soil salinization impacts on the agricultural productivity by causing disruptions to the processes of nitrogen uptake and plant growth development. An increase in soil salinity further deteriorates soil ecosystem services and decreases revenues for farmers and smallholders (Handbook…, 2018).

The diffusion process in response to the concentration gradient determines the distribution of salts in the soil profile, especially the distribution between large and small pores. Additional simultaneous interaction of dissolved salts with other ions in the solution or with the soil surface changes the composition of the solution (Soil Salinity…, 1984).

Our researches on Merefa stationar were established in automorphic conditions on irrigation during 24 years by sweet water with a mineralization of less than 1 g/dm^3, the salts storage in the aeration zone practically did not change (Table 2). The content of water-soluble salts in the soil (up to a depth of 1 m) was 0.04%. The content of toxic salts in the upper 0-30 cm layer was 0.03%, and deeper - 0.02%. Therefore, chernozem typical of stationary is characterized as non-saline.

In the long-term cycle a stable salt regime is formed in the seasonal cycle it is seasonally reversible with insignificant (0.01-0.05%) dynamics. Irrigation causes a noticeable transformation of the qualitative composition of salts. The total toxic alkalinity (HCO_3-Ca) increases slightly (on 0.01-0.14 meq/100 g). As a result the pH of the soil increased from 6.6-6.7 (0-25, 25-50 cm) to 7.3-7.4. The content of water-soluble sodium increases throughout the soil profile. The calcium content decreases or tends to decrease.

Table 2. Salt regime in chernozem typical of Merefa stationar

Irrigation/ non-irrigation	Depth, cm	Total soluble salt, %	Toxic salts, %	pH	Ca^{2+}, mmol (equiv.) /100 g of soil	Na^{+}, mmol (equiv.) /100 g of soil	Ca/Na
Non-irrigation	0-25	0.04	0.02	6.6	**0.37**	0.06	**6.2**
	25-50	0.04	0.02	6.7	0.35	0.07	5.0
	50-75	0.04	0.02	6.7	0.32	0.06	5.3
	75-100	0.04	0.02	6.8	0.33	0.07	4.7
Irrigation	0-25	0.04	0.03	7.4	**0.28**	0.17	**1.6**
	25-50	0.04	0.02	7.3	0.23	0.11	2.1
	50-75	0.04	0.02	7.3	0.25	0.10	2.5
	75-100	0.04	0.02	7.3	0.25	0.10	2.5

The ratio of calcium to sodium (Ca/Na) in the soil profile progressively narrows from 4-6:1 to 1-3:1 in the soil of Merefa stasionar. Sodium content usually increases in the first 3-5 years of irrigation and then it relatively stabilizes. The decrease of the calcium content has a stable progressive character, especially in layer 0-50 and 0-100 cm.

On Pervomaysk stationar in long irrigation with saline water changes in the salt composition of the soil were significant. The total content of water-soluble salts increased from 0.04-0.08 % (non-irrigated soil, layers 0-25, 25-50 cm) to 0.16% in the irrigated soil (Figure 2).

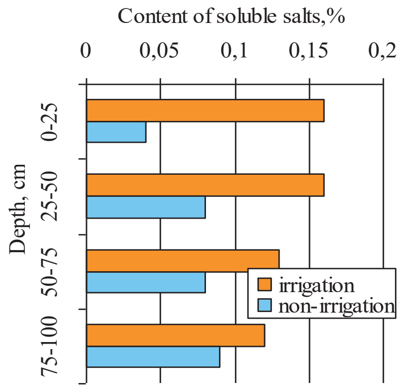

Figure 2. Content of soluble salts in chernozem ordinary (Pervomaysk stationar)

The concentration of toxic salts in the 0-50 cm layer rises to 0.10-0.11%. In this case the degree of soil salinity increases to slightly saline (Figure 3).

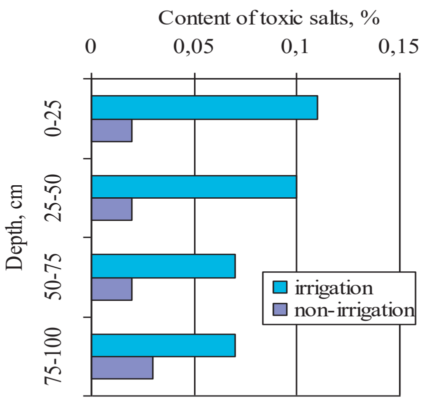

Figure 3. Content of toxic salts in chernozem ordinary (Pervomaysk stationar)

The pH of the soil increased from 7.4-7.6 (layer 0-25, 25-50 cm) to 7.9-8.0. The reaction of water extract of chernozem ordinary is alkaline. On irrigation with saline waters the content of water-soluble sodium in the soil increased from 0.10-0.13 mmol/100 g to 1.35-1.37 mmol/100 g in 0-50 cm layer. The ratio of water-soluble cations Ca/Na in the soil profile decreases from 4.4-10.3:1 (non-irrigation) to 0.4-0.6:1 (irrigation) (Table 3). This indicates the development of the process of irrigation alkalinization and its gradual advance deeper along the profile with systematic irrigation by saline waters.

Table 3. Salt regime in chernozem ordinary of Pervomaysk stationar

Option	Depth, cm	pH	Ca^{2+}, mmol (equiv.) / 100 g	Na^{+}, mmol (equiv.) / 100 g	Ca/Na
Non-irri-gation	0-25	7.4	**0.86**	**0.13**	**6.6**
	25-50	7.6	0.82	0.10	8.2
	50-75	7.7	0.74	0.09	8.2
	75-100	7.7	0.79	0.17	4.6
Irriga-tion	0-25	7.9	**0.58**	**1.37**	**0.4**
	25-50	8.0	0.67	1.35	0.5
	50-75	8.1	0.62	0.94	0.7
	75-100	8.0	0.53	0.98	0.5

According to the results of statistical analysis a high inverse correlation between the mineralization of the irrigation water and the Ca/Na ratio in the water extract from the soil was established - r = (–0.92). Regression models of the dependence of the toxic salts content in the soil from the irrigation water mineralization and their total content were constructed.

In the long-term dynamics for the irrigated chernozem ordinary of Pervomaysk stationar the type of salt accumulation is seasonally reverse with a tendency to an increase in their concentration in certain years. This is due to the intensity of irrigation and the irrigation rates of vegetable crops.

Under the influence of irrigation changes in the composition of the soil absorbing complex occur. The degree of change in the qualitative composition of the soil absorbing complex cations is determined by the irrigation water quality irrigation norms, initial soil properties, ecology-agroameliorative condition of irrigated lands. Alkalinization is the most widespread degradation process on irrigated soils.

The content and composition of soil absorbing complex cations allows us to evaluate changes in soil quality. Changes in the composition of the soil-absorption complex are caused by irrigation water of any composition, however, the severity of these changes is different.

On irrigated with sweet waters (Merefa stationar), the content of exchangeable calcium in the chernozem typical tends to gradually decrease in absolute and relative content.

The content of exchangeable magnesium tends to increase somewhat. In this case, the content of exchangeable sodium increases in absolute and relative content - from 0.6% to 1.5-1.6% from the sum of all cations (Figure 4, Table 4). The potassium content remains practically unchanged. The total content of exchangeable sodium and potassium cations increases from 1.5 % to 2.5-2.6 % from the sum of all cations.

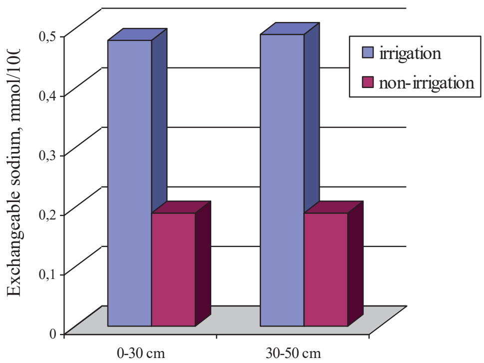

Figure 4. Content of exchangeable sodium in chernozem typical (Merefa stationar)

Table 4. Percentage content of exchangeable cations in chernozem typical (Merefa stationar)

Option	Depth, cm	% from the sum of cations			
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
Non-irrigation	0-30	83.5	14.8	0.6	0.9
	30-50	83.5	15.0	0.6	0.8
Irrigation	0-30	77.6	19.9	1.5	1.0
	30-50	77.8	19.7	1.6	1.0

On long irrigation by saline waters the transformation of the soil exchangeable complex is more intense, and the degree of manifestation of degradation processes increases. On the Pervomaysk stationar with prolonged irrigation with unsuitable water and the use of chernozem ordinary in an intensive vegetable-fodder crop rotation the intensity of alkalinization increases.

The composition of the absorbed complex changed significantly. The concentration of exchangeable sodium in chernozem ordinary increased from 0.30-0.37 to 1.28-1.70 mmol/100 g (Figure 5). The content of exchangeable calcium was decreased (Table 5).

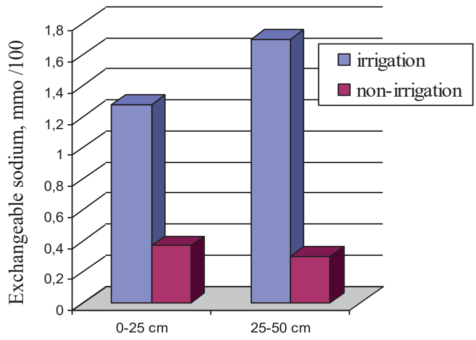

Figure 5. Content of exchangeable sodium in chernozem ordinary (Pervomaysk stationar)

Table 5. Percentage content of exchangeable cations in chernozem ordinary (Pervomaysk stationar)

Option	Depth, cm	% from the sum of cations			
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
Non-irrigation	0-25	71.2	26.4	0.9	1.4
	25-50	70.7	27.1	0.8	1.4
Irrigation	0-25	68.2	26.4	3.6	1.9
	25-50	62.7	30.6	4.5	2.1

The content of exchangeable sodium and potassium increased to 5.3-6.6% of the total contents of cations in the 0-25 and 25-50 cm layers (Figure 6). The soil was characterized by a weak and medium degree of alkalinization. A tendency towards more intensive progression of alkalization down the profile was established.

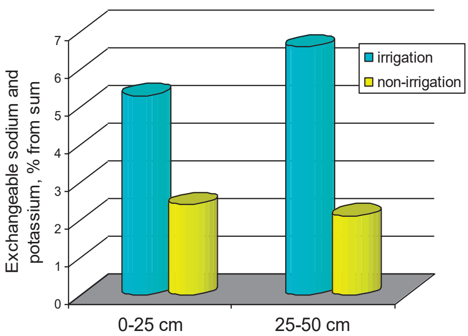

Figure 6. Content of exchangeable sodium and potassium in chernozem ordinary, % sum exchangeable cations (Pervomaysk stationar)

Changes in the soil absorbing complex are due by increasing of the activity of sodium ions and decreasing of the activity of calcium ions in the soil solution especially in the plowing layer on irrigation with unsuitable waters. Non-irrigated soil on to the activity of calcium ions was characterized as medium-buffered to alkalization (7.5-7.9 meq/dm^3). On long-term irrigation with unsuitable waters the buffering capacity of chernozem ordinary decreased to a low level due to an increase in the activity of sodium ions (from 0.85 to 4.36 meq/dm^3) and a decrease in the activity of calcium ions to 4.73 meq/dm^3 (Naydyonova, 2015).
The regression model of the dependence between the exchangeable sodium and potassium cations content in chernozem ordinary and the salinity of irrigation water and the Ca/Na ratio in the water extract was developed (Figure 7).
It is described by the equation:

$$B_{Na+K} = 14.07 - 7.85\cdot x - 6.69\cdot b + 1.26\cdot x^2 + 2.05\cdot xy + 1.32\cdot y^2;$$

B_{Na+K} - the content of exchangeable sodium and potassium cations, meq/100 g of soil;
x - mineralization of irrigation water, g/dm^3;
c - Ca/Na ratio in water extract.
The multiple correlation coefficient (r) is 0.90, the determination coefficient is $R^2 = 0.81$.

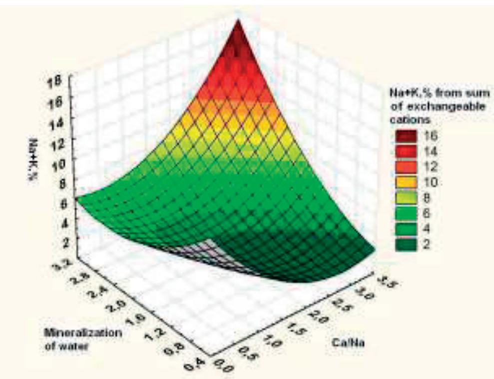

Figure 7. Dependence of the soil alkalinisation from the mineralization of irrigation water and Ca/Na ratio in the water extract

Changes in the cationic composition of the soil absorbing complex affect the agrophysical, physicochemical properties of irrigated soils and determine the level of their fertility, soil buffering to anthropogenic loads, the fulfillment of ecosystem services by the soil (provisioning, regulating and others).

CONCLUSIONS

Long-term irrigation in vegetable-fodder crop rotation by sweet water did not lead to an increase in the total content of water-soluble salts in chernozem typical. The soil in layers 0-25 and 25-50 cm is classified as unsalted. The transformation of the cation-anionic composition of the salts was established. In typical chernozem total toxic alkalinity, pH of the soil increased. The content of water-soluble sodium increases throughout the soil profile. The calcium content decreases or tends to decrease. The ratio of calcium to sodium (Ca/Na) in the soil profile progressively narrows from 4-6:1 to 1-3:1. The content of exchangeable calcium in the chernozem typical tends to gradually decrease. The total content of exchangeable sodium and potassium cations increases from 1.5% to 2.5-2.6% from the sum of all cations.
Long-term irrigation with saline waters ("unsuitable") led to significant changes in the salt composition of the water extract and the composition of the soil absorbing complex. The total content of salts and toxic salts in chernozem ordinary has increased in comparison with non-irrigated soil. The 0-50 cm layer was classified as slightly saline. The content of water-soluble sodium in the soil has increased significantly. pH is increased.
On long irrigation by saline waters the transformation of the soil exchangeable complex is more intense, and the degree of manifestation of degradation processes increases. The content of absorbed sodium and potassium in the soil-absorbing complex increased to 5.6-6.6%. Alkalinization in the soil was developed in a weak and medium degree.
Changes in the soil absorbing complex are due by increasing of the activity of sodium ions and decreasing of the activity of calcium ions in the soil solution especially in the plowing layer on irrigation with unsuitable waters.
The regression model of the dependence between the exchangeable sodium and potassium cations content in chernozem ordinary and the salinity of irrigation water and the Ca/Na ratio in the water extract was

developed. She showed a close relationship between these indicators.

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RESEARCH ON THE INFLUENCE OF TECHNOLOGICAL SYSTEMS ON MAIZE CULTIVATION IN THE SOUTH OF DOLJ COUNTY, ROMANIA

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Abstract

Conservation agriculture implies practices that are applied in such a way as to cause minimum damage to the environment. Conservation practices, the most important aspect of conservation agriculture, are widely supported worldwide, and it is considered that they take care of soil health, plant growth and the environment. The growing concern for food security through improved soil management techniques requires the identification of a sustainable, environmentally friendly and ecological system that would also ensure a high yield for the cultivated plants. Research reports have identified more benefits of conservation practices, than of conventional practices, in terms of crop yields. Moreover, while no less than 25% of greenhouse gas emmissions released into the atmosphere are attributed to agriculture, research in relation to the processes of climate change mitigation and adaptation found that the zero tillage (ZT) practice is the most environmentally friendly among different processing techniques. Therefore, conservation practices involving zero tillage and minimal tillage, which have the potential to break up the compact surface area of the soil with reduced soil disturbance, provide a better soil environment and a crop yield with minimum impact on the environment. In this context, in 2019, an experiment was performed in order to analyze the influence of cultivation practices on maize yield in Urzicuta locality, Dolj County, in the Sourthern Oltenia Region of Romania. In this experiment, two tillage systems were applied, with A representing the applied soil practices (a1-conventional tillage and a2 - minimum tillage), each with control and fertilized variants, as following - b1-control, b2-$N_{100}P_{50}K_0$, b3-$N_{100}P_{50}K_{50}$, b4-$N_{150}P_{50}K_{50}$ and b5-$N_{100}P_0K_0$. While following the development of maize in both systems and for each variant, this paper aims to identify the best system that would ensure an increased yield for the cultivation of maize in the specified region.

***Key words**: minimum tillage, sustainability, fertilisation, soil resources.*

INTRODUCTION

The decrease in the soil's fertility, which has repercussions on the health and on the quality of agricultural yields, should be a warning that must compel us to limit the degradation of soils and to rehabilitate them. Changing production technologies, by using high-performance machines, requires, as we all know, extensive research in order to understand their long-term effects on the physical, chemical and biological properties of soils (Mihalache, 2012; 2015; Marin, 2012; 2015).

When choosing which technological systems should be applied, one must take into account the properties of the soil and its degree of supply in nutrients. The experiments that were conducted have shown that soils respond differently to the application of different technological systems and the soil's properties that can influence these responses are: soil humus content, soil texture, soil structure, the calcium carbonate content, the degree of nutrient supply, etc. An important factor is also given by the application of fertilizers that contain nitrogen, phosphorus and potassium.

Soil conservation practices are considered among the most important components of conservation agriculture. Therefore, by applying minimum tillage systems, the water available for plants in the soil is better preserved and water losses are reduced (Kopek, 2015; Simon, 2018).

Other than that, research conducted with different technological systems has shown that by applying minimum tillage systems the degree of weeding in maize crops is 116% higher than in the case of applying conventional systems (Chițoi, 2018).

Regardless, the yields obtained at minimum tillage and without tillage depend on the soil's characteristics and on the characteristics of each plant (Rusu, 2015).

MATERIALS AND METHODS

In order to follow the influence of technological systems and the influence of fertilization applyed on the maize crops, in 2019, an experimental field was arranged in the southern part of Dolj County, on Chernozem soils (Figure 1).

The representative soil in the research area is a typical Chernozem with a loamy texture, having a neutral reaction of 7.12-7.40, humus of 3.30%, total nitrogen of 0.135%, P_{AL} 15 mg/kg, K_{AL} 90 mg/kg, Zn 0.4 mg/kg, Cu 1.5 mg/kg, Fe 25.3 mg/kg and Mn 33.7%.

Figure 1. Chernozem profile - Urzicuța locality, Dolj County

Table 1. Characterization of the main chemical properties of the soil

System technological	pH	H %	N total %	P_{AL} mg/kg	K_{AL} mg/kg
Conventional	7.12	3.40	0.135	15	94
Minimum tillage	7.51	3.32	0.107	30	92

The preparation of the land for the plots cultivated in the minimum tillage system was carried out with a disc at a depth of 25 cm in autumn, on the 20th of September, 2018. When the germinative bed was prepared, one disc passing took place, while in the conventional system the plowing was made at 25 cm depth with discs.

The cultivated maize hybrid was PR 9911, a semi-late hybrid, from the FAO 410 group. It is characterized by an excellent production capacity, and offers the future plant an extremelly developed root system. For the experiment described in this paper around 65,000 germinating plants/ha were sown in an irrigated system with the precursor plant being also maize.

This experiment included conventional practices and minimum tillage practices (part A) with the following variants: a1-conventional and a2 - minimum tillage, with control and fertilized variants, as following: b1-unfertilized, b2-$N_{100}P_{50}K_0$, b3-$N_{100}P_{50}K_{50}$, b4-$N_{150}P_{50}K_{50}$ and b5-$N_{100}P_0K_0$. Each variant had three repetitions and for the statistical data processing the variation analysis was used.

RESULTS AND DISCUSSIONS

In order to measure the influence of technological systems and fertilization on the maize crop, the following analyzes were made for 10 plants from each repetition and variant: plant height, plant weight, root system weight (Table 2), leaf weight and the maize production was calculated in tonnes/ha.

In Table 1 the highest weight of the root system was recorded in the b4 variant with $N_{150}P_{50}K_{50}$ kg/ha. It can be seen, in this variant, that the application of complex fertilizers containing nitrogen, phosphorus and potassium have contributed to a better distribution of the root system of the plants in the soil.

In the b5 variant, where only 100 kg of nitrogen were applied, the root system weight was close to the b1-unfertilized variant. Nevertheless, the differences are significant in all variants, when compared to the control variant.

Table 2. The influence of fertilization on the root system weight of maize

Variants	Production, g/10 plants	%	g/plant	%	Significance
b1-Control	243.66	100	Mt	-	-
b2-$N_{100}P_{50}K_0$	321.66	132.01	78	32.01	***
b3-$N_{100}P_{50}K_{50}$	440.66	238.73	197	138.73	***
b4-$N_{150}P_{50}K_{50}$	549.50	538.38	305.83	438.38	***
b5-$N_{100}P_0K_0$	263.16	581.47	19.5	481.47	***
LSD 5%=4.42 g/plants LSD 1%=6.10 g/plants LSD 0.1%=8.38 g/plants					

The tillage practices highlighted a higher distribution of the root system in the variants in the conventional system, given by a better soil processing and a better aeration of the soil, than in the case of the minimum tillage system.

In the case of both the conventional system and the minimum tillage system, the differences obtained, in comparision to the control variant, are significant.

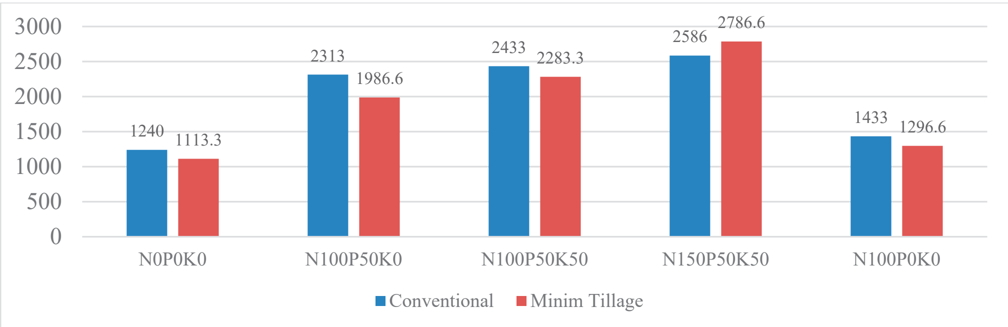

Figure 2. The weight of the maize stalk in conventional and minimum tillage systems (g)

In Figure 2 we can see a tendency to increase the corn stalk, a higher weight for the conventional system compared to the minimum tillage for the variant to which nitrogen 150, phosphorus 50 and potassium 50 were applied.
The values obtained were higher than in the conventional system. In the minimum tillage system, the weight values of the root system are lower than in the conventional system, which indicates the fact that, by applying the minimum tillage system practices, a more accentuated compaction is registered, which can influence the distribution of the root system.

Table 2. The influence of tillage practices on the root system weight of maize

Variants	a1-Conventional			a2-Minimum tillage		
	g/plant	Difference	Significance	g/plant	Difference	Significance
b1-Control	251.66	-		235.66	-	-
b2-$N_{100}P_{50}K_0$	322.66	71	***	320.66	85	***
b3-$N_{100}P_{50}K_{50}$	443.33	191.6	***	438	202.33	***
b4-$N_{150}P_{50}K_{50}$	551.66	300	***	547.33	311.66	***
b5-$N_{100}P_0K_0$	273.66	22	***	252.66	17	*
LSD 5%=6.262797 LSD 1%=8.628812 LSD 0.1%=11.86143				LSD 5%=10.20789 LSD 1%=20.30318 LSD 0.1%=55.93887		

The weight of the maize leaves at harvest increased with the application of different doses of nitrogen, phosphorus and potassium in both systems, but in the case of the conven-

tional system, a higher quantity of leaves was produced, when compared to the minimum tillage system.
The highest amount of leaves was obtained in variant b4 of 626.6 g in the conventional system and 596.6 in the minimum tillage system. In variant b5, where the 100 kg of nitrogen were applied, did not lead to the increase of leaf weight, compared to the respective control value, of 395 g in the conventional variant and 291.6 g in the minimum tillage system (Figure 3).

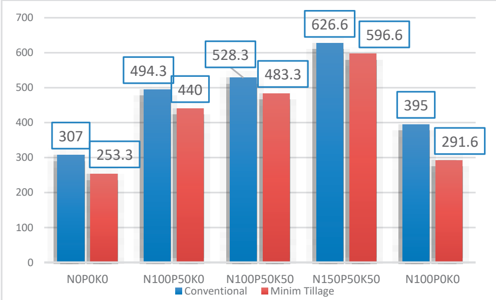

Figure 3. Weight of maize leafs in conventional and minimum tillage system (g)

Table 3. The influence of fertilizers on the height of maize plants

Variants	Height	Difference	Height	Difference	Significance
	cm	%	cm	%	
b1-Control	184.16	100	Mt	-	-
b2-$N_{100}P_{50}K_0$	271.83	147.60	87.66	47.60	***
b3-$N_{100}P_{50}K_{50}$	307.08	166.74	122.91	66.74	***
b4-$N_{150}P_{50}K_{50}$	316.00	171.58	131,83	71.58	***
b5-$N_{100}P_0K_0$	214.25	116.33	30.08	16.33	***
LSD 5%=3.184271 LSD 1%=4.387253 LSD 0.1%=6.030853					

At harvest, the average height of the plants ranged from 184 cm, in the control variant, to 316 cm in the b4 variant, with $N_{150}P_{50}K_{50}$. It can be seen how only applying a dose of 100 kg of nitrogen can only lead to an increase of height of up to 214.25 cm. On the other hand, under irrigation conditions, the application of complex fertilizers with nitrogen, phosphorus and potassium greatly influences the development of maize plants (Table 3).

The height of the plants did not show significant differences regardless of the technological system applied, however, in both the conventional and the minimum tillage system, the highest height of maize plants was recorded by applying variant b4 - $N_{150}P_{50}K_{50}$, that led to having results with a height of 321.66 cm and 310.66 cm respectively, as shown in Table 4.

Table 4. The influence of tillage practices on the height of maize plants

Variants	a1-Conventional			a2-Minimum tillage		
	cm	Difference	Significance	cm	Difference	Significance
b1-Control	187.5	Mt	-	180.83	Mt	-
b2-$N_{100}P_{50}K_0$	274.33	86.83	***	269.33	88.5	***
b3-$N_{100}P_{50}K_{50}$	311.66	124.16	***	302.5	121.66	***
b4-$N_{150}P_{50}K_{50}$	321.33	133.83	***	310.66	129.83	***
b5-$N_{100}P_0K_0$	217.83	30.33	***	210.66	29.83	***
LSD 5%=4.50324 LSD 1%=6.204513 LSD 0.1%=8.528914				LSD 5%=7.429184 LSD 1%=14.83235 LSD 0.1%=41.03982		

The maize production in the experimental field, varied, in 2019, from 6.22 tonnes/ha in the control variant, to 14.13 tonnes/ha in the b4 variant, in which $N_{150}P_{50}K_{50}$ was applied. This dosage is recommended for the maize hybrid used in the experimental field, in the conditions of the Chernozem soils from the southern part of Oltenia, Romania and in accordance with the soil's degree of supply in nutrients (Table 5).

Although there are many claims related to the good potassium supply of Chernozems, the undertaken experiments and the results obtained for maize crops, under irrigation conditions, highlighted the importance of using complex mineral fertilizers (Table 5).

Table 5. The influence of fertilizers on maize yield

Variants	a1-Conventional		a2-Minimum tillage		Significance
	Yield, t/ha	%	Yield, t/ha	%	
b1-Control	6.22	100	Mt	-	-
b2-$N_{100}P_{50}K_0$	11.86	190.43	5.63	90.43	***
b3-$N_{100}P_{50}K_{50}$	12.69	388.13	6.46	288.13	***
b4-$N_{150}P_{50}K_{50}$	14.13	880.88	7.90	780.88	***
b5-$N_{100}P_0K_0$	8.01	1133.64	1.78	1033.64	***
LSD 5%=0.515511 LSD 1%=0.710265 LSD 0.1%=0.976352					

Regarding the influence of the two technological systems applied on maize production, it can be shown that, in both the conventional and minimum tillage system, the production increased significantly when compared to the control variant. In the b4 variant, where $N_{150}P_{50}K_{50}$ was applied, the production was of 13.9 t/ha in the conventional system and of 14.30 t/ha in the minimum tillage system. For all other variants and doses applied, maize yields were lower in the minimum tillage system, compared to the conventional system, as shown in Table 6.

Table 6. The influence of tillage practices and fertilizers on maize yield

Variants	**a1-Conventional**			**a2-Minimum tillage**		
	t/ha	Difference	Significance	t/ha	Difference	Significance
b1-Control	6.28	Mt	-	6.175	Mt	-
b2-$N_{100}P_{50}K_0$	12.28	6.00	***	11.44	5.26	***
b3-$N_{100}P_{50}K_{50}$	12.89	6.60	***	12.50	6.32	***
b4-$N_{150}P_{50}K_{50}$	13.97	7.69	***	14.30	8.12	***
b5-$N_{100}P_0K_0$	8.66	2.38	***	7.366	1.19	*
LSD 5%=0.729042 LSD 1%=1.00466 LSD 0.1%=1.38077				LSD 5%=1.04416 LSD 1%=1.981983 LSD 0.1%=5.165623		

CONCLUSIONS

The experiments carried out in 2019 in the ecopedological conditions found in the South of Dolj County, that tried to verify the influence of the conventional system and the minimum tillage system might have on maize production and characteristics, allowed to fundamentate the choice, in terms of which would be the best technological system and the best doses of fertilizers to be applied for maize cultivation.

The application of the conventional system, that ensures a better aeration of the soil, led to significant differences when compared to the minimum tillage system, especially regarding the distribution of the root system of the obtained biomass and regarding the height of the plants.

The highest maize production of 14.30 t/ha was obtained by applying the minimum tillage system and the b4 variant, with a dose of $N_{150}P_{50}K_{50}$, as compared to a production of 13.97 t/ha in the conventional system.

Therefore, taking into consideration the pedoclimatic conditions, the properties and the clay texture of the Chernozem from the Southern Oltenia, Romania, it is concluded that the minimum tillage system can be applied for maize cultivation in irrigated system.

Based on this research, the optimum doses of fertilizers that would obtain the highest maize production, were 150 kg/ha nitrogen, 50 kg/ha phosphorus and 50 kg/ha potassium.

Given the low potassium supply of Chernozems in the area of research, it is necessary to apply complex fertilizers that also contain potassium.

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MAPPING REAL EVAPOTRANSPIRATION IN WINTER WHEAT AND NON-IRRIGATED MAIZE CROPS DURING THE AGRICULTURAL SEASON 2019-2020

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Abstract

Evapotranspiration in agricultural crops is represented by water losses (unproductive) by evaporation from the soil surface and consumption (productive) by transpiration of plants. This study will try to highlight the importance of evapotranspiration (potential and real) in assessing the water consumption of plants, this being an essential parameter, with a particularly important impact on the crops obtained. This study was performed by daily analysis of the evolution of potential evapotranspiration calculated by the Penman-Monteith method (FAO-56 PM), as well as the real evapotranspiration performed in winter wheat and unirrigated maize crops for soil on different depths, in weekly and monthly intervals during the agricultural year September 1st, 2019 - August 31st, 2020. The specialized data analyzed included meteorological parameters: minimum and maximum diurnal temperatures, wind speed, sunlight duration, relative air humidity, crop coefficient, recorded precipitation and soil moisture reserve during the vegetation period of field crops with the most significant share in Romania, respectively winter wheat and unirrigated maize.

Key words: *real evapotranspiration, crop coefficient, agricultural season, water deficit, pedological drought.*

INTRODUCTION

In nature, evaporation is a much more complex process, because in addition to the real physical evaporation, there is also physiological evaporation from plant perspiration. Water evaporated from the soil, together with plant perspiration forms evapotranspiration. At the surface of the soil, evaporation depends on the water content of the soil, capillary water, caloric energy of the soil, atmospheric pressure, air temperature, solar radiation, wind speed and atmospheric humidity.

At the surface of the water, evaporation depends on the concentration in salts, the extent and depth, the dynamism of the surfaces. At the physiological evaporation the main role is played by the water reserve in the soil. There is a big difference between real and potential evaporation, i.e. maximum evaporation under certain conditions. Known as FAO 56 PM, this method is a global standard based on meteorological data (Allen et al., 1998), and it has been found to study well in numerous locations if the required data are available (Allen et al., 1989; Garcia et al., 2004; López-Urrea et al., 2006; Xing et al., 2008).

Evapotranspiration is affected by a number of factors, including weather parameters, crop characteristics, management and environmental issues (Allen et al., 1998; Wang et al., 2014). Evapotranspiration depends primarily on the air temperature and the amount of water in the soil. Evapotranspiration in agricultural crops is represented by water losses (unproductive) by evaporation from the soil surface and consumption (productive) by transpiration of plants. Potential and real evapotranspiration are agrometeorological indicators that provide new information necessary to identify agricultural areas affected by the phenomenon of pedological drought with different degrees of intensity (moderate, strong and extreme pedological drought) and negative effects with direct impact on major crops in Romania, in the context of current climate change.

The study highlights the importance of evapotranspiration (potential and real) in assessing the water consumption of plants, this being an essential parameter, with a particularly important impact on the crops obtained.

MATERIALS AND METHODS

The conversion of water into vapors returning to the atmosphere through the process of evapotranspiration (evaporation and transpiration) is decisively influenced by the amount of water available, which is why two parameters have been defined. ***Real evapotranspiration*** (ETR), which occurs in conditions of natural humidity and ***Potential evapotranspiration*** (ETP) representing the amount of water that can be evaporated and transpired under conditions of sufficient water reserves to compensate for maximum losses.

The Penman-Monteith method is the standard for estimating reference evapotranspiration and requires several meteorological elements (Dias et al., 2021).

The standard conditions refer to crops grown in large fields under excellent agronomic and soil water conditions. The crop evapotranspiration differs distinctly from the reference evapotranspiration (ET_o) as the ground cover, canopy properties and aerodynamic resistance of the crop are different from grass. The effects of characteristics that distinguish field crops from grass are integrated into the crop coefficient (K_c). In the crop coefficient approach, crop evapotranspiration is calculated by multiplying ET_o by K_c (Guidelines for computing crop water requirements - FAO, 1998).

Reference evapotranspiration is a term more frequently used for potential evapotranspiration in the usual literature in our country (Păltineanu et al., 2007).

The need for irrigation water for a given crop structure is determined proportionally on the basis of what is needed for each crop. The influence of climate on crop water needs: this influence is given by the evapotranspiration of reference crops (ETR).

Crop type and the stage of growth related to the water needs of the field plants give the expressed term crop coefficient (K_c) (Figure 1).

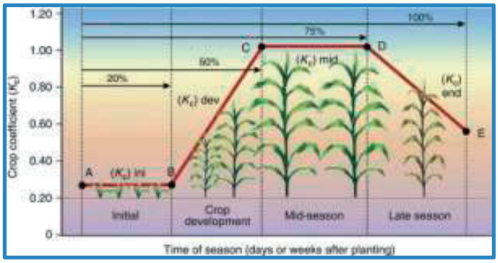


Figure 1. Crop coefficient -K_c-season (Source: Jan Pokorny, in Encyclopedia of Ecology, Second Edition), 2019

The real evapotranspiration (ETR) is determined for each crop, and with the obtained values the soil water balance calculations are performed and the monthly water requirement for the crops within the plant assortment specific to the area is determined. Crop water requirement (ETR) is defined as the depth (or amount) of water required to meet water loss through evapotranspiration. In other words, it is the amount of water needed by different crops to grow optimally.

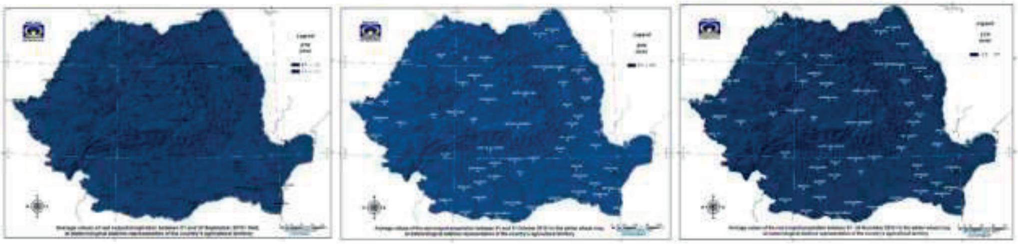
This study was performed by daily monitoring of the evolution of potential evapotranspiration (ETP) calculated by the Penman-Monteith method (FAO-56 PM), as well as of the real evapotranspiration (ETR) performed in winter wheat and non-irrigated maize crops, using meteorological data from NMA network, in weekly and monthly intervals during the agricultural season September 1st, 2019 - August 31st, 2020.

RESULTS AND DISCUSSIONS

The evolution of real evapotranspiration in the winter wheat crop in the agricultural year 2019-2020

In the autumn of 2019, the real evapotranspiration (ETR) dynamics in the winter wheat crop, showed low values throughout the country, being between 0.7-1.2 mm in September 2019, 0.4-0.6 mm in October 2019 and 0.2-0.7 mm in November 2019 (Figure 2 a, b, c).

During winter season and first month of the spring time (march), real evapotranspiration values are very low (close to zero), because of reduced activities of the crops.



a) b) c)

Figure 2. Average Real evapotranspiration (ETR) in the winter wheat crop, in September 2019 (a), October 2019 (b) and November 2019 (c)

The average values of this agrometeorological indicator on April 2020, in the winter wheat crop, on the soil depth 0-100 cm, ranged between 2.9-3.0 mm, isolated in northern and eastern Transylvania, southeastern Dobrogea. Locally in the south, northeast and center of Transylvania, isolated north of Oltenia, east of Dobrogea, central Moldova there were limits between 3.1-3.5 mm. In Maramureș, on large agricultural areas in Banat, Crișana, locally south and east of Oltenia, north, west, center, southwest and south of Muntenia, south, southwest, southeast, northwest and center of Transylvania, north, center, east, isolated southwest of Moldova, southwest of Dobrogea, values ranged from 3.6-4.0 mm. Locally in the south, southeast, east, center and west of Moldova, north and center of Dobrogea, east and center of Oltenia, south and southeast of Muntenia, isolated west of Crișana, northwest of Banat, the recorded data were between 4.1 - 4.5 mm. Higher limits (4.6-5.1 mm) were reported locally in northern, northeastern and eastern of Muntenia, isolated in Moldova area (Figure 3).

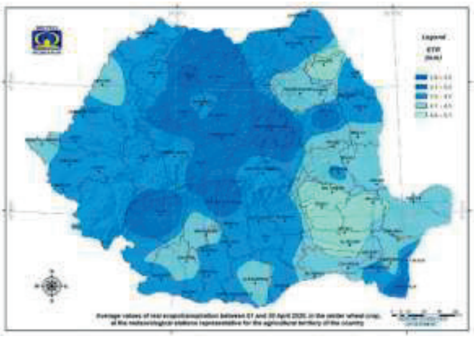


Figure 3. Average real evapotranspiration (ETR) - winter wheat crop, April 2020

Figure 4 shows the real evapotranspiration (ETR) in the winter wheat crop, between 01st-30 June 2020, with the lowest values between 3.3-4.0 mm, in Maramureș, Transylvania, Banat, most of Crișana, locally north, isolated center of Moldova, north and east of Oltenia, southeast of Dobrogea, northeast of Muntenia. On large agriculture areas in Moldova, Muntenia, locally south and east of Oltenia, center, east, isolated southwest of Dobrogea, south of Crișana, the limits ranged between 4.1-4.5 mm. Locally in the north, east and south of Muntenia, isolated north and center of Dobrogea, central and southwest of Oltenia, values between 4.6-4.9 mm were recorded.

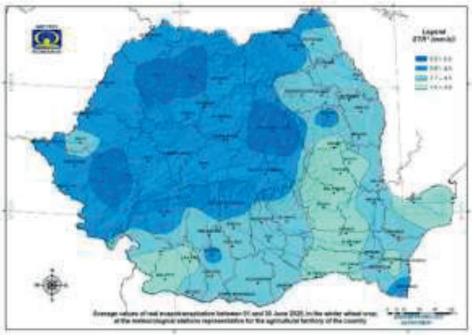


Figure 4. Average real evapotranspiration (ETR) - winter wheat crop, June 2020

The evolution of the daily average of the real evapotranspiration ETR (mm/day), calculated for the winter wheat crop, between 01-30 June 2020 at the meteorological stations with agrometeorological program, showed a tendency to increase the values, the daily averages being between 2.9-6.0 mm (Figure 5).

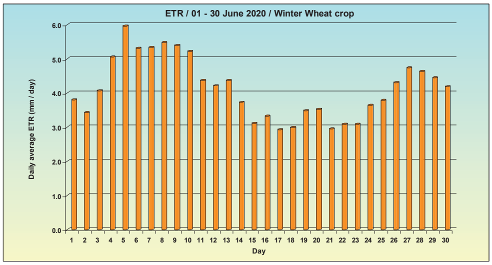


Figure 5. The evolution of real evapotranspiration 01st-30 June 2020

The comparative analysis of the values of potential evapotranspiration (ETP) with the average monthly rainfall regime, from September 1st, 2019 to June 30, 2020, shows that water consumption by evapotranspiration frequently exceeded the amount of precipitation recorded in September 2019, October 2019, January 2020, March 2020, April 2020, May 2020 and June 2020, except for the months of November 2019, December 2019 and February 2020, where the monthly average of the recorded precipitation quantities exceeded the evapotranspiration values (Figure 6).

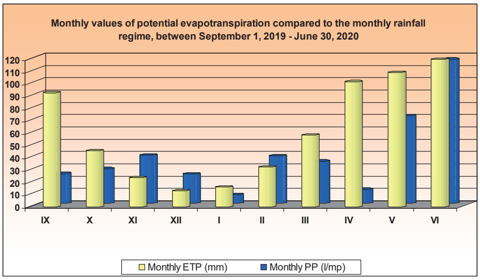


Figure 6. The evolution of potential evapotranspiration between September 1st, 2019 - June 30, 2020

The evolution of real evapotranspiration in non-irrigated maize crop in the agricultural season 2019-2020

Figure 7 shows the spatial zonation of the average real evapotranspiration (ETR) in the non-irrigated maize crop, on the soil profile 0-50 cm, between 01st-31st May 2020. Average values were recorded between 1.8-2.1 mm, locally in the north, northeast, center and east of Transylvania, isolated northwest, east and center of Moldova, southeast of Dobrogea. In Maramureș, Banat, Crișana, most of Transylvania, Oltenia, Muntenia and Moldova, locally the center, the east, isolated southwest of Dobrogea, the limits were between 2.1-2.5 mm. Locally in the north, east and south of Muntenia, east and center of Oltenia, isolated north and center of Dobrogea, average values between 2.6-3.0 mm were reported.

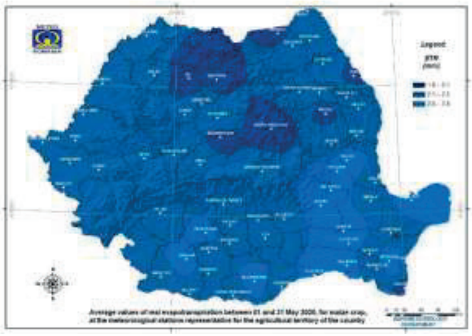


Figure 7. Average real evapotranspiration (ETR), non-irrigated maize crop, between 01-31 May 2020

The average values of the real evapotranspiration from 01-30 June 2020, on the soil depth 0-100 cm, in the non-irrigated maize culture, were between 3.3-3.5 mm, in Maramureș, Transylvania, locally west of Crișana, isolated north- west and northeast of Banat, northwest of Muntenia, southeast of Dobrogea. On large agricultural areas in Muntenia, Oltenia and Moldova, locally north and west of Banat, center, east, isolated southwest of Dobrogea, there were limits between 3.6-4.0 mm. Locally in the north, east and south of Muntenia, central and southwest of Oltenia, isolated north and center of Dobrogea, the values ranged between 4.1-4.4 mm (Figure 8).

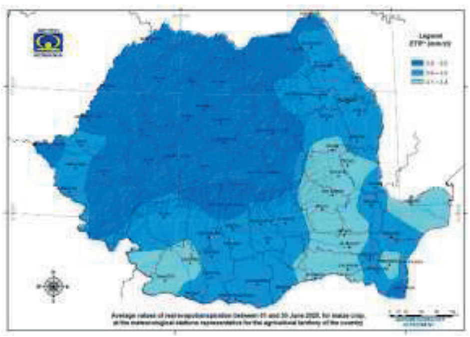
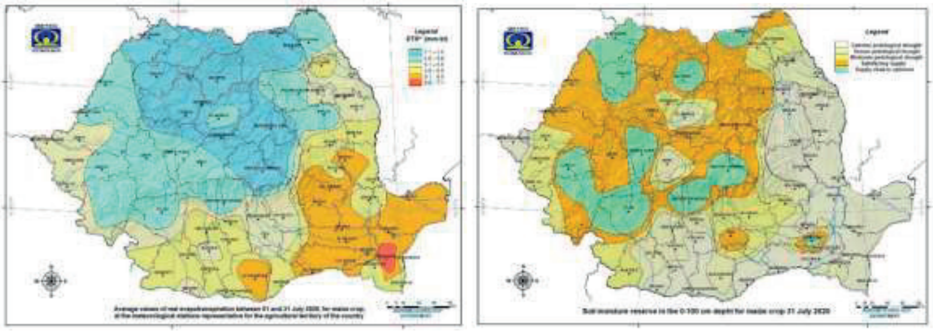


Figure 8. Average real evapotranspiration (ETR), non-irrigated maize crop, between 01-30 June 2020

In the non-irrigated maize crop, on the soil profile 0-100 cm, the values of real evapotranspiration between 01-31 July 2020, were between 4.1-4.5 mm in Maramureș, local north, center, east and southeast of Transylvania, north and northwestern Moldova. In most of Crișana, locally northeast, north, west and center of Moldova, south, southwest, isolated center of Transylvania, north of Oltenia, northwest of Muntenia, limits between 4.6-5.0 mm were reported. The highest values (5.1-7.1 mm) were recorded in Dobrogea, most of Oltenia, Muntenia, Moldova, Banut and isolated areas from southern Crișana, Figure 9 (a), thus resulting in a pedological drought with different degrees of intensity (moderate, strong and extreme), in these agricultural areas (Figure 9 b).



a) b)

Figure 9. Average real evapotranspiration (ETR) - (a) and soil moisture depth 0-100 cm (b) non-irrigated maize crop, 01-31 July 2020

The evolution of the daily average of the real ETR evapotranspiration (mm/day), calculated for the non-irrigated maize culture, between 01-31 July 2020 at the meteorological stations with agrometeorological program, shows a tendency to increase the values, the daily averages being between 3.9-6.9 mm (Figure 10).

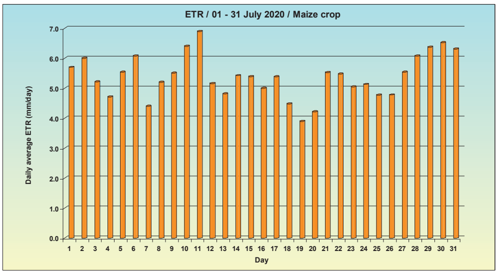


Figure 10. Real evapotranspiration 01-31 July 2020

The lowest values of real evapotranspiration (ETR) in the culture of unirrigated maize, on the soil depth 0-100 cm, between 01-31 August 2020, were between 3.1-4.0 mm, in Maramureș, Transylvania, the largest part of Crișana and Banat, locally north, northwest, west and center of Moldova, isolated northwest of Muntenia. Locally in the north, west, center, northeast, southwest and south of Muntenia, north, south and east of Dobrogea, east, center, northeast, southeast and southwest of Moldova, south, isolated east of Oltenia, west and the northwest of Banat, the southwest and the west of Crișana, limits between 4.1-4.5 mm were reported. Values between 4.6-5.0 mm were registered, locally in the east and center of Oltenia, north, south, southeast and west of Muntenia, south, east and center of Moldova. Locally in the center of Dobrogea, north, east, isolated south of Muntenia, the limits ranged between 5.1-5.4 mm (Figure 11).

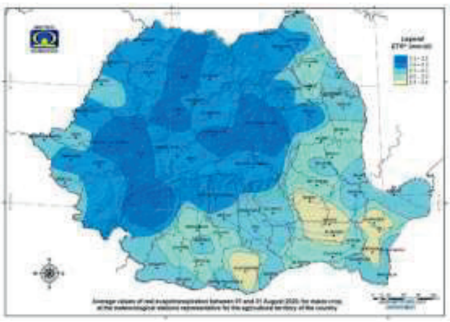


Figure 11. Average real evapotranspiration (ETR), non-irrigated maize crop, 01-31 August 2020

Figure 12 shows the evolution of the daily average of the real ETR evapotranspiration (mm/day), calculated for the non-irrigated maize crop, between 01-31 August 2020 at the meteorological stations with agrometeorological program. This shows an increasing trend of values, the daily averages being between 2.3-6.7 mm.

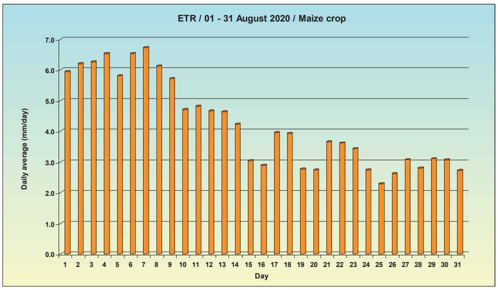


Figure 12. Real evapotranspiration 01 and 31 August 2020

In the culture of non-irrigated maize, on the soil profile 0-100 cm, between 03-09 August 2020, the real evapotranspiration showed limits between 4.6-5.0 mm, isolated in eastern Transylvania. In Maramureș, Banat, local north, northwest, center, south, southwest and southeast of Transylvania, isolated northwest and center of Moldova, west of Crișana, north of Oltenia, northwest of Muntenia, the values ranged between 5.1-5.5 mm. Locally in the northeast, center and south of Transylvania, north, west and east of Moldova, southeast, east, isolated southwest and north of Dobrogea, south of Crișana, there were limits between 5.6-6.0 mm. The highest values of real evapotranspiration were between 6.1-8.4 mm, in most of Muntenia, Oltenia, Dobrogea and Moldova, isolated west of Crișana (Figure 13).

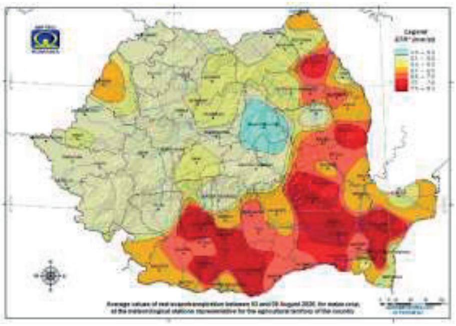


Figure 13. Average real evapotranspiration (ETR), non-irrigated maize crop, 03-09 August 2020

The comparative analysis of the values of potential evapotranspiration (ETP) with the average monthly rainfall regime, from September 1, 2019 to August 31, 2020, shows that water consumption by evapotranspiration frequently exceeded the amount of precipitation recorded in September 2019, October 2019, January 2020, March 2020, April 2020, May 2020, June 2020, July 2020 and August 2020, except for November 2019, December 2019 and February 2020, where the monthly average of precipitation exceeded the evapotranspiration values (Figure 14).

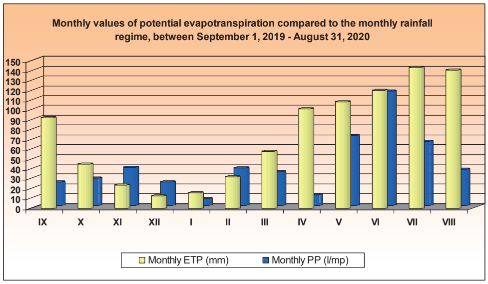


Figure 14. Potential evapotranspiration September 1, 2019 and August 31, 2020

CONCLUSIONS

Evapotranspiration in agricultural crops is represented by water losses (unproductive) by evaporation from the soil surface and consumption (productive) by transpiration of plants.

The study was performed by daily monitoring of the evolution of the calculated potential evapotranspiration (ETP), as well as the real evapotranspiration (ETR) performed in winter wheat and non-irrigated maize crops for soil depths 0-20 cm, 0-50 cm and 0-100 cm, in weekly and monthly intervals during the agricultural year 01 September 2019 - 31 August 2020.

For this purpose, the potential of the agro-climatic resources available for agriculture at the level of the country's agricultural territory was analyzed and agrometeorological data were processed based on the values registered at the meteorological stations with agrometeorological program in Romania.

For the spatial zoning of the average real evapotranspiration (ETR), the monthly maps were represented, during the entire agricultural year for each of the two crops analyzed.

August was characterized by deficient rainfall in most parts of the country, moisture reserve on the soil depth 0-100 cm, in the non-irrigated maize crop, showing low and particularly low values (moderate, strong and extreme pedological drought), especially in the south, east and south-east of the country, resulting in high ETR values in these areas.

In this study we tried to highlight the importance of evapotranspiration (potential and real) in assessing the water consumption of plants, this being an essential parameter, with a particularly important impact on the crops obtained.

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SELECTIVITY AND EFFECTIVENESS OF HERBICIDES APPLIED TO CHICKPEAS CULTURE UNDER THE CONDITIONS OF S.C.D.A. TELEORMAN

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Abstract

Chickpea (Cicer arietinum L.) is known to be sensitive to many chemicals and therefore the use of herbicides for the control of dicotyledonous weeds is limited. The research was conducted in 2019-2020 at A.R.D.S. Teleorman, with different applied herbicide associations being studied. The research focused on the selectivity and effectiveness degrees for the control of monocotyledonous and dicotyledonous, annual and perennial weeds, by applying combined herbicides treatments. From the analysis of the 12 variants, the experimental variant with the association of herbicides Gardoprim Plus Gold 500 SC (4.0 l) + Merlin Flex (0.3 l). Applied during pre-emergence phase, this solution registered a higher efficiency, of 96-99%, for both monocotyledonous (Avena fatua; Echinochloa crus-galli; Setaria spp.) and dicotyledons (Amaranthus retroflexus; Chenopodium album; Chenopodium polyspermum; Hibiscus trionum; Polygonum convolvulus; Sinapis arvensis) weeds. In the embodiments Gardoprim + Merlin 500 SC Plus Gold Flex Flex Leopard 50 EC + Merlin, there is a chemical weed control that significantly exceeded, provided statistically the untreated control and with production increases between 115-246 kg/ha.

***Key words**: chickpeas, herbicides, weeds, control, efficacy.*

INTRODUCTION

Chickpeas (*Cicer arietinum* L.) is considered a crop with low labor consumption, and its production requires fewer inputs compared to cereals.

Chickpeas are widely grown around the world and serve as a multifunctional crop.

It plays a significant role in improving soil fertility by fixing atmospheric nitrogen. After harvesting, it leaves a substantial amount of residual nitrogen in the soil for the forerunner cultures and adds an amount of organic matter to maintain and improve soil health and fertility.

Saves the cost of entering fertilizers, not only for chickpeas, but also for subsequent cultures.

In the period 1990-2012, chickpeas had a slight recovery, being cultivated on areas that reached up to about 10 thousand ha, mainly in the south of the Romanian Plain, Banat, Moldova and Dobrogea, the most areas being found in the culture structure of agricultural research stations (Sturzu et al., 2012).

It is known that chickpeas (*Cicer arietinum* L.) are sensitive to many herbicides and therefore the options for using herbicides for weed control are limited.

Ratnam et al. (2011) conducted a study between 2006-2007 and 2008-2009 at RARS, Lam Farm, Guntur, to find out the most suitable integrated practices for weed control in chickpeas. The results indicated that weed control treatments significantly reduced the density and dry weight of weeds in chickpeas culture. Post-emergence application of imazetapir 63 g/ha caused 20% damage to chickpeas plants.

In Romania, chickpeas are a "niche" culture, whose proportion in the structure of agricultural plants is low, but increasing, due to its advantages: the crop is made with relatively low costs, obtains economic productions, having a high content of protein substances (23.6-31.2%).

Agricultural systems that include legumes increase soil fertility and prevent erosion and desertification, are of major interest in many

countries around the world, leading to increased plant productivity (Egamberdieva et al., 2014).
Increasing the yield in chemical control may depend on the appropriate use of moisture, nutrients, light and sowing density of chickpeas, in the absence of competition with weeds, as shown by experimental data obtained by Arya (2004) and Patel et al., 2006.
The best weed control in chickpeas was obtained by applying pendimethalin and trifluralin (Hassan et al., 1995).
Another study shows that weeds produce a loss of chickpea yield of 40-90% (Solh and Pala, 1990).
Simultaneous and rapid growth of weeds with chickpeas plants leads to severe competition for light, moisture, space and nutrients, which leads to a drastic reduction in yield. Production losses ranged between 40 and 94% (Bhan and Kukula, 1987).
To date, not enough weed control strategies have been found, so the main purpose of the research is to establish and extend weed control strategies in chickpeas culture, by selecting and testing an assortment of selective herbicides with increased effectiveness, environmentally friendly.
Weeds competition affects space, air, water, light and nutrients in culture plants. Moreover, they increase production costs, favour the attack of pests and plant diseases, decrease the quality of agricultural production and reduce soil fertility. Harvest losses are due to weeding (45%), pest attack (30%), disease attack (20%) and other causes (5%) (Rao, 1983). Effective control increased chickpeas production by 97%, and the first 4-6 weeks after sowing were the most critical period for competition in weed control (Ahlawat et al., 1981).
Singh and Sharma reported in 2013 that pendimethalin is an effective herbicide applied in pre-emergence (0.50-0.75 kg/ha) to control annual dicotyledonous weeds in chickpeas culture. They also reported that the application of oxyfluorfen (0.25 kg/ha) in pre-emergence is effective for the management of dicotyledonous weeds, especially for *Medicago* sp. from central India. The reduction of chickpea production due to the presence of weeds in proportion of 75% was also observed by Chaudhary et al. (2005).
Whish et al. (2002) highlighted the fact that with the increase of weed density, a loss of chickpea production and a decrease of production components were reported. Even low weed densities (<10 m^2 plants) caused a large reduction in production (by about 50%) and higher production losses at longer distances between plant rows.

MATERIALS AND METHODS

The research took place in the years 2019-2020, at A.R.D.S. Teleorman, being studied the combinations and associations of herbicides applied to chickpea culture. The experiment was located on a soil of chernozem vertric type with good fertility (over 3.1% humus, clay content over 42% in the horizon 0-24 cm, pH -> 5.9), using the variety of chickpeas Burnas. The forerunner plant was wheat. The experiment was performed according to the method of randomized blocks, with a plot area of 25 m^2, in four repetitions.
The calculation and interpretation of the results was made based on the analysis of the variance of the experiments placed in the subdivided plots (Săulescu and Săulescu, 1967).
Research has shown the degree of selectivity and effectiveness in controlling annual and perennial monocotyledonous and dicotyledonous weeds by applying herbicide treatments in combinations and combinations (Table 1).
Good seed germination and the appearance of plants are important preconditions for a successful cultivation, and soil temperature and humidity are important factors for their achievement in optimal parameters.
In optimal conditions of humidity and temperature, chickpea seeds quickly absorb water and germinate in a few days, provided that the temperatures are > 0°C. Chickpeas will not germinate in soils below 0°C.
In terms of water, in 2019, chickpeas benefited from 376.6 mm of rainfall over the entire vegetation period, being 76.6 mm more than the crop's requirements for humidity, but their distribution was unfavourable to chickpeas.

Table 1. Experimental variants in chickpea culture. ARDS Teleorman, 2019 - 2020

No. variant	Active substance content	Dose g.s.a./ha	Herbicide treatment	Period of application
V1	Unhoeing	-	Control – untreated	
V2	2 mechanical hoeing	-	Untreated control 2 mechanical hoeing	
V3	50 g/l quizalofop-p-ethyl isoxaflutole 240 g/l Cyprosulfamide (safener): 240 g/l	1.20 l/ha 0.15 l/ha	Leopard 50EC + Merlin Flex	Postem.
V4	960 g/l S-Metolachlor isoxaflutole 240 g/l Cyprosulfamide (safener) 240 g/l	1.50 l/ha + 0,20 l/ha	Dual Gold 960EC+ Merlin Flex	Preem.
V5	960 g/l S-Metolachlor isoxaflutole 240 g/l Cyprosulfamide (safener) 240 g/l	1.20 l/ha + 0.30 l/ha	Dual Gold 960 EC + Merlin Flex	Preem.
V6	312.5 g/l S-metolachlor 187.5 g/l terbutylazine	4.0 l/ha	Gardoprim Plus Gold 500 SC	Preem.
V7	312.5 g/l S-metolachlor 187.5 g/l terbutylazine isoxaflutole 240 g/l Cyprosulfamide (safener): 240 g/l	4.0 l/ha + 0.20 l/ha	Gardoprim Plus Gold 500 SC + Merlin Flex	Preem.
V8	312.5 g/l S-metolachlor 187.5 g/l terbutylazine isoxaflutole 240 g/l Cyprosulfamide (safener): 240 g/l	4.0 l/ha + 0.300 l/ha	Gardoprim Plus Gold 500 SC + Merlin Flex	Preem
V9	312.5 g/l S-metolachlor 187.5 g/l terbutylazine pyridate 450 g/kg	4.0 l/ha + 1.0 l/ha + 1.0 l/ha	Gardoprim Plus Gold 500 SC + Lentagran+ Lentagran	Preem. Post. I Post. II
V10	960 g/l S-metolachlor aclonifen	1.5 l/ha + 2.5 l/ha	Dual Gold 960EC + Challenge 600 SC	Preem.
V11	960 g/l S-metolachlor 40 g/l imazamox	1.5 l/ha + 0.7 l/ha	Dual Gold 960EC+ Pulsar 40	Preem. Post.
V12	960 g/l S-metolachlor metribuzin 700 g/kg	1.5 l/ha + 0.3 l/ha	Dual Gold 960EC + Sencor 600 SC	Preem.

Thus, in the first part of the vegetation period the precipitations were quantitatively higher than the multiannual average with (+27.2 mm) in April, (+48.1 mm) in May and (+99.3 mm) in June. During the harvest formation period, there was an accentuated water deficit in July (-27.1 mm) and August (–47.2 mm), a month in which no precipitation was registered (Figure 2).

In 2020, there were excess rainfall in May (+7.8 mm) and June (+11.6 mm) and deficit in April (–21.8 mm), July and August (cumulative –92.9) mm), compared to the multiannual averages of the area (Figure 2).

In July, it can be said that the total drought was installed, only 2.8 mm of rainfall was recorded, the rainfall being practically absent, the deficit of the month being 58.6 mm, and in August 12.6 mm, of which 12.2 mm in the second decade, and the deficit was 34.4 mm.

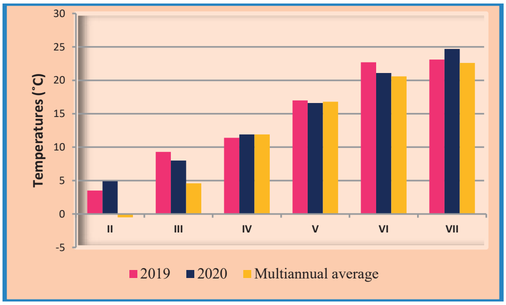

Figure 1. Evolution of average monthly temperatures at ARDS Teleorman 2019-2020

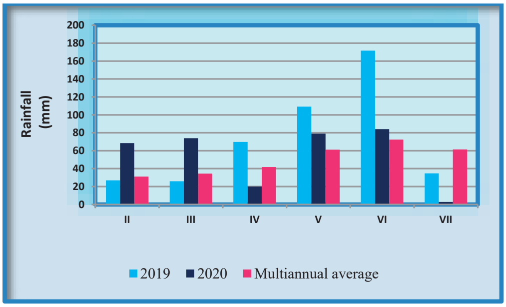

Figure 2. Evolution of rainfall at ARDS Teleorman 2019-2020

The abundant precipitations from May and June 2019, in the presence of relatively high temperatures, had an unfavorable influence on the foliar apparatus through the explosion of foliar diseases, implicitly reducing the assimilation surface of the plants. As a result, the yields obtained are low compared to the genetic capacity, the values of useful agronomic indicators (weight of 1000 seed and hectolitre mass) do not live up to expectations.
The herbicide treatments, mentioned in table 1, were applied: pre-emergent and post-emergent, in the stage of 10-12 cm height at the chickpea plant and the growth stage of monocotyledonous (BBCH 11-14) and dicotyledonous (BBCH 11-15) weeds.
After the application of herbicide treatments, observations were made on the degree of selectivity (%) at intervals of 7 - 14 - 28 days after treatment and the effectiveness of weed control (%) at the interval of 14 and 28 days after treatment.
During the research, specific determinations were made to establish the degree of weeding, weeding characteristics, effectiveness of tested control strategies and herbicide phytotoxicity.
Determinations regarding the weeding characteristics specific to chickpeas in the study area: determination of the annual and average weeding degree (numerical and gravimetric), for chickpeas cultivated in the ARDS Teleorman area.

RESULTS AND DISCUSSIONS

As part of the chickpea experiment located in the experimental field at A.R.D.S. Teleorman, the culture presented a high degree of infestation with annual monocotyledonous and dicotyledonous weeds, depending on the local pedoclimatic conditions, of the years 2019 - 2020.
The most common species of annual monocotyledonous weeds: *Echinochloa crus-galli*, *Setaria* spp., *Avena fatua* and annual dicotyledons: *Amaranthus retroflexus*, *Chenopodium album*, *Chenopodium polyspermum*, *Hibiscus trionum*, *Polygonum convolvulus*, *Sinapis arvensis*, *Stellaria media*, *Veronica* spp., *Xanthium italicum*, as well as perennials: *Cirsium arvense*, *Convolvulus arvensis*, *Sonchus arvensis* (Table 2).
The following problem species were identified on the basis of frequency: *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Setaria spp.*, *Amaranthus retroflexus*, *Chenopodium album*, *Solanum nigrum*, *Hibiscus trionum*, *Polygonum convolvulus*, *Sinapis arvensis*, *Stellaria media, Veronica spp.*, *Xanthium italicum*, *Cirsium arvense*, *Convolvulus arvensis*, *Sonchus arvensis.*
The causes that determined the appearance with frequency and high weight of the above weed species are related to: temporary excess of humidity, short rotations, etc.
The succession of culture and culture-specific technologies in a crop rotation is one of the most effective measures to control weeds, in close connection with the application of treatments.
From the comparative analysis of the average weeding present in the control plots of chickpeas, 14 days after the emergence of the plants, results the obvious role of the need to apply herbicides in weed control in chickpeas, very sensitive to weeds at this stage, through high competition weeds with chickpea plant (Table 2).

Table 2. The structure of the segetal flora from the chickpea culture from ARDS Teleorman, 2019 -2020

No crt	Species	Average pl/m^2		Participation %	
		2019	2020	2019	2020
1.	*Digitaria sanguinalis* (am)	1.0	18.5	0.8	14.7
2.	*Echinochloa crus-galli* (am)	18.0	31.7	14.1	25.2
3.	*Setaria* spp. (am)	18.5	14.5	14.5	11.5
4.	*Amaranthus retroflexus* (ad)	14.5	12.5	11.4	9.9
5.	*Chenopodium album* (ad)	31.7	3.0	24.9	2.4
6.	*Solanum nigrum* (ad)	2.7	1.2	2.1	1.0
7.	*Hibiscus trionum* (ad)	5.5	7.0	4.3	5.6
8.	*Polygonum convolvulus* (ad)	7.2	7.6	6.0	6.0
10.	*Sinapis arvensis* (ad)	0.7	5.5	0.5	4,4
11.	*Stellaria media* (ad)	1.4	1.2	1.1	1.0
12.	*Veronica* spp. (ad)	1.2	2.7	0.9	2.2
13.	*Xanthium italicum* (ad)	7.0	7.6	5.5	6.0
14.	*Cirsium arvense* (ad)	4.6	4.0	3.6	3.1
15.	*Convolvulus arvensis* (pd)	11.0	6.4	8.6	5.0
16.	*Sonchus arvensis* (pd)	2.2	2.4	1.7	2.0
	Total	**127.2**	**125.8**	**100**	**100**
	Annual monocotiles (am)	37.5	64.7	29.4	51.4
	Perennial monocotile	-	-	-	-
	Annual dicotyledonate (ad)	71.9	48.3	56.5	38.3
	Perennial dicotyledonate (pd)	17.8	12.8	13.9	10.1

If effective methods are not applied to control weed weeding in pre-emergence, as it increases, the weeding potential of the crop increases due to the development of annual and perennial monocotyledonous and dicotyledonous species present in chickpea culture.

Under these infestation conditions, the application of herbicides and herbicide combinations has shown good results in controlling the control of monocotyledonous and dicotyledonous weed species, depending on the degree of infestation, the spectrum and the dominance of the species present in the chickpea culture. The influence of climatic conditions (precipitation recorded before and after treatment).

During the experimentation period, the application of herbicide treatments shows a significant control of annual and perennial weed species, in the herbicidal variants, in accordance with the products used compared to the untreated control.

In the years 2019-2020 for the pre-emergence and post-emergence application (Tables 3 and 4) of the new combinations and combinations of herbicides, tested in the experimental field in chickpea culture, the results obtained 14 days after treatment showed superior efficacy (95-99%) for some monocotyledonous weeds (*Digitaria sanguinalis*, *Echinochloa crus-galli*, *Setaria* spp.) and annual dicotyledons (*Amaranthus retroflexus*, *Chenopodium album*, *Solanum nigrum*, *Hibiscus trionum*, *Polygonum convolvulus*, *Sinapis arvensis*, *Stellicum X*, as.). Exceptions are the annual dicotyledonous weeds *Amaranthus retroflexus*, *Xanthium strumarium*, *Chenopodium album*, *Sinapis arvensis*, *Solanum nigrum*, which showed a lower control effect in the variants treated with the herbicides Dual Gold 960 EC + Sencor 600 SC and Dual Gold 960 EC + P, these registering a lower control effect and the presence of phytitoxicity in the chickpea plant.

In the experimental version treated with the herbicide combination: Gardoprim Plus Gold 500 SC (4.0 l/ha) + Merlin Flex (0.3 l/ha) a superior control efficiency of 96-99% was obtained, both for monocotyledonous weeds and dicotyledonous, annual and perennial.

Analyzing the average results obtained 28 days after treatment, on the effectiveness of the herbicides used (Tables 3 and 4), an optimal level of weed control is found, depending on the control spectrum specific to each product. The Leopard 50 EC variant 1.2 l/ha postem + Merlin Flex 0.150 l/ha, applied post-emergence, achieves a high efficacy on dicotyledonous weeds, with a very good degree of control of (92-98%).

Average results obtained for *Amaranthus retroflexus* species, *Chenopodium album*, *Solanum nigrum*, *Hibiscus trionum*, *Polygonum convolvulus*, *Sinapis arvensis*, *Stellaria media*, *Veronica* spp., *Xanthium italicum*, were low (68%), on average over the experimental years in some combinations and combinations, and in the experimental variants treated with the combination of herbicides, Gardoprim Plus Gold 500 SC (4.0 l/ha) + Merlin Flex (0.3 l/ha) pre-emergent and Leopard 50 EC 1.2 l/ha postemergent + Merlin Flex 0.150 l/ha postemergent, a superior control efficiency was registered.

Table 3. Biomass of annual and perennial monocotyledonous and dicotyledonous weeds in chickpeas. ARDS Teleorman, 2019

No. field	The experimental variant	Total weight at harvest (kg/ha)				Monocotyledonous		Dicotyledonous	
						Green weight (kg/ha)	Dry weight (kg/ha)	Green weight (kg/ha)	Dry weight (kg/ha)
		Green weight (kg/ha)	Dry weight			at harvest	at harvest	at harvest	at harvest
			(kg/ha)	%	%				
V1	Control – Untreated	10192	2312	100	1895.1	4152	784	6040	1528
V2	Untreated control 2 - 2 mechanical hoeing	614	122	5.3	100	230	46	384	76
V3	Leopard 50 EC + Merlin Flex	2885	576	24.9	472.1	436	71	2449	505
V4	Dual Gold 960 EC + Merlin Flex	1644	322	13.9	263.9	90	12	1554	310
V5	Dual Gold 960 EC + Merlin Flex	1134	268	11.6	219.7	78	9	1056	259
V6	Gardoprim Plus Gold 500 SC	2247	472	20.4	386.9	71	61	2176	411
V7	Gardoprim Plus Gold 500 SC + Merlin Flex	1074	230	9.9	188.5	50	10	1024	220
V8	Gardoprim Plus Gold 500 SC + Merlin Flex	747	185	8.0	151.6	38	7	709	178
V9	Gardoprim Plus Gold 500 SC + Lentagran +Lentagran	1846	366	15.8	300.0	182	34	1664	332
V10	Dual Gold 960 EC + Challenge 600 SC	2039	441	19.1	361.5	252	50	1787	391
V11	Dual Gold 960 EC + Pulsar 40	2321	500	21.6	409.8	189	34	2132	466
V12	Dual Gold 960 EC + Sencor 600 SC	2682	587	25.39	481.1	330	54	2352	533

Table 4. Biomass of annual and perennial monocotyledonous and dicotyledonous weeds in chickpeas. ARDS Teleorman, 2020

No. field	he experimental variant	Total weight at harvest (kg/ha)				Monocotyledonous		Dicotyledonous	
						Green weight (kg/ha)	Dry weight (kg/ha)	Dry weight (kg/ha)	Dry weight (kg/ha)
		Green weight (kg/ha)	Dry weight			at harvest	at harvest	at harvest	at harvest
			(kg/ha)	%	%				
V1	Control 1– Untreated	9043	1834	100	2183.3	3943	800	5100	1034
V2	Untreated control 2 - 2 mechanical hoeing	416	84	4.6	100	80	36	336	48
V3	Leopard 50 EC + Merlin Flex	642	130	7.0	154.8	35	7	607	123
V4	Dual Gold 960 EC + Merlin Flex	1460	296	16.1	352.4	210	42.5	1250	253.5
V5	Dual Gold 960 EC + Merlin Flex	1230	233	12.7	277.4	70	14.0	1080	219
V6	Gardoprim Plus Gold 500 SC	1962	398	21.6	473.8	82	16.6	1880	381.3
V7	Gardoprim Plus Gold 500 SC + Merlin Flex	980	199	10.8	236.9	60	12.0	920	186.6
V8	Gardoprim Plus Gold 500 SC + Merlin Flex	536	109	5.9	129.8	26	5.2	510	103.4
V9	Gardoprim Plus Gold 500 SC + Lentagran + Lentagran	1330	270	14.7	321.4	130	26.3	1200	243.4
V10	Dual Gold 960 EC + Challenge 600 SC	1860	377	20.5	448.8	280	56.7	1580	320
V11	Dual Gold 960 EC + Pulsar 40	2260	458	24.9	545.2	60	12.0	2200	446
V12	Dual Gold 960 EC + Sencor 600 SC	2780	1258	68.5	1497.6	380	771	2400	486.8

The average results on the effectiveness of herbicide application obtained at the last evaluation (28 days after treatment) of the experimental variants Dual Gold 960 EC (1.5 l/ha) pre-emergent + Pulsar 40 (0.7 l/ha), showed a low level of control, which led to reinfestations and regenerations caused by the reserve of weed seeds in the soil and the presence of precipitation.

The efficacy of herbicide treatments applied at this time in the experimental version Gardoprim Plus Gold 50 SC (4.0 l/ha) pre-emergent + Lentagran (1.0 l/ha) postemergent I + Lentagran (1.0 l/ha) postemergent II was 89-95% (Table 5). The treatment was applied depending on the observance of the optimal phase of the chickpea plant, the weed species at the time of treatment, the dominance and spectrum of the two groups of weeds (annual and perennial monocotyledons and dicotyledons) and the zonal climatic conditions. During the research period, observations were made on the selectivity at 7 - 14 - 28 days from the treatments applied with combined and associated herbicides, to the chickpea culture. In the experimental variant Dual Gold 960 EC (1.5 l/ha) + Chellenge 600 SC (2.5 l/ha), the presence of phytotoxic phenomena manifested temporarily was observed (according to the EWRS scale = 0), for a period of 12-18 days, with stagnation from plant growth and loss of vigor. After 28 days from the emergence of chickpeas, the effectiveness of the tested herbicides was good and very good. Among the combinations of herbicides studied for weed control in chickpeas, the variant (V3) Leopard 50 EC (1.2 l/ha) + Merlin Flex (0.150 l/ha) applied in early post-emergence, together with the variant (V8) Gardoprim Plus Gold 500 SC (4.0 l/ha) + Merlin Flex (0.3 l/ha preem.), Which were appreciated as having very good efficacy, being close as a percentage of control.

Table 5. Degree of weed control according to the treatments applied. A.R.D.S. Teleorman, 2019-2020

No. Var.	Combat / dose variant		Sele-ctivit EWRS	Percentage of weeds controlled Effectiveness (%)			Note EWRS	Appre-ciation
				2019	2020	Average 28 days		
1.	Control – untreated		-	-	-	Mt	***9***	***NS***
2.	Untreated control 2 mechanical hoeing		-	92	94	93^{000}	***3.0***	***B***
3.	Leopard 50 EC + Merlin Flex	1.20 l/ha post 0.15 l/ha post	**1**	92	98	95^{000}	*3.0*	***B***
4.	Dual Gold 960EC+ Merlin Flex	1.5 l/ha preem 0.2 l/ha preem	**1**	94	98,4	96.2^{000}	***2.8***	***FB***
5.	Dual Gold 960 EC + Merlin Flex	1.2 l/ha 0.3 l/ha preem	**1**	95	97	96^{000}	***2.8***	***FB***
6.	Gardoprim Plus Gold 500 SC	4.0 l/ha preem	**1**	89	90	89.5^{000}	***3.7***	***B/S***
7.	Gardoprim Plus Gold 500 SC + Merlin Flex	4.0 l/ha preem 0.2 l/ha preem	**1**	91	93	92^{000}	***3.0***	***B***
8.	Gardoprim Plus Gold 500 SC + Merlin Flex	4.0 l/ha preem 0.3 l/ha preem	**1**	96	99	97.5^{000}	***2.5***	***FB***
9.	Gardoprim Plus Gold 500 SC + Lentagran + Lentagran	4.0 l/ha preem 1.0 l/ha post I 1.0 l/ha post II	**1**	89	95	92^{000}	***3.0***	***B***
10.	Dual Gold 960EC + Challenge 600 SC	1.5 l/ha preem 2.5 l/ha post	**2**	87	89	88^{000}	***3.7***	***B/S***
11.	Dual Gold 960EC+ Pulsar 40	1.5 l/ha preem 0.7 l/ha post	**4**	75	80	77.5^{000}	***4.5***	***NS***
12.	Dual Gold 960EC + Sencor 600 SC	1.5 l/ha preem 0.3 l/ha preem	**1**	86	87	86.5^{000}	***4.0***	***NS***

FB= very good effect; B = good effect; S = satisfactory effect; NS = unsatisfactory

For the comparative analysis of the effectiveness of the tested control variants, the averages of the control percentages recorded for all the observations during the chickpea vegetation were also calculated, with the help of which the combat degree ensured in each experimental combat variant tested can be defined (Table 5).

In 2019 and 2020, the most effective combination of herbicides was Gardoprim Plus Gold 500 SC 4.0 l/ha + Merlin Flex 0.3 l/ha, applied pre-emergently, 10 days after sowing the chickpeas. It had a very good efficacy, due to the precipitation that fell after sowing. In the years with less precipitation, as was the case in 2020, we can speak of a good efficiency in postemergence at the variant (V3) Leopard 50 EC 1.2 l/ha + Merlin Flex 0.150 l/ha.

The average yields obtained are closely correlated with the degree of weed control.

Thus, in 2019, in the case of pre-emergence control, the highest production levels were achieved for Gardoprim Plus Gold 500 SC + Merlin Flex treatments, with a very significant production increase, statistically assured, of 1089 kg / ha compared to Mt. 1 and 76 kg/ha compared to Mt. 2, not statistically insured, and in the case of post-emergence control at the treatments with Leopard 50 EC + Merlin Flex (V3), the increase was very significant, statistically assured, of 791 kg/ha compared to Mt.1 (Table 6).

In 2020, compared to Mt.1, all experimental variants had very significant production increases, statistically assured, and compared to Mt.2 very significant production increase, statistically assured (246 kg/ha) was obtained for the Gardoprim Plus variant Gold 500 SC + Merlin Flex (V8), in post-emergence treatments with Leopard 50 EC + Merlin Flex (V3) obtained a distinctly significant production increase (186 kg/ha). Significant production increase (115 kg/ha) compared to Mt.2 was obtained for Gardoprim Plus Gold 500 SC + Merlin Flex (V7) (Table 8).

Table 6. The influence of herbicide combinations on chickpea yields, SCDA Teleorman 2019

No. crt.	Variant experimental	Average yield kg/ha	Relative yield compared to Mt.1	Relative yield compared to Mt.2	The difference ±Mt.1	The difference ±Mt.2	Significance	
							compared to Mt.1	compared to Mt.2
1	Control –untreated	397	100	28.17	MT	-1013	-	000
2	Untreated control 2 mechanical hoeing	1411	355.03	100	1013	MT	***	-
3	Leopard 50 EC + Merlin Flex	1188	298.99	84.22	791	-223	***	000
4	Dual Gold 960 EC + Merlin Flex	1143	287.58	81.00	745	-268	***	000
5	Dual Gold 960 EC + Merlin Flex	1243	312.84	88.11	846	-168	***	00
6	Gardoprim Plus Gold 500 SC	1060	266.69	75.12	662	-351	***	000
7	Gardoprim Plus Gold 500 SC + Merlin Flex	1191	299.66	84.40	793	-220	***	000
8	Gardoprim Plus Gold 500 SC + Merlin Flex	1487	374.16	105.39	1089	76	***	-
9	Gardoprim Plus Gold 500 SC + Lentagran I + Lentagran II	1207	303.69	85.54	809	-204	***	000
10	Dual Gold 960 EC + Challenge 600 SC	1177	296.31	83.46	780	-233	***	000
11	Dual Gold 960 EC + Pulsar 40	400	100.59	28.33	2	-1011	-	000
12	Dual Gold 960 EC + Sencor 600 SC	851	214.09	60.30	453	-560	***	000

LSD 5% = 92.52 kg/ha LSD 1% = 126.11 kg/ha LSD 0.1% =169.49 kg/ha

Table 7. ANOVA for the influence of herbicide combinations on chickpea yields, ARDS Teleorman

The cause of variability	Analysis of variance			
	SP	GL	s^2	Test F
TOTAL	4.13	35		
Rehearsal	0.07	2		
Variants	4.00	11	3.999	69.98***(3.12)
Error	0.06	22	0.057	

Table 8. The influence of herbicide combinations on chickpea yields, SCDA Teleorman 2020

No. crt.	Variant experimental	Average yield kg/ha	Relative yield compared to Mt.1	Relative yield compared to Mt.2	The difference ± Mt.1	The difference ± Mt.2	Significance	
							compared to Mt.1	compared to Mt.2
1	Control –untreated	564	100	31.94	MT	-1201	-	000
2	Untreated control 2 mechanical hoeing	1765	313.07	100	1201	MT	***	-
3	Leopard 50 EC + Merlin Flex	1950	346.01	110.52	1387	186	***	**
4	Dual Gold 960 EC + Merlin Flex	1680	298.05	95.20	1116	-85	***	-
5	Dual Gold 960 EC + Merlin Flex	1761	312.36	99.77	1197	-4	***	-
6	Gardoprim Plus Gold 500 SC	1520	269.66	86.14	956	-245	***	000
7	Gardoprim Plus Gold 500 SC + Merlin Flex	1880	333.47	106.52	1316	115	***	*
8	Gardoprim Plus Gold 500 SC + Merlin Flex	2010	356.65	113.92	1447	246	***	***
9	Gardoprim Plus Gold 500 SC + Lentagran I + Lentagran II	1620	287.46	91.82	1057	-144	***	0
10	Dual Gold 960 EC + Challenge 600 SC	1710	303.43	96.92	1147	-54	***	-
11	Dual Gold 960 EC + Pulsar 40	870	154.29	49.28	306	-895	***	000
12	Dual Gold 960 EC + Sencor 600 SC	1280	227.14	72.55	717	-484	***	000

LSD 5% = 106.84 kg/ha LSD 1% = 145.62 kg/ha LSD 0.1% =195.71 kg/ha

Table 9. Analysis of variance for the influence of herbicide combinations on chickpea yields, ARDS Teleorman 2020

The cause of variability	Analysis of variance			
	SP	GL	s^2	Test F
TOTAL	7.19	35		
Rehearsal	0.02	2		
Variants	7.08	11	6.385	74.39***(3.12)
Error	0.09	22	0.086	

Figure 3. Plus Gold 500 SC (4.0 l/ha) + Merlin Flex (0.3 l/ha) - 14.05.2019

Figure 4. Untreated control 14.05.2019

Figure 5. Leopard 50 EC (1.2 l/ha) +Merlin Flex (0.150 l/ha)

Table 10. Comparative analysis of chickpea production in weed control variants. S.C.D.A. Teleorman, 2019–2020

No. var.	Combat variant	Average yields kg/ha		Classification	
		2019	2020	2019	2020
V8	Gardoprim Plus Gold 500 SC 4.0 l/ha preem + Merlin Flex 0.3 l/ha preem	**1487**	**2010**	**A**	**A**
V5	Dual Gold 960 EC 1.2 l/ha preem + Merlin Flex 0.3 l/ha preem	**1243**	**1761**	**AB**	**CD**
V9	Gardoprim Plus Gold 500 SC + 4.0 l/ha preem Lentagran + 1.0 l/ha post I + Lentagran 1.0 l/ha post II	**1207**	**1620**	**BC**	**D**
V7	Gardoprim Plus Gold 500 SC 4.0 l/ha preem + Merlin Flex 0.2 l/ha preem	**1191**	**1880**	**CD**	**BC**
V3	Leopard 50 EC 1.2 l/ha postem + Merlin Flex 0.150 l/ha postem	**1188**	**1950**	**D**	**AB**

CONCLUSIONS

The combined herbicides and the association of herbicides, applied pre-emergence and post-emergence to the chickpea culture had a good control effect, highlighting their effectiveness by a single application only, in some variants.

Combined herbicides Gardoprim Plus Gold 500 SC 4.0 l/ha + Merlin Flex 0.3 l/ha applied pre-emergence save the weed culture, with maximum efficiency and very good persistence, by reactivating with rainfall after sowing.

The effectiveness of herbicide application depends on the level of infestation, dominance, weed spectrum, applied dose and climatic conditions, having a high productivity by applying the two mechanical hoeing.

Regarding the effectiveness of weed control in chickpeas, the Leopard 50EC variant (V3) (1.2 l/ha) + Merlin Flex (0.150 l/ha) applied in early post-emergence together with the variant (V8) Gardoprim Plus Gold 500 SC (4.0 l/ha) + Merlin Flex (0.3 l/h preem.), were appreciated as having very good efficacy, being very close as a control percentage registering a superior efficacy of 95-97%, both for weeds monocotyledons (*Echinochloa crus-galli*, *Setaria* spp.) and dicotyledons (*Amaranthus retroflexus*, *Chenopodium album*, *Solanum nigrum*, *Hibiscus trionum*, *Polygonum convolvulus*, *Sinapis arvensis*, *Stellaria media*, *Veronica* spp., *Xanthium italic*, *Sonchus arvensis*), as well as for the resistant ones (perennial dicotyledons: *Convolvulus arvensis*, *Sonchus arvensis*).

When applying the Gardoprim Plus Gold 500 SC + Merlin Flex herbicide combination, a chickpea production of 2010 kg/ha and a very significant increase of 246 kg/ha are obtained, compared to the control with the two mechanical hoeing, in 2020.

The productions made after the application of herbicides are in direct correlation with the degree of weed control, being differentiated according to the degree of infestation, and the effectiveness of herbicide combinations and combinations, have achieved a degree of control of 95-99%, being selective for chickpeas plants.

In the two years of research, the most effective combination of herbicides was Gardoprim Plus Gold 500 SC 4.0 l/ha + Merlin Flex 0.3 l/ha, applied pre-emergently, 10 days after sowing the chickpeas, which were effective very good, due to the precipitation that fell after sowing.

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BEHAVIOR OF SOME EXPERIMENTAL SUNFLOWER HYBRIDS IN DIFFERENT LOCATION

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Abstract

In year 2020, we tested experimental sunflower hybrids at herbicide Pulsar Plus and Express 50SG in three locations, Fundulea1, Fundulea2 and Braila for resistance at biotic factors. Oil content was between 41.24% at H4CL+, treated with herbicide Pulsar Plus and 53.54% at H3CL+, treated with herbicide Pulsar Plus. Seed yield of sunflower hybrids, was between 2258 kg/ha at H4CL+, treated with herbicide Pulsar Plus and 3975 kg/ha at H7E, treated with herbicide Express 50SG. Hectolitre weight (kg/100 l) was between 62.8 kg/hl at H6E, treated with herbicide Express 50SG and 69.1 kg/hl at H5CL+, treated with Pulsar Plus. In location Fundulea 1, sunflower plants infected with pathogen Plasmopara hastedii, was between 0% at sunflower hybrid H3CL+, and 17.5% at sunflower hybrid H4CL+. In location Fundulea 2, sunflower plants infected with pathogen Plasmopara hastedii, was between 4.81% at sunflower hybrid H3CL+, and 73.7% at H5CL+. In Braila location, in year 2020, sunflower plants infested with broomrape, was between 0%, at sunflower hybrid H3CL+ treated with herbicide Pulsar Plus and 100% untreated with herbicide.

***Key words**: sunflower, herbicide, downy mildew, broomrape, resistance.*

INTRODUCTION

To register a sunflower hybrid, it must be tested before in different condition of medium to see his behavior, agronomic traits, resistance at abiotic and biotic factors**.** The time to obtain a sunflower hybrids is 7-8 years and after that is tested for another three years at The State Institute for Testing and Registration of Varieties (I.S.T.I.S.) and after that is register in National Official Catalog.

Sunflower hybrids must have uniformity, stability, distinctiveness and value added per unit, to be tolerant at new downy mildew races, tolerant at broomrape races G, H, tolerant at verticillium wilt, rust, resistant to lodging, must have a high seed yield, a high oil content, resistant a herbicide imidazolinone in ClearField plus technology or at herbicide sulfonilureea in ExpessSun technology (Delchev, 2019; Stanciu et al., 2019; Risnoveanu et al., 2019).

MATERIALS AND METHODS

In year 2020, we tested three experimental sunflower hybrids H3CL+, H4CL+ and H5CL+ in ClearField plus technology and two experimental sunflower hybrids H6E and H7E, in ExpessSun technology.

These sunflower hybrids, were sowing in micro parcels of four rows, with seven meters long, in three replications in three location, Fundulea 1, Fundulea 2, in South Romania and Braila, in South East Romania. Sowing date at Fundulea 1 and Fundulea 2, was April 29, 2020 and at Braila, was May 12, 2020 (Table 1).

Table 1. Sunflower hybrids tested in three locations, in year 2020, for resistance at biotic factors

Sunflower hybrid	Location		
	Fundulea 1	Fundulea 2	Brăila
H3CL+	Date of herbicide treatment 11.05.2020	Date of herbicide treatment 11.05.2020	Date of herbicide treatment 15.06.2020
H4CL+			
H5CL+			
H6E			
H7E			

All seeds of sunflower hybrids was treated with fungicide Apron XL (2 ml/kg) with active substance metalaxil-M against downy mildew from all three locations.

Sunflower hybrids H3CL+, H4CL+ and H5CL+, was sprayed in phenophase of six true leaves, with herbicide Pulsar Plus, who have active substance imazamox 25 g/l (Arda and Alyürük, 2020), in concentration of 1.6 l/ha.

Sunflower hybrids H6SU and H7SU, was sprayed in phenophase of six true leaves, with herbicide Express 50SG, who have active substance 50% tribenuron methyl (Christov and Hristova-Cherbadzhi, 2020), in concentration of 30 g/ha.
At sunflower hybrids tested in all three locations, in year 2020, was made notation about sunflower downy mildew, broomrape, the oil content was analyzed with Oxford Instruments Magnetic Resonance.

RESULTS AND DISCUSSIONS

The 58 mm precipitation, recorded in May, in 2020, in the Fundulea location, was ideal for the development of the pathogen *Plasmopara hastedii* who causes sunflower downy mildew. The precipitations registered in 2020, in Fundulea, during the period of sunflower development, from April to August, were lower than the average of 60 years (Figure 1).
In 2020, in the Fundulea location, the amount of precipitation was 423.2 mm and the average amount of precipitation over 60 years was 584.3 mm.

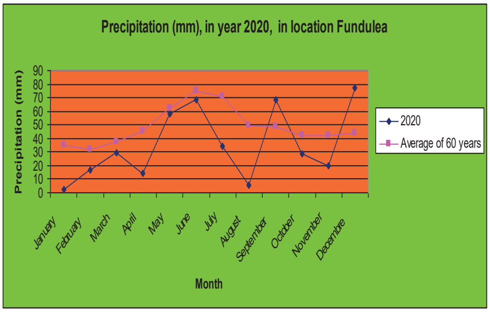

Figure 1. Precipitations (mm) registered in year 2020, in location Fundulea

The temperature of 17°C, recorded in May, in 2020, in the Fundulea 1 location, was ideal for the development of the pathogen *Plasmopara hastedii*. The temperature recorded in year 2020, in Fundulea 1, was higher than the average of 60 years (Figure 2).
In 2020, in the Fundulea location, the average annual temperature was 13.5°C and the average multiannual temperature for 60 years was 10.9°C

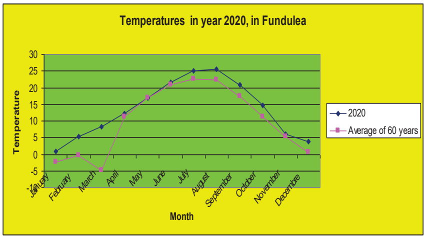

Figure 2. Temperature (°C) registered in year 2020, in location Fundulea

In Fundulea 1 location, was used six differential lines (Table 2), for identifying races of downy mildew present in this area.

Table 2. Behaviour of differential lines in Fundulea 1 location, in year 2020

Number	Differential lines for downy mildew	*Pl* genes	Sunflower plants with downy mildew/Total sunflower plants
D3	RHA274	Pl_2/Pl_{21}	0/13
D4	PMI3	Pl_{PMI3}	20/72
D4	DM2	Pl_5	5/30
D7	HAR-4	Pl_{15}	0/2
D8	QHP1	Pl_1/Pl_{15}	10/18
D9	HA335	Pl_6	3/9
D17	RHA340	Pl_8	4/18

(Differential lines after Gascuel et al, 2015; Gilley, 2017)

Differential line PMI3, is sensible at downy mildew races 314, 330, 334, 710, 714, 717, 730, 774. Differential line DM2, is sensible at downy mildew races 314, 710, 714, 730, 774. Differential line QHP1, is sensible at downy mildew races 307, 703, 707, 717. Differential line HA335, is sensible at downy mildew races 304, 307, 314, 334, 704, 707, 714 717, 774. Differential line RHA340 is sensible at downy mildew races 700 and 710 (Gascuel et al, 2016; Türkmen and Çalışkan, 2016).
In Fundulea location is identifying downy mildew races 304, 314, 330, 334, 710, 714.
In location Fundulea 1, sunflower plants infected with pathogen *Plasmopara hastedii*, was between 0% at sunflower hybrid H3CL+, and 17.5% at sunflower hybrid H4CL+ (Table 3).
In location Fundulea 2, sunflower plants infected with pathogen *Plasmopara hastedii*, was between 4.81% at sunflower hybrid H3CL+, and 73.7% at H5CL+ (Figure 3).

Table 3. Sunflower plants infected with pathogen *Plasmopara hastedii*, in three location, in year 2020

Sunflower hybrid	Fundulea 1	Fundulea2	Brăila
H3CL+	0%	4 ,81%	0 %
H4CL+	17.5%	72 %	0 %
H5CL+	14.47%	73.7%	0 %
H6SU	4.28%	55 %	0 %
H7SU	6.66%	73.4%	0 %

Figure 3. Aspects from location Fundulea 2, with the highest infection with pathogen *Plasmopara halstedii*

In locations Brăila, sunflower plants infected with pathogen *Plasmopara hastedii* was 0%, at all five sunflower hybrids.

In Braila area (Figure 4), is the most virulent races of broomrape (races G and H), who causes low seed yield (Risnoveanu et al, 2016 a, b).

Figure 4. Aspects from location Braila, with the highest infestation with parasite *Orobanche cumana*

In Brăila location, in year 2020, sunflower plants infested with broomrape caused by parasite *Orobanche cumana*, was between 0%, at sunflower hybrid H3CL+ treated with herbicide Pulsar Plus (1.6l/ha) and 100% at sunflower hybrids H3CL+, untreated with herbicide (Table 4).

Table 4. Sunflower plants infested with parasite *Orobanche cumana*, in Brăila location, in year 2020

Sunflower hybrid	Treated with herbicide Pulsar Plus		Untreated with herbicide	
	Infested plants with broomrape (%)	The average number of broomrape stalks per sunflower plant	Infested plants with broomrape (%)	The average number of broomrape stalks per sunflower plant
H3CL+	0	0	100	1.2
H4CL+	6	1.4	43	1.1
H5CL+	3	1	63	1
Sunflower hybrid	Treated with herbicide Express SG50		Untreated with herbicide	
	Infested plants with broomrape (%)	The average number of broomrape stalks per sunflower plant	Infested plants with broomrape (%)	The average number of broomrape stalks per sunflower plant
H6SU	100	1.8	100	1.3
H7SU	31	1.2	30	1.2

In the Fundulea 1 and Fundulea 2 locations, no broomrape was observed.

Oil content, was between 41.24% at H4CL+, treated with herbicide Pulsar Plus and 53.54% at H3CL+, treated with Pulsar Plus, in location Fundulea 1 (Table 5).

Table 5. Oil content of sunflower hybrids, in Fundulea 1 location, in year 2020

Sunflower hybrid	Oil content %	
	Treated with herbicide Pulsar Plus	Untreated with herbicide
H3CL+	53.54	45.61
H4CL+	41.24	45.44
H5CL+	41.77	44.70
Sunflower hybrid	Oil content %	
	Treated with herbicide Express SG50	Untreated with herbicide
H6SU	42.90	45.30
H7SU	42.80	44.90

The year 2020, with low rainfall, especially in the vegetation phase of seed filling, affected the seed yield of tested sunflower hybrid. Seed yield, was between 2258 kg/ha at H4CL+, treated with Pulsar Plus and 3975 kg/ha at H7SU, treated with herbicide Express, in location Fundulea 1 and was calculate at density of 48000 plants/ha and humidity of 5.8.

Regarding seed yield of sunflower hybrids treated with herbicide and untreated, there were no major differences (Table 6).

Table 6. Seed yield, of sunflower hybrids, in Fundulea 1 location, in year 2020

Sunflower hybrid	Seed yield (kg/ha)			
	Treated with herbicide Pulsar Plus		Untreated with herbicide	
H3CL+	73 plants / 3720g	2446 kg/ha	36 plants / 2190g	2920 kg/ha
H4CL+	71plants / 3340g	2258 kg/ha	73 plants / 3440g	2261 kg/ha
H5CL+	57plants / 3170g	2669 kg/ha	85 plants / 4740g	2676 kg/ha
Sunflower hybrid	Seed yield (kg/ha)			
	Treated with herbicide Express SG50		Untreated with herbicide	
H6SU	51plants / 3230g	3040 kg/ha	50plants / 3240g	3110 kg/ha
H7SU	32plants / 2650g	3975 kg/ha	36plants / 2980g	3973 kg/ha

Sunflower hybrids, in the Fundulea 1 location, in a field without irrigation and with low rainfall, recorded a lower seed yield than in a year with higher rainfall.

Hectolitre weight (kg/100L) was between 62.8 kg/hl at H6SU, treated with herbicide Express and 69.1 kg/hl at H5CL+, treated with herbicide Pulsar Plus, in location Fundulea 1 (Table 7).

Table 7. Hectolitre weight, of sunflower hybrids, in Fundulea 1 location, in year 2020

Sunflower hybrid	Hectolitre weight (kg/ 100L)	
	Treated with herbicide Pulsar Plus	Untreated with herbicide
H3CL+	68.6	61.6
H4CL+	62.8	69.6
H5CL+	69.1	73.3
Sunflower hybrid	Hectolitre weight (kg/ 100L)	
	Treated with herbicide Express SG50	Untreated with herbicide
H6SU	62.8	68.5
H7SU	63.8	67.7

CONCLUSIONS

All five sunflower hybrids behave differently in all three locations we tested. Regarding sunflower downy mildew, sunflower hybrid H3CL+ has a good genetic resistance in all three locations. In location Fundulea 2, was the biggest attack of pathogen *Plasmopara hastedii*. Regarding sunflower broomrape *(Orobanche cumana)*, sunflower hybrid H3CL+ who has incorporated in mother line gene *Or5 (*resistance at race F of broomrape), if is treated with herbicide Pulsar Plus can be cultivated in area infested with this parasite with race G, G+. In location Braila, was the biggest attack of parasite *Orobanche cumana* with race G and G+ of broomrape. Sunflower hybrid H3CL +, has the biggest oil content (53.54%) than other sunflower hybrids tested and can be send to The State Institute for Testing and Registration of Varieties (I.S.T.I.S.) to be tested for another three years.

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NUTRITIVE QUALITY OF CAMELINA VARIETIES WITH SPECIAL FOCUS ON OIL

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Abstract

The oil extracted from Camelina sativa seeds is underused and not well known, making the camelina crop a niche one. However, the oil is a potential source of omega-3, omega-6, and omega-9 fatty acids, so the crop has started to draw attention and occupy increasingly larger areas. This is owed to the multiple uses of the oil (in medicine, chemistry, energy), as well as the economic efficiency of the crop. The objective of this study was to determine which of the three different varieties, each one with different provenience and from different pedoclimatic conditions, has a better fatty acid ratio in the extracted oil. This study covers the results obtained in the laboratory research phase on a selection of three camelina varieties (Camelia, created by the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Calena, with Austrian origins and a local population by BUASVM Timisoara) regarding fatty acid content. The oil extraction and further determinations were made in the research platform of BUASVM Timisoara. Results showed that there are no major differences between the camelina varieties, with omega-3 fatty acid content ranging from 28.48% to 33.62%, omega-6 values between 21.28% and 23.62% and omega-9 between 30.11% and 31.64%, although their origin is from different.

Key words*: camelina, fatty acid profile, oil.*

INTRODUCTION

Camelina (*Camelina sativa* L. Crantz) or false flax is a crop from *Brassicaceae* family. In the last year crops have gained attention, especially due to their economic efficiency and multiple uses of the oil (medicine, chemistry, energy).

Speaking of economic efficiency, camelina does not have great requirements in matter of temperature and soil, as it is often grown on marginal land (Ehrensing et al., 2008). Furthermore, it is relatively easy to grow and requires few agricultural inputs compared to other crops (Ehrensing et al., 2008). This makes it a promising crop even in areas that are not generally favourable for agriculture.

Camelina's adaptation to vast areas of the world, combined with its unique oil composition and properties useful for the production of biofuels, jet fuel, bio-based products, feed and food and more recent, a potential medical use as novel antimicrobian has resurfaced interest in this ancient crop (Marisol, 2016; Bătrîna, 2021).

Different from other cultivated *Brassicaceae*, camelina has a unique seed oil composition (Vollmann & Eynck, 2015), with a high content of α-linolenic acid (20-35%), eicosenoic acid (11-19%) and tocopherols (Vitamin E) (Abramovič et al., 2007) as well as a naturally low content of the undesirable fatty acid erucic acid (<4%), rendering camelina oil well-suited for a variety of food, feed or non-food applications (Zubr, 1997; Faure, 2016; Zanetti, 2017).

The aim of this paper is to study the nutritive quality of three varieties of camelina, each from a different area, with different climate and of course different soil conditions. Increased emphasis is placed on oil content, more specifically on fatty acids and their percentage in oil composition for each of the varieties. It is important to mention that a similar fatty acid ratio for each variety, regardless of soil and weather conditions, would make Camelina a versatile plant, potentially laying a foundation for satisfactory crops and predictable, high quality outcomes.

MATERIALS AND METHODS

There were three different varieties of camelina seeds taken into observation. First was Calena variety, produced by Saatbau Linz in Austria and provided by a subsidiary here in Romania; the second is Camelia variety produced by University of Agronomical Sciences and Veterinary Medicine Bucharest and the third variety which is a local population of camelina from the Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara (BUASVMT), provided by Crop Science Department.

The oil extraction is the same as described by Domil (2015). In a brief description, camelina seeds (100 g) were ground in a coffee grinder and oil was extracted with hexane for 24 h in a Soxhlet apparatus. Hexane was removed by rotary evaporation (10 mbar, 30ºC). For determination of total oil content, 10.0 g triplicates of ground seed were extracted (Soxhlet) for 24 h, and after hexane was removed by rotary evaporation (10mbar, 30ºC) the weight of the residual oil was calculated. The percentage of oil recovered from the samples was around 39.8 wt. % (rM ± 0.5%), with slight differences between the three varieties. This percentage is in the same range as in other studies (for example in Imbrea et al., 2011, oil content varied between 38.7-42.5%).

Fatty acids of oils were derivatised in their methyl esters (FAMEs) before gas chromatography (GC) analyses using Boron trifluoride methanol complex 20%from Merck Germany. Further, 3 ml of 20% boron trifluoride solution was added to methylate 0.10 g of the samples. Derivatisation was performed in an ultrasonic bath at 80ºC for 1 hour. After cooling, 2.5 ml of 10% sodium chloride solution and 2 ml of hexane were added and the mixture was shaken. Organic phases were separated by centrifugation at 3000 rpm for 10 minutes and transferred to vials for GC analysis.

FAMEs were determined using a Shimadzu GCMS QP 2010Plus Apparatus (Shimadzu Corporation, Tokyo, Japan) with a mass Spectrometer detector (MS) and equipped with a capillary column AT-Wax (30 m x 0.32 mm x 1 μm). The injection volume was 1.0 μL, the temperature of the injection port was 250ºC. The carrier gas (Helium) flow rate was 1.8 ml min^{-1} and the split ratio was 1:10. Oven temperature was set at 110ºC (2 minutes) increasing with 8ºC/min to 250ºC. The final oven temperature was maintained 7.5 minutes. MS parameters (Ion Source and Interface Temperature) were set at 210ºC and 255ºC respectively. For confirmation of identified and determined FAMEs in oils, the NIST05 library and area normalization method was used. The results of FAs were expressed as percentages of total FAMEs.

RESULTS AND DISCUSSIONS

Camelina oil is rich in oleic acid (18:1) with values ranging between 14-16%, linoleic acid (18:2) between 15-23%, α-linolenic acid (18:3) between 31-40% and gondoic acid (20:1) between 12-15%. Other minor fatty acids include palmitic (16:0), stearic (18:0), and erucic acid (22:1) (Singh et al., 2014). Camelina seed oil composition varies with cultivar, location, environment and extraction method (Berti et al., 2016).

As shown in Table 1, the three camelina varieties used in this study were richest in oleic acid, linoleic acid, linolenic acid and gondoic acid. Other fatty acids found include palmitic, stearic, eicosadienoic, eicosatrienoic, arachidic and erucic acid. In between varieties, there were minimal differences regarding fatty acid content.

The saturated fatty acids (SFA) found in the oil extracted from all three varieties were: palmitic acid, stearic acid and arachidicc acid. Out of these, the highest concentration was that of palmitic acid: 9.53% for 7C/1, 9.77% for 7C/2 and 9.13% for 7C/3, followed by stearic acid with 3.46% highest value for 7C/1, 3.34% for 7C/2 and 3.26% for 7C/3 and finally arachidic with a maximum value of 1.32 for 7C/3 and a minimum value of 0.41 for 7C/1.

Table 1. Fatty acid content in the three studied varieties

	Fatty acid	7c/1	7c/2	7c/3
1	palmitic acid C16:0	9.53	9.77	9.13
2	stearic acid C18:0	3.46	3.34	3.26
3	acid oleic C18:1	17.04	17.60	17.14
4	linoleic acid C18:2	19.78	19.75	21.38
5	α-linolenic acid C18:3	33.28	30.86	27.07
6	arachidic acid C20:0	0.41	1.02	1.32
7	gondoic acid C20:1	13.61	13.32	14.46
8	eicosadienoic acid C20:2	1.61	2.02	2.31
9	eicosatrienoic acid C20:3	0.24	1.35	1.45
10	erucic acid C22:1	1.07	0.92	2.41
11	SFA	13.40	14.13	13.71
12	MUFA	31.72	31.74	34.01
13	PUFA	54.81	53.98	52.21

The extracted monounsaturated fatty acids (MUFA) were oleic acid with a maximum of 17.60% for 7C/2, gondoic acid peaking at 14.46% for 7C/3 and erucic acid with the highest value of 2.41% for 7C/3 and the minimum of 0.92% for 7C/2.

The extracted polyunsaturated fatty acids (PUFA) were: linoleic, α-linolenic, eicosadienoic, eicosatrienoic. The highest concentrations were as follows: α-linolenic 33.28% for 7C/1, eicosadienoic 2.31% for 7C/3 and eicosatrienoic 1.45% in 7C/3.

Out of the total quantity of oil that was extracted, Calena variety had the highest amount of PUFA (54,8%), with 31.72% MUFA and 13.4% SFA. In comparison, Camelia variety had just 1% less PUFA (53.98%), with 31.74% MUFA and 14.13% SFA. The BUASVMT variety had the lowest PUFA content (52.21%), which is 2% less than Calena, while yielding a slightly higher value for MUFA (34.01%) and similar SFA content (13.71%).

Table 2. Comparative data between presented study and Abramovič et al. (2005) and Zubr & Matthaus (2002) studies

Fatty acid	Fatty acid content				
	This study			Abramovič et al. (2005)	Zubr et al. (2002)
	7C/1	7C/2	7C/3		
palmitic acid 16:0	9.53	9.77	9.13	6.43 ± 0.01	5.3 - 5.6
stearic acid 18:0	3.46	3.34	3.26	2.57 ± 0.01	2.3 – 2.7
oleic acid 18:1	17.04	17.60	17.14	17.40 ± 0.30	14.0 – 16.9
linoleic acid 18:2	19.78	19.75	21.38	16.90 ± 0.10	13.5 - 16.5
α-linolenic acid 18:3	33.28	30.86	27.07	35.20 ± 0.40	34.9 - 39.7
arachidic acid 20:0	0.414	1.02	1.32	1.24 ± 0.05	1.2 - 1.5
gondoic acid 20:1	13.61	13.32	14.46	14.90 ± 0.20	15.1 - 15.8
eicosadienoic acid 20:2	1.61	2.02	2.31	2.12 ± 0.02	1.7 - 2.0
eicosatrienoic acid 20:3	0.24	1.35	1.45	1.61 ± 0.03	1.3 - 1.7
erucic acid 22:1	1.07	0.92	2.41	1.62 ± 0.03	2.6 - 3.0

Legend: 7C/1 = Calena oil; 7C/2 = Camelia oil; 7C/3 = BUASVMT local camelina variety

At a first glance all results appear to be similar. Resemblances stand out especially in between the varieties included in this study, while the amount of fatty acids obtained in this study are akin to those obtained by Abramovič et al. in 2005 and Zubr & Matthaus in 2002.

Out of all the fatty acids, a-linolenic acid was obtained in the highest quantities both in this study (33.28% in the Camelia variety), as well as in the studies of Abramovič et al. in 2005 (35.2%) and Zubr & Matthaus in 2002 (between 34.9-39.7%). These numbers, more specifically their similarity, may bear significant importance for future studies, as it is known that a-linolenic content is influenced by environmental conditions (Zubr & Matthaus, 2002). It is essential to remind that two of the varieties used in this study were of Romanian origin and one was of Austrian origin, while the study carried out by Zubr & Matthaus was in Denmark and the study of Helena Abramovic took place in Slovenia, considerably closer to the Mediterranean Basin.

Other notable similarities between results include the percentage of oleic acid (about 17% in all varieties of this study, respectively an average of 17.4% obtained by Abramovič et al. (2005). The case is similar for gondoic acid, with a maximum of 14.46% in the BUASMVT variety, comparable to 14.9% (Abramovič et al., 2005) and 15% (Zubr & Matthaus, 2002).

Arachidic acid results were approximately 1% or a little above this value in most of the measurements: 1.02% 7C/2, 1.32 7C/3, comparable to 1.24% (Abramovič et al., 2005) and 1.2-1.5% (Zubr & Matthaus, 2002). There was a significant difference between these figures and the concentration of arachidic acid found in the oil extracted from AT, a mere 0.414%.

However, there are some subtle differences in the results that are worth mentioning. The highest difference can be seen in linoleic acid content, with an approximate difference of 3-4% between the highest values of this study and the results obtained by Abramovic. Another difference can be seen in the content of palmitic acid, with values above 9% in all three varieties taken under observation and in the studies chosen as comparison a mere 6.43% (maximum for Abramovic). The same situation goes for stearic acid, which can be found in more than 3.25%, which is almost 1% higher than the content found by Abramovic or Zubr.

As far as the practical uses of these oils are concerned, a distinction must be made between human consumption and industrial use.

Erucic acid is known to be potentially harmful if present in food (*Zealand, F. S. A. N.*), therefore the lowest possible content in oil is desirable. All three Camelina variants included in this study have shown a reduced content of erucic acid (0.92-2.41%), more specifically below the limits imposed by EU regulations (*The Commission of the European Communities (1980). "Commission Directive 80/891/EEC of 25 July 1980 relating to the Community method of analysis for determining the erucic acid content in oils and fats intended to be used as such for human consumption and foodstuffs containing added oils or fats". EurLex Official Journal. p.254*; *U.S. Dept. of Health and Human Services, CFR - Code of Federal Regulations Title 21 1 April 2010*). As a result, Calena oil, Camelia oil and BUASVMT local camelina variety are all sources of oils that are suitable for use in the food industry.

In order to be appropriate for biokerosene production, oils with an increased presence of saturated fatty acids have shown a proportional increase in both CFPP and biofuel viscosity (Golimowski et al., 2017). CFPP or cold filter plugging point is the minimal temperature expressed in degrees Celsius at which liquid fuel plugs the filters through which it must pass, and it is preferable to have it as low as possible. An inversed proportionality was observed in oils high in unsaturated fatty acids, therefore biokerosene obtained from oils rich in monounsaturated fatty acids and polyunsaturated fatty acids will have a lower CFPP and will be less viscous – more appropriate for use in this situation. The oil from camelina seeds contains over 50% polyunsaturated essential fatty acids, particularly linoleic and alpha-linolenic acids. Camelina oil is 10 times richer in these acids than many other vegetal oils (Putnam, 1993; Tabără, 2007; Bătrîna, 2020). Out of the three camelina varieties included in this study, Calena had the highest content of mono- and polyunsaturated acids combined (approximately 86%), making it the most suitable for biokerosene production.

CONCLUSIONS

There are no major differences between the three camelina varieties included in this study, although they were cultivated in different climates. Our results were similar to those obtained by Abramovic et al. (2005) and Zubr & Matthäus (2002), with the most notable differences in linoleic acid content, by about 3-4%, followed by stearic and palmitic acid.

This study of the oil content ultimately has its purpose in determining potential practical uses. Due to a reduced content of erucic acid, all three varieties yielded oil that is appropriate for human consumption, according to EU and US regulations. In terms of industrial use for the production of biokerosene, a high content of mono- and polyunsaturated fatty acids is desirable, thus making the Calena variety the most appropriate out of the three.

All in all, the results of this study may provide an interesting starting point for further research, with oils having a fatty acid ratio with specific characteristics, based on their intended use.

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SIMILARITY ANALYSIS OF THE POLYPHENOLIC PROFILE TO SPONTANEOUS SPECIES OF THE *THYMUS* GENUS FROM BANAT (ROMANIA)

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Abstract

This study analyses the amount of total polyphenolic content (TPC) in dry plant materials collected from different populations of Thymus belonging to several spontaneous species from multiple regions of the Banat area. The aim of the research is to identify the quantitative differences of TPC in the thyme populations studied, regarding both the area and the species, or potentially subspecies. The TPC was determined with Folin-Ciocâlteu reagent using gallic acid as standard, and the concentration of ethanolic extracts was expressed in mg/g dry matter. The results obtained were statistically analysed using proximity matrices that indicate the similarity of the polyphenolic profile in the populations studied. The populations were classified clustered according to the similarity of the polyphenolic profile. The data obtained indicate significant differences in the polyphenolic profile at species and harvest area level. The possibility of using this indicator as a chemical fingerprint for the spontaneous thyme species from Banat was analysed.

***Key words**: Thymus, polyphenolic profile, wild populations.*

INTRODUCTION

Plants are an inexhaustible source of unique chemical compounds. In the last years there been was a growing need for plants used as a potential resource of new products with biological and medicinal properties. The species of the genus *Thymus* are recognized for the large amount of biologically active compounds. (Tohidi et al., 2019)

According to the latest data, about 6% of the superior plants were studied for their pharmacological potential and only 15% were evaluated for their phytochemical potential. (Cragg & Newman, 2013; Espinosa-Leal et al., 2018).

Biologically active chemical compounds are secondary metabolites in plants, chemical compounds produced by plants but which are not a condition for their growth and development. (Pickens et al., 2011) Secondary metabolites play an important role both ecologically and biologically in plant defense mechanisms often having oxidative and antimicrobial properties (Najafian, 2014; Rus et al., 2016). For humans, these compounds are of great interest, being used for various purposes since the 19th century. (Patwardhan, 2005; Cragg & Newman, 2013).

Natural antioxidants such as phenolic compounds can have significant antioxidant, anti-inflammatory and anti-proliferative properties; thus, plants can be used to prevent diseases, both as a spice and as medicinal preparations (infusions, decoctions, essential oils) (Leal et al., 2017; Rus et al., 2016; Ghitea et al., 2020).

The genus *Thymus* is taxonomically classified in the Lamiaceae Family (Labiatae), a group of medicinal and aromatic plants with a high level of polyphenolic compounds (Jovanović et al., 2017) this parameter being a characteristic of the chemical profile of species of this type (Raudone et al., 2017). It is proven that there is a direct connection between the content of polyphenols in plants and antioxidant activity with their curative effects. Polyphenols play an important role in protecting the body from the harmful effects of free radicals (Öztürk, 2015), as well as in reducing the viability of some cell lines involved in the emergence of three cancers (Martins-Gomes et al., 2018).

MATERIALS AND METHODS

The analyzed material, i.e. the above-ground vegetative part (herba), was harvested in 2018 and 2019 from several areas of Banat, located at different altitude (80-1430 m). 20 populations were analyzed, with individuals randomly selected within each population, at least 20 individuals (Table 1).
Plants were morphologically analyzed in the laboratory (phenotypic aspects). The determination was carried out with the help of specialized works (Guşuleac, 1961 in Flora României vol. VIII, Sârbu et al., 2013).
Specimen samples for each population were stored after identification in the herbarium of the Biology Department of the Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timișoara.

Table 1. Populations of the genus *Thymus* analyzed and their location

Thymus species	Sample name	Location	Altitude (m)	GPS coordinates (degree, minutes)	
Th. comosus	P1	Dobraia	890	N 44° 59'	E 22° 28'
Th. dacicus	P2	Lescovița	146	N 44° 52'	E 21° 32'
Th. dacicus	P3	Ostrov	119	N 44° 42'	E 21° 37'
Th. glabrescens	P4	Silagiu	191	N 45° 36'	E 21° 36'
Th. glabrescens	P5	Valea Iuți	119	N 44° 29'	E 22° 10'
Th. pannonicus ssp. auctus	P6	Pojejena	172	N 44° 47'	E 21° 35'
Th. pannonicus ssp. auctus	P7	Silagiu	192	N 45° 36'	E 21° 36'
Th. pannonicus ssp. auctus	P8	Tricule	82	N 44° 29'	E 22° 08'
Th. praecox ssp. janke	P9	Domogled	897	N 44° 53'	E 22° 25'
Th. praecox ssp. polytrychus	P10	Gozna	1.429	N 45° 10'	E 22° 03'
Th. praecox ssp. polytrychus	P11	Semenic	1.399	N 45° 11'	E 22° 04'
Th. praecox ssp. polytrychus	P12	Semenic	1.395	N 45° 11'	E 22° 04'
Th. pulegioides ssp. chamaedrys	P13	Carasova	593	N 45° 09'	E 21° 52'
Th. pulegioides ssp. chamaedrys	P14	Dobraia	924	N 44° 59'	E 22° 28'
Th. pulegioides ssp. chamaedrys	P15	Pojejena	194	N 44° 47'	E 21° 35'
Th. pulegioides ssp. montanus	P16	Carasova	511	N 45° 09'	E 21° 52'
Th. pulegioides ssp. montanus	P17	Pojejena	170	N 44° 47'	E 21° 35'
Th. pulegioides ssp. pulegioides	P18	Carasova	582	N 45° 09'	E 21° 52'
Th. pulegioides ssp. pulegioides	P19	Semenic	998	N 45° 13'	E 22° 04'
Th. pulegioides ssp. pulegioides	P20	Nermet	389	N 45° 14'	E 21° 53'

Sample preparation and determination of total phenolics content (TPC)
The extraction method used was conducted according to the Cocan et al., 2018 method with modification regarding extraction time and conditions. Sample of 0.5 g of herb was weighed accurately on analytical balance. 20 mL ethylic alcohol 70% (1:10, w/v) were added to the flask and vortexed for 2 min (Vortex Genie 2, Scientific Industries Portland, Oregon United States). The extracts obtained were filtered and stored at room temperature (24-25°C) until used. TPC was determined with Folin-Ciocâlteu reagent using gallic acid as standard (Dumbrava et al., 2020). Briefly, 0.5 mL of ethanolic extract was mixed with 1.25 mL of Folin-Ciocâlteu reagent (Merck, Germania) 1:10 diluted with distilled water. This mixture stands for 5 min at room temperature. Therefore, was added 1.0 mL of Na_2CO_3 (60g/L). That mixture stands for 30 min at 50°C in darkness. The absorbance at 750 nm was measured using a UV-VIS Analytic Jena Specord 205 Spectrophotometer in triplicate. TPC was calculated based on the standard curve of gallic acid, and results were expressed as milligrams of gallic acid equivalent per gram of extract (mgGAE/g extract).

Statistical methods used
Statistical analysis of experimental data has been carried out with IBM SPSS Statistics Version 21. Cluster analysis of polyphenolic profiles can be used to determine significant differences between the chemotypes of the different *Thymus* populations analyzed. *Thymus* populations were analyzed statistically and clustered according to the similarity of the polyphenolic profile, using the function

"Hierarchic cluster Analysis". The method used is "between-groups linkage" and the distance is the Euclidean one. On the basis of these distance coefficients, UPGMA dendrograms were generated for cluster analysis of polyphenolic profiles in the populations studied. Average values, standard deviations, maximum and minimum content of all results obtained were determined.

RESULTS AND DISCUSSIONS

The evaluation of biochemical diversity between some *Thymus* populations based on the total polyphenol content of the alcoholic extract of dry plant material revealed at the 20 populations studied, an average of 0.779 ± 0.006 mgGAE/g dry vegetable mass (herba).

The median of TPC values determined in the studied populations is of 0.580 ± 0.334 mgGAE/g dry vegetable mass (herba). The amount of TPC in the *Thymus* populations studied, can be seen in Figure 1.

The total values of the polyphenol determined by our study in *Thymus* species were found to be lower than those reported in the literature for plants collected from natural habitats or grown under field conditions (Petrović et al. 2017; Tohidi et al., 2019; Guriță et al., 2019; Golkar et al., 2020).

Climatic and pedological conditions, sampling and extraction methods, the genetic background of the plants is responsible for the differences in the TPC content reported in various studies.

In our conditions, the extraction time (2 min.) and the extraction method (vortexing) were adapted to the purpose of the study, namely to compare the TPC content of different species prelevated from spontaneous flora and not to quantify the TPC content. These changes in the extraction technique were one of the factors responsible for the lower amounts of TPC reported in existing literature studies.

A maximum of 2.253 ± 0.020 mgGAE/g dry vegetable mass (herba) was determined at the population level, at population 6, species *Th. pannonicus* ssp. *actus* – identified in the area of Pojejena and a minimum quantity of 0.204 ± 0.004 mgGAE/g dry vegetable mass (herba) in population 2, *Thymus dacicus* – identified in the area of Lescovița.

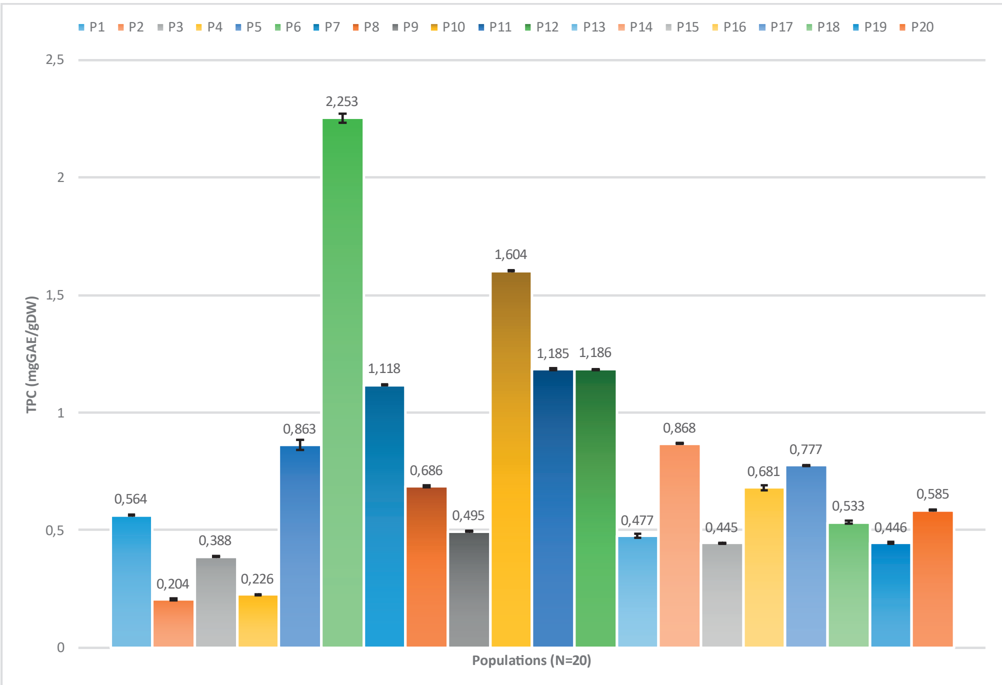

Figure 1. The amount of TPC in *Thymus* populations studied (mgGAE/gDW)

At the species level, within the six species evaluated in our study, the largest differences were obtained in the species *Th. pannonicus* ssp. *actus* (three populations), where the average amount of TPC determined was 1.352 ±0.809 mgGAE/g dry vegetable mass (herba). The largest amount of TPC 2.253 ± 0.020 mgGAE/g dry vegetable mass (herba) was determined at the population 6, identified in the area of Pojejena locality and the lowest amount of TPC 0.686 ± 0.003 mgGAE/g dry vegetable mass (herba), was determined in population 8 identified in the area of Tricule.

The genus *Thymus* presents a high ecological plasticity, occupying various areas, which explains the high chemical variability (Varga et al., 2015; Raudome et al., 2017).

An interesting situation occurs at populations 9, 10, 11 and 12 respectively, belonging to the same species, *Th. praecox,* where the average quantity of TPC determined was 1.117 ± 0.459 mgGAE/g dry vegetable mass (herba), where a difference at subspecies level occurs: a significantly higher amount of polyphenols was determined in populations 10, 12 and 11 of *Th. praecox* ssp. *polytrychus,* with an average of 1.325 ± 0.242 mgGAE/g dry vegetable mass (herba), harvested in the Gozna-Semenic area, compared to *Th. praecox* ssp. *janke*, harvested in the area of the Domogled massif, where the average amount of TPC determined was 0.495 ± 0.001 mgGAE/g dry vegetable mass (herba). Being an endemic species (Boros et al. 2010; Raudome et al., 2017), with limited spread, these differences could provide additional chemotaxonomic characteristics with possible use in subspecies taxonomic determination. There may also be a possible link between the significantly increased quantity determined in these populations and the altitude at which these species grow, but further studies are needed on this issue.

Significant variations were also identified in the two populations of *Th. glabrescens,* with an average TPC of 0.545 ± 0.450 mgGAE/g dry vegetable mass (herba), where the amount of TPC determined at population 18, harvested in the Iuti Valley was 0.863 ± 0.049 mgGAE/g dry vegetable mass (herba) is significantly higher than that determined in population 2, harvested in the area of Silagiu locality, of 0.226 ± 0.001 mgGAE/g dry vegetable mass (herba). Here we can also discuss a possible zonal influence, the distance between the harvesting areas being the largest.

For the two populations of *Th. dacicus,* the quantity of TPC determined had the lowest values, with an average quantity of 0.296 ± 0.130 mgGAE/g dry vegetable mass (herba), and a population difference of 0.184 ± 0.004 mgGAE/g dry vegetable mass (herba). Previous studies have indicated in this species a quantity of TPC of 178.83 ±1.09 mgGAE/g dry vegetable mass (Petrović et al. 2017). These values are superior to the values obtained by us, but the parameters used in the determination were different, in particular the method of extraction of polyphenols, which differ essentially (enriched and concentrated methanolic extract).

At the population of *Th. comosus*, identified in the area of Dobraia, the quantity of TPC determined was of 0.564 ± 0.002 mgGAE/g dry vegetable mass (herba). As an endemic species, this result may have significance, but further studies are needed to validate the result obtained.

The eight populations of *Th. pulegioides* were analyzed, where the average quantity of TPC determined was of 0.601 ± 0.159 mgGAE/g dry vegetable mass (herba), where a smaller differentiation in subspecies from *Th. praecox*. Thus, in the case of the three populations of *Th. pulegioides* ssp. *pulegioides*, there were recorded values below the other two subspecies, with an average of 0.521 ± 0.070 mgGAE/g dry vegetable mass (herba), followed by the three populations of *Th. pulegioides* ssp. *chamaedrys*, with an average of 0.597 ± 0.236 mgGAE/g dry vegetable mass (herba). The highest values were determined at *Th. pulegioides* ssp. *montanus* with an average of 0.729 ± 0.068 mgGAE/g dry vegetable mass (herba). At the subspecies level, the largest value differences were recorded at *Th. pulegioides* ssp. *chamaedrys* with a range of variation in the amount of TPC between 0.445 ± 0.001 and 0.868 ± 0.002 mgGAE/g dry vegetable mass (herba). In this case, a possible altitudinal influence was also observed. The smaller differences in value were recorded at *Th. pulegioides* ssp. *montanus* with a range of variation in the amount of TPC between 0.681 ± 0.010 and 0.777 ± 0.002 mgGAE/g dry vegetable mass (herba).

The results obtained give a significant variation in the content of TPC by species and harvesting area. Quantitative differences in the altitude of the harvesting area have been identified and further studies are needed, with high variability in the data obtained.

There are numerous studies showing that these quantitative variations of TPC are mainly determined by species, genotypic differences, extraction method, phenological period, sample processing conditions, climatic and environmental factors (Tohidi et al., 2017; Tohidi et al., 2019; Golkar et al., 2020).

Depending on the total amount of polyphenols determined, the matrix of dissimilarity of the populations taken in the study was drawn up, then used to obtain the dendrogram (Figure 2) according to the method of the mean of the groups (clusters). A number of two major clusters of *Thymus* populations were obtained by cluster analysis relative to the amount of TPC determined in this study in the 20 *Thymus* populations, to which was added population 6, which by the much higher amount of TPC determined was framed separately.

Two subclusters were identified at the level of the first major cluster (cluster 1). The first subcluster (1.1) unites a number of 10 populations with an average content of 0.436 ± 0.130 mgGAE/g dry vegetable mass (herba), with a minimum quantity of 0.204 ± 0. 004 mgGAE/g dry vegetable mass (herba) in second population and a maximum quantity of 0.585 ± 0.002 mgGAE/g dry vegetable mass (herba) in population 20.

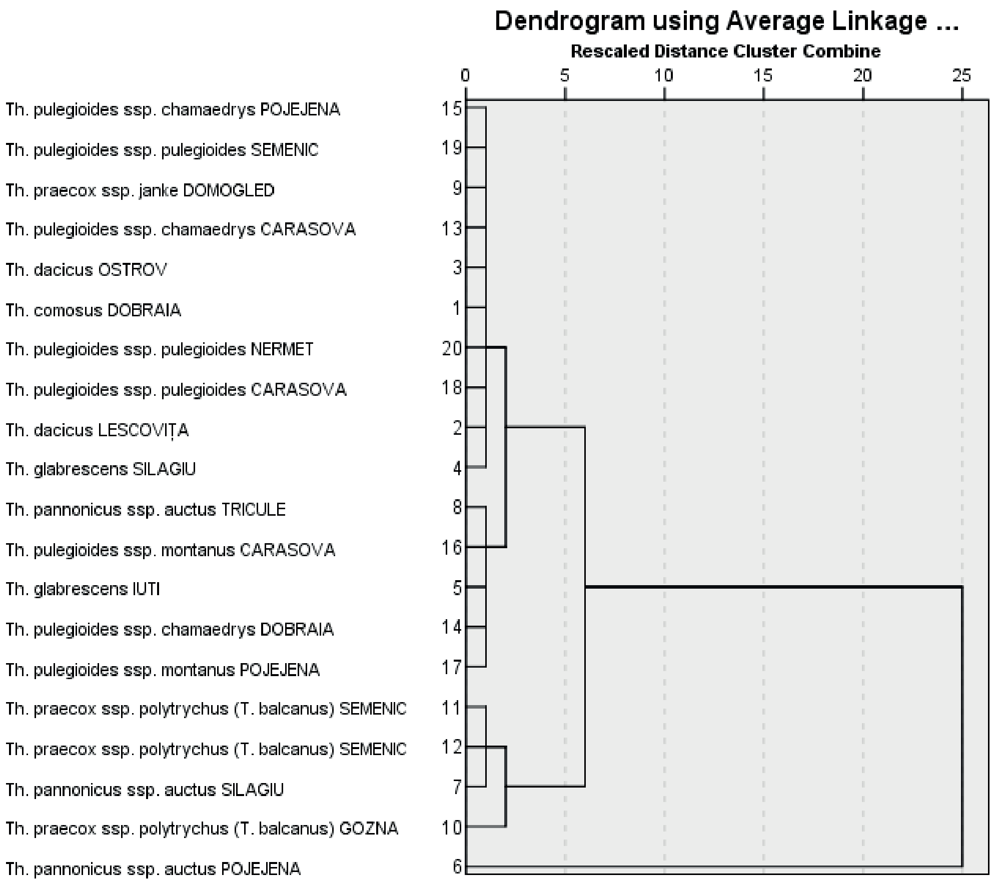

Figure 2. Two-dimensional dendrogram obtained by cluster analysis of the amount of TPC in *Thymus* populations (N=20)

The second subcluster (1.2) of the first major cluster, unites five populations, with an average content of 0.775 ± 0.091 mgGAE/g dry vegetable mass (herba), with a minimum amount of 0.681 ± 0.010 mgGAE/g dry vegetable mass (herba) at population 16 and a maximum quantity of 0.868 ± 0.002 mgGAE/g dry vegetable mass (herba) at population 14. The TPC content of populations in major cluster one is lower than populations in major cluster two.
Major Cluster two is distinguished by higher values in terms of the amount of TPC and comprises a subcluster (2.1) with three populations, with an average quantity of 1,163 ± 0,039 mgGAE/g dry vegetable mass (herba), of which a minimum amount of 1,118 ± 0,001 mgGAE/g dry vegetable mass (herba) at population seven and a maximum quantity of1,186 ± 0,001 mgGAE/g dry vegetable mass (herba) at population twelve, to which population ten is added, with a significantly higher amount of TPC 1.604 ± 0.001 mgGAE/g dry vegetable mass (herba).
Statistical data on the amount of TPC in population clusters of *Thymus* are given in Table 2.
A significant aspect of this study is the positioning in different clusters of two subspecies of the same species *Thymus praecox*, a result that may represent a valid starting point for the development of biochemical fingerprinting methods of *Thymus praecox* subspecies. Thus, Population 9 - *Th. praecox* ssp. *janke,* identified in the area of the Domogled massif was classified in cluster 1, and population 10, respectively population11 and 12 - *Th. praecox* ssp. *polytrychus (T. balcanus),* in the Gozna-Semenic area was placed in cluster 2.

Table 2. Statistical data of clusters for Thymus populations studied, based on total polyphenol content (N=20)

Cluster/ subcluster	Mean	STD[1]	Min.[2]	Max.[2]	CV[3] (%)
Subcluster 1.1 (N=10)	0.436	0.130	0.204 ± 0.004	0.585 ± 0.002	29.8
Subcluster 1.2 (N=5)	0.775	0.091	0.681 ± 0.010	0.868 ± 0.002	11.7
Subcluster 2.1 (N=3)	1.163	0.039	1.118 ± 0.001	1.186 ± 0.001	3.3
Subcluster 2.2 Popultion 10	1.604	0.001	-	-	-
Population 6	2.253	0.020	-	-	-

[1]STD = standard deviations.
[2]Min./Max. = minimum/maximum TPC content (means ± standard deviations mgGAE/g).
[3]CV=coefficient of variation.

CONCLUSIONS

The results obtained highlight a significant variation in the content of TPC in *Thymus* populations from Banat area by species and harvesting area.
The degree of variability of the values obtained in widespread species indicates that further development of genotypic studies is necessary to allow a possible correlation between the species of the genus *Thymus* at chemical and genetic level.
A validation of these studies could provide additional taxonomic characteristics with possible use in the development of faster taxonomic determination tools.
Depending on the similarity of the quantity determined by total polyphenols the populations of *Thymus* were classified hierarchically into two main clusters; positioning in different clusters of two subspecies of the same species *Thymus praecox*, a result that may represent a valid starting point for the development of biochemical fingerprinting methods of *Thymus* subspecies.

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STUDY OF THE INFLUENCE OF INTERACTION VARIETY × YEAR × LOCATION ON WINTER WHEAT YIELD CULTIVATED IN DIFFERENT LOCATIONS IN THE PERIOD 2018-2020

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Abstract

The paper aims to analyze the influence of the interaction variety x year x location at 15 varieties of winter wheat of different origins, grown in Romania for 3 years (2018-2020) in three different locations: University of Craiova - ARDS Caracal, NARDI Fundulea and Mircea Vodă - Brăila county. The highest yield was recorded by the Sothys variety in 2020 at Caracal location - 13128 kg/ha and the lowest yield by the Avenue variety in 2020 at Mircea Vodă location - 2600 kg/ha. The analyzed data highlighted the fact that in all the years at the varieties Airbus, Alcantara, Anapurna, Koreli, Lennox, Sothys and Otilia, the yield was very low in Fundulea and Mircea Vodă compared to Caracal. In 2018, the varieties Exotic, Glosa, Izalco and Sofru presented statistically undifferentiated yields, when they were cultivated in Caracal and MirceaVodă. The results showed that the studied interaction strongly influenced the yield. Taken separately, the factors studied also influenced yield. The yields obtained in 2018 and 2020 were distinct significantly and very significantly higher than the average yield of the three years (7109 kg/ha), while the yield of the year 2019 was very significantly lower than average. The yields obtained at Fundulea and Mircea Vodă were much lower than those obtained at Caracal on average over the three years and the 15 varieties tested. The stability of yield was analyzed in terms of the coefficient of variation. The lowest values of the coefficient of variation were recorded by the varieties Lennox (11.57%) and Exotic (13.66%) - the most stable, and the highest value by the variety Izalco (21.13%) - the most unstable.

Key words: *winter wheat, variety, year, location, interaction.*

INTRODUCTION

The choice of variety is an important link in wheat cultivation technology, anywhere in the world (Nicolescu et al., 2007). This must take into account the interaction between pedo-climatic conditions and cultural practices (Baranger and Bonneau, 2020). Romania's accession to the European Union on January 1, 2007 led to the invasion of the market with many varieties of cereals of which nothing was known about. Until their widespread testing, they were used by farmers, who often suffered significant material damage due to ignorance of their adaptability, productivity and the quality of yield obtained through their use.

In most European countries, there is a network or an institute that deals with testing of varieties and hybrids that aims to disseminate the information obtained to the main beneficiaries - farmers and farmers' associations. For example, in France there is the Arvalis Institute which is considered the main provider of agricultural information (www.arvalis-infos.fr).

In Romania, 2019 was the fourth year in which APPR (Romania's Association of Corn Producers) tested straw cereal crops. The testing was performed scientifically, in a system of microplots with three repetitions, having 70 varieties of wheat in 5 locations in non-irrigated conditions, one of the locations being SCDA Caracal - the non-teaching department of the University of Craiova. R.I.T.A.C. is the only Independent Field Testing and Analysis Network, scientifically developed in collaboration with service providers, research institutes and in direct partnership with seed companies and that have commercial activity in Romania.The testing followed and continues to follow, first of all, the levels of productivity and humidity at

harvest, but also the other aspects of crop management. The tests are performed in cooperation with seed companies or independent testing companies, and the seeds used for testing are prepared and coded by the APPR technical team (APPR Catalogue, 2020).
Farmers are urged to check the results of the APPR in the area most similar in terms of soil and climate to the area of cultivation in which they operate.
Wheat is one of the main plants consumed worldwide and one of the main sources of calories and protein. Approximately 82-85% of the world's population depends on wheat to provide the necessary calories and protein (Chaves et al., 2013).
It is frequently grown in temperate areas, with drought occurring in early summer and limiting grain yield, as stress corresponds to the grain filling period at most cereals, including wheat (Frorgóné, 2009).
Also, the variation of production and quality is influenced by the agrotechnical measures and the genetic characteristics of the varieties or hybrids, both in the case of wheatand other species (Cotuna et al., 2015; Paraschivu et al., 2015; Partal and Paraschivu, 2020; Paraschivu et al., 2020).
The results obtained in wheat testing are greatly influenced by climatic conditions. Half of the area where wheat is cultivated in developed countries and over 70% of the area in developing countries suffers from long periods of drought. Drought can occur throughout the growing season of the crop in areas with low rainfall. Plants that are exposed to water and heat stress reduce their yield all over the world. The combined effect of the two on yield is stronger than the effect of each stress at a time (Dreesen et al., 2012; Rollins et al., 2013).Although Romania's climate is generally characterized as "moderately continental", over the recent years there have been extremely large variations, both in the total amount of rainfall from one year to another and in their distribution during the year, which determines water deficits (often associated with heat) frequent during the vegetation of agricultural crops, in almost all areas of the country (Munial and Dhanda, 2005; Păunescu Aida, 2017).
The response of plants to drought is also influenced by the intensity, duration and frequency of stress, as well as by the plant-soil-atmosphere interaction. Many morphological and physiological strategies have been identified in response to water deficiency, ranging from avoiding dehydration to tolerance to dehydration (Carolina et al., 2012). Research on the influence of water stress on plants (Ciulu, 2005; Păunescu and Boghici, 2008, Boghici, 2008; Urechean et al., 2011; Borleanu, 2012; Păunescu, 2017) was based on numerous observations made in the Oltenia county, with the purpose of identifying drought-resistant genotypes to use in breeding programs.
This paper was made possible by the concept of "open science", which makes available to researchers, on the one hand, and users of the research results, on the other hand, data to which there is open access.

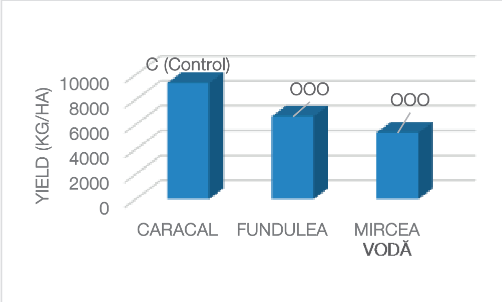
MATERIALS AND METHODS

The paper aims to analyze the influence of the variety x year x location interaction at 15 varieties of winter wheat of different origins (factor A), grown in Romania for 3 years (2018-2020) (factor B) in three different locations (factor C): Caracal (University of Craiova - non-teaching department SCDA Caracal - data from the experience field), Fundulea (INCDA Fundulea - data published in the APPR catalogue) and MirceaVodă -jud. Brăila (data published in the APPR catalogue).
The tested varieties entered the network following the chain: Limagrain (Airbus, Alcantara, Anapurna, Avenue), Caussade (Izalco, Sofru, Sosthene, Sothys, Solveig), INCDA Fundulea (Glosa, Otilia), Ciproma (Koreli), RWA (Lennox), APPR (Exotic and Silverio).
The pedoclimatic conditions available at SCDA Caracal are favorable for the cultivation of hail cereals, as they possess a soil of baticalcariccambicchernozem type with a high potential for fertility and a good yield capacity. In general, chernozems do not pose any particular problem in terms of cultivation with a comprehensive assortment of agricultural plants (Roșculete et al., 2019).
The varieties were tested in microplots of 7 m^2 in 3 repetitions, in each location and in each of

the years under a coded name that was revealed after the delivery of the results. For each location and for each year, the technology used is specified in the APPR catalogs (www.apprs.ro).
Climatic conditions were very differentiated depending on the year of experimentation and location. In Caracal, the year 2020 was an extremely favorable year for wheat cultivation, a fact reflected in the very high yields obtained. In MirceaVoda, 2020 was considered the driest year in the last 60 years.
The yields obtained were calculated at STAS humidity (14%) and reported in kg / ha. The calculation of the limit differences was performed by the program of plots subdivided with 3 factors.

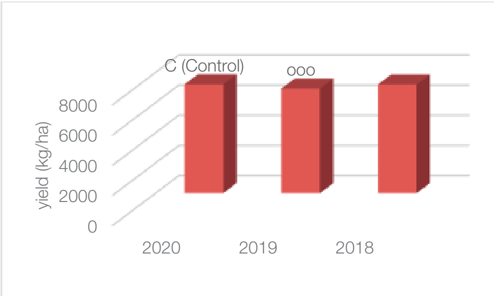
RESULTS AND DISCUSSIONS

The highest yield was recorded for the Sothys variety in 2020 in Caracal - 13128 kg/ha and the lowest yield for the Avenue variety in 2020 in Mircea Vodă - 2600 kg/ha. All tested varieties obtained the lowest yields in Mircea Voda in 2020 and the highest yields in Caracal in the same year. This highlights the particular importance of climatic factors. Excessive drought in the southeast of the country and extremely favorable conditions in the southwest of the country (Oltenia County), both recorded in 2020, were the basis for the polarization of these results.
Taken separately, the factors studied (location, year, variety) also influenced yield.
The yields obtained in Fundulea and Mircea Vodă was very significantly lower than those obtained in Caracal on average over the three years and for the 15 tested varieties (Figure 1).
The yields obtained in 2018 and 2020 were distinctly significant and significantly higher than the average yield of the three years (7109 kg / ha), while the yield of 2019 was significantly lower than average.
Taking as control 2020, the year with the highest yield, it was observed that only 2019 was significantly inferior in terms of yield, while 2018 was at the same level as 2020 (Figure 2).



LSD 5% = 52 kg/ha; LSD 1% = 69 kg/ha; LSD 0.1% = 90 kg/ha

Figure 1.Yields of varieties tested according to the location (average 2018-2020)



LSD 5% = 62 kg/ha; LSD 1% = 88 kg/ha; LSD 0.1% = 110 kg/ha

Figure 2.Yields of varieties tested according to the year of experimentation (average 2018-2020)

The most productive variety, on average for 3 years and 3 locations was Sothys (7730 kg/ha) and the least productive was Izalco (6442 kg/ha). All tested varieties were superior to the control variety Glosa, with statistical assurance, except for the Avenue variety which was at control level and the Izalco variety which was significantly inferior to the control (Table 1).
When analyzing the lowest yields obtained, all those obtained in MirceaVoda location in 2020, varieties Airbus, Alcantara, Avenue, Izalco, Koreli, Sofru, Sosthene, Otilia, were inferior with statistical assurance to the control variety Glosa.
However, the varieties Anapurna, Exotic, Lennox, Silverio and Sothys, even under these harsh conditions were significantly superior to the Glosa variety (Table 2).

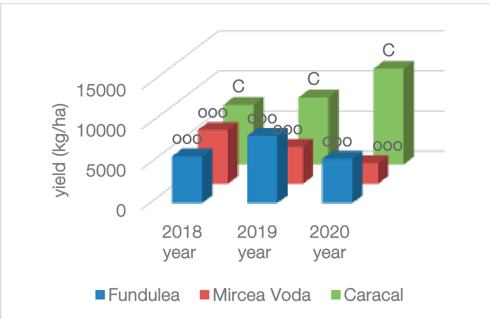
Table 1. Yield of tested varieties - average of the three locations (Caracal, Fundulea, MirceaVodă) and three years (2018-2020)

Variety	Yield (kg/ha)	Diff.	Significance
GLOSA	6809	0	
AIRBUS	6973	164	*
ALCANTARA	7170	361	***
ANAPURNA	7285	476	***
AVENUE	6845	36	
EXOTIC	7261	452	***
IZALCO	6442	-367	ooo
KORELI	6949	140	*
LENNOX	7178	369	***
SILVERIO	7321	512	***
SOFRU	7174	365	***
SOLVEIG	7241	432	***
SOSTHENE	7241	432	***
SOTHYS	7730	921	***
OTILIA	7010	201	**

LSD 5% = 132 kg/ha; LSD 1% = 177 kg/ha; LSD 0.1%=236 kg/ha

To highlight the variety x year x location interaction and its influence on yield, two averages of factor C (location) were compared to the same factor A (variety) and to the same factor B (year).

The analyzed data highlighted the fact that in all the years for varieties Airbus (Figure 3), Alcantara, Anapurna, Koreli, Lennox, Sothys and Otilia, the yields were very low in Fundulea and Mircea Vodă compared to Caracal.



LSD 5% = 353 kg/ha; LSD 1% = 466 kg/ha; LSD 0.,1% = 600 kg/ha

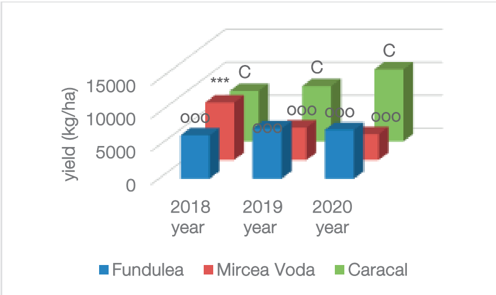
Figure 3. Behavior of the Airbus variety in 3 locations (average 2018-2020)

Only the varieties Silverio (Figure 4) and Solveig had significant yield increases for MirceaVodă compared to Caracal, in 2018.

Table 2.Minimum yield of tested varieties (location MirceaVodă in the driest year -2020)

Variety	Yield (kg/ha)	Diff.	Significance
GLOSA	3597	0	
AIRBUS	3038	-559	ooo
ALCANTARA	2647	-950	ooo
ANAPURNA	4220	623	***
AVENUE	2600	-997	ooo
EXOTIC	4591	994	***
IZALCO	2800	-797	ooo
KORELI	3411	-186	oo
LENNOX	4301	704	***
SILVERIO	3897	300	***
SOFRU	3394	-203	oo
SOLVEIG	3474	-123	
SOSTHENE	2891	-706	ooo
SOTHYS	4754	1157	***
OTILIA	3264	-333	ooo

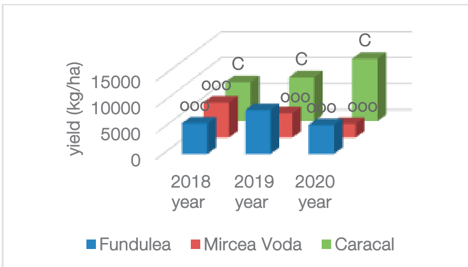
LSD 5% = 132 kg/ha; LSD 1% = 177 kg/ha; LSD 0.1% = 236 kg/ha



LSD 5% = 353 kg/ha; LSD 1% = 466 kg/ha; LSD 0,1% = 600 kg/ha

Figure 4. Behavior of the Silverio variety in 3 locations (average 2018-2020)

In 2019, the Avenue variety (Figure 5), presented a statistically undifferentiated yield, when it was cultivated at Fundulea in relation to Caracal.



LSD5% = 353 kg/ha; LSD 1% = 466 kg/ha; LSD 0.1% = 600 kg/ha

Figure 5. Behavior of the Avenue variety in 3 locations (average 2018-2020)

The varieties Exotic, Glosa, Izalco, Sofru and Sosthene presented statistically undifferentiated yields, when they were cultivated at MirceaVodă in 2019, in relation to Caracal.
The results showed that the studied interaction strongly influenced the yield.
On average over the three years, the varieties studied, depending on the location, had very differentiated yields.
The varieties Alcantara, Avenue and Otilia had minimum average yields below 5000 kg/ha (4958 kg/ha, 4655 kg/ha, respectively 4997 kg/ha) given maximum average yields over 9000 kg/ha (9483 kg/ha, 9267 kg/ha respectively 9326 kg/ha).
The Glosa and Izalco varieties also recorded minimum average yields below 5000 kg / ha but the maximum average yields did not exceed 9000 kg / ha. The limits were 4967-8758 kg/ ha for Glosa and 4455-8508 kg/ha for Izalco. Most varieties had values of minimum average yield over 5000 kg / ha and maximum average yields over 9000 kg / ha - Airbus (5241-9483 kg/ha); Anapurna (5618 kg/ha); Exotic (5964-9152 kg / ha); Koreli (5366-9106 kg/ha); Sulfur (5292-9468 kg / ha); Solveig (5402-9517 kg / ha); Sosthene (5499-9457 kg /ha).
The following varieties had a distinct behavior: Silverio with a minimum average yield over 5000 kg / ha but a maximum average yield over 9000 kg/ha (4958-9571 kg/ha); Lennox (minimum average yield of 6000 kg/ha and maximum of 9130 kg/ha); Sothys variety (minimum average yield of 5918 kg/ha and maximum of 10499 kg/ha). For all these varieties, without the exception of the average yield for three years with the minimum value, it was obtained at Mircea Vodă and the maximum average yield at Caracal.
The stability of yield was analyzed in terms of the coefficient of variability. It was calculated, eliminating the extreme values (Caracal and Mircea Voda locations in 2020). It was estimated that in a set of three years the probability of finding the worst year in history for one location and the best for another location in the same year is very small. The lowest values of the variability coefficient were recorded by the varieties Lennox (11.57%) and Exotic (13.66%) - the most stable, and the highest value by the variety Izalco (21.13 %) - the most unstable (Figure 6).

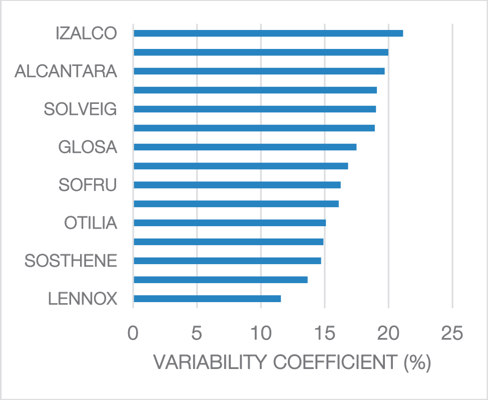


Figure 6.Variability coefficient for the tested varieties (average 2018-2020)

CONCLUSIONS

The choice of variety is an important link in wheat cultivation technology, anywhere in the world.
The highest yield was recorded by the Sothys variety in 2020 at Caracal location - 13128 kg / ha and the lowest yield by the Avenue variety in 2020 at MirceaVodă location - 2600 kg / ha.
All tested varieties obtained the lowest yields at MirceaVoda in 2020 and the highest yields at Caracal in the same year. This highlights the particular importance of climatic factors. Excessive drought in the southeast of the country and extremely favorable conditions in the southwest of the country (Oltenia County), both recorded in 2020, were the basis for the polarization of these results.
Taken separately, the studied factors (location, year, variety) also influenced yield.
The most productive variety, on average for 3 years and 3 locations was Sothys (7730 kg / ha) and the least productive was Izalco (6442 kg / ha). All tested varieties were superior to the control variety Glosa, with statistical assurance, except for the Avenue variety which was at control level, and the Izalco variety which was significantly inferior to the control.
The stability of yield was analyzed in terms of the variability coefficient. The lowest values were recorded by the varieties Lennox (11.57%) and Exotic (13.66%) - the most stable, and the highest value by the variety Izalco (21.13 %) - the most unstable.

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IMPACT OF THERMAL REQUIREMENT ON GROWTH AND GRAIN YIELD OF MAIZE HYBRIDS UNDER RAINFED CONDITIONS

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Abstract

In Oltenia region, maize productivity is highly affected by increasing average temperature and the uneven distribution of rainfall. The suitable hybrid is one of the main adaptation strategies for maize crop for managing the climatic changes. In this study, a field experiment was conducted in rain-fed conditions during 2018 and 2019 at ARDS Simnic to evaluate the accumulated growing degree days (GDD), phenothermal index (PTI) and heat use efficiencies (HUE) at different phenological stages (emergence, tasseling, silking and physiological maturity) and grain yield (GY) of three maize hybrids. The results revealed that among different thermal indices, GDD, PTI and HUE at physiological maturity stage better explained the variations in grain yield across the different growing seasons (96.6%, 93.1% and 99.7%, respectively). Therefore, these thermal indices could be used as the best indices for selection of heat tolerant hybrids for this region. Among the studied hybrids, Pioneer P0216 and Monsanto DK 5068 produced higher yields while accumulated maximum GDD, PTI and HUE at different phenological stages.

***Key words**: growing degree days, heat use efficiencies, phenological stages, phenothermal index.*

INTRODUCTION

Maize (*Zea mays* L.) is the second most important cereal crop in the world after wheat and is mainly grown for food, feed and as an industrial raw material. Global climate changes are increasingly affecting maize yield and raising future food security concerns.

In Romania, maize occupies around 2,442 thousand hectares with total production of 18,664 thousand tons and grain yield of 7 641 kg/ha (Bonea, 2020a). In the central part of the Oltenia region, drought and heat stresses causes substantial yield reductions of up to 60.5% in maize (Bonea & Urechean, 2017).

Characterizing and better understanding crop phenology is crucial for field crop management practices. Adaptation strategies, such as the use of suitable cultivars can help in reducing impacts of climate change (Aggarwal, 2008).

The phenological stages could be used to indicate whether a cultivar can be grown commercially in a certain area (Tojo-Soler et al., 2005). The rate of crop growth through the different phenological stages is a function of its response to temperature and rainfall (Bonea, 2020b; Kamal et al., 2017; Urechean & Bonea, 2017).

Temperature, photoperiod and genetic response to the environmental factors, greatly influence growth, development and production of a crop. The knowledge on the calculation of the heat unit requirement under field conditions, mostly called the growing degree days (GDD) and their further mathematical derivations like phenothermal index (PTI) and heat use efficiencies (HUE) are the basic principles to describing and understand the phenological behaviour of different crops in specific environment (Fealy and Fealy, 2008; Matzarakis et al., 2007; Rajput et al. 1987; Sreenivas et al., 2010; Tojo-Soler et al., 2005). The PTI helps in evaluating relative performance of different cultivars under high temperature conditions (Rathod & Chimmad, 2016). Pramanik & Sikder (2020) reported that for evaluation of crop yield potential in different growing conditions, quantification of HUE is necessary.

Thus, due to climatic changes, is crucial to have knowledge of exact duration of phenological stages in a particular crop growing environment and their impact on yield of crop (Dangi & Shrestha, 2018). Also, little is known about the relationship between thermal

indices and maize yield under current environmental conditions of the Oltenia region.
In present study, investigation was carried out to evaluate the performances of different maize hybrids in relation with duration of phenological stages, thermal indices and grain yield, as well as to select the most suitable hybrids for this region.

MATERIALS AND METHODS

The field experiments were conducted during growing seasons of 2018 and 2019 at Agricultural Research and Development Station (ARDS) Simnic, Craiova. The study area is located at $44^{0}19'$ N latitude, $23^{0}48'$ E longitude, and 182 m altitude in the central part of the Oltenia region, Romania.
The experiment was laid out in a randomized block design with three replications. Three semi-late maize hybrids: Fundulea F 376, Pioneer P0216 and Monsanto DK 5068 were used in this study. These maize hybrids are among the most cultivated ones in this region due to high productivity. The crop was sown on 24 April 2018 and 17 April 2019, respectively. The soil of the experiment area is a reddish preluvosoil with pH=5.8. All the recommended agronomic practices for this area were followed.
Whole growth cycle of maize hybrids was categorized into four different phenological stages *viz*., emergence stage, tasseling stage (appearance of tassels in 75% of the plants in each plot), silking stage (appearance of silks in 75% of the plants in each plot) and physiological maturity stage (appearance of a black layer at base of the grain).
The daily weather data records were collected from the Craiova Meteorological Station.
The thermal indices at different phenological stages based on grain yield were calculated according to the following formulas (Amgain, 2011):

Growing degree days (GDD):

$$GDD = \sum_{i=1}^{n}\left(\frac{Tmax+Tmin}{2}\right) - Tb$$

were Tmax =Daily maximum temperature (°C);
Tmin = Daily minimum temperature (°C);
Tb = Base temperature = 10°C

Phenothermal index (PTI):

$$PTI = \frac{GDD}{growth\ days}$$

Heat use efficiency (HUE):

$$HUE = \frac{GY}{GDD}$$

were GY = grain yield (kg/ha)
The recorded data for grain yield were subjected to Fisher's analysis of variance technique (ANOVA) and means were compared for significance using Duncan's multiple range tests at 5% level of probability. The relationship between the thermal indices with the grain yield was described by Pearson's correlation coefficients and by linear regression.

RESULTS AND DISCUSSIONS

Weather conditions

The weather data showed more favorable environmental conditions for the growth and development of maize in 2018 as compared to 2019 (Table 1). In 2018, April, May and August were dry, but June and July were wet with frequent rainfall. Temperature in July was below multiannual average (-1.2°C), but in the rest of the growing season it was significantly warmer than the multiannual average, especially in April (+4.4°C). The growing season ended with a surplus of rainfall (+38.5 mm) and was 1.6°C warmer than the multiannual average. In 2019, the rainfall deficiency was of -49.6 mm as compared to multiannual average, largely pronounced in April, May, July and August. The mean monthly temperatures were over the multiannual average in August (+2.6°C).

Table 1. Rainfall distribution and average temperature for 2018 and 2019 at ARDS Simnic

Item	Year	April	May	June	July	August	Sum/average 1.04 – 31.07
Rainfall (mm)	2018	-42.0	-20.7	+67.4	+52.8	-19.0	366.1
	2019	-11.1	-39.7	+62.4	-23.2	-38.0	278.0
	Multiannual average	53.1	71.7	73.6	82.2	47.0	327.6
Temperature (°C)	2018	+4.4	+1.7	+0.1	-1.2	+1.2	21.1
	2019	-0.3	-1.3	-1.2	-0.9	+2.6	19.8
	Multiannual average	12.2	17.5	21.5	23.8	22.5	19.5

Phenology of maize hybrids

The duration of phenological stages over two-year observations are presented in the Table 2. The results indicated that the duration of the growing cycle (number of days from sowing to physiological maturity) was affected by the growing conditions of the years, but also by the hybrids.

In the growing conditions of 2018, the time from sowing to emergence was similar in two hybrids, Fundulea F 376 and Pioneer P0216 (16 days).

Relatively more days to tasseling (72) and silking (74 and 73) were recorded by Fundulea F 376 and by Pioneer P0216, while lesser days were taken by Monsanto DK 5068 (69 and 70 days, respectively). Monsanto DK 5068 required the highest number of days for physiological maturity (130 days) followed by Pioneer P0216 (129 days), while Fundulea F376 required the lowest number of days (125 days).

In 2019, the time from sowing to tasseling and the time from sowing to silking were similar in hybrids Fundulea 376 and Monsanto DK 5068 (83 and 85 days, respectively), while Pioneer P0216 required 91 and 90 days, respectively.

On average, the number of days from sowing to plant emergence was 16.3 for 2018, and was slightly increased to 17.0 days for 2019.

However, the greatest effect of growing conditions was observed in the number of days from sowing to tasseling and in the number of days from sowing to silking, which have increased from 71.0 days and from 72.3 days in 2018, to 85.66 days (21% increase) and to 86.66 days, respectively (19.8% increase) for 2019. Also, the number of days from sowing to physiological maturity was reduced from 128 days in 2018, to 124.33 days in 2019.

The drought and heat stresses during July and August 2019, hastened physiological maturity reducing the duration of phenological development during grain filling period (from silking to physiological maturity). Similar results for maize under high temperatures conditions were reported by Castro-Nava et al. (2011) and Bonea (2020b). High temperatures accelerates growth rate shortening growth stages and reducing grain yield (Hamid et al., 2020). White and Reynolds (2003) found that reduction in the length of the growth cycle, especially the grain-filling period, is the most important factor in explaining reduced yields at warmer temperatures.

Table 2. Phenological calendar of maize hybrids (days)

Year	Hybrids	Emergence	Tasseling	Silking	Physiological maturity
2018	Fundulea F 376	16	72	74	125
	Pioneer P0216	17	72	73	129
	Monsanto DK 5068	16	69	70	130
	Mean	16.3	71.0	72.3	128.0
2019	Fundulea F 376	16	83	85	123
	Pioneer P0216	18	91	90	126
	Monsanto DK 5068	17	83	85	124
	Mean	17	85.7	86.7	124.3

Growing degree days (ºC days)

The accumulated heat units (GDD) of maize at different phenological stages by different hybrids are presented in Table 3. The GDD was increasing from early stages to late stages. Similar results were reported by Amgain (2011) and Bonea (2020b).

In 2018, the Monsanto DK 5068 had the highest GDD requirement for attaining physiological maturity (1483.5 ºC day) followed by Pioneer P0216 (1469.5 ºC day) and Fundulea F 376 (1415.5 ºC day), but had the lowest GDD requirement for tasseling (685.0 ºC day) and silking (698.0 ºC day).

Due to the large deficit of rainfall and temperatures above the multiannual average in May and April, the germination and emergence of plants were severely affected, thus leading to an increase in GDD requirement for all hybrids.

In 2019, the highest GDD requirement for attaining all the phenological stages was observed in Pioneer P0216 (82.5 ºC day for emergence, 804.5 ºC day for tasseling, 795.5 ºC day for silking and 1263.5 ºC day for physiological maturity).

On average, the greatest effects of growing conditions were observed in the GDD requirement for emergence, which have reduced from 159.5 in 2018, to 75.7 for 2019 (52% reduction) and in the GDD requirement for physiological maturity which have reduced from 1456.2 in 2018, to 1240.8 for 2019 (15% reduction).

The lower heat units (GDD) requirement for attaining the physiological maturity in the growing condition of 2019 as compared to 2018 were due to drought and heat stresses which shortened the period from silking to physiological maturity (grain-filling period). Pramanik and Sikder (2020) also reported that stress condition reduced GDD requirement in wheat genotypes.

Table 3. Growing degree days (GDD) at different phenological stages of maize hybrids

Year	Hybrids	Emergence	Tasseling	Silking	Physiological maturity
2018	Fundulea F 376	156.5	716.0	741.0	1415.5
	Pioneer P0216	165.5	716.0	727.5	1469.5
	Monsanto DK 5068	156.5	685.0	698.0	1483.5
	Mean	159.5	705.7	722.2	1456.2
2019	Fundulea F 376	69.0	735.0	753.5	1222.5
	Pioneer P0216	82.5	804.5	795.5	1263.5
	Monsanto DK 5068	75.5	735.0	753.5	1236.5
	Mean	75.7	758.2	767.5	1240.8

Phenothermal index (°C days/day)

Among the different phenological stages, the highest PTI was observed during sowing to physiological maturity, while the PTI was lowest during sowing to emergence (Table 4). Similar results were observed by Amgain (2011) at maize planted during spring.

In the growing conditions of 2018, the maximum PTI was observed at physiological maturity stage in Monsanto DK 5068 (11.41) followed by Pioneer P0216 (11.40), while Fundulea F 376 required the minimum PTI (11.32).

In the growing conditions of 2019, the maximum PTI was also observed at physiological maturity in Pioneer P0216 (10.03) followed by Monsanto DK 5068 (9.97).

On average, the greatest effect of growing conditions was observed in the PTI requirement for emergence, which have reduced from 9.76 in 2018, to 4.44 for 2019 (55% reduction). These results indicate that, even for a short period, changes in the air temperature are reflected in the PTI during the individual growth stages.

The higher PTI in 2018 suggested a longer growing period of each phenophase which led to the accumulation of more thermal units and therefore, a higher GY.

Similar results were noted in wheat by Ram et al. (2016). Sikder (2009) and Gill et al. (2014) also mentioned that the PTI is affected by the growing conditions and the studied cultivars.

Table 4. Phenothermal index (PTI) at different phenological stages of maize hybrids

Year	Hybrids	Emergence	Tasseling	Silking	Physiological maturity
2018	Fundulea F 376	9.78	9.94	10.01	11.32
	Pioneer P0216	9.73	9.94	9.96	11.40
	Monsanto DK 5068	9.78	9.93	9.97	11.41
	Mean	9.76	9.94	9.98	11.38
2019	Fundulea F 376	4.31	8.85	8.86	9.94
	Pioneer P0216	4.58	8.84	8.84	10.03
	Monsanto DK 5068	4.44	8.85	8.86	9.97
	Mean	4.44	8.85	8.85	9.98

Heat use efficiency (t/ha/°C days)
The quantification of HUE is important for determination of grain yield performance in different growing conditions. It was observed that all the hybrids were more heat use efficient in 2018 (Figure 1).
In the growing condition of 2018, Pioneer P0216 had highest HUE (6.73) followed by Monsanto DK 5068 (6.61), while Fundulea F 376 had the lowest HUE (5.85).
In the growing condition of 2019, Monsanto DK 5068 had highest HUE (4.90) followed by Fundulea F 376 (4.73), while Pioneer P0216 had the lowest HUE (4.51).
On average, the value of HUE at physiological maturity has reduced from 6.40 in 2018, to 4.71 for 2019 (26% reduction).
This result indicates that the thermal indices were more favorable in 2018 as compared to 2019 when plants increased the physiological activities through more efficient use of accumulated heat units, which resulted in a higher HUE and a higher GY, respectively. Pramanik and Sikder (2020) confirmed that the stress conditions reduced HUE in wheat.

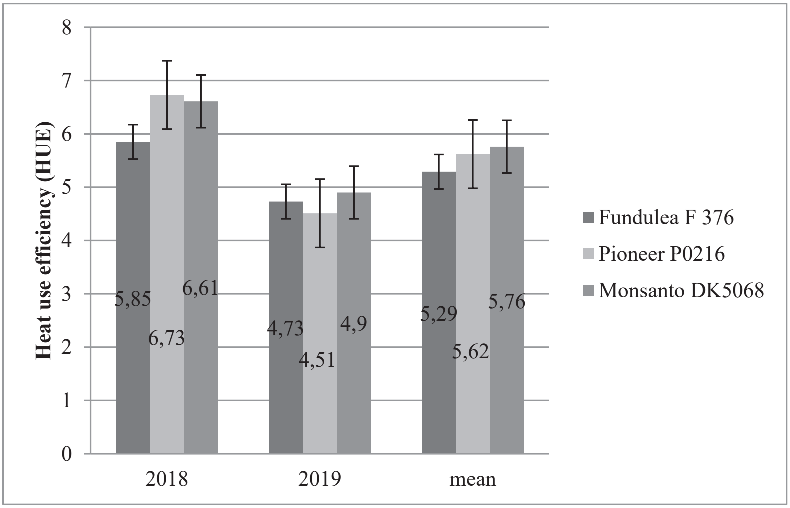
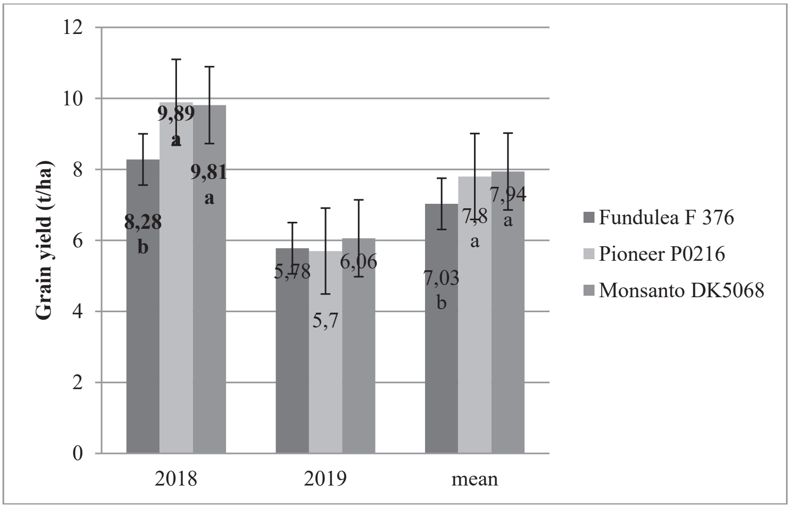


Figure 1. Heat use efficiency (HUE) of maize hybrids

Maize grain yield
Drought and heat stresses at different phenological stages have caused an array of morphological, physiological and biochemical changes that have reduced GY of maize hybrids.
In 2018, the GY was significantly higher (P≤5%) in Pioneer P0216 (9.89 t/ha) and Monsanto DK 5068 (9.81 t/ha), while the minimum GY was recorded in Fundulea F 376 (8.28 t/ha).
The same ranking of hybrids was observed for the average of the two-years (Figure 2).
In 2019, non-significant differences (P≤5%) were observed between hybrids for GY.
On average, the GY has reduced from 9.33 t/ha in 2018, to 5.85 t/ha for 2019 (37% reduction) due to the drought and heat stresses during grain–filling period.
According to Shao et al. (2008) the yield loss depends not only on the severity and duration of the stress, but also on when the stress occurs. Bonea and Urechean (2020) mentioned that air temperatures during grain-filling period are correlated strongly negative with the level of maize yield.



Means followed by different latters in each column are significantly different from each other at 5% level of significance

Figure 2. Grain yield (GY) of maize hybrids

Relationship between thermal indices and grain yield

The Pearson correlations of GY with GDD (Table 5) were highly significant positive (p=1%) at emergence stage (+0.958) and at physiological maturity stage (+0.983).

The significant negative relationship (p=5%) between GY and GDD at silking stage (-0.841) revealed that with increase in GDD requirement in this stage, the yield of maize decreased.

The relationship of GY with PTI was highly significant positive (p=1%) at all the phenological stages of maize hybrids (+0.954, +0.955, +0.948 and +0.965 respectively). It showed that as thermal indices are increased the grain yield is also linearly increased.

On the contrary, Singh et al. (2014) observed negative correlation between seed yield of mustard and PTI at anthesis stage and at maturity stage.

GY and HUE was registered a strongly positive relationship (+0.997). The results revealed that GY increased with the increased of HUE. Similar results were reported by Pramanik and Sikder (2020) in wheat.

Singh et al., (2014) reported significant positive correlations between seed yield of mustard and GDD (at emergence, anthesis, 50% flowering and at maturity) and between seed yield and HUE (at maturity), therefore they recommended these thermal indices for use in selection of genotypes for high temperature tolerance.

The linear regression equations were developed between the thermal indices and GY to find out the extent of variability in GY (Table 5).

It was observed that GDD at different phenological stages was able to explain the variability of GY from 56.3% at tasseling stage, to 96.6% at physiological maturity stage; PTI was able to explain the variability of grain yield from 89.9% at silking stage, to 93.1% at physiological maturity stage, and HUE (at maturity) was able to explain 99.3% variation in GY.

Choudhary et al. (2018) reported that GDD and PTI at maturity of mustard was able to explain variation in seed yield to the value of 83.2% and 85.3% respectively, while HUE could explain 72.5% of variation.

Also, Srivastava et al. (2011) observed that 66% variation in yield of oilseed *Brassica* could be explained through the accumulated GDD when crop was sown in variable weather conditions.

Table 5. Correlation and regression results between grain yield (GY) and thermal indices at different phenological stages (mean two-years)

Item	Regression equation	R^2	r
GDD at emergence	Y = 23.23x – 51.09	0.959	0.958**
GDD at tasseling	Y = -15.04x + 846	0.563	-0.750
GDD at silking	Y = -13.63x + 848.3	0.707	-0.841*
GDD at physiological maturity	Y = 59.56x +896.6	0.966	0.983**
PTI at emergence	Y = 1.394x – 3.477	0.910	0.954**
PTI at tasseling	Y = 0.286x + 7.22	0.912	0.955**
PTI at silking	Y = 0.293x + 7.189	0.899	0.948**
PTI at physiological maturity	Y = 0.370x + 7.865	0.931	0.965**
HUE	Y = 0.488x + 1.846	0.993	0.997**

*, ** significant 0.05 and 0.01 levels

CONCLUSIONS

Drought and heat stresses in maize for Oltenia region can be mitigated in two ways: agronomical practices or the development of the tolerant hybrids. Breeding for genotypes that are tolerant to stress at grain-filling period is an effective strategy to overcome this problem.

The present study showed that, the stress conditions of 2019 during grain-filing period lowered the GDD requirement and PTI at physiological maturity stage and reduced the HUE and GY in all the hybrids.

Among different thermal indices, GDD, PTI and HUE at physiological maturity stage better explained the variations in GY across two growing conditions (96.6%, 93.1% and 99.7%, respectively). Therefore, these thermal indices could be used as the best indices for selection of heat tolerant hybrids.

The hybrids Pioneer P0216 and Monsanto DK 5068 which have had highest GDD, PTI, HUE and GY, are the most suitable hybrids for the cultivation in this region and can be recommended for breeding programme for developing of tolerant genotypes.

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THE MAIZE AND SUNFLOWER CROPS, STUDIED IN CENTRAL MOLDAVIA AREA, IN DIFFERENT CLIMATIC CONDITIONS

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Abstract

The breeding activity for obtaining maize and sunflower hybrids, having high genetic potential for the grain yield, also good stability, in different environment conditions is of great importance for the management of these crops. The yield potential brings the highest contribution to the crop efficiency. To increase the grain yield it is necessary to have a good developing of the plants in different growing conditions, respectively the plants need to have favourable growing conditions to express their yielding potential. The objective of the present paper is to present the results obtained at 4 maize and 4 sunflower hybrids studied in the field conditions of the Central Moldavia (East Romania), during three years (2018, 2019 and 2020). It was intended to find out the influence of climatic conditions on the grain yield of the studied hybrids, but also on the oil and protein content of the grains. Comparing the three years, regarding the air temperatures and the amount of rainfall in the growth period of the two crops (maize and sunflower), the year 2018 was dryer comparing with 2019 year, and the 2020 year was the driest from all three at the beginning of the growing period despite the fact that later there were registered important rainfall. The studding of genotypes under different climatic conditions allows to identify the genotypes with good adaptation capacity. The grain yields of the maize and sunflower hybrids were influenced significantly by year, respectively by the climatic conditions of the year. The protein content in the maize hybrids and the oil content both for maize and sunflower hybrids are much more stable than the grain yield in different growing conditions.

Key words: *maize; sunflower; yield; oil content; protein content.*

INTRODUCTION

Maize (*Zea mays* L.) is among the most important crop worldwide, and it can be considered a reference crop for the benefits of different management patterns characterized by different degrees of intensification.

Romania plays an important role in the international maize market, being in full ascendency in terms of production and export (Popescu et al., 2018).

So, the breeding activity for obtaining maize hybrids having high genetic potential for the grain yield, but also with a very good stability, in different environmental conditions is of a great importance for the management of this crop (Iordan et al., 2016). To increase the grain yield it is necessary to have a good developing of the plants in different conditions of growing, good tolerance to biotic and abiotic stress, adaptability to the cultivation technology (Cristea et al., 2004; Horhocea et al., 2020).

Very important are the local biotic factors, which can produce high seed yield losses (Bărbulescu, 2000).

Sunflower (*Helianthus annuus* L.) crop has an important place in the world agriculture, due to many advantages, the most important being the

capacity to produce high seed yield and good oil content (Anton et al., 2015).
Sunflower grains are used in industry for obtaining good oil for human consumption, as well as the secondary matter used in animal feeding (Vrânceanu, 2000).
Sunflower oil has a very good quality, with high percent of the unsaturated acids and capacity to maintain stability and long-time conservation (Skoric et al., 2012).
The open pollinated varieties, with oil contents up to 50%, are well recognised as the main genetic base of modern sunflower breeding (Vear, 2016).
In the practical selection, which is part of the production of hybrids with high yielding potential, as well as high adaptive potential, a strong influence belongs to the adaptive reactions to the ecological environment they are located (Hera et al., 1989).
The achievements related to the sunflower hybrids in terms of number and performances are excellent (Pacureanu-Joita et al., 2007).
Weed and Orobanche control with HT-tolerant sunflowers offers an excellent opportunity to increase sunflower yield in different countries (Kaya, 2015).
The objective of the present paper is to present the results obtained at 4 maize and 4 sunflower hybrids studied in the field conditions of the Central Moldavia (East Romania), during three years (2018, 2019 and 2020).

MATERIALS AND METHODS

We studied 4 maize and 4 sunflower hybrids cultivated in 3 years, at the Agro Tora society, in Negresti, Vaslui county, eastern Romania.
Negreşti is situated in central Moldavia, being characterized by cambic chernozem soil, well supplied with potassium and mid supplied with phosphorus and nitrogen.
The climate is characterized by an annual average of 9.5ºC and 420 mm rainfall.
The maize hybrids which have been cultivated and studied are: P 9911, Olt, P 9241 and F 423.
The studied sunflower hybrids are: PR64LE99, FD15E27, PR64LE25, and Neoma.
The climatic conditions were very different, from year to year in terms of temperatures and rainfall. We made our study in 2018, 2019 and 2020 years.
The sowing was performed in all studied years and both for maize and sunflower in the first decade of April. The hybrids were cultivated in the farming system. The plants density was 65,000 plants/hectares for maize and 55,000 plants/hectares, for sunflower.
Both for maize and sunflower hybrids, before sowing there was applied the herbicide Dual Gold 960 EC (960 g/l S-metolaclor) in a rate of 1.5 l/ha. In vegetation, the weed control (dicotyledonous and monocotyledonous) in the maize plots was performed by applying the herbicide Adengo 465 SC (isoxaflutol 225 g/l + tiencarbazon-metil 90 g/l + ciprosulfamide (safener) 150 g/l) in a rate of 0.35 l/ha and in the 2 leaves growth stage, while in the sunflower plots the weed control (monocotyledonous) was performed by applying the herbicide Select Super (cletodim 120 g/l) in a rate of 2.0 l/ha and in the 6 leaves growth stage.
Also both for maize and sunflower hybrids, before seed bed preparation there were applied 70 kg of nitrogen/ha and 70 kg of P_2O_5/ha as complex fertilizer 20:20:0. For maize, it was applied supplementary in the 6 leaves growth stage 34 kg of nitrogen/ha as ammonium nitrate.
There have been determined, for the studied hybrids, the grain yield, the oil content and the protein content of the grains.

RESULTS AND DISCUSSIONS

In the three years the climatic conditions were different in Negresti location. The highest air temperature was registered in 2020 year followed by 2018 year (Figure 1).
The rainfall registered in 2019 year were moderate in all vegetation period of maize and sunflower, comparing with 2018 when the most quantity was registered in June-July (Figure 2).
In 2020 year it was registered a small rainfall in the first period of vegetation of the studied crops (maize and sunflower), having influenced the amount of the grain yields, despite the fact that later in the vegetation period there were registered important rainfall.

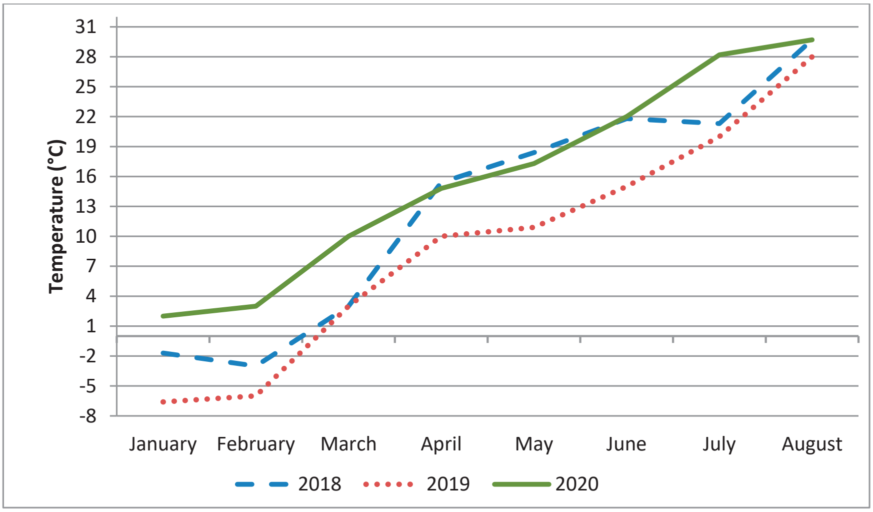

Figure 1. The average of monthly air temperature in three years, Negresti

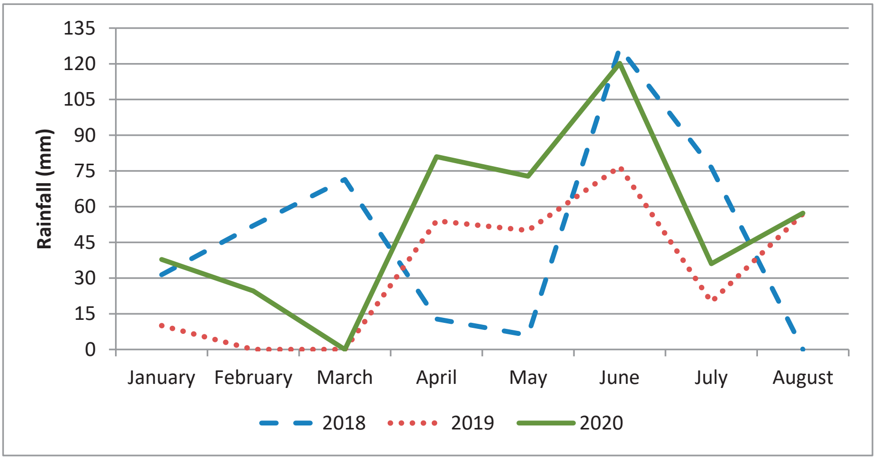

Figure 2. Monthly rainfall in three years, Negresti

Grain Yield

Looking at the average yield of the maize studied genotypes, there are obviously differences in the three years (2018, 2019 and 2020) (Table 1). That means the year had significant effect on the grain yield level.

In the year 2019, for all maize hybrids there were registered the highest yields. The hybrids P 9911, Olt, and P 9241 recorded greater yield variability, the variation coefficient being higher than 17%. The highest yield stability was registered at hybrid F 423 (coefficient of variation less than 10%, respectively of 8.32%), but this hybrid being characterised by the smallest yield obtained in all three years.

In Table 2, there is presented the grain yield for sunflower hybrids, in the three years. The genotypes FD15E27 and Neoma have the best stability (the variation coefficient less than 15%, respectively 12.84% for FD15E27 hybrid and 13.38% for Neoma hybrid). The hybrids PR64LE99 registered the highest variability (coefficient of variation of 18.95%). As in the case of maize, the highest grain yields were registered in the climatic conditions of the year 2019, and the smallest grain yields ere registered in the year 2020.

Table 1. The grain yield (kg/ha) and maize hybrids stability (%)

No.	Genotype	Yield (kg/ha)			Average	C.V. (%)
		2018	2019	2020	2018-2020	
1.	P 9911	10340	12630	8340	10436	21.38
2.	Olt	10254	11945	8735	10312	17.24
3.	P 9241	9785	11200	7650	9545	18.45
4.	F 423	8058	9890	6580	8176	8.32
Average		9610	11416	7826	9617	16.84

Table 2. The seed yield (kg/ha) and sunflower hybrids stability (%)

No.	Genotype	Yield (kg/ha)			Average	C.V. (%)
		2018	2019	2020	2018-2020	
1.	PR64LE99	2890	3340	2050	2760	18.95
2.	FD15E27	3145	3860	2985	3330	12.84
3.	PR64LE25	3230	3895	2780	3300	15.76
4.	Neoma	2985	3650	2550	3060	13.38
Average		3062	3685	2590	3112	15.23

Table 3. The protein content (%) and maize hybrids stability (%)

No.	Genotype	Protein content (%)			Average	C.V. (%)
		2018	2019	2020	2018-2020	
1.	P 9911	11.7	11.9	11.0	11.5	1.82
2.	Olt	11.6	11.3	11.3	11.4	0.54
3.	P 9241	12.0	12.4	11.3	11.9	0.76
4.	F 423	11.2	11.8	11.0	11.3	0.89
Average		11.6	11.8	11.1	11.5	1.00

Table 4. The oil content (%) and maize hybrids stability (%)

No.	Genotype	Oil content (%)			Average	C.V. (%)
		2018	2019	2020	2018-2020	
1.	P 9911	5.1	5.6	5.8	5.5	0.94
2.	Olt	5.9	5.8	6.2	5.9	1.28
3.	P 9241	5.7	5.0	5.4	5.3	0.79
4.	F 423	4.9	5.2	5.0	5.0	0.68
Average		5.4	5.4	5.6	5.4	0.92

Table 5. The oil content (%) and sunflower hybrids stability (%)

No.	Genotype	Oil content (%)			Average	C.V. (%)
		2018	2019	2020	2018-2020	
1.	PR64LE99	49.7	48.4	49.8	49.3	1.67
2.	FD15E27	50.3	49.7	51.4	50.4	2.45
3.	PR64LE25	50.8	50.0	51.9	50.9	1.88
4.	Neoma	51.2	49.8	50.8	50.6	2.21
Average		50.5	49.4	50.9	50.3	2.05

Protein content

The protein content for the studied maize hybrids in the three years varied between 11.0 and 12.4%, and the average protein content for all the maize hybrids in all the studied years was of 11.5% (Table 3).

The highest protein content was registered in the year 2019, when it was also registered the highest grain yields.

Compared to the grain yield, the protein content was much more stable, the coefficient of variation being more than 1% only for one hybrid, respectively P9911 hybrid with a value of 1.82%. The highest stability for the protein content was registered for the Olt hybrid (coefficient of variation of 0.54%).

Oil content

The oil content for the studied maize hybrids in the three years varied between 4.9 and 6.2%, and the average oil content for all the maize hybrids in all the studied years was of 5.4% (Table 4).

The highest oil content was registered in the year 2020, when it was registered the smallest grain yields, which was on the contrary to the protein content.

As in the case of the protein content, the coefficient of variation was more than 1% only for one hybrid, respectively Olt hybrid with a value of 1.28%, this hybrid having the highest variability for oil content but the highest stability for the protein content.

The oil content for the studied sunflower hybrids in the three years varied between 48.4 and 51.9%, and the average oil content for all the sunflower hybrids in all the studied years was of 50.3% (Table 5).

As in the case of maize, the highest oil content of the sunflower hybrids was registered in the year 2020 (50.9% in average), when it was registered the smallest grain yields.

In average for the three years, the oil content was less than 50% only for one hybrid (49.3% for PR64LE99 hybrid).

The coefficient of variation registered higher values for the oil content of the sunflower hybrids compared to the maize hybrids. Thus, whether in the case of maize only one hybrid registered a value higher than 1%, in the case of sunflower hybrids the coefficient of variation registered values between 1.67 and 2.45%.

CONCLUSIONS

On the bases of the obtained results, the following conclusions can be drawing:

- The grain yields of the maize and sunflower hybrids were influenced by year, respectively by the climatic conditions of the year. The most favourable year was 2019, when the average yields was of 11,416 kg/ha for maize hybrids and 3,685 kg/ha for sunflower hybrids.
- The studding of genotypes under different climatic conditions allows to identify the genotypes with good adaptation capacity.
- The most suitable genotype for the grain yield for the studied area was P 9911 maize hybrid and FD15E27 sunflower hybrid.
- The most favourable maize genotype for the protein content in the studied area was P 9241 hybrid, while for the oil content was the Olt hybrid.
- The most favourable sunflower genotype for the oil content in the studied area was PR64LE25 hybrid.
- Compared to the grain yield, the protein content in the maize hybrids and the oil content both for maize and sunflower hybrids registered much more stable values.

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THE INFLUENCE OF CLIMATIC CONDITIONS ON THE GROWTH STAGES OF SEVERAL MAIZE HYBRIDS IN THE OSMANCEA - CONSTANȚA AREA

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Abstract

Maize occupies about 46% of Romania's arable land, which is why it has been constantly in the attention of researchers who try to maximize production by various agrophytotechnical methods adapted to the climatic conditions so different in recent years. The aim of the research is to determine the influence of precipitation and temperatures on the growth stages of maize depending on the hybrid. To achieve the research objectives, a 3 x 3 x 3 three-factor experiment was established, sown in three repetitions according to the method of subdivided plots, with the following study factors: factor A - tillage system (a_1-ploughing; a_2-tiger; a_3-disc), factor B - fertilization formula (b_1-N0P0K0; b_2-N90P40K40; b_3- N90P40K40 + Greenstart (N4P10Mg0.5S1.25Zn0.5) and factor C - hybrid (c_1-Mas 40 F; c_2-Dartone; c_3-P9911). The growth stages of maize are largely influenced by the climatic conditions of the study years. The influence of climatic conditions is also reflected in the level of production/yield. The amount of water in precipitation is used differently depending on the tillage, fertilization formula and hybrid used. Of the hybrids tested in the 3 years of experimentation, the P9911 hybrid is the most productive.

Key words: *production/yield, maize, fertilizers, hybrids, tillage, temperature, precipitation.*

INTRODUCTION

Sustainable, conservative agriculture seeks to meet the needs of the present times without compromising the ability of future generations to meet their own needs. For this, solutions must be found for the rational exploitation of resources and for reducing the degradation of the environment (Guș et al., 2011; Rusu et al., 2009).

Tillage produces physical, chemical, mechanical changes in its mass, influencing the development of chemical and biological processes. Applied correctly, they favour self-restoration processes and the increase of the productive potential of the soil (Ankwe MA, 2007).

Excessive tillage of agricultural land, with various mechanical equipment and first of all the basic work, the ploughing, which mobilizes the soil in depth, in addition to penetration, destruction of diseases and pests also has negative effects through greater water loss, a weaker mineralization of vegetation residues, the creation of the hardpan, the rupture of the continuity of the capillarity, and if the ploughing on sloping lands is done along the line of the highest slope, it will favour erosion (Bogdan et al., 2007; Cociu, 2011; Ibanez et al., 2008; Moraru and Rusu, 2010; Pop et al., 2013).

When choosing the soil tillage system, the long-term consequences must be taken into account. In this respect, the system of minimal works has multiple advantages and it must be applied in its different variants, depending on the pedoclimatic conditions of the area (Jităreanu G., 1998).

The unconventional tillage system means the total or periodic abandonment of ploughing with the overturning of the furrow, the rationalization of the number of works and the keeping on the surface of the soil of at least 15-30% of the total vegetal residues, the aim being to reduce energy consumption, soil and water conservation, organization of adequate crop rotation, environmental protection, reduction of soil erosion and compaction (Marin et al., 2012).

The quantitative and qualitative growth of production at the level of current requirements is not possible without the use of chemical fertilizers (Mihăilă et al., 1996; Bîlteanu, 1998).
The multitude of factors that influence the effectiveness of fertilizers create difficulties when it comes to determining the doses, all the more so as some factors are more difficult to control, such as large variations in weather conditions from year to year.
Also, Cociu I. Al. (2011) confirms that traditional agriculture, based on intensive tillage by ploughing with the overturning of the furrow and removal of plant remainings followed by numerous secondary works, has the disadvantage of high cost and disproportionate distribution of inputs from crop technology in relation to expected efficiency, high consumption energy and labour, low productivity as well as major risks of soil degradation and environmental pollution.
In the context of global warming soil conservation works are different depending on the possibilities of mechanization and increase along with the increase in the capacity of tractors and agricultural machinery and diversification of loosening, tillage and sowing equipment. The use of fertilizers for profitable production and soil conservation must be made on the basis of realistic forecasts, which take into account local soil and climatic conditions, the productive potential of crops and the technological level. In this respect, the system of minimal works has multiple advantages and it must be applied in its different variants, depending on the pedoclimatic conditions of the area (Guş, 2004).
Water recovery by plants can be assessed by indicators that express the use by the plant of the entire amount of water consumed or only the efficiency of recovery of irrigation water (Domuţa, 2009). In the Romanian specialized literature, the indicators for assessing water recovery are approached from two perspectives: one that highlights the production, showing the amount of main yield obtained from consumption or use.
Water recovery is different from one crop to another, being influenced by soil and climatic conditions (Grumezan et al., 1989; Grumezan and Kleps, 2005) and by elements of technology: crop rotation, variety or hybrid, density, tillage, fertilization, control of weeds, diseases and pests, the degree of water supply.
Minimum tillage has many advantages, including: the soil is better protected from erosion, soil aggregates are more stable, organic matter increases, fertility levels and biodiversity increase, soil compaction decreases. There is also a better water retention in the soil. This is due to the reduction of temperature, by up to 6 °C, in the surface layer due to the thermoregulatory capacity and the high degree of reflection of the sun's rays by the plant residues retained on the soil surface (Phillips, 1984).
We must not lose sight of the hybrid used as a technological measure, knowing that the choice of the hybrid is a technological measure that does not generate costs, but can contribute by about 30% to the achievement of expected results.
Sin Gh., (2007) specifies that by replacing the ploughing with the disc harrowing at 10-12 cm, for 1-2 years in maize, the production is not significantly affected, but there is an increase in the number of weeds, especially perennials.
Long-term experiences play a key role in understanding complex plant x soil x climate interactions and their effect on plant productivity. At the same time, it provides data on changes that occur as a result of fertilizer application, constituting a rich source of scientific information on agronomic conditions over a long period of time (Petcu et al., 2003).

MATERIALS AND METHODS

To achieve the research objectives, a 3 x 3 x 3 three-factor experiment was established, sown in three repetitions based on the method of subdivided plots, with the following study factors: factor A - tillage system (a_1-ploughing; a_2-tiger; a_3-disc); factor B - fertilization formula (b_1-$N_0P_0K_0$; b_2-$N_{90}P_{40}K_{40}$; b_3-$N_{90}P_{40}K_{40}$ + Greenstart ($N_4P_{10}Mg_{0.5}S_{1.25}Zn_{0.5}$) and factor C - hybrid (c_1-Mas 40 F; c_2-Dartona; c_3-P9911).
The main purpose of the paper was the study of the influence of precipitation and temperatures on the growth stages of maize depending on the

hybrid, reflected in the level of production obtained in the Osmancea - Constanța area.
The forerunner was autumn wheat (*Triticum aestivum L.*). The tillage of the germination bed was carried out by disc harrowing one day before the sowing day at a depth of 5-6 cm.
The sowing was carried out on 2.04.2017, 3.04.2018 and 2.04.2019, respectively, at a depth of approximately 5-6 cm, at a density of 63,000 germinating grains /ha.
Before using herbicides as a technological method, it is necessary to know the structure of crops in crop rotation and the rotation thereof, the main weed species and the establishment of doses, epochs and methods of application thereof (Geoffroy et al., 2013; Vasileiadis et al., 2013).
Weed control in maize crop was carried out by pre-emergent application of the commercial product Wing P (212.5 g/l *Dimethenamid*-P and 250 g/l *Pendimethalin*), at a dose of 3.5 l / ha. In the 4-6 leaf stage to control weeds, maize was treated with herbicides using the REKOR package (Callam®: *tritosulfuron* and *dicamba* and Samson® Extra 6 OD: 60 g/l nicosulphuron) + DASH adjuvant.
Phenological observations were made from spurting to panicle emergence (vegetative phenophases until entering the generative phase), following the thermal gradient achieved from spurting to each phenophase, as well as the thermal gradient recorded for each phenophase. The calculation and interpretation of the production results was made based on the analysis of the variance of the experiments placed in the subdivided plots (Săulescu and Săulescu, 1967).

RESULTS AND DISCUSSIONS:

Analysing the agricultural years 2017-2019 from the point of view of the evolution of temperatures and precipitations we can characterize the experimental years as follows:

- agricultural year 2017 - very warm and normal in terms of rainfall;
- agricultural year 2018 - very hot and rainy;
- agricultural year 2019 - excessively hot and rainy.

In the three years of experiments, the monthly averages were higher as compared to the multiannual average of the reference area, regardless of the year of experimentation, as this is the trend of recent years. The sum of the average monthly temperatures for the studied agricultural years exceeds the multiannual average for 77 years (4174.1°C) by 609.6°C in 2017, by 524.5°C in 2018, the record being held by 2019 (+ 1030.7°C), which demonstrates the arid trend of the south-eastern area of Romania.
The largest positive deviations of the average monthly temperatures as compared to the multiannual average are registered in the months: June 2019 (+ 5.5°C), October 2019 (+ 5.1°C), November 2019 (+ 5.0°C), March 2019 (+4.4°C), March 2017 (+ 4.1°C), June 2018 (+ 3.7°C), December 2017 (+ 3.6°C), September 2017 (+ 3.5°C), December 2019 and October 2018 (3.4°C), August 2019 and August 2018 (3.3°C), February 2019 (+ 2.9°C), May 2018 (+ 2.8°C), and January and September 2018 (+ 2.6°C) (Figure 1).

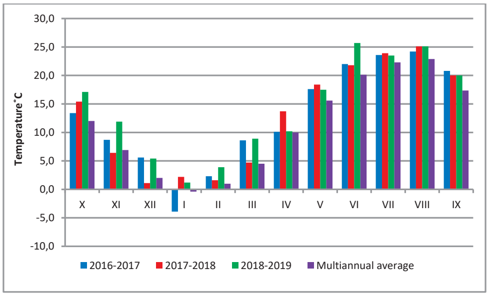

Figure 1. The evolution of the average monthly temperatures in the experimental years at Osmancea - Constanța

From a rainfall point of view, only 2017 differs, which can be considered a normal year in terms of accumulated rainfall, 28.5 mm less than the multiannual average (433.8 mm). The years 2018 and 2019 exceed the multiannual average (433.8 mm) by 289.1 mm, respectively 255.3 mm, but the distribution of precipitation is unevenly distributed compared to the requirements of the maize plant.
Thus, in 2018, compared to the multiannual average, the months with excess precipitation alternated (+52.4 mm in March, +34.2 mm in May, +88.2 mm in June, +71.1 mm in July) with dry months (-31.2 mm in April, -36.6 mm in August, -25.7 mm in September and -34.0 mm in October). In 2019, the alternation of rainy and dry months was more prominent. Thus, in March they recorded +53.1 mm,

followed by April -31.2 mm, excess rainfall in June (+88.2 mm) and July (+71.1 mm), followed by August when there was no rainfall (-36.6 mm = value of the multiannual average) and September (-25.7 mm) (Figure 2).

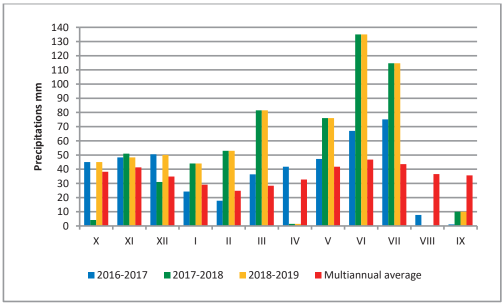

Figure 2. The evolution of precipitation in the experimental years at Osmancea - Constanța

To trigger the processes of germination and emergence, maize needs certain requirements of temperature ($8\text{-}10^0C$) and humidity (grains need at least 27-34% water by weight), correlated so as to ensure a good emergence of plants.

In addition to ensuring the optimal conditions of temperature and humidity at the time of sowing, the applied agrophytotechnical measures (soil tillage system, fertilization formula and hybrid) also play an important role.

If the soil moisture is sufficient for germination, the emergence of maize takes place in 16-20 days at temperatures of 10-12°C, in 13-15 days at temperatures of 12-15°C, in 8-10 days at temperatures of 15-18°C and in 5 -6 days at a temperature of 21°C.

In 2017, maize was sown on 2.04. and the emergence took place 23-25 days after sowing, the tested hybrids having the best reaction to the fertilization formula $N_{90}P_{40}K_{40}$ + Greenstart ($N_4P_{10}Mg_{0.5}S_{1.25}Zn_{0.5}$), regardless of the tillage system.

In 2018, maize was sown on 3.05. and the emergence took place 12-13 days after sowing, and the tested hybrids reacted similarly as in 2017 in terms of the fertilization formula.

In 2019, maize was sown on 2.04. and emergence took place 19-21 days after sowing. The influence of the application of the fertilization formula $N_{90}P_{40}K_{40}$ + Greenstart ($N_4P_{10}Mg_{0.5}S_{1.25}Zn_{0.5}$) is relevant regardless of the tillage system and the tested hybrid.

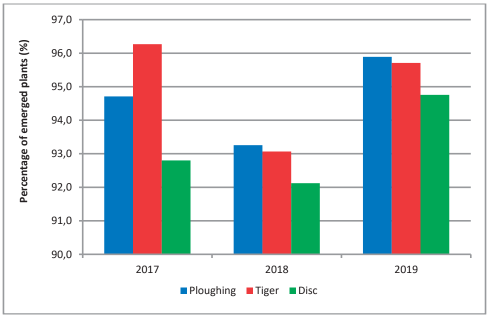

Figure 3. Percentage of plants emerged on average on tillage in experimental years

The evolution of climatic conditions after sowing and the tillage system influence the percentage of emerged plants. The highest percentage of emergence was recorded in the work performed with tiger in 2017 (93.3%), when maize benefited from temperature and humidity at the level of specific crop requirements (Figure 3).

In 2018 and 2019, the best percentage of emergence is registered in the tillage system by ploughing (93.3%, 95.9%), (Figure 4) a phenomenon explainable by the higher storage capacity of precipitation during the winter compared to the tiger or disc tillage system, when the soil is less modified in depth.

In addition to the influence of the climatic conditions of the experimental years, the percentage of emerged plants was also influenced by the fertilization formula.

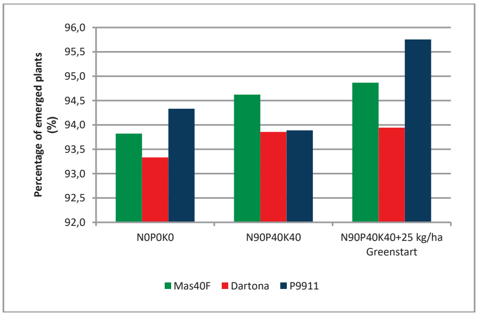

Figure 4. Percentage of plants emerged on average per hybrid depending on the fertilization formula

In 2017, the largest emergence process was recorded by the hybrid P9911, when the fertilization formula $N_{90}P_{40}K_{40}$ + Greenstart ($N_4P_{10}Mg_{0.5}S_{1.25}Zn_{0.5}$) was applied. Hybrid P9911 behaved similarly in 2018 (Figure 5).

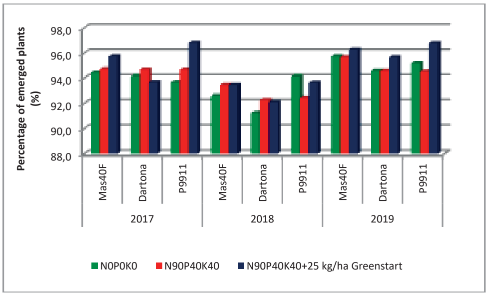

Figure 5. Percentage of plants emerged on average per hybrid depending on the fertilization formula and the experimental year

In 2019, when sowing was carried out very late, in May due to the absence of soil moisture, a higher percentage of emergence is recorded in case of hybrid P9911 for the non-fertilized, which can be explained by the inability of the applied fertilizers to mobilize.

Phenological observations made in the vegetative phase of maize per years of experimentation show that the tillage and fertilization formula do not significantly influence their duration and the sum of the temperature degrees required for the development of phenophases (Table 1).

Table 1. The growth stages of maze in the experimental years, Osmancea

Phenophase	2017			2018			2019		
	Date	Number of days	$\sum t^0$	Date	Number of days	$\sum t^0$	Date	Number of days	$\sum t^0$
Emergence	26.04	24	242.4	15.05	12	220.8	22.04	20	204.0
2 leaves	4.05	8	140.8	20.05	5	92.0	29.04	7	71.4
4 leaves	11.05	7	123.2	5.06	16	293.0	7.05	10	248.8
6 leaves	17.05	6	105.6	11.06	6	130.8	12.05	6	110.4
8 leaves	29.05	13	228.8	17.06	6	130.8	24.05	12	210.0
Panicle emergence	30.06	32	695.2	9.07	24	520.3	27.06	33	812.9

Date of sowing: 2.04.2017; 3.05.2018; 2.04.2019

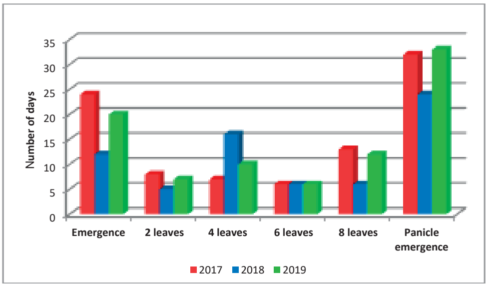

Figure 6. Number of days spent by maize hybrids on growth phenophases

Regarding the number of days necessary for the development of vegetative phenophases, it can be mentioned that (Figure 6):
- the emergence took place the latest in 2017 (24 days);
- the 2-leaf phenophase was registered 5-8 days after emergence, regardless of the years of experimentation;
- the 4-leaf phenophase took place 7 days after the emergence of the 2nd leaf in 2017, 10 days in 2019 and 16 days in 2018;
- 6-leaf phenophase, regardless of the experimental year, the hybrids needed 6 days to move to this phenophase;
- 8-leaf phenophase - the hybrids needed 6 days in 2018, 12 days in 2019 and 13 days in 2017, to reach this phenophase;
- the panicle emergence took place the fastest in 2018 at only 24 days from the previous phenophase, and in 2017 and 2018 at 32-33 days.

Regardless of the number of days from sowing to emergence, the hybrids needed on average over the experimental years a sum of temperatures of 224.4°C (Figure 7).

In order to go through the growth stages, the maize hybrids reacted differently in the years of experimentation regarding the accumulation of the thermal gradient necessary for each phenophase and in close connection with the presence or absence of precipitations in the analysed period. A solid example is the year 2018, when at the 4-leaf stage it was necessary to accumulate 293°C in 16 days from the previous phenophase, a period in which 76.0 mm of precipitation fell, which negatively influenced the growth of maize plants.

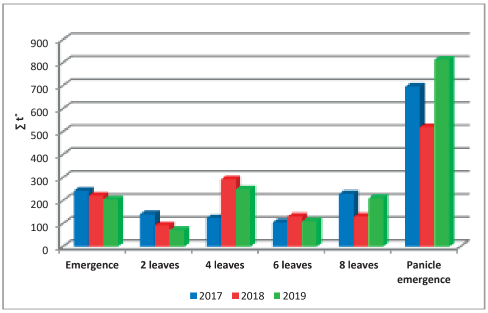

Figure 7. The sum of the temperatures achieved by maize hybrids on the growth phenophases

The influence of climatic factors (temperature and precipitation) on the vegetative phase of maize was also reflected in the yields obtained.
On average for the experimental years, in 2017, a normal agricultural year in terms of rainfall compared to the multiannual average (-27 mm), the level of yield was higher (10804 kg/ha) than in other experimental years.
Analysing the influence of climatic conditions on the yields of maize hybrids on average on tillage, it is observed that the highest yields are obtained for the tillage performed with tiger in 2017 and 2018 (11719 kg/ha and 11189 kg/ha respectively). The lowest productions were obtained in 2019. In the conditions of extreme drought of 2019, the yields obtained for the tested maize hybrids were higher in case of the ploughed tillage (5394 kg/ha) (Table 2).
On average during the three experimental years, the highest yields in case of tested maize hybrids were obtained by using the fertilization formula N90P40K40 + Greenstart (N4P10Mg0.5S1.25Zn0.5) regardless of the tillage system (9704 kg/ha).
In 2017, on average on tillage systems, hybrid P9911 is highlighted in case of the fertilization formula N90P40K40 + Greenstart (N4P10Mg0.5S1.25Zn0.5) (12306 kg/ha).
On average over experimental years, the highest yields are obtained by the hybrid P9911 in case of all fertilization formulas. The highest production is obtained by the Mas40F hybrid in 2017 for the tiger tillage system.
Analysing the average yields on the fertilization formula obtained for the hybrids tested in the three years of experimentation in all tillage systems, we can conclude:

- in case of ploughing, the highest yield is obtained by the hybrid P9911 (10682 kg/ha);
- in case of Tiger the highest yield is obtained by the hybrid Mas40F (10780 kg/ha);
- in case of disc the highest yield is obtained by the hybrid P9911 (7975 kg/ha);

Table 2. Yields obtained in case of maize hybrids at the 3x3x3 multifactorial experiment, Osmancea, Constanța 2017-2019

Tillage	Fertilization formula	Hybrid	Year of experimentation			Hybrids average
			2017	2018	2019	
Ploughing	$N_0P_0K_0$	Mas40F	8706	9197	4093	7332
		Darton	8246	9373	3897	7172
		P9911	9103	9223	4070	7465
	N90P40K40	Mas40F	10812	10577	5813	9067
		Darton	11086	9923	5713	8907
		P9911	11504	11043	6073	9540
	N90P40K40 + Greenstart (N4P10Mg0.5S1.25Zn0.5)	Mas40F	13401	11973	6533	10636
		Darton	12922	12793	6017	10577
		P9911	13638	12070	6337	10682
Average on plough			**11046**	**10686**	**5394**	**9042**
Tiger	$N_0P_0K_0$	Mas40F	9166	9537	4000	7568
		Darton	8560	9997	4083	7547
		P9911	9047	9993	4053	7698
	N90P40K40	Mas40F	13236	12070	5057	10121
		Darton	13259	11967	5147	10124

		P9911	12854	11550	5077	9827
	N90P40K40 + Greenstart (N4P10Mg0.5S1.25Zn0.5)	Mas40F	13208	13208	5923	10780
		Darton	12717	12717	5647	10360
		P9911	13102	13102	5923	10709
Average on tiger			**11719**	**11189**	**4744**	**9217**
Disc	$N_0P_0K_0$	Mas40F	7739	7493	3317	6183
		Darton	7495	7433	3670	6199
		P9911	7973	6890	3360	6074
	N90P40K40	Mas40F	11048	8237	3927	7737
		Darton	11329	7980	4073	7794
		P9911	11284	8320	4097	7900
	N90P40K40 + Greenstart (N4P10Mg0.5S1.25Zn0.5)	Mas40F	9578	9223	4343	7715
		Darton	10191	9047	4480	7906
		P9911	10178	9320	4427	7975
Average on disc			**9646**	**8216**	**3966**	**7276**
Average per experimental years			**10804**	**10030**	**4701**	

In order to highlight the role of precipitation in crop formation, the precipitation recovery coefficient was determined (yield kg /precipitation mm), relating the yield obtained to the amount of precipitation during the vegetation period of maize.

The technological elements can influence the way water from precipitations is recovered, and in order to determine the influence of the studied experimental factors (tillage, fertilization formula and hybrid) the precipitation recovery coefficients were calculated according to each factor.

In recent years there are growing problems regarding drought, so it is necessary to conserve water from the soil, and the choice of equipment and technology has the most important role (Rusu et al., 2014). Limiting the effects of drought can also be achieved through agro-phytotechnical measures for the accumulation, conservation and efficient recovery of rainwater.

By analysing Table 3, it can be observed that in 2017 the highest precipitation recovery coefficient is registered in case of the ploughing tillage system (Control). When working with the tiger and the disc, the difference from the control is negative in both cases, which leads us to the conclusion that in years with moderate rainfall, water is best used by plants when the soil is ploughed.

Table 3. Precipitation recovery coefficient according to tillage
Osmancea, Constanța 2017-2019

Tillage system	Precipitation recovery coefficient kg / mm			%			Dif. ± Control kg / mm			Significance		
	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
Ploughing	56.8	31.7	26.6	100			Control					
Tiger	48.7	34.3	24.6	85.7	108.2	92.5	-8.1	2.6	-2	000	*	-
Disc	40.2	24.4	19.6	70.8	77.0	73.7	-16.6	-7.3	-7	000	00	00
DL 5%							2.0	3.4	2.9			
DL 1%							3.4	5.6	4.9			
DL 0.1							6.3	10.4	9.2			

In 2018, a rainy year (+100 mm as compared to the multiannual average), the rainwater was best used for the tillage performed with the tiger, this one being significant.

2019 is characterized by a water deficit of 34 mm as compared to the multiannual average, the rainfall being very unevenly distributed as compared to the maize moisture requirements (0 mm in August and only 10 mm in September). Under these conditions, the highest precipitation recovery coefficient is recorded in case of the ploughed tillage (Control), due to the capacity of the plants to use the water reserve accumulated in the deeper layers of the soil (Table 3).

Soil fertilization presents an effective measure to minimize the consequences of pedological droughts by using moisture reserves by 20-25% more efficiently. This is because by ensuring a balanced fertilization ratio, with macroelements the efficiency of water recovery saving water /product unit increases (Săndoiu, 2012).

The precipitation recovery coefficients were influenced by the fertilization formula, 2017 being very statistically significant for the formula N90P40K40 and Greenstart (N4P10Mg0.5S1.25Zn0.5) for N90P40K40 (Table 4). The positive effect of the fertilization formula N90P40K40 + Greenstart (N4P10Mg0.5S1.25Zn0.5) is observed in 2018 when the application of Greenstart (N4P10Mg0.5S1.25Zn0.5) resulted in an increase in the precipitation recovery coefficient from significant to very significant statistically. In 2019, both fertilization formulas brought a distinctly significant increase in the precipitation recovery coefficient (table 4).

The application of fertilization formulas determines an optimal growth and development of plants, with a capacity to use on water from precipitation. The maize hybrids studied did not differ in terms of the manner of water recovery from precipitations in 2017 and 2018. In 2019, the hybrid P9911 registers a statistically significant recovery coefficient as compared to the control (average of tested hybrids), and Mas40F hybrid the lowest coefficient of water recovery from precipitations which indicates a sensitivity of the hybrid to drought (Table 5).

Table 4. Precipitation recovery coefficient according to the fertilization formula, Osmancea, Constanța 2017-2019

Fertilization formula	Precipitation recovery coefficient kg / mm			%			Dif. ± Control kg / mm			Significance		
	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
$N_0P_0K_0$	35.5	26.1	18.9	100			Control					
N90P40K40	49.0	30.2	24.7	138.0	115.7	130.7	13.5	4.1	5.8	***	*	**
N90P40K40 + G *	50.4	34.1	27.2	142.0	130.7	143.9	14.9	8	8.3	***	***	**
DL 5%							2.6	2.5	3.2			
DL 1%							4.4	4.2	5.3			
DL 0.1							8.2	7.8	9.9			

* Greenstart (N4P10Mg0.5S1.25Zn0.5)

Table 5. Precipitation recovery coefficient according to tested hybrids, Osmancea, Constanța 2017-2019

Hybrid	Precipitation recovery coefficient kg / mm			%			Dif. ± Control kg / mm			Significance		
	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
Mas40F	44.9	30.2	18.9	99.8	100.1	80.3	-0.1	0.03	-4.7			
Darton	44.4	30.1	24.7	98.6	99.8	104.5	-0.6	-0.06	1.1			
P9911	45.7	30.2	27.2	101.6	100.1	115.3	0.7	0.03	3.6			*
Average (MT)	44.9	30.1	23.6	100			Control			-	-	-
DL 5%							8.8	1.6	3.1			
DL 1%							14.6	2.6	5.1			
DL 0.1							27.4	4.8	9.6			

CONCLUSIONS

The growth stages of maize are largely influenced by the climatic conditions of the study years while the influences due to the tillage method, the fertilization formula and the hybrid do not show significant differences.

The influence of climatic conditions is also reflected in the level of production.

On average, the highest yields on tillage are obtained for the one performed with tiger in 2017 and 2018 (11719 kg/ha and 11189 kg/ha, respectively).

The lowest yields were obtained in 2019. In the conditions of extreme drought of 2019, the yields obtained for the tested maize hybrids were higher in case of ploughed tillage (5394 kg/ha).

In the three experimental years, the highest yields of tested maize hybrids were obtained with the fertilization formula N90P40K40 + Greenstart (N4P10Mg0.5S1.25Zn0.5) regardless of the tillage system. Of the hybrids tested in the 3 experimental years, the P9911 hybrid is the most productive.

Rainwater is used differently by maize plants depending on the technology used. In years with lower rainfall, the plants make the best use of water when sown by ploughing, and in rainy years when sown with the tiger. Regardless of the amount of rainfall the precipitation recovery coefficient is higher when the plants receive additional fertilization.

Under normal water supply conditions, the differences between the precipitation recovery coefficients depending on the hybrid are not statistically assured, but in 2019, a year with accentuated water deficit, the P9911 hybrid stands out, which obtains a statistically significant difference as compared to the tested hybrid average.

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RESEARCH ON EFFECTIVENESS OF SOME FUNGICIDES TREATMENTS ON THE ATTACK OF *Phomopsis/Diaporthe helianthi* ON SUNFLOWER IN BRAILA, BRAILA COUNTY

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Abstract

The paper aims to present the results obtained concerning the effectiveness of fungicides in controlling the attack of Phomopsis/Diaporthe helianthi. Was followed the behavior of some sunflower hybrids against the attack, were applied solid fertilizers (urea 46% N) and liquids fertilizers in two doses (Last N 250 g/l N) on a constant agricultural fund N:P:K. and the pathogen was controlled by applying two fungicides, Sfera 535 SC (0.4 l/ha) and Tanos 50 WG (0.4 kg/ha). Regarding the hybrid ES JANIS, the fungicide Tanos (0.4 kg/ha) recorded the best value for the effectiveness, with a percentage of 95.56% in the fertilized plot with Last N (1 dose). In the case of the hybrid SY BACARDI CLP, in 2018, the fungicide Tanos (0.4 kg/ha) recorded the best value concerning the effectiveness, with a percentage of 92.27% in the fertilized plot with urea 46% N (90 kg/ha N a.s.). In 2019, both Sfera (0.4 l/ha) and Tanos (0.4 kg/ha) recorded the best values concerning the effectiveness, with a percentage of 94.10% in the fertilized plots with Last N (2 doses).

Key words*: sunflower, degree of attack, effectiveness, fungicides.*

INTRODUCTION

Sunflower is the main oil plant in Romania, with productions to ensure the need for oil in human food (Chiriac et al., 2018 a; Chiriac et al., 2018 b). In the coming years, Romania will continue to become a more and more important oilseeds producer and exporter in the European Community (Arghiroiu et al., 2015; Chiriac et al., 2019).

The first appearance of the fungus *Diaporthe helianthi* was reported in 1980 in Voevodina - Yugoslavia, from where it expanded and appeared in Hungary, Romania, Bulgaria, Italy and France (Vranceanu et al., 1983). *Diaporthe helianthi* was assumed to be the single causal agent of *Phomopsis* stem canker, although several researchers suspected that the disease might be caused by more than one species of *Diaporthe* (Mathew et al., 2018).

The *Phomopsis* attack has serious repercussions on seeds and oil production when it appears early. Later infections, before flowering, destroy the vessels leading plants, but when the attack occurs at the end of flowering, the losses are substantially reduced. Production losses are estimated at over 50% in the case of infections before flowering, at 20-30% for those during flowering and at 10-20% for the attack at the end of flowering. At physiological maturity, the losses are negligible (Davet et al., 1991).

The symptoms following *Phomopsis* attack are characterized by the appearance of spots on the leaves, petioles, stems and under certain conditions, also on the capitulum.

On the stem, around the insertion point of the petioles, spots appear ellipsoidal, brown, extending to surround the stem. The tissues of the spots rot and the stem it breaks. Pycnidia appear in the attacked tissues as small, brown spots. It manifests itself on the leaves by brown lesions, bordered by yellow areas, which from the extremities and the tip of the leaves, advances to the triangle-shaped petiole. The spots may cover the entire leaf. In case of a strong attack, the plants browns and dries, having a charred appearance (Cristea, 2005).

Dabaeke et al. (2009), quoting Jouffret et al. (2005), recommends the application of fungicides by conventional sprayers, in crops 55 - 60 cm high, at the end of the month May and until mid-June.

The measures to prevent this pathogen consist of cultivating tolerant hybrids, respecting a rotation in which the sunflower does not return sooner than 5-6 years, respecting the optimum sowing period, using a suitable density as well as balanced fertilization that will include all macro-elements in approximately equal proportions (Popescu, 1999). The fertilization of the sunflower necessarily needs to take into account the economic component of this technological step (Franzen, 2016). With all the existing concerns in obtaining products with activity in controlling the pathogens (Ichim et al., 2017; Cristea et al., 2017), the application of fungicides treatments is an important measure against diseases in the cultivated plants (Balasu et al., 2014; Buzatu et al., 2018; Jaloba et al., 2019; Alexandru et al., 2019).

The agricultural sector will continue to keep up with the changes, to adapt, to remain competitive because having ability to produce goods and to provide services means economic growth (Chiriac et al., 2018b).

MATERIALS AND METHODS

The research has followed the behavior of some sunflower hybrids against *Phomopsis/Diaporthe helianthi* in terms of application of solid and liquid fertilizers on a constant agriculture fund N:P:K and the effectiveness of the treatments applied against the monitored pathogen. A three-factor experience, the location of the plots was done randomly, by the method of incomplete blocks, each variant having 3 repetitions. The results were obtained in 2018 and 2019.

Factor 1 (genotype): ES JANIS CLP, MAS 92.CP, SY NEOSTAR CLP, SY BACARDI CLP, ES GENESIS.

Factor 2 (fertilization): unfertilized plot; urea fertilizer 46% N (90 kg/ha active substance); liquid fertilizer Last N (250 g/l N – one dose: 15 l/ha in 200 l water); liquid fertilizer Last N (250 g/l N – two doses: first application 15 l/ha in 200 l water and the second application, with the same dose, 14 days after).

Factor 3 (fungicides): Sfera 535 SC (0,4 l/ha); Tanos 50 WG (0,4 kg/ha).

The pathogen was controlled with two fungicides, with Sfera 535 SC (160 g/litre Cyproconazole+375 g/litre Trifloxystrobin) in dose of 0.4 l/ha and Tanos 50 WG (250 g/kg Cymoxanil+250 g/kg Famoxadone) in dose of 0.4 kg/ha.

Assessments were made on the frequency (F%) and the intensity (I%) of pathogen attack, the degree of attack (DA%) and effectiveness (E%).

The degree of attack was calculated using the formula: $DA\% = \frac{F\% \cdot I\%}{100}$.

The effectiveness was calculated using the Abbott formula:

$E\% = 1 - \frac{DA\%Tp}{DA\%Up} \cdot 100$, where DA%Tp = degree of attack in treated plot; DA%Up = degree of attack in the untreated plot.

RESULTS AND DISCUSSIONS

The applied treatments considerably reduced the attack of *Phomopsis/Diaporthe helianthi* in the treated plots (Table 1.). Regarding the hybrid ES JANIS, it registered an attack of *Phomopsis/Diaporthe helianthi* only in 2018, with a degree of the attack of 0.45% for the untreated plot. For the other variants, the degree of attack varied from 0.02% for the fertilized plot with Last N (1 dose) to 0.11% for the fertilized plot with urea 46% N (90 kg/ha N a.s.). Concerning those two fungicides used in the control of this pathogen, the fungicide Tanos (0.4 kg/ha) recorded the best value for the effectiveness, with a percentage of 95.56% in the fertilized plot with Last N (1 dose).

In the case of the hybrid MAS 92.CP, for the untreated plot, in 2018 the degree of the attack was 0.56% and in 2019 0.83%. The most effective fungicide in controlling the pathogen *Phomopsis/Diaporthe helianthi,* proved to be the fungicide Tanos (0.4 kg/ha), with an effectiveness of 92.86% for the fertilized plot with Last N (1 dose) in 2018 and an effectiveness of 95.18% for the fertilized plot with urea 46% N (90 kg/ha N a.s.) in 2019.

The hybrid SY NEOSTAR, registered a degree of the attack of 7.03% in 2018 and 5.18% in 2019, for the untreated plot. The fungicide Tanos (0.4 kg/ha) recorded the best value concerning the efficacy, with a percentage of 96.87% in the fertilized plot with Last N (1 dose) in 2018 and an effectiveness of 94.59% for the fertilized plot with Last N (2 doses) in 2019.

In the case of the hybrid SY BACARDI CLP, for the untreated plots, in 2018 the degree of the attack was 6.73% and in 2019, 5.2%. In 2018, the fungicide Tanos (0.4 kg/ha) recorded the best value concerning the efficacy, with a percentage of 92.27% in the the fertilized plot with urea 46% N (90 kg/ha N a.s.). In 2019, both Sfera (0.4 l/ha) and Tanos (0.4 kg/ha) recorded the best values concerning the effectiveness, with a percentage of 94.10% in the fertilized plot with Last N (2 doses). Toth and Cristea (2020) shows also that the fungicide Sfera 535 SC had a high effectivenes in controlling cercosporiosis.

Table 1. Effectiveness of the tretments applied in the control of the pathogen *Phomopsis/Diaporthe helianthi* (2018 – 2019) in Braila, Braila county

			Phomopsis/Diaporthe helianthi			
			2018		**2019**	
Hybrid	**Chemical fungicides/ fertilization**		DA(%)	**E(%)**	DA(%)	**E(%)**
ES JANIS	Untreated		**0.45**	-	-	-
	Sfera 535 SC (0.4 l/ha)	urea 46% N (90 kg/ha N a.s.)	0.11	**75.56**	-	-
		Last N (1 dose)	0.07	**84.44**	-	-
		Last N (2 doses)	0.07	**84.44**	-	
	Tanos (0.4 kg/ha)	urea 46% N (90 kg/ha N a.s.)	0.05	**88.89**	-	-
		Last N (1 dose)	0.02	**95.56**	-	-
		Last N (2 doses)	0.07	**84.44**	-	-
MAS 92. CP	Untreated		**0.56**	-	**0.83**	-
	Sfera 535 SC (0.4 l/ha)	urea 46% N (90 kg/ha N a.s.)	0.12	**78.57**	0.16	**80.72**
		Last N (1 dose)	0.12	**78.57**	0.13	**84.34**
		Last N (2 doses)	0.17	**69.64**	0.15	**81.93**
	Tanos (0.4 kg/ha)	urea 46% N (90 kg/ha N a.s.)	0.06	**89.29**	0.04	**95.18**
		Last N (1 dose)	0.04	**92.86**	0.07	**91.57**
		Last N (2 doses)	0.22	**60.71**	0.21	**74.70**
SY NEOSTAR CLP	Untreated		**7.03**	-	5.18	-
	Sfera 535 SC (0.4 l/ha)	urea 46% N (90 kg/ha N a.s.)	1.51	**78.52**	1.27	**75.48**
		Last N (1 dose)	0.96	**86.34**	1.34	**74.13**
		Last N (2 doses)	0.78	**88.90**	0.73	**85.91**
	Tanos (0.4 kg/ha)	urea 46% N (90 kg/ha N a.s.)	0.56	**92.03**	0.37	**92.86**
		Last N (1 dose)	0.22	**96.87**	0.60	**88.42**
		Last N (2 doses)	0.54	**92.32**	0.28	**94.59**
SY BACARDI CLP	Untreated		**6.73**	-	**5.42**	-
	Sfera 535 SC (0.4 l/ha)	urea 46% N (90 kg/ha N a.s.)	1.38	**79.49**	1.64	**69.74**
		Last N (1 dose)	1.68	**75.04**	1.14	**78.97**
		Last N (2 doses)	1.34	**80.09**	0.32	**94.10**
	Tanos (0.4 kg/ha)	urea 46% N (90 kg/ha N a.s.)	0.52	**92.27**	0.64	**88.19**
		Last N (1 dose)	0.33	**95.10**	0.60	**88.93**
		Last N (2 doses)	0.30	**95.54**	0.32	**94.10**
ES GENESIS	Untreated		**0.68**	-	-	-
	Sfera 535 SC (0.4 l/ha)	urea 46% N (90 kg/ha N a.s.)	0.18	**73.53**	-	-
		Last N (1 dose)	0.19	**72.06**	-	-
		Last N (2 doses)	0.10	**85.29**	-	-
	Tanos (0.4 kg/ha)	urea 46% N (90 kg/ha N a.s.)	0.08	**88.24**	-	-
		Last N (1 dose)	0.06	**91.18**	-	-
		Last N (2 doses)	0.22	**67.65**	-	-

Regarding the hybrid ES GENESIS, it registered an attack of *Phomopsis/Diaporthe helianthi* only in 2018, with a degree of attack of 0.68% for the untreated plot. For the other variants, the degree of attack varied from 0.06% for the fertilized plot with Last N (1 dose) to 0.22% for the fertilized plot with Last N (2 doses). Also, for this hybrid, the fungicide Tanos (0.4 kg/ha) proved to be effective, with an effectiveness of 91.18% for the fertilized plot with Last N (1 dose).

Research on the behavior of some sunflower hybrids to the attack of the pathogen *Diaporthe helianthi* were made also by Manole et al. (2018).

CONCLUSIONS

The treatments applied have considerably reduced the attack of *Phomopsis/Diaporthe helianthi* in the treated plots.

The fungicide Tanos (0.4 kg/ha) proved to be effective for all the analyzed variants.

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STRUCTURAL ANALYSIS AND SEED PRODUCTIVITY OF POPULATIONS OF BIRD'S FOOT TREFOIL GROWN UNDER CONDITIONS OF CENTRAL NORTHERN BULGARIA

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Abstract

During the period 20016-2018 in the experimental field of RIMSA-Troyan were tested selection populations of bird's foot trefoil Syn_1, Syn_2, Syn_3, Syn_4, Syn_5, compared with to the Bulgarian variety 'Targovishte 1'. Seed yield and its structural elements were determined in the second and third years of the development of grassland and on the basis of studied reproductive indicators interrelations among them and productivity were established. It was found that the maximum yield of seeds was obtained by the population Syn_1 - 0.37 t .ha^{-1}, with an excess over the standard by 23.73%. High productivity is determined by the large number of seeds per pod (18.6). The lowest degree of variability according to the variation coefficients was found in the number of racemes per stem (5.89), and the highest in the number of pods per plant (19.40). The established very strong correlation between the number of pods per raceme and the weight of 1000 seeds (r = 0.7939), allows to derive the regression equation - y = 0.0794x + 0.9588. Thus, the presented statistical analysis of the data from the seed productivity allows to make a complete characterization of the populations, which will serve for the further selection process.

Key words: *Lotus corniculatus L., seed yield, structural elements, correlation coefficients Regression dependence.*

INTRODUCTION

Bird's foot trefoil (*Lotus corniculatus* L.) is an excellent component for creating artificial meadows and pastures (Bozhanska, 2017). It is considered as one of the most important fodder legumes along with alfalfa and clover (Bozhanska & Naydenova, 2020; Vasileva, 2017; Naydenova & Bozhanska, 2020). Variation in the quantitative and qualitative characteristics of the genetic resources of plants grown in different habitats is important for seed productivity (Kyuchukova, 2009; Uzun et al., 2015).

In the last few years, bird's foot trefoil has gained popularity due to its nitrogen-fixing ability (Vasileva & Ilieva, 2017), which is important for reducing nitrogen costs and defines its cultivation as economically efficient (Waghorn, 2008). On the other hand, its resistance to pathogens reduces rooting of roots (Beuselincket et al., 2005). A disadvantage of bird's foot trefoil is its low seed productivity (Garcia-Diaz & Steiner, 2000). This is due to the uneven cracking of the pods, which prevents its spreading (Sareen, 2004). The seed yield of bird's foot trefoil depends on the duration of sunlight and therefore the long summer days in some parts of the world are ideal for its production (Ferguson, 2017). The determined negative correlation among some characteristics (yields of dry matter and seeds, morphological, as well as disease resistance) and the different altitudes of collected seeds prove significant inherent variations in all traits and thus determine the huge genetic diversity of the region (Uzun & Donmez, 2016).

The objective of the present research was to study 5 bird's foot trefoil populations and to determine the seeding potential of their seeds, their structural elements determining yield and the relationship among them.

MATERIALS AND METHODS

During the period 2016-2018 in the experimental field of RIMSA-Troyan were tested selection populations of bird's foot trefoil, compared with 'Targovishte 1' - a Bulgarian cultivar: Syn_1 (limited free pollination of genotypes of ‘Bright’ and ‘Georgia 1’ cultivars), Syn_2 (pre-pollinated plants of local population with an origin from the village of Staro Selo and the Hungarian cultivar ‘Ergechi’), Syn_3 (synthetic population of local genotypes originating from Central Northern Bulgaria), Syn_4 (synthetic population of the Hungarian cultivars ‘Pecoli’ and ‘Gjiki’), Syn_5 (synthetic population of genotypes originating from Shumen, Nessebar, Kiten) Sowing was carried out in 2016, and the seeds were harvested over the next two years (2017 and 2018).

The sowing was performed manually, at a depth of 0.5-1.0 cm and a sowing rate of 12.0 t ha^{-1}. The phosphorous and potassium fertilizers were applied as reserve at a rate of 240 t ha^{-1} a. i., while nitrogen was applied at one fertilization with 60 t ha^{-1} a. i., before sowing. The birds foot trefoil was cultivated under the agricultural techniques, adopted in the area. The seeds were harvested from second cuttings as 65-70% of them became ripen.

The following characteristics were observed from the structural elements of the seed yield: number of racemes per plant; number seeds per pod; number of pods per plant; seed yield (t ha^{-1}). The study included average data of the different characteristics over the years. The mean values (X), minimum (min) and maximum (max) limits of the structural elements and seed yield were calculated (Lidanski, 1988). The degree of variation (VC) of parameters was determined through variation coefficient according to the scheme of Mamaev: up to 7% - very low, 7.1 to 12.0 % low, 12.1 to 20.0 % moderate; 20.1 – 40.0 % high: over 40.0 % - very high. Correlations (r) of Brave and Pirson were calculated to prove the relations between the different characteristics and their impact on productivity as well as among them. The data was processed by Microsoft Excel. The significant differences were determined by the methods of dispersion analysis.

RESULTS AND DISCUSSIONS

The 2016 is considered to be favorable in terms of meteorological conditions (Table 1), when the precipitation was evenly distributed during the months of the vegetation period. The average daily air temperature of 11.1 C° and the amount of precipitation of 115.2 l/m^2 in April in the year of sowing (2016) had a favourable effect on the normal sprouting of bird's foot trefoil. Phenophase 2-4 leaves occurred normally in mid-April, and bud formation in mid-June, when the first regrowth was harvested. The low soil moisture due to rainfall amount of 56.9 and 42.2 l/m^2 in June and July slowed down the growth and development of bird's-foot-trefoil and the formation of a second regrowth.

Table 1. Climatic characteristic for the period of 2016-2018

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average temperature	Average precipitation amount
Mounthly precipitation amount (l/m^2)														
2016	88,8	59,9	58,5	115,2	161,5	56,9	42,2	82,8	26,2	51,3	46,9	46,8	837,0	594,6
2017	70,1	21,1	60,7	90,4	133,1	113,2	186,6	13,2	38,9	126,3	53,7	75,9	983,2	762,4
2018	29,6	96,9	83,2	22,8	82,5	174,3	241,1	9,4	30,0	56,2	45,2	48,2	919,4	699,5
Average monthly air temperature (^{0}C)														
2016	-1,0	7,4	7,4	13,4	14,0	20,0	21,1	20,0	16,2	9,5	5,4	-0,7	11,1	15,2
2017	-5,3	2,1	8,1	10,1	14,5	19,9	20,6	20,4	16,4	9,8	6,3	3,5	10,5	15,0
2018	1,5	1,7	5,1	14,5	16,7	18,7	20,4	20,3	15,8	11,2	4,9	1,0	11,0	15,3

The second year of plant development (first seed productive year) was characterized by a significantly higher amount of precipitation in June (113.2 l/m^2), July (186.6 l/m^2), when the seed regrowth is formed. The lower humidity in August accelerated the harvesting of seeds, which was done during the first ten days of the month, but did not prevent high seed productivity from the grassland.

The agrometeorological characteristics of the third year of grassland development and the second for seed harvesting are quite different from the other two.

The significantly higher amount of precipitation in June (174.3 l/m^2), July (241.1 l/m^2) is obvious, as a very low moisture content was registered in August (9.4 l/m^2) and high average monthly air temperature (20.3°C). This was a prerequisite for the rapid cracking of the seeds and obtaining a lower yield.

The average annual results and the average ones on the structural analysis of seed yield for the whole study period are shown in Table 2.

Table 2. Structural elements of the seed yield year and per Mean for the period

populations	Number of pods per racemes			Number of racemes per stems			Number of seeds in pod			1000 seed weight		
	2017	2018	Mean	2017	2018	Mean	2017	2018	Mean	2017	2018	Mean
Targovishte 1	3,1	2,9	3,0	12,9	10,8	11,9	15,3	19,4	17,4	1,08	1,24	1,16
Syn_1	3,8	3,5	3,7	13,5	11,2	12,4	16,4	20,8	18,6	1,25	1,31	1,28
Syn_2	3,5	3,3	3,4	13,3	11,8	12,6	12,7	17,3	15,0	1,14	1,28	1,21
Syn_3	3,0	3,3	3,2	13,3	13,5	13,4	19,5	15,1	17,3	1,25	1,18	1,22
Syn_4	4,1	4,6	4,4	14,6	12,1	13,4	20,4	14,7	17,6	1,50	1,25	1,38
Syn_5	4,8	4,9	4,9	14,9	12,8	13,9	17,1	13,3	15,2	1,30	1,28	1,29
X	3,72	3,75	3,73	13,75	12,03	12,89	16,90	16,77	16,83	1,25	1,26	1,26
SD	0,67	0,80	0,72	0,80	1,00	0,76	2,81	2,92	1,42	0,15	0,05	0,08
VC	18,12	21,45	19,40	5,85	8,32	5,89	16,63	17,40	8,46	11,61	3,58	6,06
Min	3,00	2,90	3,00	12,90	10,80	11,85	12,70	13,30	15,00	1,08	1,18	1,16
Max	4,80	4,90	4,85	14,90	13,50	13,85	20,40	20,80	18,60	1,50	1,31	1,38

The impact of all factors involved in the experiment reflects on the structural elements of seed yield. In the first seed production year, data show that Syn_5 population had the highest number of pods per racemes - 4.8 and number of racemes per stem -14.9, compared to other bird's foot trefoil populations tested. The difference in the values of the number of pods per racemes is insignificant in terms of the standard and populations Syn_1, Syn_2, Syn_3. The value of this indicator is 1 point higher in Syn_4 population. Of the indicators determining the structural seed yield, the number of pods per racemes had the highest degree of variability, which can be seen from the coefficient of variation (CV = 18.12). The values for the number of racemes per stem vary within the narrowest limits in the individual populations, respectively from 12.9 to 14.9, which is due to their low coefficient of variation CV = 5.85. The mean value for the number of pods per racemes of the test populations was X = 3.72 with a standard deviation SD = 0.67. The average number of racemes per stem was 13.75, and the standard deviation SD = 0.80. The highest number of seeds per pod was found in Sin_1 population, as degree of variability according to the variation coefficient (CV=16.63) was average. A minimum value was reported in Syn_2 population. The mean value for this indicator is X = 16.90 and the standard deviation is 2.81. The weight of 1000 seeds per year in all tested populations varied from 1.08 to 1.50. The degree of variability is low, which is due to the insignificant difference between the minimum (1.08) and maximum values (1.50). The mean value of the number of seeds per pod is X = 1.25, and the standard deviation SD = 0.15.

In the second seed production year, the number of beans per racemes was from 2.9 in the standard to 4.9 in Syn_5 population, with an mean value of X = 3.75. This indicator has the highest value of the coefficient of variation (VC = 21.45) compared to all indicators characterizing seed productivity. In general, there is some correspondence between Number of pods per racemes and Number of racemes per stems over the years of study. All populations, slightly exceeded the standard in the number of racemes per stem. On this basis, however, the variation is within narrow limits (VC = 8.32). The average data for the year show that the populations formed an average of 16.77 Number of seeds per pod with a minimum value of 13.30 and a maximum of 20.80. The average weight of 1000 seeds were 1.26 g, which is a very low variability according to the coefficient of variation VC = 3.58 and the standard deviation SD = 0.05. The low values of seed weight are due to the fact that the seed undergrowth was formed in the absence of precipitation in August (9.4 l/m^2), which impeded the increasement of their weight.

The highest average number of pods per plant and number of racemes per stem was registered in population Syn_5, respectively 4.9 and 13.9. The number of pods per plant had an average degree of variability according to variation coefficient CV% - 19.40, and the number of racemes per stem had a very low degree of variability - 5.89. The highest number of seeds per pod was in Syn_1 population (18.6), while the highest weight of 1000 seeds was registered in Syn_4 population (1.38 g). There were not any significant differences in the number of pods per plant and the number of racemes per stem in the tested populations compared to the standard. The lowest degree of variability according to the variation coefficients was found in the number of racemes per stem (5.89), and the highest one in the number of pods per plant (19.40).

The values for seed yield in the studied cultivars and populations (Table 3) varied widely over years and on average for the study period. On average for the study period the seed yield in the tested populations, with the exception of the population Syn_2, exceeded the standard cultivar 'Targovishte 1'. The highest seed yield was obtained from population Syn_1 - 0.37 t.ha^{-1}, as the excess compared to the standard was 23.73%. A relatively high yield was also shown by Sin_3 population, which exceeded the standard by 11.54%. Syn_1 population had very well-proven differences in seed yield. Good evidence of differences was also reported in the yield of Syn_3 population.

Table 3. Seed yield year and per Mean for the period, t ha^{-1}

populations	2017 г.		2018 г.		Mean for the period	
	t ha^{-1}	% to St	t ha^{-1}	% to St	t ha^{-1}	% to St
Targovishte 1	0. 29	100.00	0.31	100.00	0.30	100.00
Syn_1	0.38	129.91+++	0.36	117.90++	0.37	123.73+++
Syn_2	0.30	102.56-	0.28	91.13-	0.29	96.68-
Syn_3	0.34	118.29+	0.32	105.16-	0.33	111.54+
Syn_4	0.32	111.79-	0.32	103.23-	0.32	107.39-
Syn_5	0.33	114.70+	0.28	91.77-	0.31	102.90-
LSD 5%	0.37	13.58	0.33	10.69	0.24	8.25
LSD 1%	0.55	18.80	0.45	14.80	0.34	11.43
LSD 0.1%	0.75	25.94	0.63	20.42	0.47	15.76

LSD – Fisher's least significant difference

Seed yield in the first seed production year varied from 0.30 t ha^{-1} to 0.38 t ha^{-1}. The maximum value was obtained in the population Syn_1, as the excess compared to the standard was 29.91%. All tested populations exceeded the seed yield standard. The insemination of plants in the second year of their development could be explained by the formation of a larger number of generative stems. The excess is from 2.56 to 29.91%. The low seed yields that year were due to unfavorable weather conditions. The seed yields in Syn_4 and Sin_5 populations are approximately the same, 0.32 and 0.33 t ha^{-1}, respectively.

In the second year, the seed productivity in most of the populations was lower than the first year. The highest seed yield was gathered from population Syn_1 (0.36 t ha^{-1}), which exceeded the standard by 17.90%. The high seed yield in the second year was due to the good supply of moisture of the grassland in June and July and the higher values for the number of seeds per pod and the number of racemes per stem. Seed yield exceeding the standard was reported in Syn_3 and Syn_4 populations (0.32 t ha^{-1}). In Syn_1 population, the differences are well established. In all other populations, there was a negative proof of the differences.

Populations from different habitats play a key role in terms of their main characteristics - adaptability and resistance to biotic and abiotic stress. Due to the impact of extreme weather conditions on the adaptability and productivity of bird's foot trefoil, according to Churkova (2013) the synthetic population that serves as a starting material for selection should be determined before it is approved as a cultivar in order to show its potential before spreading. The different yields obtained over the years show that the populations have shown their productive potential depending on the climatic conditions during the years of study as a result of their origin.

Theoretically, each plant has the potential to produce seeds equivalent to fertile ova. Of the five populations studied, it was found that none of the populations produced as many seeds as expected. The difference between the theoretical and the actual seed yield is large (Churkova, 2002). Pods cracking is a serious problem in bird's-foot-trefoil. The development of the seeds and the cracking of the pods depend on the relative air humidity, as the most important factor. The period of pod cracking is related to the maturity of the legumes, environmental conditions and individual genetic differences of plants (Sareen, 2004). The difference in the yield of the studied populations can be attributed to the uneven flowering period, due to which during the harvesting of seeds all pods do not have the same stage of maturity. In the present study, all populations began to bloom in late June in both years, but due to different weather conditions over the years, the seeds in the first production year was harvested in early August and for the second year in the last ten days of the same month. Other factors that could be a prerequisite for low seed yields are abortion of flower buds, lack of pollination and genetic factors. The presented structural analysis shows the great variability of the indicators in the individual populations. In the future, this can be used to isolate lines with less pod cracking and higher seed yield.

The performed correlation data analysis (Table 4) shows that the seed yield is positively correlated with all indicators of its structural elements. A strong correlation was found between seed yield and number of seeds/ pods (r = 0.7690).

Very strong correlation was established between the number of pods per racemes and the weight of 1000 seeds (r = 0.7939), which allows to derive the regression equation - y = 0.0794x + 0.9588 (Figure 1) and a strong correlation between the number of racemes per stem and number of seeds per pod (r = 0.7462). A good positive correlation was observed between the weight of 1000 seeds and the number of racemes per stem (r = 0.6052). A slight positive correlation of seed yield with Number of pods / raceme (r = 0.0297) and Number of racemes per stems (r = 0.0023) was found.

Table 4. Correlation coefficients between the Seed yield and Structural elements

indicators	Seed yield	Number of pods per racemes	Number of racemes per stems	Number of seeds in pod	1000 seed weight
Seed yield	1				
Number of pods per racemes	0.0297	1			
Number of racemes per stems	0.0023	0.7462	1		
Number of seeds in pod	0.7690	-0.2849	-0.3558	1	
1000 seed weight	0.3449	0.7939	0.6052	0.1719	1

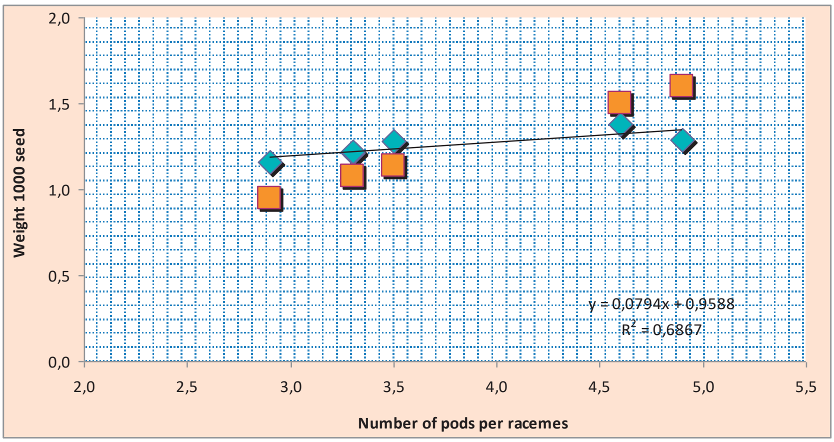

Figure 1. Regression dependence between 1000 seed Weight (g) and the Number of pods per racemes (Number)

CONCLUSIONS

Under the conditions of light gray pseudo-podzolic soils, from the studied 5 bird's-foot-trefoil population, the maximum seed yield was achieved by population Syn_1 - 0.37 $t.ha^{-1}$, with an excess over the standard by 23.73%. High productivity is determined by the large number of seeds per pod (18.6). The lowest degree of variability according to the variation coefficients was found in the number of racemes per stem (5.89), and the highest in the number of pods per plant (19.40). The established very strong correlation between the number of pods per raceme and the weight of 1000 seeds ($r = 0.7939$), allows to derive the regression equation - $y = 0.0794x + 0.9588$. The obtained data show that the genetic capabilities of the populations are best expressed in a certain area, with a combination of appropriate soil and climatic conditions and are important for further selection work.

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VIRULENCE AND AGGRESSIVENESS OF SOME SUNFLOWER BROOMRAPE POPULATIONS BELONGING TO DIFFERENT COUNTRIES

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Abstract

Broomrape (Orobanche cumana Wallr.) is a non-photosynthetic, obligatory, root parasitic plant, that specifically infects sunflower crop, causing significant yield losses. The parasite is widely spread in the majority of sunflower growing countries from Europe, Middle East and Asia. In the last years broomrape rapidly evolves, developing more aggressive races, which spread to new areas. In order to understand dynamics of pathogen populations and their adaptation to environment the knowledge related the virulence and aggressiveness of plant pathogens are required. Contrary to virulence which reveal the qualitative component of pathogenicity that is expressed vertically, aggressiveness refers to the quantitative variation of pathogenicity on susceptible hosts, without any restriction related to specificity. Present study was focused on the evaluation of aggressiveness of different broomrape populations belonging to different countries, such as Republic of Moldova, Bulgaria, Serbia, Romania, Ukraine, Spain, Turkey and China. Experience was carried out in green house using different sunflower genotypes (without qualitative resistance or genes or caring some or genes). The numbers of aerial and underground broomrape shoots, as well as of tubercles were counted and the frequency, intensity and degree of attack were calculated. The significant differences between broomrape populations for all analysed parameters were found. Thus, the most aggressive populations were those belonging to Soroca, Republic of Moldova and Ismail, Ukraine, followed by some populations from Turkey and Romania.

Key words: *sunflower, broomrape, aggressiveness, virulence.*

INTRODUCTION

Virulence and aggressiveness are key characteristics of pathogens. Virulence, defined as the ability of a particular genotype of parasite to infect a susceptible host, is the qualitative component of pathogenicity and determines the specialization of parasites within host families. Contrary to virulence, aggressiveness is a quantitative component, representing the degree of pathogenicity of the virulent strain. These two traits are interdependent. Thus, if the parasite is avirulent to a particular species or variety of plants, it is not aggressive towards these hosts, while a virulent strain of parasite could manifest different level of aggressivity (high, moderate or low), causing disease to a strong or weak degree (Shaner et al., 1992; Dyakov et al., 2007).

Host plant-pathogen association coevolves, so that, resistance and pathogenicity depend on specific interactions between single or multiple genes, determining qualitative or quantitative resistance in hosts, with the specific genes in parasites. Hosts and parasites may modulate the dynamics and genetic structure of each other, so in response to disease emergence, hosts evolve resistance, which reduces pathogen invasion, while the pathogens evolve greater virulence and aggressiveness as response to the selection imposed by qualitative and quantitative resistance of host (Woolhouse et al., 2002).

In many pathosystems interaction between host and parasite follows gene-for-gene model, with complete resistance in the host and avirulence in parasite, determined by *R* and, respectively, *Avr* dominant genes (Flor, 1971). According to Van der Plank, virulence is expressed vertically and it is linked to the presence or absence of *R* genes, whereas aggressiveness, mostly determined polygenic, is expressed horizontally, without specificity to host genotypes (Van der Plank, 1968). The products of *Avr* genes may act as effectors of pathogen virulence (Mundt, 2014; Jones and Dangl, 2006). Recent reports suggest that aggressiveness is also conditioned by minor gene-for-gene interactions (Niks et al., 2015).

The evolution of virulence ensures the emergence of pathogens, extension of hosts range, overcoming of specific host resistance genes and development of new pathotypes/

physiological races able to successful invasion and may compromise the effectiveness of control strategies. The phenotypic variation in aggressiveness is a key factor for pathogen adaptation to host and environment (Sacristan et al., 2008). It has to be mentioned that parasites present significant differences in aggressiveness, even they belong to the same pathotype, which are apparently homogenous (Pariaud et al., 2009; Geffroy et al., 2000). Large variation in aggressiveness has been detected within and between populations of different agriculturally important fungal pathogens, such as *Puccinia striiformis f. sp. tritici* (Milus et al., 2006), *P. triticina* (Pariaud et al., 2007), *Phytophthora infestans* (Andrivon et al., 2007), as well as parasitic angiosperm (Molinero-Ruiz and Melero-Vara, 2004).

In order to understand dynamics of pathogen populations and their adaptation the knowledge related the aggressiveness and virulence of plant pathogens are required (Leach et al., 2001).

Parasitic angiosperm *Orobanche cumana* Wallr. is one of the most critical constraints for sunflower production in the majority of European countries, as well as in the Middle East and Asia. In the last two decades, the aggressiveness and virulence of sunflower broomrape, naturally distributed from central Asia to south-eastern Europe, increased significantly and highly virulent races have appeared and spread very quickly to new areas outside the natural distribution area, such as France, Northern Spain, United Kingdom, Tunisia, Morocco and Portugal (Parker, 2014; Jestin et al., 2014; González-Cantón et al., 2019; Pineda-Martos et al, 2014).

The continuous introduction of new sunflower hybrids with monogenic race-specific resistance exert a selection pressure on broomrape populations evolution and contribute to the development of new more virulent races that overcome sunflower genetic resistance of cultivated genotypes (Rubiales, 2018). Thus, until now, eight races of *O. cumana*, A through H, have been identified.

Present study was focused on the evaluation of virulence and aggressiveness of different broomrape populations belonging to different countries, such as China, Turkey, Bulgaria, Serbia, Spain, Ukraine, Romania and Republic of Moldova.

MATERIALS AND METHODS

The virulence and aggressiveness of 27 populations of *O. cumana* was assessed and compared after infection of five sunflower inbred lines and hybrids. Particularly, as biological materials were used broomrape populations belonging to China (Inner Mongolia – nr. 1 and 6; He Bei); Turkey (Edirne, Merkez; Edirne, Kesan; Kirklareli, Luleburgaz; Adana); Bulgaria (Debovo); Serbia (conventionally noted as – ORSR 04; ORSR 07; ORSR 11; ORSR 14; ORSR 24; ORSR 25; ORSR 43); Spain (Seville); Ukraine (Ismail); România (Braila; Tulcea); Republic of Moldova (Cazanesti; Singera; Carabetovca; Rassvet; Chisinau; Popeasca; Sarata-Mereseni; Soroca).

The virulence of *O. cumana* populations was evaluated on a set of differential lines and sunflower commercial hybrids carrying different genes of resistance, including one susceptible (Performer from National Agricultural Research and Development Institute Fundulea – NARDI) and four resistant genotypes, as follow: LC1003A (from NARDI) – resistant to race E; LC1093A (NARDI) – resistant to race F; H_1E – resistant to race G (NARDI) and H_2Lg (Limagrain) – resistant to race H. Thirty plants for each sunflower line, separated into two replications of fifteen plants each, have been planted in boxes, which contained a mix of sand and peat in ratio 1:1, v/v, uniformly infected with broomrape seeds (30 mg seeds at 200 g of mix) from each population. The sunflower lines were grown in the greenhouse for 80 days at 18/24°C (night/day) temperatures with a 14h/10h photoperiod.

After the cultivation period, the plant root system was removed from the substrate and washed. To establish the aggressiveness of broomrape populations the total number of infested plants, as well as the number of broomrape attachments (tubercles, aerial and underground shoots) per host was quantified. The incidence of broomrape (attack frequency) and the average number of broomrape attachments per plant (attack intensity) have been established using the following formulas:

$F_{(\%)}$ = (N/ Nt) x 100, where

N – number of infected plants,

Nt – total number of observed plants;

I = a / N, where
a – total number of broomrape attachments,
N – number of infected plants.

RESULTS AND DISCUSSIONS

Resistance to *O. cumana* in sunflower is controlled by a combination of qualitative, race-specific resistance, associated with *Or*, dominant genes, and quantitative, non-race specific resistance, determined by multiple genes. The first type of resistance affects the presence or absence of infection with broomrape, while the second one affects the number of *O. cumana* attachments per host plant (Pérez-Vich et al., 2004).

Broomrape populations analysed in present study shown different pattern of virulence. All populations have infected susceptible hybrid Performer and were avirulent against the genotype H_2Lg carrying genes of resistance to race H (Table). The most virulent were the populations from Turkey, Romania, Ukraine, one population collected from China (Inner Mongolia region) and two from the Republic of Moldova (Soroca and Sarata Mereseni), which affected all sunflower genotypes, excepting the hybrid H_2Lg. Such results are not surprising, as it is known that more virulent broomrape races were found especially in the countries around the Black Sea (Molinero-Ruiz et al., 2015). Also, according to recent studies highly virulent race G was identified in China, being mainly limited to the western part of Inner Mongolia (Shi et al., 2015).

Six of analysed populations (Inner Mongolia nr. 1, He Bei, China; Debovo, Bulgaria; Seville, Spain; Popeasca and Chisinau, Moldova) parasitized only the deferential lines LC1003 and LC1093 carrying *Or5* and *Or6* genes conferring resistance to race E and F, and two populations belonging from Moldova (Carabetovca and Rassvet) affected only the line LC1003. The results are supported by previously reported data. Thus, the presence of new virulent populations of broomrape able to parasitize most of the race F resistant hybrids has been revealed in sunflower fields from Bulgaria and Spain (Shindrova and Penchev, 2012; Martin-Sanz et al., 2016).

Moldovan broomrape populations from Chisinau and Stefan-Voda (Popeasca) have affected inclusively LC1093 line resistant to race F, contrary to previously reported data, when they showed virulence only on the susceptible genotype and LC1003 line (Duca et al., 2017). These results suggest the existence of pathotypes with different virulence in the same or nearly situated fields. A high heterogeneity in virulence of broomrape and presence in the same field of an admixture of biotypes (races), where one or other type is dominant, was reported by Antonova et al. (Antonova et al., 2020).

Table. Virulence of *O. cumana* belonging to different countries on sunflower differential lines

Broomrape populations	Performer (sensible)	LC1003A (resistant to race E)	LC1093A (resistant to race F)	H_1E (resistant to race G)	H_2Lg (resistant to race H)
China					
Inner Mongolia nr. 1	+	+	+	-	-
Inner Mongolia nr. 6	+	+	+	+	-
He Bei	+	+	+	-	-
Turkey					
Edirne, Merkez	+	+	+	+	-
Edirne, Keşan	+	+	+	+	-
Kirklareli, Luleburgaz	+	+	+	+	-
Adana	+	+	+	+	-
Bulgaria					
Debovo	+	+	+	-	-
Serbia					
ORSR 04	+	-	-	-	-
ORSR 07	+	-	-	-	-
ORSR 11	+	-	-	-	-
ORSR 14	+	-	-	-	-
ORSR 24	+	-	-	-	-
ORSR 25	+	-	-	-	-
ORSR 43	+	-	-	-	-
Spain					
Seville	+	+	+	-	-
Ukraine					
Ismail	+	+	+	+	-
Romania					
Braila	+	+	+	+	-
Tulcea	+	+	+	+	-
Republic of Moldova					
Cazanesti	+	-	-	-	-
Singera	+	-	-	-	-
Carabetovca	+	+	-	-	-
Rassvet	+	+	-	-	-
Chisinau	+	+	+	-	-
Popeasca	+	+	+	-	-
Sarata Mereseni	+	+	+	+	-
Soroca	+	+	+	+	-

"+" indicates compatible interaction (presence of infection) and "–" indicates incompatible interaction (absence of infection).

The less virulent were the *O. cumana* belonging from Serbia, which infested only the susceptible hybrid, being highly aggressive against it (number of broomrape shoots per plant ranged from 7.3 to 14.0, with the incidence of attack

50.0-93.8%). In contrast to frequent changes in race composition and rapid appearance of new more virulent pathotypes of broomrape reported in Russia, Ukraine, Moldova, Romania, Turkey and Spain, data regarding racial status of this parasite in Serbia supported the opinion of slow evolution of *O. cumana.* Even the first report on broomrape in Serbian sunflower growing areas dates from 1951, until now only two races have been identified, the most virulent being race E (Miladinovic et al., 2014). Recently the presence of race F on small infestation spots has been established (Dedic et al., 2018).

Unlike virulence, aggressivity as a quantitative trait can be discussed in terms of a higher or lower level (Dyakov et al., 2007). In many cases, plant parasites manifest different levels of aggressiveness even within the same race. Thus, significant differences in aggressiveness were found among *P. halstedii* pathotypes (Sakr, 2013) and *O. cumana* race F (Molinero-Ruiz et al., 2009).

The analysed broomrape populations showed different aggressiveness on the susceptible sunflower hybrid and genotypes resistant to different *O. cumana* races. The incidence of parasite ranged between 40.0-100.0%; 26.7-100.0%; 11.0-100.0% and 5.0-100.0%, with average numbers of attachments per plant between 1.4-22.9; 1.0-9.4; 1.0-5.0 and 1.0-5.9, on susceptible hybrid Performer, LC 1003, LC1093 and H_1E, respectively (Figure 1-4).

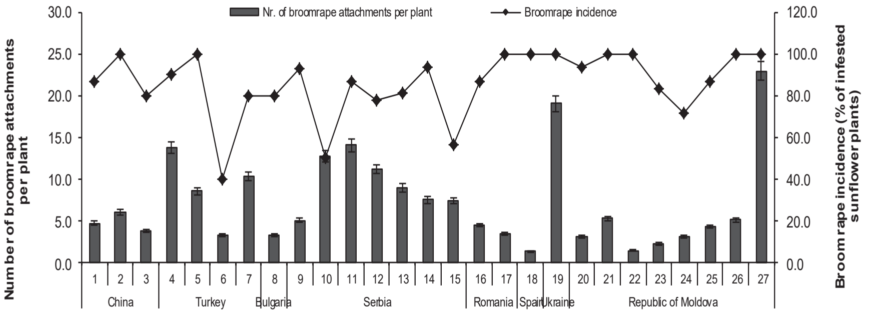

Figure 1. The broomrape incidence and average number of *Orobanche cumana* attachments per plant on the sunflower susceptible hybrid Performer

1 – Inner Mongolia, nr. 1; **2** – Inner Mongolia nr. 6; **3** – He Bei; **4** – Edirne, Merkez; **5** – Edirne, Kesan; **6** – Adana; **7** – Kirklareli, Luleburgaz; **8** – Debovo; **9** – ORSR 04; **10** – ORSR 07; **11** – ORSR 11; **12** – ORSR 14; **13** – ORSR 25; **14** – ORSR 26; **15** – ORSR 43; **16** – Braila; **17** – Tulcea; **18** – Seville; **19** –Ismail; **20** – Cazanesti; **21** – Sangera; **22** – Carabetovca; **23** – Rassvet; **24** – Chisinau; **25** – Popeasca; **26** – SarataMereseni; **27 –** Soroca.

Hybrid Performer was most severely affected, with high level of incidence (80 to 100%) and a high number of attachments per plant (10.3 to 22.9), by two broomrape populations from Turkey (Edirne, Merkez and Kirkareli, Luleburgaz), one populations from Serbia (ORSR11), one collected from Soroca, Moldova and Ukrainian broomrape. The highest level of aggressivity was observed in the case of broomrape from Soroca (Figure 1).

A moderate incidence of attack (40.0 and 50.0%), with moderate and high number of broomrape shoots was revealed in the Adana (Turkey) and ORSR07 populations. A low number of attachments per plant (1.3 to 2.2) and high level of incidence (83.3 to 100.0%) was showed by two populations from Moldova (Carabetovca and Rassvet) and Spanish *O. cumana.*

About a half of populations virulent against the sunflower line LC1003 with resistance to race E manifested a high level of attack (75.0 to 100.0%) and moderate number of attachments per host (3.8 to 9.4) (Figure 2).

The maximal values were observed in the case of Ukrainian population, followed by the populations from Chisinau and Soroca (Moldova), Tulcea (Romania) and broomrape belonging from Edirne, Kesan and Kirkareli, Luleburgaz (Turkey). The less aggressive populations were those collected from Inner Mongolia, China (conventionally noted as nr. 6) and Popeasca, Moldova, with 1-2 attachment per plant and moderate level of incidence (31.3 and 26.7%, respectively).

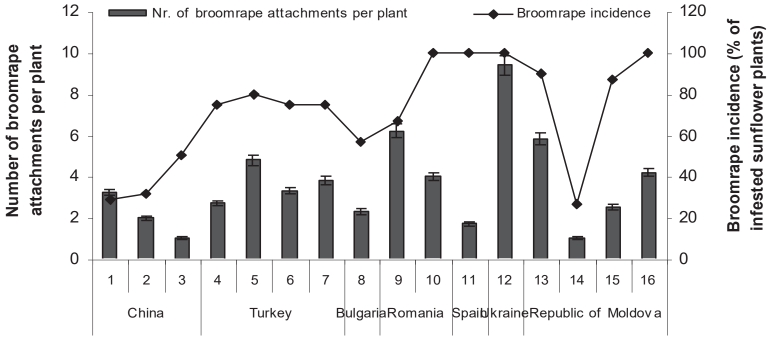

Figure 2. The broomrape incidence and average number of *Orobanche cumana* attachments per plant on the sunflower line LC1003A resistant to race E

1 – Inner Mongolia, nr. 1; **2** – Inner Mongolia nr. 6; **3** – He Bei; **4** – Edirne, Merkez; **5** – Edirne, Kesan; **6** – Adana; **7 -** Kirklareli, Luleburgaz; **8** – Debovo; **9** – Braila; **10** – Tulcea; **11** – Seville; **12** – Ismail; **13**– Chisinau; **14** – Popeasca; **15** – Sarata Mereseni; **16 –** Soroca.

O. cumana belonging from Turkey, Romania (Braila), Ukraine and Moldova (Soroca) were the most aggressive inclusive against line LC1093 resistant to broomrape race F (Figure 3). The number of detected broomrape shoots ranged from 3.3 to 5.0, with high number of parasitized plants (80.0 to 100.0%). Sunflower genotype was least affected, with an average of 1.2 to 2.0 broomrape attachments per host and incidence of attack 11.0-35.7%, by populations from Adana (Turkey), Popeasca (Moldova) and Inner Mongolia nr. 1 (China).

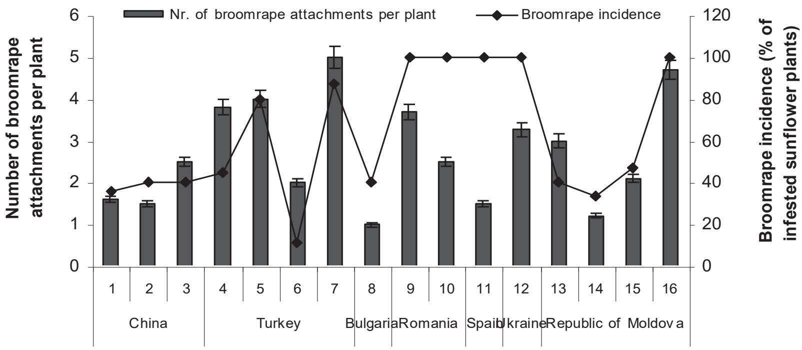

Figure 3. The broomrape incidence and average number of *Orobanche cumana* attachments per plant on the sunflower line LC1093A resistant to race F

1 – Inner Mongolia, nr. 1; **2** – Inner Mongolia nr. 6; **3** – He Bei; **4** – Edirne, Merkez; **5** – Edirne, Kesan; **6** – Adana; **7 -** Kirklareli, Luleburgaz; **8** – Debovo; **9** – Braila; **10** – Tulcea; **11** – Seville; **12** – Ismail; **13** – Chisinau; **14** – Popeasca; **15** – Sarata Mereseni; **16 –** Soroca.

Only 10 from 27 *O. cumana* populations presented virulence on the H_1E hybrid with resistance to race G (Figure 4). Broomrape belonging from Luleburgaz, Edirne Merkez and Kesan (Turkey), Ismail (Ukraine) and Tulcea (Romania) manifested a similar pattern of aggressivity – 80.0-100.0% of parasitized host plants and a moderate number of shoots per plant (3.6 to 5.9).

There were significant differences in aggressivity between populations of *O. cumana* with similar virulence (that infect the same host genotypes). Thus, a part of populations, which attacked all sunflower genotypes (excepting H_2Lg) and supposed to belong to race H, are characterized by low or moderate level of aggressivity (ex. Inner Mongolia nr. 6, Adana, Sarata Mereseni), while other were highly aggressive

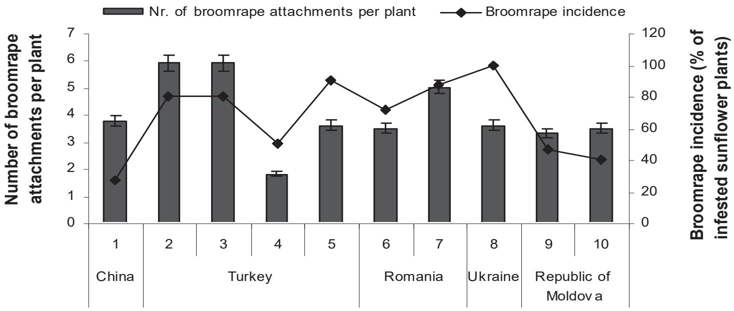

Figure 4. The broomrape incidence and average number of *Orobanche cumana* attachments per plant on the sunflower hybrid H_1E resistant to race G

1 – Inner Mongolia, nr. 6; **2** – Edirne, Merkez; **3** – Edirne, Kesan; **4** – Adana; **5 -** Kirklareli, Luleburgaz; **6** – Braila; **7** – Tulcea; **8** – Ismail; **9** – Sarata Mereseni; **10 –** Soroca.

The highest level of aggressivity against all host genotypes was observed in populations from Turkey (Kesan and Luleburgaz), Romania, Ukraine and Moldova (Soroca), the data being in accordance with those reported by other researchers (Pacureanu et al., 2012; Molinero-Ruiz et al., 2013; Rîşnoveanu et al., 2016). According to obtained data the populations from China are less aggressive as European and Turkish broomrape.

CONCLUSIONS

The results of the study of twenty-seven populations of broomrape belonging to different sunflower growing countries (China, Turkey, Bulgaria, Serbia, Spain, Ukraine, Romania and Republic of Moldova) showed their heterogeneity in virulence and aggressiveness. The most aggressive populations with a wide virulence were those from Turkey, Ukraine, Romania and Republic of Moldova.
Knowledge related sunflower parasite *O. cumana* and periodic monitoring of pathogenicity of broomrape from different fields are important to design feasible and long-term control strategies.

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THE REACTION OF SOME WINTER BARLEY GENOTYPES TO THE NORTH-WEST PEDOCLIMATIC CONDITIONS OF ROMANIA

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Abstract

This paper is aimed to evaluate the behaviour of 25 winter barley genotypes, tested on the acid soils at ARDS Livada, during the 2016-2018 period, in two ways of sowing: one at a distance of 12.5 cm between the rows and the other one at the distance of 25cm. The variance analysis led to the fact that the sowing distance (D), as a variation source, does not significantly influence the yield. On the other hand, the variety (S) and the interaction of distance and years between the barley varieties, had a significant influence on the yield. The regression analysis and the variation coefficient led to the identification of the most productive, stable and adaptable genotypes, specific to this area (Ametist, Cardinal, Artemis, Smarald). The larger sowing distance positively influenced the thousand grain weight, the hectolitre mass and the number and weight of the barley grains/ears. The attacks of the pathogen agents have been reduced (with 3-6%) and the resistance to lodging has been increased (with over 30%).

Key words: *barley genotypes, acid soil, sowing distance, yield, stability.*

INTRODUCTION

The barley's importance in the cultivation's structure is given by its multiple uses: fodder, human food and as raw material in the food industry. According to the statistics (FAO.STAT) the areas cultivated with barley in Romania have been stable in the last ten years fluctuating between 450-500 thousand hectares. The results obtained through breeding programs that have taken place at NARDI Fundulea and in other research centres have led to the introduction of some efficient genotypes regarding the production capacity, stability and quality, in the crop production. Thus, it has been found a growing tendency of the average yield from the surface unit, from 2148 kg/ha in 2008 to an average yield of 4424 kg/ha in 2018.

Barley yield is always influenced by the variable climate conditions, which is why it is important that the barley varieties be adaptive to the various growing conditions (Vasilescu et al., 2017). One of the most important causes of crop decline is represented by the abiotic stress factors which produce a decrease yield that can sometimes reach till the 70% level (Săulescu et al., 1996, quoted by Petcu et al., 2007). The limitative environmental factors of yield vary in intensity from year to year. Harsh winter conditions, frequent drought, high temperatures during the growing season of barley grains, rain during the harvest time or the technological mistakes that limit potential productions that can be obtained through normal growing conditions (Mustățea et al., 2008). A safe way to reduce these losses is creating varieties resistant to unfavourable environmental factors. Regarding these aspects, the winter barley breeding programs that have taken place at NARDI Fundulea have had as a main objective the improving of resistance to the biotic and abiotic stress due to some significant genetic progress concerning the technological quality (Bude and Vasilescu, 2007). The yield stability is given by the resistance sum of the soil to the unfavourable environmental conditions (Săulescu, 1984) and by the interaction of traits with a compensatory effect (Timariu, 1975). The use of genetic diversity on a territorial level, through the growing of multiple different varieties in each zone, represents the simplest and most accessible way of reducing the crop's fluctuations (Săulescu et al., 1980). Also, the growing of varieties with high adaptability to contrasting environmental conditions may reduce the risks of yield loss in the unfavourable years (Mustățea et al., 2008).

In the North-West part of Romania beside the climate change consisting of annual average rising temperatures and high fluctuations of the water regime, a specific characteristic is given by the acid soils. The testing of different crop species genotypes in this area offers pertinent information to the breeders and farmers regarding their stability and adaptability.

This paper is aimed to evaluate the behaviour of 25 winter barley genotypes created at NARDI Fundulea and tested at the ARDS Livada both in the pedo-climatic conditions that are specific to this area but also under two different testing conditions (different sowing distances).

MATERIALS AND METHODS

The 25 winter barley and two row barley genotypes, created at NARDI Fundulea were tested in comparative crops at the ARDS Livada between 2016-2018 period with different features concerning both the thermal and pluviometric regime, in two technological ways of sowing: one at a distance of 12.5 cm row wide and the other one at the distance of 25 cm wide. The climate data was registered at the Weather Station of ARDS Livada. The sowing was realised with the seeding machine for the experimental parcels Wintersteiger in rows of 12 m^2 of which 10 m^2 on a harvestable area. The preceeding plant of the crop rotation was the field pea. The area's winter barley recommended technology was applied. The genotypes characterization took into account the following: grain yield, resistance to disease, resistance to lodging, morpho-productive features and quality. The experiments were conducted in a typical reddish preluvosol (the brown-red soils) which is part of the argiloiluvial soil class. The main characteristics of this soil are: the B horizon presence, more or less developed with a clay content between 30-35%; decreased levels of hydraulic conductivity which determine the surface water stagnation during the heavy rainfall periods; the pH situated in the weak acid and acid soil, the trend being in the acidification direction; low hummus content; as a tendency of the organic matter content evolution it is noticed a quantitative and qualitative setback; and last but not least the presence of aluminum ions, due to the potential acidity actualization. All of these impose an urgent periodical amending.

The acid soil, poorly provided with nutritious elements represent another stress factor alongside the known climate ones, biotic and abiotic. The obtained experimental results were processed through the analysis of variance (N.A. Săulescu, N.N. Săulescu, 1967), as a series of experiences in the same location with 25 genotypes, 2 sowing distances (D1=12.5 cm and D2=25 cm between rows) in three years of testing: 2016, 2017, 2018. The high fluctuation of the production's stability, appreciated through the variation coefficient (CV%), was determined by the genotypes different answer to the climate conditions from the testing period. Each genotypes's reaction to the environmental conditions was determined through the regression analysis of each variety in the three environmental conditions as opposed to the average yield of all the varieties. Keim and Kronstad 1979, (quoted by P. Mustățea et al., 2008) suggested that by using the regression analysis method a variety which is adapted to unfavourable environmental conditions when $b< 1$ and *a* (the regression's constant) has high values; adapted to favorable environmental conditions when $b>1$; largely adapted to different environmental conditions when $b> 1$ and *a* has high values. Moldovan et al. 2003, (quoted by Racz et al., 2014) suggests that the use of the coefficient of determination (r^2) instead of the deviation from regression, offers direct information related to the predictability of genotypes behaviour in different environmental conditions. The main productivity elements were determined from samples of 25 barley spikes harvested each year from both of the sowing options, resistance to disease and lodging through observations in the field during the vegetation period.

RESULTS AND DISCUSSIONS

Due to the relatively short vegetation period as well as to other physiological features, barley adapts well to natural climate conditions and to soil from different geographical areas. In general, the winter barley's most favourable climate is the one with a prolonged autumn, with not so harsh winter conditions and with a thickly enough layer of snow (Drăghici, 1975). From a geographical point of view, the ARDS Livada is situated on the following coordinates: 47º51' North latitude, 23º08' East longitude. In the North-West of Romania, the annual average temperature is of 9.7-9.8ºC. In relation to this value it has been noticed that in recent years, average temperatures go beyond 11-12ºC. The rainfall regime is extremely fluctuant from year to year and from month to month during the vegetation period. The thermal and rainfall regime from the three years of testing (crop years) is shown in figure 1. Warm autumns, mild winters, extremely high temperatures beginning with March, extremely hot in May-June and unevenly distributed rainfall during the vegetation period are the main characteristics of the experimenting period.

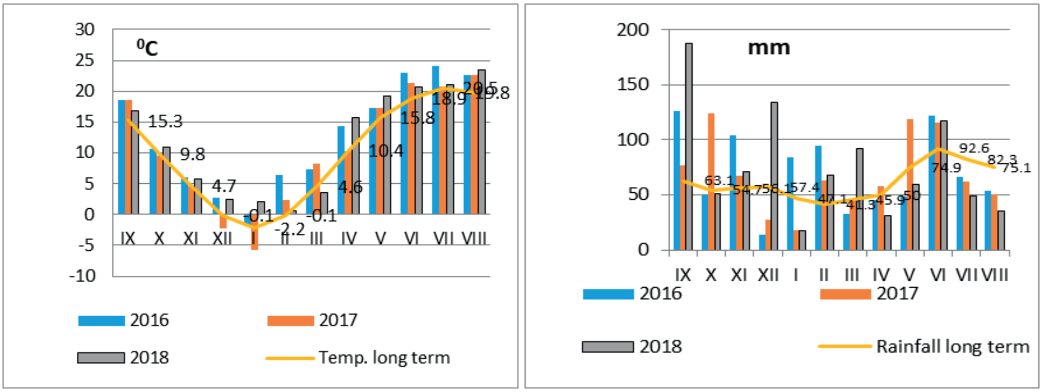

Figure 1. Average temperatures (ºC) and rainfall distribution (mm) during winter barley vegetation, ARDS Livada, 2016-2018

Reported to this diversity of climate conditions, the average level of yield was high in 2016, 2017 and quite low in 2018 (Figure 2 and 3). In 2016 year, the yield was fluctuating between 33,19 q/ha (six row barley line F8-3-01) and 54,73 q/ha (two row barley line DH 375-4), in 2017 to a few varieties and lines, the yield exceeded 60,0 q/ha, the highest being 68,37 q/ha for the Smarald variety. With a mean of 40,95 q/ha experience in 2018, were registered the lowest yields, the main cause being the lack of water during the ear development, flowering and grain filling period. Under the 25 cm distance between the rows condition, yields fluctuated between 35,01 q/ha (two row barley line DH 375-4) and 58,76 q/ha (Ametist variety). The lack of water during the maximum consumption, signalled in 2018, determined the registration of some modest yield and in this variant of sowing with a minimum of 23,2 q/ha (Standard 1) and a maximum of 47,84 q/ha (Artemis variety).

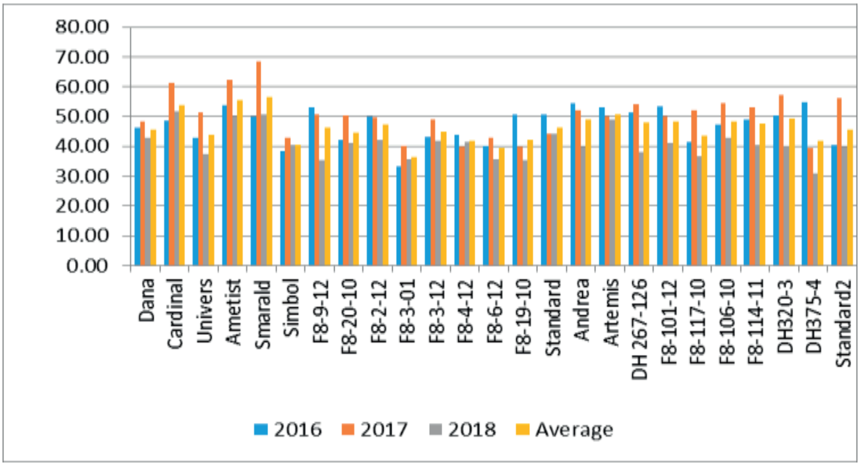

Figure 2. Annual and average yields of barley genotypes sown at 12.5 cm (q/ha), ARDS Livada, 2016-2018

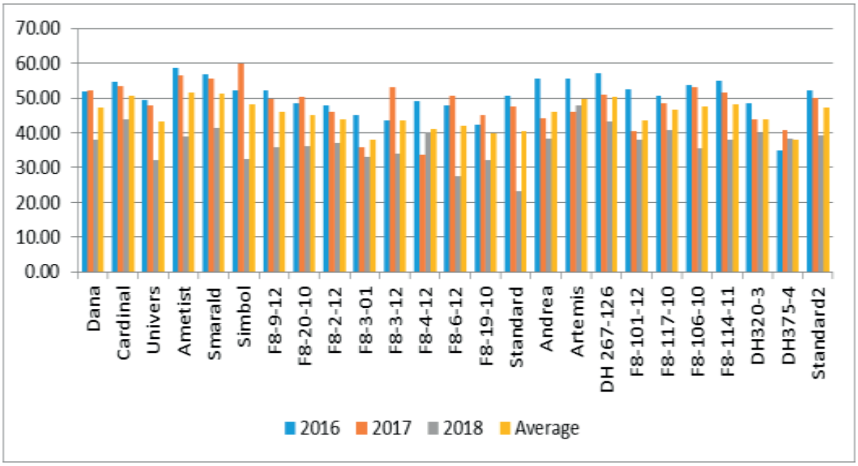

Figure 3. Annual and average yields of barley genotypes sown at 25 cm (q/ha), ARDS Livada, 2016-2018

The constancy of the obtained yield was rated upon the base of variation coefficients (Figure 4 and 5). Varieties like Ametist, Cardinal and Artemis, are characterised by high yields, more than 50q/ha and good stability, with a coefficient of variation of 11-12 %. Smarald, the variety with the highest yield (56,31 q/ha) represents a medium stability (17%). With good stability but low yield, below 45 q/ha are the following genotypes: Dana, Simbol and barley lines F8-3-12 and F8-20-10 (Figure 4).

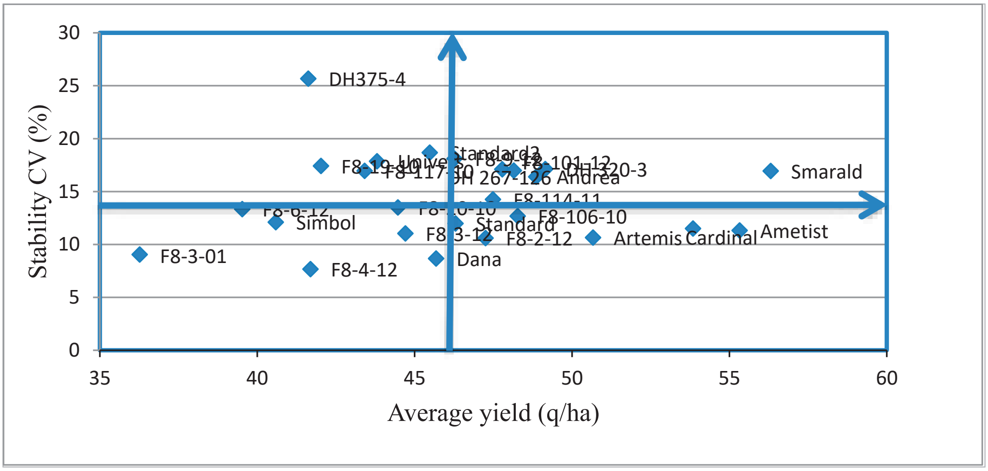

Figure 4. Relation between grain yield (q/ha) and CV/% to 25 barley genotypes sown in regular rows of 12.5 cm, ARDS Livada, 2016-2018

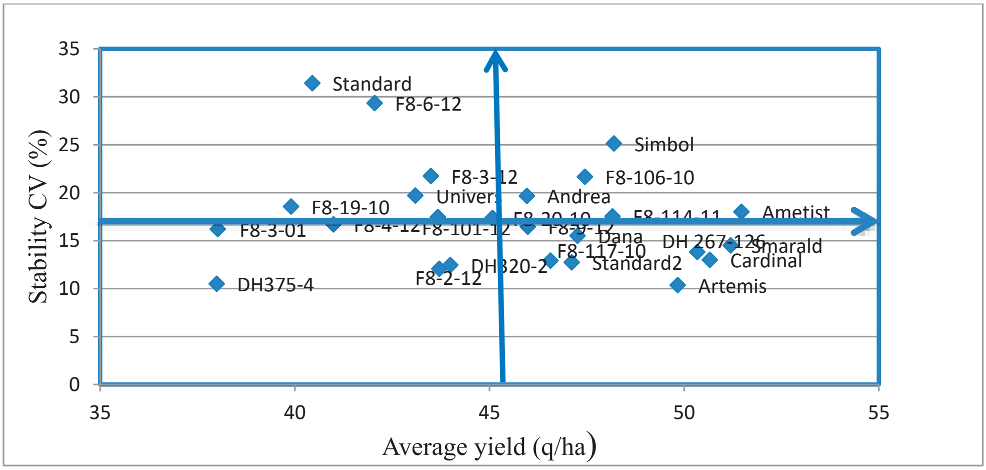

Figure 5. Relation between grain yield (q/ha) and CV/% to 25 barley genotypes sown in rare rows of 25 cm, ARDS Livada, 2016-2018

Artemis, Cardinal and Smarald varieties and two row line DH 267-126 confirm a great yield capacity but also a good stability under the North-West conditions of Romania and in the variant of sowing in rare rows (Figure 5).

The subunitar regression slope and the high value of parameter „a" suggest a good adaptation to less favourable conditions for the majority of tested genotypes (Table 1), conditions which are met in the North-West part of the country as a consequence of the evolution of climate factors but also due to specific acid soils.

Table 1. Yield stability parameters (b, a, r^2) of 25 winter barley genotypes (varieties and lines) sown to D1 (12.5 cm) and D2 (25 cm), ARDS Livada, 2016-2018

No.	Variety/line	Average grain yield - D1 (q/ha)	b	a	r^2	Average grain yield - D2 (q/ha)	b	a	r^2
1	Dana	45.68	0.57	20.14	0.34	47.26	0.72	11.21	0.79
2	Cardinal	**53.84**	0.31	29.45	0.24	**50.66**	0.67	11.33	0.55
3	Univers	43.80	0.35	30.79	0.49	43.10	0.65	17.38	0.85
4	Ametist	**55.32**	0.47	20.40	0.56	**51.47**	0.61	13.75	0.90
5	Smarald	**56.31**	0.27	31.06	0.42	**51.19**	0.75	7.02	0.86
6	Simbol	40.59	0.10	**42.07**	0.02	48.20	0.43	**24.75**	0.76
7	F8-9-12	46.27	0.39	28.03	0.71	45.98	0.75	10.87	0.90
8	F8-20-10	44.47	0.36	30.23	0.30	45.08	0.59	18.79	0.59
9	F8-2-12	47.25	0.53	21.11	0.46	43.71	1.00	1.59	0.77
10	F8-3-01	36.26	0.51	27.69	0.18	38.02	0.65	20.52	0.45
11	F8-3-12	44.71	0.43	**27.01**	0.29	43.49	0.41	**27.64**	0.41
12	F8-4-12	41.69	-0.15	**52.46**	0.01	40.99	0.21	**36.72**	0.06
13	F8-6-12	39.52	0.53	25.15	0.41	42.05	0.39	28.78	0.66
14	F8-19-10	42.02	0.22	36.91	0.17	39.90	0.57	22.75	0.49
15	Standard 1	46.30	0.06	**43.21**	0.01	40.45	0.45	27.03	0.92
16	Andrea	48.86	0.35	29.21	0.50	45.96	0.45	24.71	0.46
17	Artemis	**50.67**	0.09	**41.66**	0.01	49.83	0.45	22.73	0.15
18	DH 267-126	47.78	0.40	26.86	0.71	**50.33**	0.67	11.74	0.61
19	F8-101-12	48.15	0.28	**32.48**	0.30	43.67	0.48	**24.19**	0.38
20	F8-117-10	43.41	0.43	27.54	0.66	46.57	0.69	13.06	0.49
21	F8-106-10	48.27	0.48	23.23	0.55	47.45	0.47	23.02	0.66
22	F8-114-11	47.49	0.45	24.93	0.59	48.16	0.61	16.04	0.74
23	DH 320-3	49.15	0.39	27.03	0.71	43.99	0.65	16.88	0.35
24	DH 375-4	41.62	0.18	**38.62**	0.25	37.99	-0.20	**52.86**	0.02
25	Standard 2	45.48	0.32	31.42	0.49	47.11	0.94	1.19	0.88

The analysis of variance highlighted the fact that the sowing distance (D1 and D2) as a variation source does not significantly influence the yield, instead the genotype (G) and the interactions GxY, GxD and GxDxY had a significant influence on the yield level (Table 2).

Table 2. ANOVA for grain yield of 25 winter barley genotypes under different distance between rows

Source of variation	SS	df	MS	F
Years (Y)	10353.51	2		
Replication	540.71	6		
Distance (D)	89.3	1	89.3	1.10 (5.99)
Distance x Years (DxY)	1091.12	2	545.56	6.72 (5.14) *
Error (a)	487.18	6	81.20	
Genotype (G)	7247.67	24	301.99	13.9**(1.57;1.88)
Genotype x Years (GxY)	3206.39	48	66.80	3.07** (1.42;1.62)
Genotype x Distance (GxD)	1100.26	24	45.84	2.10 **(1.57;1.88)
GxDxY	3457.37	48	72.03	3.31** (1.42;1.62)
Error (b)	6263.40	288	21.75	

As experiment average, registered genotypes averages yield between the two technological variants of sowing are very close, and as it has been revealed by ANOVA (Table 2) there haven't been any significant differences registered (Table 3).

Table 3. The influence the sowing distance on the genotypes average yield, ARDS Livada, 2016-2018

No.	Distance	q/ha	the difference +;-	significance
1	D1 = 12.5 cm	46.20	-	-
2	D2 = 25.0 cm	45.31	-0.89	-

LSD (p 5%) = 2.08 q/ha

Cardinal, Ametist and Smarald winter barley varieties registered the highest yield in both sowing conditions, but with a significant plus to the standard sowing at 12.5 cm between rows. A similar behaviour has been noticed in the case of two rows winter barley varieties Andrea and Artemis (Table 4).

Table 4. The sowing distance influence on grain yield, ARDS Livada, 2016-2018

Genotype	Average grain yield D1 (q/ha)	Difference +,- (q/ha) *	Average grain yield D2 (q/ha)	Difference +,- (q/ha) *	Difference D1-D2 (q/ha)	Genotype classification according yield difference**
Dana	45.68	0.52	**47.26**	1.95	-1.58	25 cm
Cardinal	53.84	7.64*	50.66	5.35*	3.18	12.5 cm
Univers	43.80	-2.4	43.10	-2.21	0.71	12.5/25 cm
Ametist	55.32	9.12*	51.47	6.16*	3.85	12.5 cm
Smarald	56.31	10.11*	51.19	5.88*	5.12	12.5 cm
Simbol	40.59	-5.61^0	**48.20**	2.89	-7.61	25 cm
F8-9-12	46.27	0.07	45.98	0.67	0.29	12.5/25 cm
F8-20-10	44.47	-1.73	45.08	-0.23	-0.61	12.5/25 cm
F8-2-12	47.25	1.05	43.71	-1.6	3.54	12.5 cm
F8-3-01	36.26	-9.94^0	**38.02**	-7.29^0	-1.76	25 cm
F8-3-12	44.71	-1.49	43.49	-1.82	1.21	12.5 cm
F8-4-12	41.69	-4.51^0	40.99	-4.32^0	0.70	12.5/25 cm
F8-6-12	39.52	-6.68^0	**42.05**	-3.26^0	-2.53	25 cm
F8-19-10	42.02	-4.18^0	39.90	-5.41^0	2.12	12.5 cm
Standard 1	46.30	0.1	40.45	-4.86^0	5.85	12.5 cm
Andrea	48.86	2.66	45.96	0.65	2.90	12.5 cm
Artemis	50.67	4.47*	49.83	4.52*	0.83	12.5/25 cm
DH 267-126	47.78	1.58	**50.33**	5.02*	-2.55	25 cm
F8-101-12	48.15	1.95	43.67	-1.64	4.47	12.5 cm
F8-117-10	43.41	-2.79	**46.57**	1.26	-3.16	25 cm
F8-106-10	48.27	2.07	47.45	2.14	0.82	12.5/25 cm
F8-114-11	47.49	1.29	48.16	2.85	-0.67	12.5/25 cm
DH 320-3	49.15	2.95	43.99	-1.32	5.15	12.5 cm
DH 375-4	41.62	-4.58^0	37.99	-7.32^0	3.63	12.5 cm
Standard 2	45.48	-0.72	**47.11**	1.8	-1.63	25 cm
	LSD (p 5%) = 3.05		LSD (p 5%) = 3.05			
Average	**46.20**		**45.31**			

^{0} – not significant, * - significant, ** - recommended distance to growing.

Dana and Simbol winter barley varieties, alongside with F8-3-01, F8-6-12, F8-117-10 lines, registered on average superior yields in the rare sowing distances. Univers, Artemis winter barley varieties and F8-9-12, F8-20-10, F8-4-12, F8-106-10 and F8-114-11 lines stand out through a neutral reaction (Table 4) to the change of the sowing distance between rows, average yields obtained during the 2016-2018 period being almost similar. The importance of this approach is that it allows barley farmers, depending on their ultimate goal, grain yield for malt (for an increase of TKW) or fodder, to opt for the cultivation of the most suitable genotypes. The choice of these genotypes that react well to the sowing distance of 25 cm allows the reduction of the quantity of seed used for sowing to the surface units with real chances of obtaining very good grain yield.

The wider sowing distance positively influenced the main elements of productivity: the hectolitre weight (HW), thousand kernel weight (TKW), the number of grains per spike (NG/S), grain weight per spike (GW/S), with significant differences concerning the thousand kernel weight which is in average 47.2 g in the case of sowing to 25 cm (Table 5). The grain weight of barley, expressed usually as thousand kernel weight, is one of the most important components of production (Hadjichristodoulou, 1990 quoted by Vasilescu et al., 2014).

Table 5. The influence of the sowing distance to some yield elements, ARDS Livada, 2016-2018
(genotypes average of the two sowing condition)

No.	Distance	HW (kg/hl)	Dif. +;- (kg/hl)	S*	TKW (g)	Dif. +;- (g)	S*	NG/S	Dif. +;-	S*	GW/S (g)	Dif. +;-	S*
1	D1=12.5 cm	57.7	-	-	42.9	-	-	25.3	-	-	1.19	-	-
2	D2=25.0 cm	59.0	1,3	ns.	47.2	4.3	*	28.4	3.1	ns.	2.33	1.14	ns.

S = significance; ns – not significant; * - significant LSD (p 5%) = 4.19

The attack of leaf pathogens on the barley field is manifested differently from year to year determining significant losses of harvest.

The study and knowledge of the barley genotypes reaction as opposed to multiple causes that determine more and more damage have a great importance in the present too due to the growing number of physiological strains of pathogenic agents.

The process of pathogenesis in plants, in general, is influenced both by their special resistance and by the climate and crop conditions (Goga and Bănățeanu, 2006). For the determining of the foliar pathogens attack incidence of winter barley crop observations were made concerning the level of attack, expressed through the degree of attack (DA%), to the main pathogens which manifest under the ARDS Livada conditions: powdery mildew (*Blumeria graminis* D.C. f.s.p. hordei March), leaf scald (*Rhynchosporium secalis* Davis), net blotch (*Pyrenophora teres* drechs. F.c. *Helminthosporium teres* Sacc), root rot (*Helminthosporium sativum* (Pam. King et Bakke), barley brown rust (*Puccinia hordei*), leaf spot of barley (*Ramularia collo-cygni*). The degree of attack produced by the leaf pathogens was different from year to year being influenced by the rainfall level, thermal regime registered during the vegetation period as well as by the tolerance of the used genotypes.

Both in the variant of regular sowing and in the rare rows, the highest average degree of attack, of the complex of barley pathogens, was registered during the conditions of 2017 while in 2018 were the lowest. During each of these three experimental years, the degree of attack was higher in the standard variant of sowing – 12.5 cm (Figure 6).

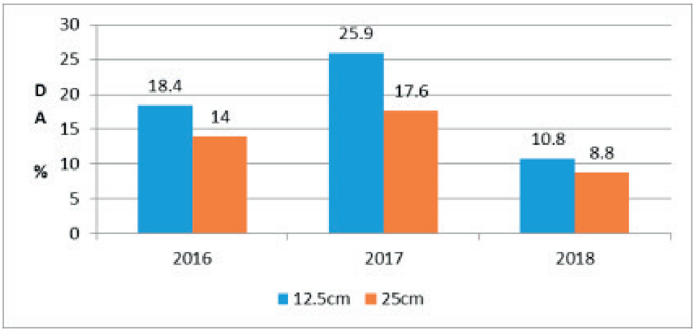

Figure 6. Average degree of attack (DA%), complex barley pathogen, ARDS Livada, 2016-2018

The difference between these two sowing variants regarding the average degree of pathogens attack specific to barley is situated in the significant limit for the 5% threshold being 4.9% (Table 6).

Table 6. The influence the sowing distance has on DA% (pathogen complex)
and of the lodging resistance (% fallen plants)

No.	Distance	DA %	Diference +;-	Significance	Lodging %	Diference +;-	Significance
1	D1=12.5 cm	18.4	-	-	72	-	-
2	D2=25.0 cm	13.5	4.9	*	41	31	*

* - significant

A factor not to be neglected which influences both the yield level and its quality is represented by the plants lodging.
The resistance to lodging is influenced both by the genetic characteristics of each genotype and by the climate conditions and the used technology. The necessity of using varieties with a good resistance to lodging in crops is given by the importance of obtaining a relatively good crop, with corresponding indices.
Crop losses registered by the lodging phenomenon can sometimes higher, especially when it takes place earlier, before ear development (Drăghici et al., 1975).
The average percentage of lodged plants fluctuated between 20-60% in the case of sowing at 25 cm while in the case of sowing in regular rows the lodging percentage has risen, the average values being in the range of 60 and 100%. On average, the percentage at the lodging plants during the 2016-2018 period was 41% for the rare sowing distance and it got to 72% for the standard sowing, the difference being significant (Table 6).
We must be aware of the fact that a great part of quality features are complex hereditary aspects, polygenic conditioned. However, it is not to be neglected that the variation of climate conditions during the vegetation period has a great impact on them.

CONCLUSIONS

Winter barley genotypes created at NARDI Fundulea are characterized by a good yield capacity, but most of all by adaptability and stability during the less favourable environmental conditions from the North-West of Romania, an area known for its acid soils.
The subunitar regression slope *(b)* and the high value of the „a” parameter suggest that has been a good adaptability to less favourable conditions for the majority of tested genotypes.
Ametist, Cardinal (six row) and Artemis (two-row) winter varieties are distinguished by a higher yield, over 50q/ha and good stability.
Artemis (two-row), Cardinal (six-row), Smarald (six-row) and DH 267-126 (two-row) line confirm a high yield capacity but also a good stability under the conditions from the North-West of the country and in the rare sowing rows.
Although there are no significant differences between them, sowing at a distance of 12.5 and 25.0 cm between rows allows the highlight of some genotypes that have similar grain yield under both growing distances: Univers, Artemis and a few advanced barley lines (F8-9-12, F8-20-10, F8-106-10, F8-114-11). These represent a good alternative for farmers, which allows them to obtain the same production performances with a cost decrease through the use of a smaller seed quantity for sowing per hectar.
Sowing in rare rows positively influenced TKW, HW and the number and weight of grains on the spike, elements of productivity influenced a lot by climate conditions.
A decrease in the degree of attack towards the main pathogenic agents specific to barley has been registered in the north-west area under rare sowing rows, which contributes to the obtaining of qualitative grain yield.

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EFFICACY OF HERBICIDES, HERBICIDE COMBINATIONS AND HERBICIDE TANK MIXTURE ON CHICKPEA (*Cicer arietinum* L.)

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Abstract

The research was conducted during 2016-2018 on pellic vertisol soil type. Under investigation was chickpea cultivar Kabule (Cicer arietinum L.). Factor A included untreated control and 4 soil-applied herbicides – Dual gold 960 EC (S-metolachlor) - 1.5 l/ha, Stomp aqua (pendimethalin) - 3 l/ha, Merlin flex 480 SC (isoxaflutole) - 420 g/ha and Pelican 50 SC (diflufenikan) - 250 ml/ha. Factor B included untreated control, 3 foliar-applied herbicides – Pulsar 40 (imazamox) - 1.2 l/ha, Challenge 600 SC (aclonifen) - 4 l/ha and Shadow 3 EC (clethodim) - 1.6 l/ha and 1 herbicide tank mixture – Challenge 600 SC (aclonifen) - 4 l/ha + Shadow 3 EC (clethodim) - 1.6 l/ha. Soil-applied herbicides were treated during the period after sowing before emergence. Foliar-applied herbicides were treated during 6 - 8 real leaf stage of the chickpea. All of herbicides, herbicide combinations and herbicide tank-mixture were applied in a working solution of 200 l/ha. Mixing of foliar-applied herbicides was done in the tank on the sprayer. Combinations of soil-applied herbicides Dual gold, Stomp aqua, Merlin flex and Pelican with foliar-applied herbicides Challenge and Shadow, as well as with herbicide tank mixture Challenge + Shadow exhibit an additive effect on herbicidal efficacy. Foliar-applied herbicide Pulsar destroys all annual and perennial broadleaved and graminaceous weeds and self-sown plants. Herbicide Challenge is the only herbicide that successfully controls Clearfield and ExpressSun sunflower self-sown plants in chickpea crops. Treatment with foliar-applied herbicide Pulsar leads to high phytotoxicity in chickpea – rate 3 according to the scale of EWRS. Herbicide combination Pelican + Pulsar leads to even higher phytotoxicity in chickpea – rate 5 according to the scale of EWRS. Soil-applied herbicide Pelican used alone or combined with foliar-applied herbicides Challenge and Shadow and herbicide tank-mixture Challenge + Shadow leads to pure phytotoxicity in chickpea – rate 2 according to the scale of EWRS. The highest yields of chickpea seeds are obtained by foliar treatment with herbicide tank-mixture Challenge + Shadow after soil-applied herbicides Pelican and Merlin flex. High yields are obtained also by foliar treatment with herbicide tank-mixture Challenge + Shadow after soil-applied herbicides Stomp aqua and Dual gold.

Key words: *chickpea, herbicides, herbicide combinations, efficacy, selectivity, seed yield.*

INTRODUCTION

Chickpea is a crop that grows slowly in the first days after germination and is easily oppressed by weeds. In its crops mainly early spring weeds develop - *Sinapis arvensis* L., *Falopia convolvulus* Leve, *Raphanus raphanistrum* L., and more limited late spring weeds develop - *Chenopodium album* L., *Amaranthus retroflexus* L., etc. From perennial weeds with the widest distribution and highest density are *Convolvulus arvensis* L. and *Cirsium arvense* Scop. An important measure in pea cultivation is the removal of weeds that carry diseases - *Anthemis arvensis* L., *Cirsium arvense* Scop., *Papaver rhoes* L., as well as those weeds that are poisonous - *Solanum nigrum* L., self-sown potatoes *Solanum tuberosum* L., *Datura stramonium* L. (Velasquez and Alonso, 1993; Soltero-Díaz et al., 2010).

It has made a huge progress in weed control during recent years. Conditions for complete elimination of manual labor were created (Marwat et al., 2004). Weed control is most successful in complex application of agro-technical and chemical methods (Şanlı et al., 2009; Ratnam and Rao, 2011). World experience shows that the possibilities for biological weed control are still small (Vaissi and Shimi, 2003). Agro-technical methods include: crop rotation – sowing of legumes after cereals; plowing and pre-sowing tillage adjusted to character and degree of weed infestation; adherence to the chickpea sowing period, etc. Chemical control is carried out with selective herbicides – soil-applied and foliar-applied. They should be selected according to the nature of the weed associations accompanying the chickpea crop (Skrobakova, 1998 and 1999; Khan et al., 2010; Tanveer et al., 2010; Delchev, 2018 and 2020).

The purpose of this investigation was to establish the efficacy and selectivity of some herbicides, herbicide combinations and a herbicide tank mixture on the chickpea by influence of different meteorological conditions.

MATERIALS AND METHODS

The research was conducted during 2016 - 2018 on pellic vertisol soil type. Under investigation was chickpea cultivar Kabule (*Cicer arietinum* L.). Two factors experiment was conducted under the block method, in 4 repetitions; the size of the crop plot was 15 m^2. Factor A included untreated control and 4 soil-applied herbicides – Dual gold 960 EC, Stomp aqua, Merlin flex 480 SC and Pelican 50 SC. Factor B included untreated control, 3 foliar-applied herbicides – Pulsar 40, Challenge 600 SC and Shadow 3 EC and 1 herbicide tank mixture – Challenge 600 SC + Shadow 3 EC. Active substances of herbicides and their doses are shown in Table 1.

Soil-applied herbicides were treated during the period after sowing before emergence.

Table 1. Investigated variants

№	Variants	Active substance	Doses
		After sowing, before emergence	
1	Control	-	-
2	Dual gold 960 EC	S-metolachlor	1.2 l/ha
3	Stomp aqua	pendimethalin	3 l/ha
4	Merlin flex 480 SC	isoxaflutole	420 g/ha
5	Pelican 50 SC	diflufenikan	250 ml/ha
		6 - 8 real leaf stage	
1	Control	-	-
2	Pulsar 40	imazamox	1.2 l/ha
3	Challenge 600 SC	aclonifen	4 l/ha
4	Shadow 3 EC	clethodim	1.6 l/ha
5	Challenge 600 SC + Shadow 3 EC	aclonifen + clethodim	4 l/ha + 1.6 l/ha

Herbicide Pulsar 40 was used in addition with adjuvant Dash HC – 1 l/ha.

Foliar-applied herbicides were treated during 6 - 8 real leaf stage of the chickpea. All of herbicides, herbicide combinations and herbicide tank-mixture were applied in a working solution of 200 l/ha. Mixing of foliar-applied herbicides was done in the tank on the sprayer. Due to of low adhesion of the herbicide Pulsar 40 was used in addition with adjuvant Dash HC – 1 l/ha.

It was investigated efficacy and selectivity of herbicides, herbicide combinations and herbicide tank mixture. It was established their influence on seed yield. Efficacy of herbicides against weeds and self-sown durum wheat was appointed according to 100 % scale of EWRS (European Weed Research Society). Selectivity of herbicides to chickpea plants was followed according to the 9-rate scale of EWRS (rating 1 - without damages, rating 9 - crop is completely destroyed). The mathematical processing is done with analysis of variance method.

RESULTS AND DISCUSSION

Annual broadleaved weeds in the experiment are represented by *Anthemis arvensis* L., *Chamomilla recutita* Rauchert, *Galium aparine*

L., *Sinapis arvensis* L., *Amaranthus retroflexus* L., *Falopia convolvulus* Leve, *Papaver rhoes* L., *Consolida regalis* Gray, *Lamium purpureum* L., *Veronica hederifolia* L., also single plants of *Capsella bursa-pastoris* L., *Lithospermum arvense* L., *Chenopodium album* L., *Viola tricolor* L., *Myagrum perfoliatum* L., *Stellaria media* Cyr.

Annual graminaceous weeds are *Avena fatua* L., *Alopecurus myosuroides* L., *Lolium multiflorum* L., *Bromus arvensis* L., also single plants of *Avena ludoviciana* Durien., *Apera spica-venti* P.B., *Lolium temulentum* L.

Perennial broadleaved weeds are *Cirsium arvense* Scop. and *Convolvulus arvensis* L. Perennial graminaceous weeds are *Sorghum helepense* Pers. and *Cynodon dactylon* Pers.

Cereal self-sown plants are represented by durum wheat (*Triticum durum* Desf.), was grown as precrop.

Broadleaved self-sown plants are Clearfield and ExpressSun sunflower hybrids (*Helianthus annuus* L.), grown two years ago as precrop of wheat.

Soil-applied herbicide Dual gold controls 100 % of *Galium aparine* L., *Sinapis arvensis* L., *Raphanus raphanistrum* L., *Anthemis arvensis* L., *Lamium purpureum* L., *Amaranthus retroflexus* L., *Chenopodium album* L. (Table 2). It controls 85 - 95 % of weeds such as *Galium aparine* L. and *Papaver rhoes* L. This herbicide has low efficacy of 30 % against *Falopia convolvulus* Leve and *Veronica hederifolia* L. and is inefficacy against *Consolida regalis* Gray.

Soil-applied herbicide Stomp aqua controls 100 % of annual broadleaved weeds such *Galium aparine* L., *Sinapis arvensis* L., *Amaranthus retroflexus* L., *Chenopodium album* L., *Anthemis arvensis* L. and *Veronica hederifolia* L. It controls 85 % of *Galium aparine* L. and *Papaver rhoes* L. This herbicide has a low efficacy of 35 – 40 % against *Falopia convolvulus* Leve and *Lamium purpureum* L.

Herbicide Merlin flex controls successfully almost all annual broadleaved weeds. This herbicide has a lower efficacity only against *Papaver rhoes* L. – 95 %.

Of the soil-applied herbicide group, the largest percentage of destruction of annual weeds was reported in herbicide Pelican. It controls successfully all early-spring and late-spring broadleaved weeds including *Amaranthus retroflexus* L. and *Chenopodium album* L.

Foliar-applied herbicide Pulsar completely controls all annual broadleaved weeds available in the experiment.

The herbicide Challenge, applied alone or as herbicide tank mixture with antigraminaceous herbicide Shadow, also completely controls all annual broadleaved weeds available in the experiment.

The seed coat of sunflower hybrids has a thick armored layer consisting of several rows of carbon cells to protect from *Homoeosoma nebulella* D. & S. For this reason, some of these seeds do not germinate next year in cereal crops, but in the year later in chickpea crops and become a major problem during the chickpea vegetation and the chickpea harvest. Herbicide Merlin flex is the only herbicide that successfully controls Clearfield and ExpressSun sunflower self-sown plants in chickpea crops.

Soil-applied herbicides Dual gold, and applied during the period after sowing before germination (ASBE) of chickpea, are inefficacy against perennial broadleaved weeds *Cirsium arvense* Scop. and *Convolvulus arvensis* L. (Table 3). Dual gold and Stomp aqua are inefficacy also against perennial graminaceous weeds *Sorghum helepense* Pers. and *Cynodon dactylon* Pers. Herbicide Merlin flex is partially efficacy only against *Sorghum helepense* Pers. (60%) – its plants become white and their growth slows significantly.

Of the soil-applied herbicide group, only herbicide Pelican has a satisfactory efficacy against perennial broadleaved and graminaceous weeds. It controls these weeds from 60 to 75 %.

Foliar-applied herbicide Pulsar completely controls all annual broadleaved weeds. Herbicide Challenge is ineffective against these weeds.

Table 2. Efficacy of some herbicides, herbicide combinations and herbicide tank mixture against annual broadleaved weeds and self-sown plants at chickpea according to the 100 % visual scale of EWRS (mean 2016 - 2018)

Herbicides		Weeds									
Soil-applied	Foliar-applied	*Galiun aparine*	*Chamomilla recutita*	*Papaver rhoes*	*Consolida regalis*	*Amaranthus retroflexus*	*Anthemis arvensis*	*Falopia convolvulus*	*Veronica hederifolia*	*Lamium purpureum*	*Helianthus annus* *
-	-	0	0	0	0	0	0	0	0	0	0
	Pulsar	100	100	100	100	100	100	100	100	100	0
	Challenge	100	100	98	98	100	100	90	95	100	0
	Shadow	0	0	0	0	0	0	0	0	0	0
	Challenge + Shadow	100	100	98	98	100	100	90	95	100	0
Dual gold	-	85	100	90	0	100	100	30	30	100	0
	Pulsar	100	100	100	100	100	100	100	100	100	0
	Challenge	100	100	98	98	100	100	90	95	100	0
	Shadow	85	100	90	0	100	100	30	30	100	0
	Challenge + Shadow	100	100	98	98	100	100	90	95	100	0
Stomp aqua	-	85	100	85	30	100	100	40	100	35	0
	Pulsar	100	100	100	100	100	100	100	100	100	0
	Challenge	100	100	98	98	100	100	90	100	100	0
	Shadow	85	100	85	30	100	100	40	100	35	0
	Challenge + Shadow	100	100	98	98	100	100	90	100	100	0
Merlin flex	-	100	100	95	100	100	100	100	100	100	100
	Pulsar	100	100	100	100	100	100	100	100	100	100
	Challenge	100	100	100	100	100	100	100	100	100	100
	Shadow	100	100	95	100	100	100	100	100	100	100
	Challenge + Shadow	100	100	100	100	100	100	100	100	100	100
Pelican	-	100	100	100	100	100	100	100	100	100	0
	Pulsar	100	100	100	100	100	100	100	100	100	0
	Challenge	100	100	100	100	100	100	100	100	100	0
	Shadow	100	100	100	100	100	100	100	100	100	0
	Challenge + Shadow	100	100	100	100	100	100	100	100	100	0

* - self-sown plants of ClearField and ExpressSun sunflower

Foliar-applied herbicides Pulsar, Shadow and herbicide tank mixture Challenge + Shadow successfully control perennial graminaceous weeds.

Soil-applied herbicide Dual gold is inefficacy against annual graminaceous weeds *Bromus arvensis* L., *Avena fatua* L. and *Avena ludoviciana* Durien. It has a low efficacy of

Table 3. Efficacy of some herbicides, herbicide combinations and herbicide tank mixture against perennial weeds, annual graminaceous weeds and self-sown plants at chickpea according to the 100 % visual scale of EWRS and selectivity according to the 9-rate scale of EWRS (mean 2016 - 2018)

Herbicides		Weeds									
Soil-applied	Foliar-applied	*Cirsium arvense*	*Convolvulus arvensis*	*Sorgum helepense*	*Cynodon dactylon*	*Avena fatua*	*Lolium multiflorum*	*Alopecurus myosoroides*	*Bromus arvensis*	*Triticum durum**	Selectivity
-	-	0	0	0	0	0	0	0	0	0	1
	Pulsar	98	100	97	100	100	100	100	98	100	3
	Challenge	0	0	0	0	40	65	80	0	70	1
	Shadow	0	0	100	100	100	100	100	100	100	1
	Challenge + Shadow	0	0	100	100	100	100	100	100	100	1
Dual gold	-	0	0	0	0	0	100	100	0	40	1
	Pulsar	98	100	97	100	100	100	100	98	100	3
	Challenge	0	0	0	0	40	100	100	0	70	1
	Shadow	0	0	100	100	100	100	100	100	100	1
	Challenge + Shadow	0	0	100	100	100	100	100	100	100	1
Stomp aqua	-	0	0	0	0	95	100	100	0	0	1
	Pulsar	98	100	97	100	100	100	100	98	100	3
	Challenge	0	0	0	0	95	100	100	0	70	1
	Shadow	0	0	100	100	100	100	100	100	100	1
	Challenge + Shadow	0	0	100	100	100	100	100	100	100	1
Merlin flex	-	0	0	60	0	100	100	100	0	55	1
	Pulsar	98	100	100	100	100	100	100	98	100	3
	Challenge	0	0	0	0	100	100	100	0	70	1
	Shadow	0	0	100	100	100	100	100	100	100	1
	Challenge + Shadow	0	0	100	100	100	100	100	100	100	1
Pelican	-	70	60	75	65	0	0	0	0	0	2
	Pulsar	98	100	100	100	100	100	100	98	100	5
	Challenge	70	60	75	75	40	65	80	0	70	2
	Shadow	70	60	100	100	100	100	100	100	100	2
	Challenge + Shadow	70	60	100	100	100	100	100	100	100	2

* - self-sown plants of durum wheat

40 % against self-sown plants of durum wheat (*Triticum durum* Desf.).

Herbicides Stomp aqua and Merlin flex are efficacy against all annual graminaceous weeds, except for *Bromus arvensis* L. The herbicides are inefficacy against self-sown plants of *Triticum durum* Desf.

Soil-applied herbicide Pelican is inefficacy against annual graminaceous weeds and self-sown plants of *Triticum durum* Desf.
In vegetation treatment, the herbicide Challenge is poorly efficacy against annual graminaceous weeds. In soil application this herbicide provides good control of these weeds. Herbicides Pulsar, Shadow and herbicide tank mixture Challenge + Shadow successfully control annual graminaceous weeds.
Combinations of soil-applied herbicides Dual gold, Stomp aqua, Merlin flex and Pelican with foliar-applied herbicides Challenge and Shadow, as well as with herbicide tank mixture Challenge + Shadow exhibit an additive effect on herbicidal efficacy.
Soil-applied herbicides Dual gold, Stomp aqua, and Merlin flex, treated after sowing before emergence and also foliar-applied herbicide Shadow, treated during 6 - 8 real leaf stage of the chickpea, have very high pea selectivity - rating 1 by scale of EWRS (Table 3).
During later vegetative treatment with herbicide Challenge, weak phytotoxicity is possible - bleaching and discoloration on the periphery of chickpea leaves. It overcomes quickly and has no effect on seed yield.
Soil-applied herbicide Pelican used alone or combined with foliar-applied herbicides Challenge and Shadow and herbicide tank-mixture Challenge + Shadow have pure phytotoxicity in chickpea – rate 2 according to the scale of EWRS. It is overcome by the 30th day after Pelican treatment.
Soil-applied herbicide Pulsar used alone or combined with soil-applied herbicides Dual gold, Stomp aqua and Merlin flex have phytotoxicity in chickpea – rate 3 according to the scale of EWRS. Although there are no strong visible signs, it overcomes slowly and leads to a reduction in seed yield.
Herbicide combination Pelican + Pulsar leads to high phytotoxicity in chickpea – rate 5 according to the scale of EWRS. It overcomes very slowly, slows the growth of chickpeas and leads to a significant reduction in seed yield compared to the alone uses of herbicides Pelican and Pulsar.
Data for the influence of investigated herbicides, herbicide combinations and the herbicide tank mixture on seed yield of chickpea (Table 4) show that the lower yield is obtained by alone use of antigraminaceous herbicide Shadow, especially during wet years. The increase in yield is unproven compared to untreated control, due to the low efficacy of Shadow over the annual and perennial broadleaved weeds that are dominant in the experiment.
The yield increase in relative to the control is also unproven by herbicide combination the Pelican + Pulsar. This is due to the strong phytotoxicity of this herbicide combination to chickpeas, despite its high efficacy against weeds.
Treatment with herbicide Pulsar showed higher yields over the untreated control during the three years. Chickpea is lagging poorly in its development, the maturing stage is delayed by 4-5 days, but however seed yields are not significantly reduced, as weeding is significantly lower than untreated control, because Pulsar destroys all available weeds and self-sown plants.
It is important to note that herbicide Pelican has an initial phytotoxic effect on chickpea, which is to inhibit plant growth during the first 20-30 days after treatment. Subsequently, chickpeas overcome this negative effect and at the vegetation end in this variant high seed yields have been obtained, which is proven mathematically. This is due to the good chemical control of herbicide Pelican against existing weeds.
The alone use of soil-applied herbicides Dual gold, Stomp aqua and Merlin flex increases less the see yields than the alone use of foliar-applied herbicide Challenge and the herbicide tank mixture Challenge + Shadow, because these herbicides cannot control the perennial weeds and part of the annual weeds.
The highest yields of chickpea seeds are obtained by foliar treatment with herbicide tank-mixture Challenge + Shadow after soil-applied herbicides Pelican and Merlin flex – respectively 120.5% and 120.2% relative to the untreated control. High yields also are obtained also by foliar treatment with herbicide tank-mixture Challenge + Shadow after soil-applied herbicides Stomp aqua and

Table 4. Influence of some herbicides, herbicide combinations and herbicide tank mixture on seed yield of chickpea (2016 - 2018)

Herbicides		2016		2017		2018		Mean	
Soil-applied	Foliar-applied	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%
-	-	2077	100	1211	100	1760	100	1683	100
	Pulsar	2278	109.7	1291	106.3	1880	106.8	1816	107.9
	Challenge	2353	113.3	1345	111.1	1955	111.0	1884	112.0
	Shadow	2125	102.3	1245	102.8	1822	103.5	1731	102.8
	Challenge + Shadow	2397	115.4	1389	114.7	1989	113.0	1925	114.4
Dual gold	-	2191	105.5	1284	106.0	1971	107.7	1815	107.9
	Pulsar	2289	110.2	1321	109.1	1918	109.0	1843	109.4
	Challenge	2399	115.5	1393	115.0	2049	116.4	1947	115.7
	Shadow	2247	108.2	1326	109.5	1918	109.0	1830	108.7
	Challenge + Shadow	2436	117.3	1405	116.0	2054	116.7	1965	116.8
Stomp aqua	-	2216	106.7	1303	107.6	1917	108.9	1812	107.7
	Pulsar	2303	110.9	1339	110.6	1989	111.1	1877	111.5
	Challenge	2410	116.0	1399	115.5	2024	115.0	1944	115.5
	Shadow	2264	109.0	1332	110.0	1941	110.3	1846	109.7
	Challenge + Shadow	2453	118.1	1423	117.5	2059	117.0	1978	117.5
Merlin flex	-	2233	107.5	1308	108.0	1932	109.8	1824	108.4
	Pulsar	2337	112.5	1368	113.0	1969	111.9	1891	112.4
	Challenge	2453	118.1	1425	117.7	2068	117.2	1982	117.8
	Shadow	2264	109.0	1339	110.6	1955	111.1	1853	110.1
	Challenge + Shadow	2517	121.2	1460	120.8	2094	119.0	2024	120.2
Pelican	-	2250	108.3	1322	109.2	1955	111.1	1842	109.5
	Pulsar	2195	105.7	1241	102.5	1833	104.2	1756	104.4
	Challenge	2467	118.8	1441	119.0	1091	118.8	1957	116.3
	Shadow	2274	109.5	1340	110.7	1964	111.6	1859	110.5
	Challenge + Shadow	2513	121.0	1465	121.1	2107	119.7	2028	120.5
	LSD 5 %	106	5.1	48	4.0	83	4.7		
	LSD 1 %	137	6.6	68	5.6	107	6.1		
	LSD 0.1 %	170	8.2	88	7.3	134	7.6		

Dual gold – respectively 116.8% and 117.5%. Combinations of soil-applied herbicides Dual gold, Stomp aqua, Merlin flex and Pelican with foliar-applied herbicides Challenge, Pulsar and Shadow always leads to higher yields compared to the alone use of the respective herbicides during the three years of the investigation.

CONCLUSIONS

Combinations of soil-applied herbicides Dual gold, Stomp aqua, Merlin flex and Pelican with foliar-applied herbicides Challenge and Shadow, as well as with herbicide tank mixture Challenge + Shadow exhibit an additive effect on herbicidal efficacy.

Foliar-applied herbicide Pulsar destroys all annual and perennial broadleaved and graminaceous weeds and self-sown plants.

Herbicide Challenge is the only herbicide that successfully controls Clearfield and ExpressSun sunflower self-sown plants in chickpea crops.

Treatment with foliar-applied herbicide Pulsar leads to high phytotoxicity in chickpea – rate 3 according to the scale of EWRS. Herbicide combination Pelican + Pulsar leads to even higher phytotoxicity in chickpea – rate 5 according to the scale of EWRS.

Soil-applied herbicide Pelican used alone or combined with foliar-applied herbicides Challenge and Shadow and herbicide tank-mixture Challenge + Shadow leads to pure phytotoxicity in chickpea – rate 2 according to the scale of EWRS.

The highest yields of chickpea seeds are obtained by foliar treatment with herbicide tank-mixture Challenge + Shadow after soil-applied herbicides Pelican and Merlin flex.

High yields are obtained also by foliar treatment with herbicide tank-mixture Challenge + Shadow after soil-applied herbicides Stomp aqua and Dual gold.

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RESEARCH ON THE NUTRITION SYSTEM FOR *Jerusalem artichoke* GROWN ON SANDY SOILS

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Abstract

From the agronomic point of view, Jerusalem artichoke is considered a drought-resistant species and can be grown in non-irrigated conditions, by capitalizing on poor soils. Research conducted at RDSPCS Dabuleni in the period 2018-2020 under irrigation conditions showed that Jerusalem artichoke is a species that has performed well in cultivation on sandy soils. The experiment was placed on a soil with a low nitrogen content, normal phosphorus, low potassium and organic matter, and the soil reaction was weakly acidic. The research results in the period 2018-2020 show that the highest production of above-ground biomass (stems + leaves) of 64779 kg/ha was obtained on the agrofund of $N_{160}P_{160}K_{80}$ *and the planting distance of 40 cm between plants in a row.*

***Key words**: sandy soil, production, density, fertilization.*

INTRODUCTION

Jerusalem artichoke is appreciated for its characteristic, sweet taste, rich in minerals (Ca, Mg, K, P), vitamins (β-carotene, thiamine, lactoflavin, niacin, biotin, ascorbic acid), amino acids (lysine, arginine, histidine ,cystine, tryptophan, aspartic acid), specific amino acids (choline, betaine, saponin, quercitrin) and enzymes (inulinase, proteinase, invertase, phosphorylase and phenolase). The biochemical composition of tubers depends mainly on the genetic structure, environmental factors and genotype (Soare et al., 2017). Over time, Jerusalem artichoke has been used successfully to treat a wide range of conditions, from diabetes, colds, atherosclerosis, colorectal cancer, cholesterol, to digestive candidiasis, constipation, lack of calcium or obesity. Jerusalem artichoke is also recommended in asthenia, as a tonic, antiseptic, gout, dyspepsia, rheumatism. Tubers are used as a raw material in the alcohol industry, but also in the manufacture of sweets. From 100 kg of Jerusalem artichoke tubers can be obtained 7-10 liters of alcohol. The alcohol fermented from the tubers is of a better quality than that of sugar beet.

From an agronomic point of view, Jerusalem artichoke is considered a drought-resistant species and can be grown in non-irrigated conditions, by capitalizing on poor soils (Monti et al., 2005). It has a very high adaptability to the extremes of unfavorable factors, resistance to extremely high temperatures (35-45°C plants and 30-45°C in the case of tubers), resistance to high concentrations of salts, heavy metals, nitrates. Studies in this species have shown that irrigation is necessary when water is insufficient and exacerbates drought. Jerusalem artichoke is also a very valuable crop for biomass that is considered a rich source of ethanol (Denoroy, 1996). Jerusalem artichoke biogas production is much higher compared to other energy crops (Emmerling, 2007). In a semi-arid region of China, Zu Xin Liu (2012) conducted a study of twenty-six Jerusalem artichoke clones to evaluate their potential as a biomass feedstock for ethanol production and demonstrated that the biomass obtained can be a feedstock, first promising material for cellulosic ethanol.

Despite the great interest in this Jerusalem artichoke crop, there are few studies showing the spread of this species in the crop, especially in terms of the behavior of different genotypes. Planting density has been little studied and there is no important research for the regions of southern Europe. In fact, in the literature, planting density varies from case to case in the range of 2 plants/m^2 to 7 plants/m^2 (Zu XL et al., 2012, Mecella G. et al., 1996, Monti A. et al., 2005, Puangbut et al., 2012). Fertilization is the most important factor influencing the quantity and quality of Jerusalem artichoke production, but research conducted by Pomares et al., 2004, Feleafel, 2005, shows that soil, climatic conditions, genotype and cultivation technology contribute to the increase in Jerusalem artichoke production. Lombardo et al. (2017) showed that nitrogen fertilization significantly influenced the quality and shelf life of fresh Jerusalem artichoke tubers in terms of nutritional and microbiological properties.

The aim of the research was to evaluate the effect of planting density and the response to nitrogen, phosphorus and potassium fertilization of Jerusalem artichoke species with energy and food potential in the conditions of sandy soils from Research Development Station for Plant Culture on Sandy Soils (RDSPCS Dabuleni).

MATERIALS AND METHODS

The research was conducted at RDSPCS Dabuleni, in the period 2018-2020, in the conditions of a sandy soil with a low nitrogen content, normal phosphorus, low potassium and organic material, and the soil reaction was weakly acidic. The experience was bifactorial, placed according to the method of subdivided plots, in three repetitions.

FACTOR A: Fertilization system

a_1– Unfertilized

a_2 – $N_{40}P_{40}K_{40}$

a_3 – $N_{80}P_{80}$ K 80

a_4 – $N_{120}P_{120}K_{80}$

a_5 - $N_{160}P_{160}K_{80}$

FACTOR B: The distance between plants in a row

b_1 - 40 cm

b_2 - 50 cm

The biological material used for planting was the Rustic variety. The preparation of the land was done by plowing at a depth of 28-30 cm, fertilization with complex fertilizer 15-15-15 at the level of N, P, K established for the variants studied, disking + worked with milling cutter. Planting was done manually on April 3 in 2018, on March 20 in 2019 and on March 13 in 2020, at a distance of 70 cm between rows and 40 cm, respectively 50 cm between tubers per row.

In order to determine the nutrient supply status of the soil from the Jerusalem artichoke culture, soil samples were collected at a depth of 0-40 cm. The samples were recorded and conditioned in the laboratory, from which the following determinations were made:

- total nitrogen - Kjeldahl method;
- extractable phosphorus (P-AL) - Egner method - Riem Domingo, by which phosphates are extracted from the soil sample with a solution of acetate - ammonium lactate at pH - 5.75, and the extracted phosphate anion is determined colorimetrically as - blue of molybdenum;
-changeable potassium (K-AL) - Egner - Riem Domingo method by which the hydrogen and ammonium ions of the extraction solution replace by exchange the potassium ions in exchangeable form from the soil sample which are passed into the solution. The potassium is determined in the solution obtained by flame emission photometry.
-organic carbon - wet oxidation method and titrimetric dosing (after Walkley - Blak in the Gogoasa modification);
- pH- of the soil, potentiometric method.

During the vegetation period, observations and determinations were made regarding: plant height, average number of shoots/plant, average number of leaves/plant, stem base diameter, leaf area. At the beginning of flowering, because the initiation of flowers is a signal of accumulation of assimilates in tubers (M.D. Curt et al., 2006) the number of tubers/plant, the weight of tubers/plant, the production of aerial and underground biomass were determined. Biomass production (stems and tubers) was expressed in kg/ha. The nutritional quality of the potato tubers was determined in the laboratory regarding:

- water and total dry matter (%) - gravimetric method;
- soluble dry matter (%) - refractometric method;
- vitamin C (mg/100 g s.p) - iodometric method;
- starch (%) - gravimetric method;

The calculation and interpretation of the results was performed in comparison with the unfertilized control, using the method of analysis of variance.

RESULTS AND DISCUSSIONS

Laboratory analyzes on the chemical composition of the soil show the existence of a soil with (Table 1) a low content of nitrogen, normal phosphorus, low to medium potassium and organic material and the soil reaction was moderately acidic to neutral.

Table 1. Chemical composition of soil in Jerusalem artichoke culture (2018-2020)

Fertilization dose	Total nitrogen (%)	Extractable phosphorus (ppm)	Exchangeable potassium (ppm)	Organic carbon (%)	pH in water
a_1- Unfertilized	0.07	45	61	0.98	6.99
a_2 - $N_{40}P_{40}K_{40}$	0.06	56	59	0.90	7.30
a_3 – $N_{80}P_{80}K_{80}$	0.04	47	58	0.69	7.25
a_4 – $N_{120}P_{120}K_{80}$	0.07	93	93	0.97	6.72
a_5--$N_{160}P_{160}K_{80}$	0.05	80	78	0.61	6.40
poorly stocked	<0.10	8.1-18	<66	<0.58	5.01-5.80 Weak acid
Middle stocked	0.11-0.15	18.1-36	66.1-132	0.59-1.16	5.81-6.8 Moderate acid
Well stocked	0.16-0.20	36.1-72	132.1-200	1.17-2.32	6.81-7.20 Neutral

Biometric measurements made during the vegetation period on the height of the plant, no. of stems/plant, no. of lateral shoots/plant are presented in Table 2.

Table 2. Biometric determinations of Jerusalem artichoke during the growing season (2018-2020)

Fertilization dose	Planting density	The average size of the plant (cm)	Average number of stems/plant	Average number of lateral shoots/plant
a_1- Unfertilized	b_1-40 cm	205.2	5.9	4.9
	b_2- 50 cm	216.5	4.6	2.5
a_2 - $N_{40}P_{40}K_{40}$	b_1-40 cm	215.8	4.1	2.8
	b_2- 50 cm	231.3	3.0	2.6
a_3 -$N_{80}P_{80}K_{80}$	b_1-40 cm	250.3	3.9	2.8
	b_2- 50 cm	271.3	2.5	3.6
a_4 -$N_{120}P_{120}K_{80}$	b_1-40 cm	224.3	1.4	3.0
	b_2- 50 cm	255.2	2.8	3.4
a_5- $N_{160}P_{160}K_{80}$	b_1-40 cm	240.3	3.3	4.5
	b_2- 50 cm	227.3	3.9	5.0

Biometric determinations performed on the waist of Jerusalem artichoke plants show an average plant height with values between 205.2 cm in the unfertilized version which can reach up to 271.3 cm in the fertilized version with a dose of $N_{80}P_{80}K_{80}$ where the distance between plants in a row was 50 cm. Compared to the unfertilized control variant the plant size increased with the increase of fertilizer doses to $N_{120}P_{120}K_{80}$, followed by a decrease of the plant size with the increase of fertilizer doses to $N_{160}P_{160}K_{80}$ (Table 2).

It is observed that depending on the planting density, the height of the plants had higher values in the variants where the distance between plants in a row was 50 cm (Figure1).

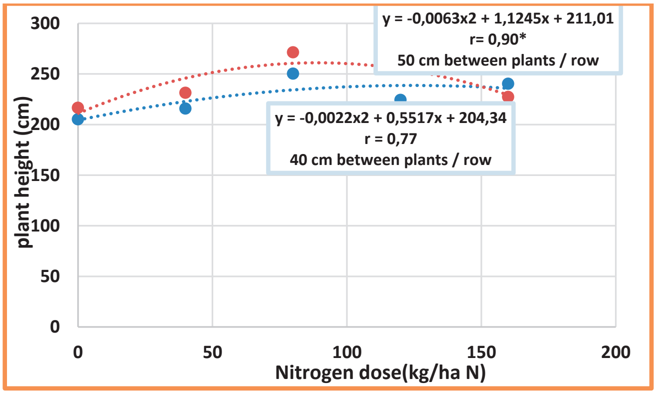

Figure1. The influence of planting density depending on the dose of nitrogen on the size of the plant

Table 3. Biometric determinations of Jerusalem artichoke during the growing season (2018-2020)

Fertilization dose	Planting density	Number of leaves/plant	Leaf area (m^2/ha)	Aboveground biomass weight (stems + leaves)(kg/ha)
a_1- Unfertilized	b_1-40 cm	444	103594.3	34250
	b_2- 50 cm	426	78933	34050
a_2 - $N_{40}P_{40}K_{40}$	b_1-40 cm	470.3	119015.3	42502
	b_2- 50 cm	454.3	96696	36589
a_3 - $N_{80}P_{80}K_{80}$	b_1-40 cm	517.3	115530.7	57487
	b_2- 50 cm	660.3	143960	46166
a_4- $N_{120}P_{120}K_{80}$	b_1-40 cm	486.6	124030	52173
	b_2- 50 cm	649.6	147810.7	54080
a_5 - $N_{160}P_{160}K_{80}$	b_1-40 cm	651.3	163860	64779
	b_2- 50 cm	512.6	105468.7	45881
LSD 5%=			67369	20960
LSD 1%=			95766	29800
LSD 0.1%=			138665	43150

The number of leaves per plant is an important component of biomass. The average results from 2018-2020 show that the average number of leaves per plant increased significantly with increasing fertilization doses, from 426 in the non-fertilized version to 660.3 in the version where the dose of $N_{80}P_{80}K_{80}$ was used (Figure 2) .

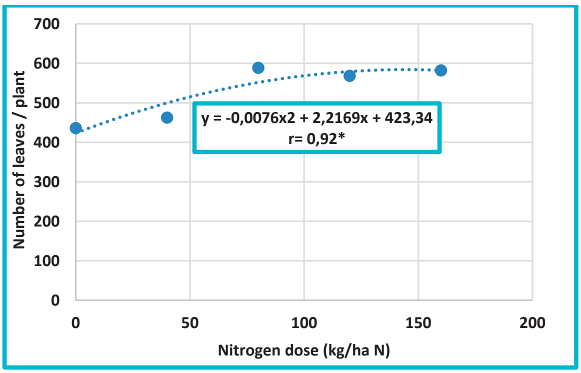

Figure 2. The influence of nitrogen dose on the number of leaves/plant

The leaf surface is a physiological indicator directly related to the amount of light that can be intercepted by plants.

Knowledge of the leaf area is a particular importance. Knowing this parameter we can approximate the primary photosynthetic production. evapotranspiration and can be used as a reference tool for crop growth. having an essential role in the ecology of theoretical production.

The leaf area/ha increased with increasing number of plants/ha by reducing the distance to 40 cm between plants/row leading to the interception of a larger amount of photosynthetically active radiation due to the higher number of plants/ha (Figure 3).

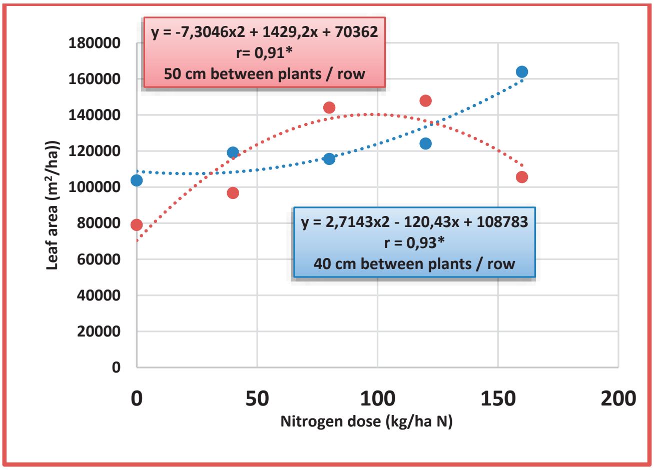

Figure 3. Influence of planting density depending on the dose of nitrogen on the leaf area

Also, the increase of fertilizer doses per unit area led to an increase in the production of stems and leaves. Increasing the amount of biomass obtained. There is a distinctly significant correlation between the leaf area and the amount of biomass obtained given by the polynomial equation of degree 2 (r = 0.99**)(Figure 4).

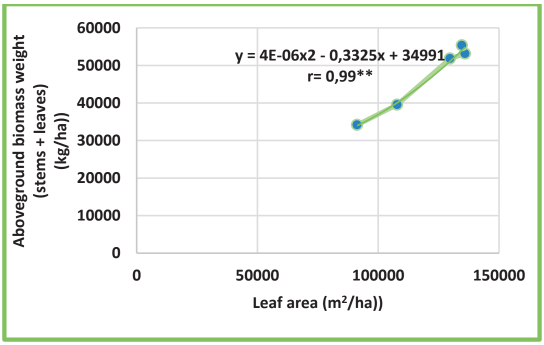

Figure 4. Correlation between leaf area and biomass production depending on nitrogen dose

From our research in the period 2018-2020 it is observed that the highest biomass production of 64779 kg/ha was obtained on the agrofund of$N_{160}P_{160}$ K_{80} and the planting distance of 40 cm between plants in a row. The functional link between the fertilization variant with N_{160} and the density of 40 cm between plants in a row is given by the polynomial function of degree 2 which shows a significant correlation (r = 0.93*) (Figure 5). In order to determine the production of tubers/ha, the number of tubers/plant, the weight of tubers/plant were determined at harvest (Table 6).

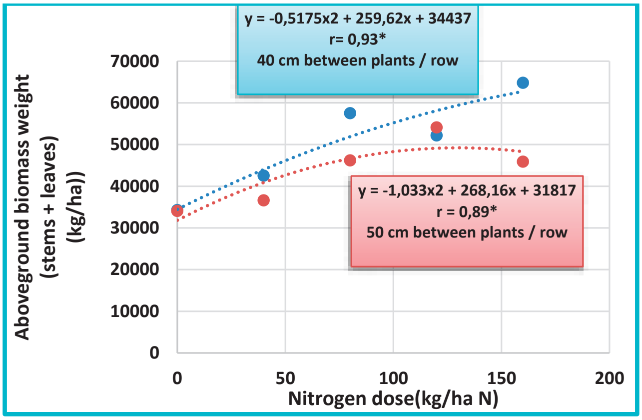

Fig. 5. Influence of planting density and nitrogen dose on aboveground biomass production (stems + leaves)

The average results of 2018-2020 on the influence of nitrogen dose on tuber production/ha show that it increased with increasing nitrogen doses to the dose of N_{120}, a significant increase compared to the unfertilized control variant. Also, there is a significant correlation (r = 0.88*) between the leaf area and the tuber production obtained/ha depending on the applied nitrogen dose (Figure 6).

Table 6. Production of Jerusalem artichoke tubers depending on fertilization and planting density (2018-2020)

Fertilization dose	Planting density	Number of tubers/ plant	Weight of tubers/ plant (kg)	Average production of tubers (kg/ha)
a_1-Unfertilized	b_1-40 cm	50.4	0.658	23036
	b_2- 50 cm	45.5	0.848	23756
a_2 - $N_{40}P_{40}K_{40}$	b_1-40 cm	46.8	0.908	31785
	b_2- 50 cm	43.5	0.913	25570
a_3 - $N_{80}P_{80}K_{80}$	b_1-40 cm	40.4	0.839	29376
	b_2- 50 cm	47.6	1.1	31039
a_4 -$N_{120}P_{120}K_{80}$	b_1-40 cm	38.7	1.19	41671
	b_2- 50 cm	55.7	1.39	39127
a_5- $N_{160}P_{160}K_{80}$	b_1-40 cm	37.5	1.08	37807
	b_2- 50 cm	38.0	1.069	29947
LSD 5%=				11200
LSD 1%=				16000
LSD 0.1%=				23200

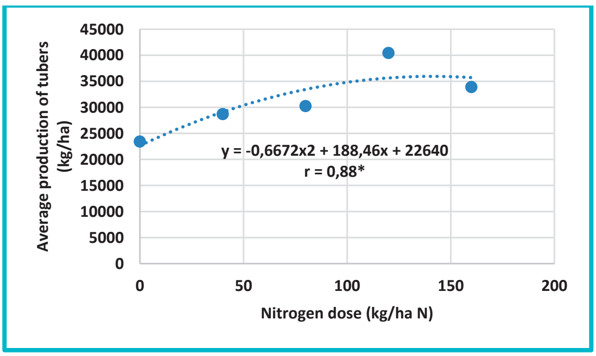

Figure 6. The influence of nitrogen dose on the production of Jerusalem artichoke tubers

Analyzing the interaction between the studied factors (planting density x fertilization doses) on the production of fresh tubers, it is found that the distance of 40 cm between plants/row and the level of fertilization of $N_{120}P_{120}K_{80}$ ensured the highest production of 41671 kg/ha fresh tubers/ha, not statistically insured (Figure 7).

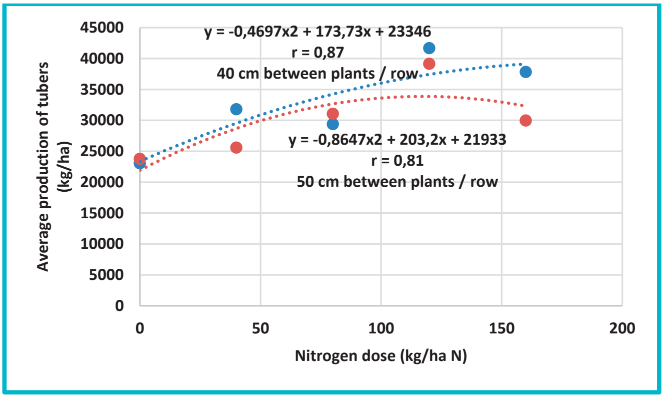

Figure 7. Influence of planting density and nitrogen dose on the production of Jerusalem artichoke tubers

The chemical composition of Jerusalem artichoke tubers depends on the cultivation technology and to a large extent on the nutritional level.The chemical composition of the tubers is also highly dependent on the type of soil, its productivity, the genetic potential of the variety and the growth stage (Meijer & Mathijssen, 1991; Mclaurin et al.,1999; Sawicka & Kalembasa, 2013), which are largely related to harvest maturity (Saengthobpinit & Sajjaanantakul, 2005). Jerusalem artichoke tubers usually contain about 80% water. They can play an important role in human nutrition as sources of protein (1-2%), carbohydrates (15%), vitamins,inulin (up to 20%) and minerals, especially iron (0.4 to 3.7 mg/100 g), calcium (14 to 37 mg/100 g) and potassium (420-657 mg/100 g) (Whitney &Rolfes, 1999).

The results obtained regarding the nutritional quality of Jerusalem artichoke tubers depending on fertilization and planting density are presented in Tables 7 and 8.

Table 7. Biochemical composition of Jerusalem artichoke tubers depending on the fertilization system

Fertilization dose	Total dry matter (%)	Water (%)	Soluble dry matter (%)	Inulin (%)	Carbohydrate content (%)	C vitamin (mg/100 g s.p)	Average tubers production (kg/ha)
a_1-Unfertilized	22.72	77.28	22.16	11.91	18.29	8.37	23396
a_2 - $N_{40}P_{40}K_{40}$	23.25	76.75	21.32	13.10	18.07	8.52	28678
a_3- $N_{80}P_{80}K_{80}$	23.64	76.36	21.22	12.38	17.99	9.12	30208
a_4 - $N_{120}P_{120}K_{80}$	23.28	76.72	20.15	12.78	17.12	8.22	40399
a_5- $N_{160}P_{160}K_{80}$	22.81	77.19	20.68	12.41	17.56	8.51	33878

Depending on the fertilization dose, in the Jerusalem artichoke tubers was determined a total dry matter between 22.72% in the unfertilized version and 23.64% in the version fertilized with $N_{80}P_{80}K_{40}$. The amount of water in the tubers that was between 76.72% and 77.28% decreases with the accumulation of dry matter.

D. De Santis, M. & Teresa Frangipane, 2017,determined in the Jerusalem artichoke tubers a water content of 79.76-81.25%. Sandra Žaldarienė et al., 2012, in a study conducted on a farm in Lithuania determined a dry matter content of 23.21%. and the average weight of the tubers was 26.1g.

Vînatoru C.. 2017, highlights the importance of water from Jerusalem artichoke tubers for the human body. Belgian chemist Aldenne B. C. together with the team of researchers from the U. B. Brussels have shown that the water present in fruits (cellular water) has other properties than that from direct sources:rivers, precipitation. The water present in Jerusalem artichoke tubers is totally different from the usual one. This cellular water synthesized and metabolized by Jerusalem artichoke has the ability to rejuvenate some cells due to its special physical properties. Studies have recently been taken up by cardiologists in the UK. And they say that there is a possibility and they think that the common Jerusalem artichoke has the ability to maintain the heart muscle.

The soluble dry matter was between 20.15% in the fertilized version with the dose of $N_{120}P_{120}K_{80}$ and 22.16% in the unfertilized version. The carbohydrate content was between 17.12% and 18.29% in the same variants.

Kays and Nottingham, 2007, in a Canadian study determined in different groups of varieties a carbohydrate content between 8.2% and 20.7%. The root of this plant stores a polymer of fructose, inulin. Compared to other plants it has been found that the inulin in Jerusalem artichoke has the lowest percentage of glucose and sucrose, thus helping patients with diabetes, helping to normalize blood sugar.

The highest inulin content (13.10%) was determined in the variant fertilized with $N_{40}P_{40}$ K_{40}.

The inulin content of artichoke tubers in Jerusalem varies from 7 to 30% of its fresh weight (Kays & Nottingham, 2008; Saengthongpinit, 2005).

The highest values of vitamin C (9.12 mg) were recorded in the version fertilized with $N_{80}P_{80}$ K_{80}. Jerusalem artichoke tubers are a source of vitamin C (4.1 mg/100 g) (Scollo et al., 2011). Ermosh et al., 2020, determined in the Jerusalem artichoke tubers an amount of vitamin C between 17 mg and 18.64 mg/100g s.p. Also, Yue Wang et al., 2015 found in Jerusalem artichoke tubers a vitamin C content between 7 and 26 mg/100 g s.p.

Analyzing the influence of fertilizer doses, the best quality results were obtained in the variants fertilized with $N_{40}P_{40}$ K_{40} and $N_{80}P_{80}$ K_{80} (Table 7) and the highest production (40399 kg/ha) was recorded in the variant fertilized with $N_{120}P_{120}$ K_{80}.

Regarding the influence of planting density on the production of tubers. as well as on their quality, the results obtained and presented in Table 8, showed an increase in the production of tubers planted at 50 cm between plants in a row compared to the distance of 40 cm by 5962 kg/ Ha. Jerusalem artichoke tubers in this variant recorded a higher content of total dry matter (23.25%), a higher amount of inulin (13.10%), as well as a higher amount of vitamin C (8.52 mg).

Table 8. Biochemical composition of Jerusalem artichoke tubers depending on the distance between plants

Varianta	Total dry matter (%)	Water (%)	Soluble dry matter (%)	Inulin (%)	Carbohydrate content (%)	C vitamin (mg/100 g s.p)	Average tubers production (kg/ha)
b_1 – 40 cm	22.72	77.28	22.16	11.91	18.29	8.37	32735
b_2 - 50 cm	23.25	76.75	21.32	13.10	18.07	8.52	29888

Multiple studies have described the effect of fertilizers on Jerusalem artichoke. For example, lack of phosphorus or potassium alters the morphogenesis, growth and yield of tubers, more pronounced compared to aerial growth. However, the influence of nitrogen on yield is stronger than that of potassium, due to differences in their original soil content; nitrogen determines the photosynthesis potential and somewhat increases the efficiency of water use (Soja and Haunold, 1991). Therefore, drought and fertilizers with nitrogen.

phosphorus and potassium can strongly affect the weight of dry matter (Monti et al., 2005). At harvest tuber production can reach up to 62 t/ha (Zhong et al., 2007).

CONCLUSIONS

Jerusalem artichoke is a species that has performed well in cultivation on sandy soils in climatic conditions from 2018-2020.

From the research carried out in 2018-2020 it is observed that the highest biomass production of 64779 kg/ha was obtained on the agrofund of $N_{160}P_{160}$ K_{80} and the planting distance of 40 cm between plants in a row.

Analyzing the interaction between the studied factors (planting density x fertilization doses) on the production of fresh tubers. it is found that the distance of 40 cm between plants/row and the level of fertilization of $N_{120}P_{120}K_{80}$ ensured the highest production of 41671 kg/ha fresh tubers / ha. not statistically insured.

The results obtained on the nutritional quality of Jerusalem artichoke tubers highlight the low requirements of the species for fertilizers.

The studied biochemical components showed a higher content at low doses of fertilizers as well as in the unfertilized version.

The planting density of the tubers did not significantly influence the quality of the Jerusalem artichoke tubers.

Jerusalem artichoke, due to the low requirements for environmental factors,but also for technological ones, can be part of the category of food safety species.

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TESTING A SELECTION OF ALFALFA VARIETIES FOR ECOLOGICAL PLASTICITY, PRODUCTIVITY AND A NUMBER OF QUALITATIVE PARAMETERS

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Abstract

The cultivation of alfalfa has an essential role in ensuring the required fodder, but the achievement of cropping performances e.g. proper yield, persistence, winter hardiness, competition capacity, ecological plasticity, depends on a multitude of technological, environmental, and genetic factors. The selection of suitable varieties for cultivation in pure stands or more perennial and productive mixtures with other perennial legumes and grasses is a key element for a successful crop. The objective of the study was to characterize the bio-productive and forage quality performances of 18 alfalfa varieties available for cultivation in Romania, originating from Romania and abroad, in the eco-climatic conditions specific to the Romanian Plain. The screening of these performances that substantiate the appreciation of the biological yield was based on detailed experiences in the field and laboratory. A set of representative indicators was selected for measurements, determinations, and observations, including growth rate, tillering, leaf-stem ratio, forage quality indicator, average crop yield, and canopy height. It was found that Romanian varieties presented improved ecological plasticity compared to the foreign varieties in the eco-climatic conditions of the experimental site.

Key words*: Medicago sativa L., dry matter, growth rate, leaf-area ratio, tillering, forage quality, yield.*

INTRODUCTION

Alfalfa is a very popular plant worldwide due to the possibility of using it for multiple purposes. The associated environmental impacts for producing the alfalfa for fodder differ depending on the type of production system and the region. It is important to take into account water and land resources together with assessing other impacts of agriculture products when performing a comparison between farming systems and improvement measures (Wang et al., 2021).

Compared to other fodder crops (Dunea et al., 2015), alfalfa is clearly superior through large fodder productions and fodder quality provided by its ability to resist for many years without being sown and optimized irrigation has an important role in achieving superior yields (Dincă et al., 2017). Cavero et al. (2017) found that the maximum alfalfa forage yield was lower in the first cropping year (17 t ha^{-1}) compared to the two following years (20-22 t ha^{-1}). In the second and third year of cropping of the Aragón variety, the increase of forage yield occurred when the applied irrigation was 115% of crop irrigation requirement compared to the first year when the yield increased linearly with the applied irrigation in a semiarid Mediterranean climate (Zaragoza, Spain). Schitea (2010) reported for Romanian alfalfa cultivars an average production of more than 17 t DM ha^{-1} of the three cropping years in irrigated conditions and application of optimal fertilization. For Romania climate, alfalfa yields were found to be responsive to irrigation level, decreasing with reductions in irrigation amount.

Phosphorous fertilization also increases the forage yield in both young and old rainfed alfalfa stands in a semiarid environment (Fan et al., 2016).

Symbiotic rhizobia provide beneficial effects on leguminous plants (Dincă and Dunea, 2017) including alfalfa. A novel effect of rhizobia on forage quality of alfalfa was found i.e., the nodulated alfalfa showed an increase in lignin content and a decrease in digestibility in comparison with non-nodulated plants due to a potential defensive response of plants to the rhizobial invasion (Zhang et al., 2016).

The forage production obtained from alfalfa cropping can be used in the form of green fodder, hay, silage, semi-hay, and dehydrated fodder (Lloveras et al., 2008).

Alfalfa also plays an important role in the rotation of crops being an excellent precursor plant. It accumulates appreciable amounts of nitrogen in the soil, and organic substances through the root system improving the physical and chemical properties of soils.

During its long history as a cultivated plant, numerous ecotypes, local populations, and alfalfa varieties have been developed under the influence of various pedo-climatic conditions that are adapted to a specific geographical area.

For many farmers, the selection of the alfalfa variety means buying the cheapest product on the market, but if we analyze this issue in more detail, we may find that the performance of the variety is more important than the price of seeds.

The price of seeds is important up to a certain level, after which, the gain per kilogram of seed means financial losses per hectare in the productivity of the variety during the exploitation of the crop.

The specialists in the cultivation of fodder plants from Romania and abroad have proven repeatedly that the results obtained in the alfalfa crops are influenced by a series of characteristics related to the yield and quality of the obtained fodder.

A series of indices can provide a ranking of the varieties regarding these performance characteristics such as:

- growth rate;
- average yield (tons dry matter ha^{-1});
- canopy height;
- tillering rate;
- indices of forage quality (crude protein, digestibility, etc.) - Sanderson (1992).

Due to the particularities of technology and economic importance, the alfalfa crop is a long-term investment (Smeal et al., 1991). To make the most of it, these investments must be followed as decisions taken to respect the correct order (e.g., pedochemical analysis of the soil, planning of the sowing densities depending on the crop destination, the choice of a suitable variety adapted to the growth and development conditions, etc.).

These few details make a difference in the success of alfalfa cultivation. The goal of seeding high-yielding varieties able to provide the required crop performance is sustained by proper researches in breeding new cultivars adapted to the climate variations of the cropping area and global warming. Li and Brummer (2012) suggest several directions to improve selection in alfalfa including diversity selection and paternity testing, introgression of quantitative trait loci, and genomic selection.

An adequate breeding program for alfalfa suitable varieties should consider the combination of high yield and production that is well distributed over the growth season, with high quality of produced forage. Furthermore, they must be hardy and adaptable, relatively with lower demands in terms of agronomic inputs. Consequently, a successful alfalfa variety should present several bio-productive features such as:

- have a medium-tall height of canopy with thin stalks and a proper leaf-stem ratio;
- to be suitable for early cuttings allowing an intensive use;
- providing a compromise between hardiness and quality: good yield in fertile plain soils and in drier hilly areas or where the winters are colder;
- have a positive fiber digestibility and protein content;
- have appropriate radiation use efficiency (Dunea et al., 2019) and water use efficiency (Dincă et al., 2017);
- have a lower environmental impact based on its life cycle assessment (Wang et al., 2021).

In this context, a breeding program is promoted by S.C. Patru Agro S.R.L. (https://samantalucerna.ro/) in cooperation with the academic researchers for developing new alfalfa varieties that are suitable for the specific Romanian eco-pedoclimatic conditions. Such varieties should have improved winter hardiness due to the cold winters with a higher incidence and duration of frost days. The program provided two varieties i.e., Dobrogea and Valahia, which have been included in the national official list of varieties (https://istis.ro/image/data/download/catalog-oficial/catalog%202020.pdf), while Dobrogea is included in the OECD list of varieties eligible for seed certification (Table 1).

The paper presents the multiannual results obtained from field-testing of a large number of alfalfa varieties from which 18 have been selected for synthetic presentation of the cropping performance based on previously mentioned indices. The screening of these varieties originating from Romania, Italy, France, and the U.S.A. can provide useful information for the farmers and specialists in fodder production.

MATERIALS AND METHODS

The screening experiments were carried out on plots in Gherghita Plain, Puchenii Mari village (N44.824060, E26.092660) – Figure 1. Each of the *18 alfalfa varieties* had three replicates and several variables have been determined for each harvest cycle: Relative Growth Rate, Tillering Rate and type (vegetative and generative), Leaf/Stem Ratio (g DM leaf m^{-2}/g DM stem m^{-2}), net yield, Forage quality (based on several parameters), canopy height, RUE, and Leaf Area Index (determined indirectly with a Delta-T Devices SunScan SS1 canopy analyzer system and checked randomly using measurements of leaf area samples with a digital planimeter).

The collection comprises varieties from Romania, other European countries, and the U.S.A.

All plots have been maintained in the same optimal conditions of fertilization and irrigation during this multiannual experiment (2017-2020).

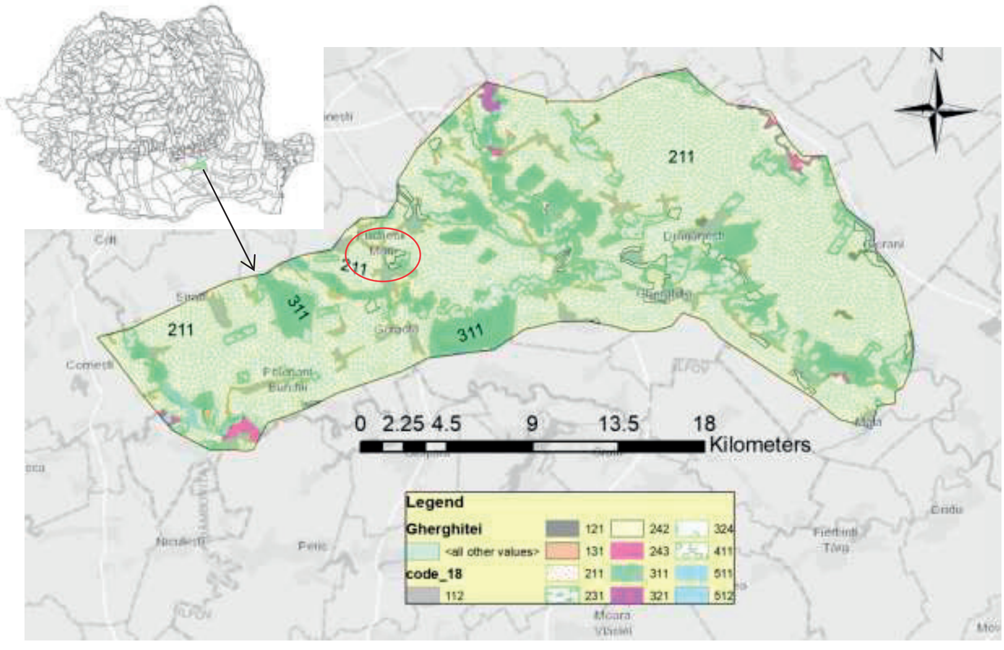

Figure 1. The study area located in Gherghitei Plain, south of Romania, in Puchenii Mari village - Land use/land cover (LULC) showing the 231 INSPIRE category attributed to Pastures; 242: Complex cultivation patterns; 321: Natural grasslands)

More details regarding the experimental setup in Gherghita Plain can be found in Dunea et al. (2019) and in Dincă and Dunea (2018) regarding the screening protocol of alfalfa varieties. Gherghita Plain is a favorable area for alfalfa cropping.

Meteorological parameters such as net radiation, air temperature, air relative humidity, wind speed, etc. have been monitored with an Adcon Telemetry dedicated system mounted near the experimental field.

Table 1 presents the tested alfalfa varieties.

Table 1. Alfalfa varieties tested in the multiannual ranking experiment (7 Romanian and 11 foreign varieties from U.S.A./Austria, Italy and France)

Variety	Maintainer name	Maintainer code*	Country
Sandra	INCDA Fundulea	1562	Romania
Mădălina	INCDA Fundulea	1562	Romania
Dobrogea	SC Patru Agro SRL	2782	Romania
Mihaela	INCDA Fundulea	1562	Romania
Valahia	SC Patru Agro SRL	2782	Romania
Roxana	INCDA Fundulea	1562	Romania
Cezara	INCDA Fundulea	1562	Romania
Dimitra	Continental Semences	724	Italy
PR55V48	S&W Seed Company/Pioneer Hi-Bred Services GmbH	3133	U.S.A., Austria
Pomposa	Gennari & Schiavi	1241	Italy
Galaxie	GIE Grass	1266	France
PR54V09	S&W Seed Company/Pioneer Hi-Bred Services GmbH	3133	U.S.A., Austria
Gea	Roberto Guarneri/ Maro Tarim Insaat ve Turizm	1359, 1988	Italy, Turkey
Harpe	GIE Grass	1266	France
Orca	RAGT 2n	2595	France
Bardine	Barenbrug Holland B.V.	401, 402	The Netherlands
Giulia	Natura Srl	2150	Italy
Letizia	Compagnia Generale Servizi	698	Italy

*https://www.oecd.org/agriculture/seeds/documents/codes-and-schemes-list-of-varieties-eligible-for-seed-certification.pdf

The following indicators were determined to characterize the biological efficiency of the tested varieties and to establish an overall ranking in the eco-pedoclimatic conditions of the experimental field:

- *Growth Rate* - the accumulation of dry matter per unit area during a day (kg DM /m^2/day) and *Relative Growth Rate* (RGR) - the average growth in length of alfalfa plants in centimeters per day (cm/day);
- *Degree of tillering* (DT) - the average of the number of generative shoots, respectively of the number of vegetative shoots per plant; the indicator is useful in estimating empirically the fodder production yield: Fodder yield/m^2 = NP × [(NLP × GL) + (NFP × GF)] (NP - number of plants/m^2, NLP - number of shoots/plant, GL - average weight of a shoot, NFP - number of leaves/plant, GF - average leaf weight);
- *Leaf/Stem Ratio* - the mass ratio between the amount of dry matter of the leaves and the amount of dry matter of the stems per unit area;
- *Forage quality* - a synthetic indicator of fodder quality using a regressive statistical model that uses as variables several indicators determined by the specific laboratory analyzes performed in an accredited laboratory, respectively dry matter content (DM), crude protein content (CP), crude fat content, crude cellulose content, crude ash content and the digestibility coefficient;
- *Estimated average forage yield* - represents the estimated amount of forage per unit area (t DM/ha);
- *Canopy height* - the average height of the canopy from ground to the average height of plants (cm).

All the characteristics were categorized using a five steps rank (1-low; 5-excellent) to screen empirically the performance of each variety in an easier way. Then, the multiannual ranks of the characteristics were used in a weighted regression in which more weight was given to the observations with smaller variance because these observations provide more reliable information about the regression function than those with large variances (Neter et al., 1996). More weights were attributed to forage yield and to forage quality as important parameters for assessing the overall crop performance.

RESULTS AND DISCUSSIONS

A selection of the most relevant characteristics determined during the multiannual screening trials performed considered yield, forage quality, tillering, and growth rate.

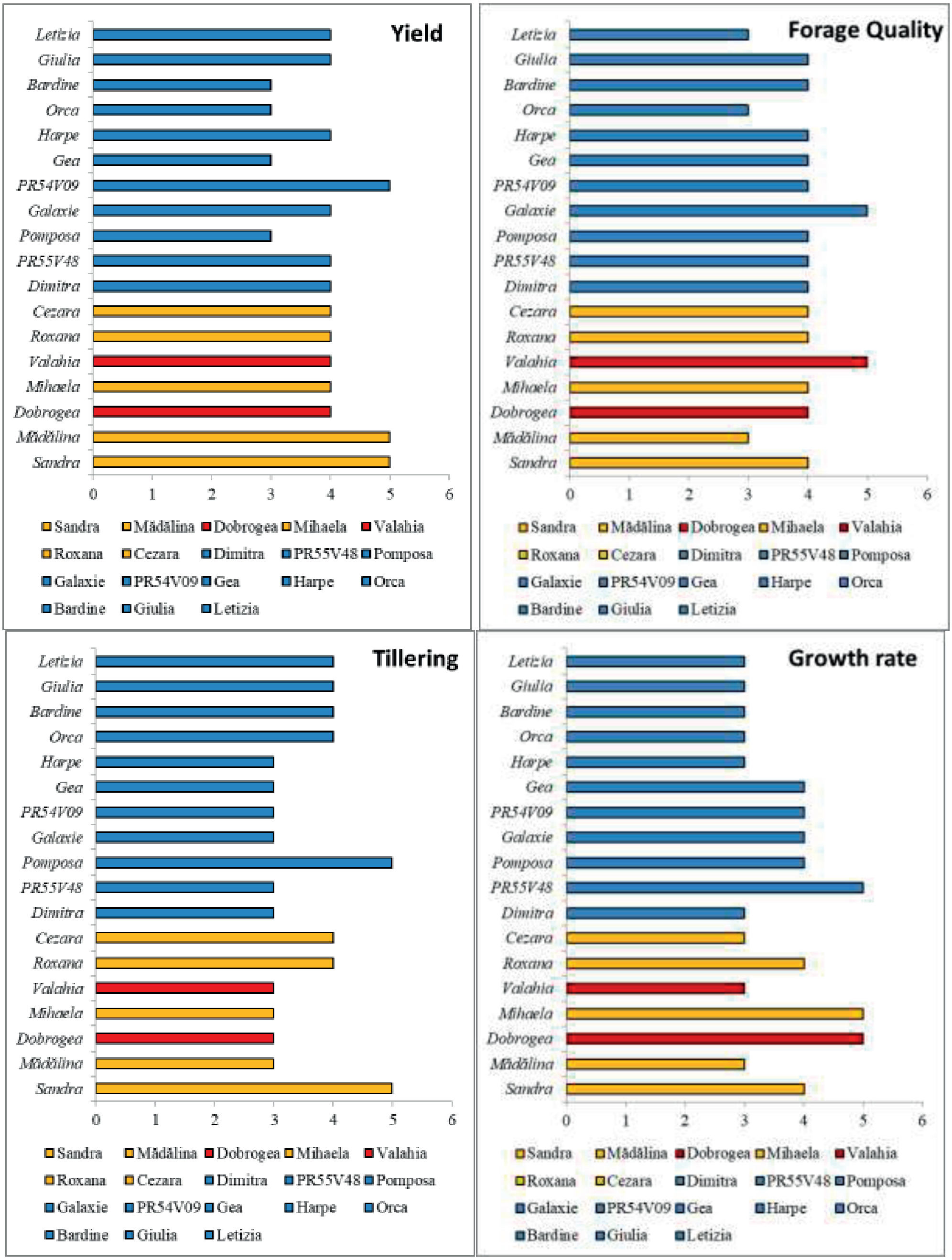

Figure 2. Evaluation of the most important parameters related to the crop performance in 18 alfalfa varieties in the eco-pedoclimatic conditions of Gherghita Plain, Romania using ranks from 1 to 5 (1-low; 5-excellent)

Some indicative values can be retrieved by using the means of all the varieties. Consequently, the RGR showed an average of 2.2 cm/day (*Standard deviation* - SD = 0.2) at the first cutting, 2.6 cm/day (SD = 0.3) at the second cutting, and 0.9 (SD = 0.3) at the third cutting. The growth rate had an average of 2.4 g DM m^{-2} (SD = 0.2) leading to an overall yield of 14 t ha^{-1} DM.

Regarding the tillering capacity, the average of varieties for all cuttings reached 16 generative shoots (SD = 6) and 13 vegetative ones (SD = 5) per plant. The L/S ratio presented a lower variability having an average of 0.7 (SD = 0.1) and the canopy height was 60 cm (SD = 6.5).
Regarding the feed value of the resulted fodder, the average of the 18 varieties for the feed unit for milk (UNL - assessed on the basis of the energy effect of barley; 1 UNL = 1700 Kcal = 7.11 MJ) was 0.9 kg/DM (SD = 0.03) and for the coefficient of organic substances digestibility was 75 (SD = 0.25).
Figure 2 shows synthetically these results. It can be seen that two Romanian varieties (Sandra and Mădălina) and one U.S. variety (PR54V09) reached the highest yields, but in terms of forage quality, Valahia and Galaxie were the most qualitative. Interestingly, most of the varieties reached good qualitative levels by rank 4. There were three exceptions (Letizia, Orca, and Mădălina). Regarding the tillering capacity, Sandra and Pomposa had the highest rank, and generally, the foreign varieties showed a better tillering than the Romanian ones. In terms of growth rate, Dobrogea, Mihaela, and PR55V48 were the most relevant between the tested varieties.
Figure 3 shows the overall results provided by the weighted regression for adjusting a hierarchy that included all the measured characteristics. It can be observed that the selected varieties both Romanian and foreign ones showed good performances in the experimental conditions under optimal irrigation and fertilization regime. The average of the synthetized rank for the Romanian varieties was 3.8 (SD = 0.27), and 3.6 (SD = 0.25) for foreign varieties.
The maximum rank was obtained by two Romanian varieties i.e. Dobrogea and Sandra followed by PR55V48. All the selected varieties have valuable bio-productive characteristics being a useful selection pool considering that the ***variety selection*** is the foremost decision growers can make.

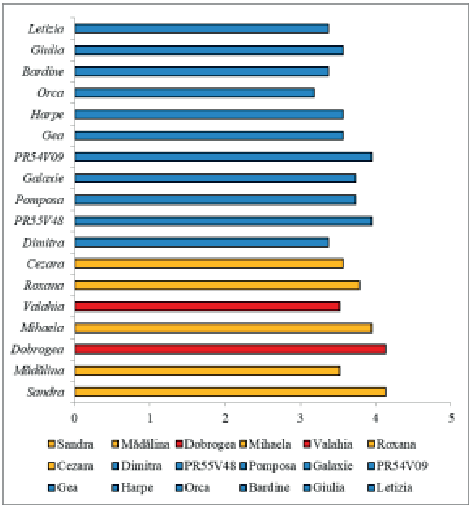

Figure 3. Results of the weighted regression showing the overall performance of the tested alfalfa varieties

Other characteristics that must be taken into account for future assessments are dormancy, resistance to diseases and pests, and the resistance to drought. In Romanian varieties, the dormancy reaches 4-4.5, being considered high, representing an important asset for the success of alfalfa cultivation, considering the frequency of frosty winters in Romania. Foreign varieties have a lower dormancy (6-7), respectively a lower frost resistance. This limitation must be carefully analyzed before considering the selection of a foreign variety.
New instruments for grassland and forage crops analysis such as multi- and hyper-spectral detection sensors are available on satellites and on aerial platforms including UAVs increasing the spatiotemporal performance for acquiring ground data from terrestrial ecosystems including forage cropping systems (Noland et al., 2018). This promising technique can provide support in validating the performance of alfalfa varieties on large-scale farms in the context of the forecasted aridity index, which is expected to increase in the south of Romania according to the simulations from the climate models.

CONCLUSIONS

By presenting the certified cultivated varieties in Romania, we tried to bring in the attention of the alfalfa growers, the main characteristics of the current certified Romanian and foreign varieties and thus providing them the possibility to choose the right variety depending on the particularities and objectives of their farm. This study can be used as a benchmark for the performances of the tested varieties.

Based on the results, the Romanian varieties presented very good ecological plasticity with drought and frost resistance combined with high biological efficiencies. Dobrogea and Sandra are very productive varieties, while the new variety Valahia showed the highest forage quality. In the eco-climatic conditions of South Romania, PR54V09 and PR55V48 have been the most high-yielding varieties from the foreign group of cultivars.

In the next period, we will continue to analyze the behavior of certain alfalfa varieties in various farm conditions by testing a selection of varieties in production fields and we will return with updated information to help the farmers in taking the substantiated decisions in choosing the proper alfalfa variety for their needs (https://samantalucerna.ro/).

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RESEARCH ON THE INFLUENCE OF LEAF AREA ON SUNFLOWER YIELD CULTIVATED ON CARACAL CHERNOZEM IN THE PERIOD 2018-2020

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Abstract

At ARDS Caracal, on chernozem type soil, for three years (2018-2020), 9 sunflower hybrids were tested to study the influence of leaf area on yield in the conditions in which the sowing was done at 3 different plant densities. The determination of the leaf area was done at 3 different moments spaced two weeks apart in June and July. The leaf area was determined on all the leaves on the plant, on 3 plants on each replication, using the formula of Rouphael et al. (2007). The correlation coefficients between the leaf area and yield were differentiated as direction depending on the year of experimentation being significantly positive in 2019 and significantly negative in 2018, given that the correlation was made between 27 values (9 hybrids x 3 densities) for each determination. When correlated, a percentage of 16-20% of leaf area variability was associated with yield variability. Although normally the leaf area is positively correlated with production, the results obtained in this experiment are supported by the fact that the production is higher at medium plant density but the leaf area is higher at low plant density. This aspect is highlighted by the leaf area / yield ratio which shows which leaf area of a plant contributes to the formation of one kilo of sunflower seeds. The highest values of this indicator (over 2 cm^2 / plant per kilo of seed) are found at the lowest density. The results suggest that the relationship between leaf area and yield at sunflower is influenced by climatic and technological factors and a large leaf area does not always lead to high yield.

Key words: *sunflower, chernozem, leaf area, correlation, ratio leaf area/yield.*

INTRODUCTION

The objectives of improving the sunflower are improve the productivity, by increasing the seed production and the oil content; genetic resistance to diseases and pests and increasing the level of ecological plasticity through adaptability to various environmental conditions (Bonciu, 2019). The leaf area is an important indicator of plant growth, being related to the accumulation of dry matter, perspiration, photosynthetic capacity. Currently, the aim is to obtain hybrids with 25-30 leaves, with large limb and grafted to increase the leaf surface index (ISF) to over 4, achieving an architecture of the leaf apparatus with a larger photosynthetic active surface. The largest foliar area of sunflower assimilation occurs during flowering, in late June - early July. The leaves have the property to withstand well the phenomenon of temporary wilting, determined by the insufficiency of water in the soil, returning to normal turgor when moisture completes. Partial or total defoliation causes a decrease in yield. If 12 leaves remain at the top of the stem, the negative influence on yield is reduced (Johnson & Tanner, 1972). The leaves and roots of the plants form the vegetative system that through specific growth processes puts in action the root and foliar sensors. The development of the leaf surface is of major importance on photosynthesis and transpiration. The total leaf surface area per plant is determined as a logistic function of the anthesis and the thermal time from sunrise, while the distribution of the solar radiation at the leaf level is determined by the ISF, the angular distribution of the leaves (Bonciu et al., 2020b). Genotype is one of the most dynamic indicators for sunflower cytogenetic research. This comes in support of genetic research for sustainable management of the agricultural system in general and sunflower crops in special. An intense mitotic activity is directly correlated

with plant growth, development and fructification at optimal parameters. However, in addition to intense mitotic activity, environmental factors also play a decisive role (Bonciu et al., 2020).
Studies on the hybrid x density x moment of determination interaction in sunflower, conducted by Dobre and Marin in 2020, showed that to the first determination, in the other three, the leaf area showed very significant increases in all densities and all hybrids. At medium and high densities, only Neoma and FD15C27 hybrids had fast leaf surface growths. The leaf area was lush at low density in Neoma and FD116M1 hybrids while Euromis and Performer hybrids showed the same leaf area in both densities: low and medium. In Performer, the leaf areas at the first two seed density and at the first 3 moments of determination are equal. The FD15C27 hybrid is the only one in which the foliar development is much reduced indifferent of the seed density, it being around 6000 cm^2.
In sunflower, experiments on morphophysiological changes due to water stress (Pârjol, 1974) showed that the sharp reduction of the assimilating surface accompanied by slowing down the growth of the root system causes a substantial decrease in seed production. Felix and Select hybrids showed better drought resistance compared to other hybrids in culture (Ţerbea, 1996). Simultaneously with the reduction of the leaf area, the number of leaves also decreased. This response can be considered as a common reaction of sunflower plants in order to limit water consumption in drought conditions (Petcu et al., 2001a, 2001b).

MATERIALS AND METHODS

At ARDS Caracal, on chernozem type soil, for three years (2018, 2019 and 2020), 9 sunflower hybrids were tested to study the correlation between yield and leaf area of three moment of leaf area determination (in 2018 at 08.06, 20.06 and 3.07; in 2019 at 18.06, 28.06 and 12.07; in 2020 at 20.06; 03.07 and 14.07). Data was collected from three densities: 43000 plants/ha, 57000 plants/ha and 71000 plants/ha).
The hybrids tested were: Euromis (1), Generalis (2), Terramis (3), Neoma (4), Diamantis (5), Subaro (6), Performer (7), FD15C27 (8), FD116M1 (9).
The experiment was placed in randomized blocks, in 3 replications. The length of the plot was 10 m and the width - 2.8 m, the equivalent of 4 rows from which the marginal ones are removed.
The leaf area was determined on all the leaves on the plant, on 3 plants on each replication, using Rouphael formula: $LA = 6.72+0.65*w^2$ - where LA = leaf area, w = width of the leaf (Rouphael et. al., 2007).
The yield was determined by harvesting the two middle rows of the 10 m long plot (harvestable area = 14 m^2), the seeds were weighed, the humidity was determined and the yield was calculated to the STAS humidity of 9%.
The correlation was made between 27 values (9 hybrids x 3 densities) for each determination. Only significant correlations were presented.
The ratio between the leaf area and the obtained yield (represents which leaf area contributes to the formation of one kg of sunflower seeds) was also calculated.

RESULTS AND DISCUSSIONS

The correlation coefficients between yield and leaf area / plant, on the one hand, and leaf area / one single leaf, on the other hand, for each year and each moment of determination highlighted its different meanings: negative in 2018 and 2020; positive in 2019 (Table 1). These differences were due to different climatic conditions from one year to another for the period of sunflower vegetation. The rainfall regime of 2018 favored the development of sunflower culture and obtaining high yields. Both at sowing and in the essential phases for development (May, June, July) there was a excess of precipitation, almost double compared to the multiannual normal for the last 30 years. The rainfall regime of 2019 partially favored the development of sunflower culture, obtaining normal productions. At sowing and then the following month, sufficient rainfall was provided for a good emergence of the crop. In the essential phases for development (May, June, July) there was a excess of precipitation, almost double compared to the multiannual normal for the last 30 years, but in August and

September, the lack of precipitation was acute. The precipitation that fell in 2020 during the vegetation period of the sunflower (April-September) - 275 mm, was the lowest of the three years experienced. Yields, as well as the leaf area, were diminished compared to the other two years, and the correlations were not present at any time of the determination.
Significance was the correlations that showed values of correlation coefficients above 0.380 at P <5% and over 0.490 for P <1%.

Table 1. Coefficients of correlation between leaf area and yield, 2018-2020 period for three time of determination

Year	first determ.		second determ.		third determ.	
	leaf area/ pl	leaf area/ single leaf	leaf area/ pl	leaf area/ single leaf	leaf area/ pl	leaf area/ single leaf
2018	-0.338	-0.155	-0.417	-0.407	-0.382	-0.438
2019	0.456	0.419	0.401	0.517	0.439	0.448
2020	-0.318	-0.325	-0.302	-0.402	-0.210	-0.365

The variability of the leaf area, in the climatic conditions of 2018, is associated with 17% of the variability of yield, in a negative sense. The increase of the leaf area by 1000 cm^2 over 3767 cm^2 leads to the decrease of the yield by 96 kg/ha, at the end of the second decade of June (Figure 1).

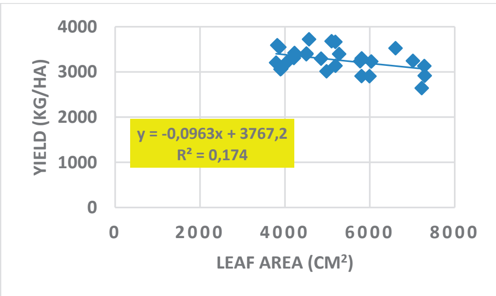

Figure 1. Relationship of leaf area with yield in 2018, second time of determination (20.06.2018)

In 2018, in the third moment of the determination, the variability of the leaf area is associated with 14% of the variability of yield, in a negative sense. The increase of the leaf area by 1000 cm^2 over 3696 cm^2 leads to the decrease of the yield by 62 kg / ha, at the end of the second decade of June (Figure 2).

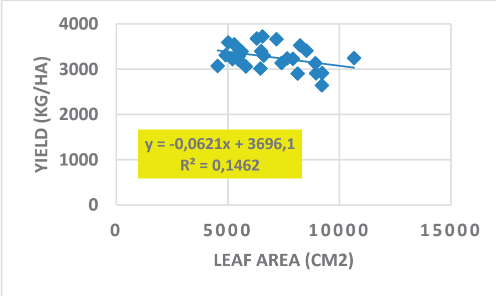

Figure 2. Relationship of leaf area with yield in 2018, third time of determination (3.07.2018)

In 2019, at the first determination, the variability of the leaf area is associated with 16% of the variability of yield, in a positive sense. The increase of the leaf area by 1000 cm^2 over 3200 cm^2 leads to the increase of the production by 82 kg / ha, at the end of the second decade of June (Figure 3).

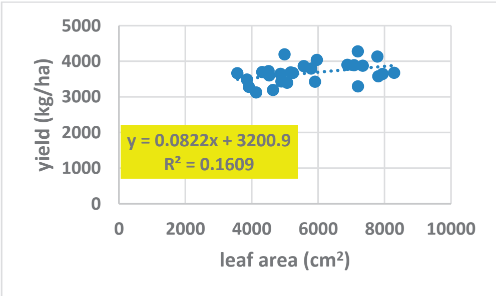

Figure 3. Relationship of leaf area with yield in 2019, first time of determination (18.06.2019)

The variability of the leaf area, in the climatic conditions of 2019, is associated with 21% of the variability of yield, in a positive sense. The increase of the leaf area by 1000 cm^2 over 3104 cm^2 leads to the increase of the yield by 78 kg/ha, at the end of June, for the studied interval (Figure 4).
The variability of the leaf area, in the climatic conditions of 2019, is associated with 19% of the variability of yield, in a positive sense. The increase of the leaf area by 1000 cm^2 over 3114 cm^2 leads to the increase of the yield by 86.5 kg/ha, in the middle of June, for the studied interval (Figure 5).

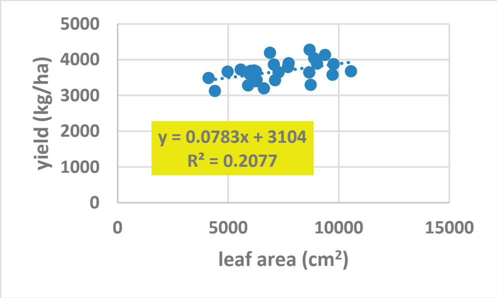

Figure 4. Relationship of leaf area with yield in 2019, second time of determination (28.06.2019)

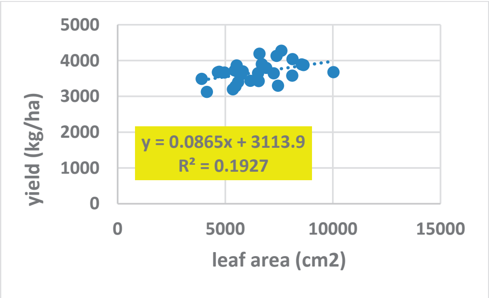

Figure 5. Relationship of leaf area with yield in 2019, third time of determination (12.07.2019)

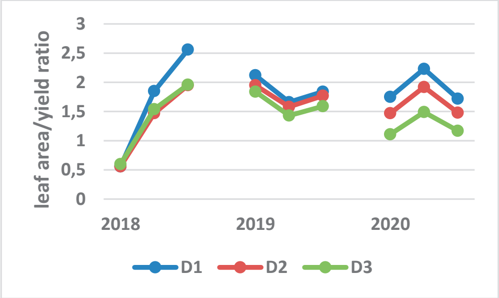

Figure 6. Leaf area / yield ratio (suprafața foliară ce contribuie la formarea unui kg de sământă) in 2018-2020 period at three densities and three moments of determination

The graphical representation of the leaf area/yield ratio highlighted three different types from one year to another, depending on the density. In 2018, when the total precipitation during the vegetation period of the sunflower was 385.6 mm, the leaf area increased more accentuated at the first density and gradually from one moment of determination to another.

In 2019 (total rainfall during the vegetation period - 462.2 mm but irregular distributed, with 63 mm in July, August and September), the leaf area has a lower growth rate at the second determination which is located at the end of the June.

The year 2020, which was the poorest in precipitation - 275 mm during the vegetation period, presented another type of development of the leaf area. The increase was more pronounced from the first determination to the second but then experienced a decrease due to premature drying of the foliar apparatus due to the manifested drought, to the third determination (mid-July) (figure 6).

The results suggest that the relationship between leaf area and yield at sunflower is influenced very strong by climatic conditions. De asemenea

When correlated leaf area and yield, a percentage of 16-20% of leaf area variability was associated with yield variability.

CONCLUSIONS

The correlation coefficients between the leaf area and yield were differentiated as direction depending on the year of experimentation being significantly positive in 2019 and significantly negative in 2018, given that the correlation was made between 27 values (9 hybrids x 3 densities) for each determination. When correlated, a percentage of 16-20% of leaf area variability was associated with yield variability. Although normally the leaf area is positively correlated with production, the results obtained in this experiment are supported by the fact that the production is higher at medium plant density but the leaf area is higher at low plant density. This aspect is highlighted by the leaf area/yield ratio which shows which leaf area of a plant contributes to the formation of one kilo of sunflower seeds. The highest values of this indicator (over 2 cm^2/plant per kilo of seed) are found at the lowest density. The results suggest that the relationship between leaf area and yield at sunflower is influenced by climatic and technological factors and a large leaf area does not always lead to high yield.

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ESTIMATION OF MAIZE YIELDS IN THE BARAGAN PLAIN (ROMANIA) - A SPATIALLY EXPLICIT APPLICATION OF A CROP GROWTH MODEL

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Abstract

Crop growth models are useful tools for in-depth analyses on agricultural productivity and resource (land and water) use under various environmental and management conditions, rendering solution-oriented information for end-users. The paper showcases a spatially explicit application of a crop growth model for simulating the cultivation of a commonly used maize cultivar in the Baragan Plain, South-East Romania. We have parameterized the EPIC⁺ model (Williams et al., 1989; Kamali et al., 2018) with geographical, agricultural practices and experimental-based crop inputs, and have designed two scenarios for crop parameter calibration and improved nutrient and irrigation application, respectively. These methodological and conceptional steps enabled model performance assessment and maize yields estimation in the study area. With adjustments of sensitive crop parameters and agricultural practices in the model setup, crop growth is particularly constrained by stress factors (e.g. nutrient stress), and potentially by model structure parameters, which also need to be calibrated. It was achieved a good agreement between areal averaged estimated yields and reported yields, suggesting that the model is suitable for further regional investigations and for supporting decisions on agricultural resources management.

Key words*: agricultural modeling, plant stress, model parametrization, EPIC⁺, SE Romania.*

INTRODUCTION

Crop growth simulation models are increasingly used for assessing a wide range of environmental problems, such as soil and water resources quality and availability, climate change impacts on crop production or soil carbon sequestration. They basically allow for designing virtual experiments to study, in a systematic and integrated way, the complex and interdependent biophysical effects of atmosphere and soil processes on crop growth and formation (Minoli et al., 2019). Coupled with a geographic information system, crop growth models have been applied particularly at regional and global-scale levels to estimate present and future crop yields, agricultural water productivity, nitrogen losses, crop vulnerability to drought, etc. under various environmental conditions and agricultural practices (Liu at al., 2007; Liu, 2009; Folberth, et al., 2012, 2016; Mauser et al., 2009; Liu et al., 2016). Termed as global gridded crop models (Rosenzweig et al., 2014) given the geospatial character of the utilized datasets, they provide evidence-based information, comprehensive overviews and comparisons among regions, being useful tools in decision making processes concerning, for instance, nature-based solutions for climate mitigation and adaptation (Balkovic et al., 2018), land and water management in agriculture (Flach et al., 2020), or sustainable agricultural productivity (Mauser et al., 2015).

The current challenging trajectories of both societal development and consumption, and climate change put a stress on water resources use. It becomes clear that sustainable management of agricultural water resources is a priority supported by deep understanding of the processes at the interface of atmosphere-soil-plant system and by evidence-based estimates of yield production and water consumption. In Romania, such investigations are particularly useful considering the new development strategies in agriculture, according to which the irrigated area is planned to increase from 0.5 million ha to almost 2 million hectares by 2020, in an ambitious objective which aims to boost agricultural activities and production, contributing also to climate change adaptation and rural communities' resilience (MADR, 2016).

In this context, the aim of this paper is to test the application and performance of a crop growth model for a pilot area in the Romanian Plain in order to analyse the effect of increasing irrigation capacities on crop production and to exemplify the utility and relevance of crop models in studies on agricultural resource management. To this end, we applied an extended version of the EPIC (Environmental Productivity Integrated Climate) crop growth model (Williams et al., 1989) which is equipped with a module for automatic calibration and parameter uncertainty assessment (Kamali et al., 2018).

The pilot area chosen for this study is the Baragan Plain, a representative agricultural region located in SE Romania (Figure 1). The area is a geographical unit of the Romanian Plain characterised by relatively homogeneous features in terms of relief, climate and soil type coverage. Generally, the soils are nutrient rich soils of the Chernozems types with good water retention capacity, while the climate is temperate-continental with increasing dry spells which intensify the already existing conditions of regional drought.

Figure 1. Cropland in the Baragan Plain, SE Romania; geospatial dataset: Dogaru & Kucsicsa, 2015

MATERIALS AND METHODS

Brief information on the crop model

EPIC⁺ is a gridded crop model that has been created by Kamali et al., 2018 as a Python-based spatial framework for the application and calibration of the field-scale, bio-physical EPIC (Environmental Productivity Integrated Climate) model. EPIC was developed by Williams et al., 1989 and constantly improved to accurately simulate process-based interactions at the interface of soil-crop-atmosphere system under various environments (Gassman et al., 2005; Izauralde et al., 2006).

In EPIC, potential crop growth is calculated daily based on intercepted photosynthetically active radiation and conversion of CO_2 to biomass (Stockle et al., 1992; Williams et al., 1989). The model simulates plant growth, yield and soil dynamics on a daily time step using a set of empirically based algorithms and climate, soil types and properties and crop management input data (Williams et al., 1989). EPIC first estimates potential plant growth and then reduces it according to the limitation due to the most dominant stresses (i.e. N and P deficit, water, temperature, aeration, salinity) by a factor between 0 and 1.

Yield is estimated using an actual harvest index (HI), which is calculated by the model within the range of a defined potential HI and a minimum HI depending on water stress. Potential evapotranspiration (ET) is calculated using the Hargreaves method (Hargreaves and Samani, 1985) and actual ET according to Ritchie (1972).

The EPIC⁺ model runs EPIC in each grid cell at a user defined resolution (Kamali et al., 2018) and implements the newest version of it, (i.e. EPICv.0810). It is coupled with the SUFI-2 automatic calibration module of SWAT (Soil and Water Assessment Tool) model, accounting for parameter uncertainty from all sources (e.g., inputs, crop parameters, and model structure) (Abbaspour, 2015). Uncertainties are expressed by distributions associated to crop growth and model structure parameters. Latin hypercube method is applied to sample the parameters, while the output uncertainty is quantified as the 95% prediction uncertainty band (95PPU) calculated at the 2.5% and 97.5% levels of the cumulative distribution function of the output variables (Abbaspour, et al., 2007). Detailed information on EPIC⁺ application and model automatic calibration is found in Kamali et al. (2018).

Model set up

Input datasets

EPIC model operates with both detailed process-based parameters of atmosphere - soil - plant interactions and comprehensive input datasets on: 1) location (longitude, latitude, elevation and slope), 2) climate, 3) soil types and properties, 4) land use, 5) cropland management such as irrigation and fertilization application, and 6) crop specific parameters. In this study all geospatial datasets were rasterized where applicable and harmonized at 1 km resolution grid cell.

DEM data were obtained from the 3 arc-seconds (approx. 90m resolution) digital elevation model of the NASA Shuttle Radar Topographic Mission provided by CGIAR-CSI GeoPortal (Consortium for Spatial Information, 2018). Terrain slopes were subsequently derived from the DEM raster based on the maximum change in the elevations between each cell and its eight neighbours.

ROCADA gridded climatic data was downloaded from PANGAEA data portal to assimilate in the crop model the daily parameters including: min. temperature, max. temperature, precipitation, and solar radiation. This dataset is based on daily observations recorded at all meteorological stations in Romania covering 1961-2013 time interval (Birsan & Dumitrescu, 2014).

The geospatial dataset of harvested area for maize was created on a 30´´x30´´ latitude-longitude grid (~ 1km) by combining locality-level reported data on land use and crop-specific harvested area from the National Institute of Statistics with CORINE Land Cover 2006 (CLC) raster data provided by European Environmental Agency (EEA, 2016). Once we re-classified the CLC maps for cropland, pasture and non-cropland areas, we calculated the proportion of cropland in each 1km grid cell (Dogaru & Kucsicsa, 2015) by applying the methodology developed by Ramankkuty, et al. (2008) for creating global geospatial datasets of cropland distribution on the basis of high-resolution satellite derived land cover data, calibrated against agricultural census data. Further on we followed the steps proposed by Monfreda, et al. (2008) to first determine the ratio of the crop area to the total cropland in each locality and then to multiply it with the proportion of cropland in each 1km grid cell for the associated locality of that grid cell. The result ultimately represents the proportion of the specific crop in each grid cell. Since the crop-specific harvested area from the National Institute of Statistics at locality level is available until 2003, the final dataset of maize harvested area was averaged around the year 2002, being though in a relatively close time scale correspondence with the CLC 2006 dataset used in this study.

Soil types and their physical-chemical characteristics were provided by the National Research and Development Institute for Soil Science, Agrochemistry and Environment – ICPA Bucharest, Romania. The following soil parameters were used to create the soil files used in EPIC: depth, percentage of silt and sand, bulk density, pH, organic carbon content, fraction of calcium carbonate, cationic exchange capacity, electrical conductivity, mobile N and mobile P. The files of soil properties were linked with the digital Soil Map of Romania existing at the scale of 1:200 000.

Fertilizer application rates for N and P on cultivated areas treated with fertilizer were obtained from the National Institute of Statistics. These data are reported as annual amounts of applied fertilizer per ha at county level, without specifying the crop for which it is applied. Given a number of socioeconomic reasons, farmers opted for rather low fertilizer inputs, at least during the last decades (Popescu, 2013). For instance, during the 2000s the average rate of N fertilization was of 85 kg / ha in Călărași county and of 82 kg / ha in Ialomița county, respectively. Here, we used a multiannual mean of N and P allocation for cultivated areas, for the entire simulation period. In spite of its coarseness, we consider that the usage of fertilizer aggregated data is a practical compromise.

The spatial dataset representing the area equipped for irrigation resulted from digitizing a publicly available map of irrigation infrastructure issued in the framework of the Irrigation Strategy in Romania (MADR, 2011). Yearly irrigated areas in each county were downloaded from the online data portal of the National Institute of Statistics (http://statistici.insse.ro:8077/tempo-online/).

To account for the areal effect of irrigation in the simulation outcomes, the irrigated and rain fed yields were weighted in each grid cell as follows:

- the irrigated and rain-fed areas were simulated separately in each grid cell
- then, the weighted yield was calculated on a grid cell basis for each year (eq. 1):

$$Y_w^{j,c} = Y_I^{j,c} \times A_{Ip}^{j} + Y_R^{j,c} \times \left(1 - A_{Ip}^{j}\right) \quad \text{(eq. 1)},$$

where $Y_w^{j,c}$ [t ha^{-1}] is the average (weighted) yield in the grid cell *c* in the county *j*, $Y_I^{j,c}$ [t ha^{-1}] is the yield on irrigated cropland in the grid cell *c* in the county *j*, A_{Ip}^{j} [.] is the percentage of irrigated area in the county *j* applied for each irrigated grid cell found in the county *j*, and $Y_R^{j,c}$ [t ha^{-1}] is the yield on rain-fed cropland in the grid cell *c* in the county *j*.

Potential heat units for F376 maize cultivar

Phenologic development of the crop depends on the number of heat units (or growing degrees days) from planting to maturity. The heat units (HU) are calculated for each day (k) according to a certain base temperature, which is crop-specific and above which the plant starts to grow, min. temperature and max. temperature in that day (eq. 2). We run the EPIC model for a maize cultivar with medium duration until maturity (i.e. F376) which is widely cultivated in the southern part of the country, including the Bărăgan Plain. To have a general, representative value of the potential heat units (PHU) for the F376 maize cultivar we used the planting and maturity dates of this cultivar obtained in the amelioration experimental fields at INCDA Fundulea during 2007-2013, and the ROCADA climatic variables in several random locations throughout the study area. Consequently, the PHU was determined as an average of the accumulated HU from planting to maturity during 2007–2013 and throughout the considered locations.

$$HU_k = \frac{T_{max,k} + T_{min,k}}{2} - T_b \quad \text{eq. (2)}$$

Crop management, simulation run-time and evaluation of model performance

Maize was assumed to be planted after fertilizer application, top-layer plowing and field preparation. In EPIC harvest is programmed to occur at 115% of the calculated PHU fraction (i.e. the default assumption), taking into account the post-maturity drying of the crop on the field. Additionally, we considered no residual biomass removal after harvest as in many places farmers incorparate it into the soil for maintaining or increasing its quality. Automatic N and P fertilizer and irrigation options were set up in the model. They are based on the plant threshold factors (e.g. if N stress exceeded 20% on a giving day, N is added up to the maximum amount of N fertilizer application rate specified here by the county-level multiannual average during the simulation period).

According to the availability of the time series of input data, especially in what regards climate and management opperations (i.e. fertilization and irrigation), the simulation period was 1997-2013.

The simulated yields were automatically calibrated against the county-level reported yields for the same period of time, using the SUFI-2 module (Kamali et al., 2018). The coefficient of determination (R^2) and the standardized root mean square error (RSR) are the two statistics that evaluated the comparison between the reported and the simulated yields (i.e. model efficiency). R^2 expresses the linear correspondence between the two variables, with 1 being the optimal result, while RSR measures the difference between the reported and the simulated yields and takes values from 0 to ∞, where 0 is the best value. During the calibration process new ranges or values of crop / model parameters are calculated in a user-defined number of iterations, forming the parameters sensitivities. These are determined on a basis of a multiple regression system where the parameters are regressed against the objective function which is considered the RSR value. Basically the sensitivities express the changes in the objective function resulting from changes in each parameter while all other parameters are changing. In this we chose study 5 influential parameters (i.e. PHU, plant density, N and P fertilization rates and irrigation volume applied) over 3 iterations to perform the sensitivity analysis and calibrate the model for the entire study area (Table 2). Besides, to have a better sense on model robustness, we performed model calibration and validation on rain-fed yields at a finer spatial scale, specifically in 4 localities in the study area for which reported data were available (Figure 3). For this latter approach we took into account the same influential parameters, but ran 10 to 50 iterations to minimize parameter interaction and then fixed the parameters to their best values (i.e. the best simulation resulting from the values that produced the smallest objective function). However, studies highlight a larger range of both model and crop parameters that are sensitive and worth being calibrated in order to obtain reliable results (Liu, 2009; Gaiser et al., 2010, Folberth et al., 2012). Given the large computational time needed for simulation (calibration), we opted here to test the model for a pilot area in order to showcase its relevance for further analyses which in most cases require adequate infrastructure as well as collaborative research frameworks for implementation.

Scenario design

To highlight the $EPIC^+$ model applicability in regional studies on cropping systems productivity, we envisaged two cases of crop management conditions which are consistent with the new development plans in the irrigation sector in Romania, specifically the rehabilitation of the primary irrigation infrastructure with the purpose of increasing the irrigated area (MADR, 2016). These cases are:

- default parameters with no irrigation increased capacities (default simulations),
- calibrated crop parameters with no increased irrigation capacities (baseline scenario). In this case the fertilization rates were as described above, while of irrigation water supply was set at 1000 mm per year per maize cultivated area, being in line with many reported values by farms in the agricultural areas where irrigation was available.
- increased irrigation capacities and improved fertilization rates (improved crop management scenario). Here, the irrigated area was enhanced according to MADR, 2016 irrigation plans, while the levels of fertilization were increased to lower plant growth nutrient limitations at values of 200 kg / ha. We assumed sufficient irrigation water supply in grid cells that are equipped for irrigation.

RESULTS AND DISCUSSIONS

The comparison between maize yield estimates resulted from simulations run with model default parameters and the reported yields shows promising results for conducting this study. Aggregating the simulated yields at county level we obtained a good agreement between the observed and the estimated yields (Figure 2).

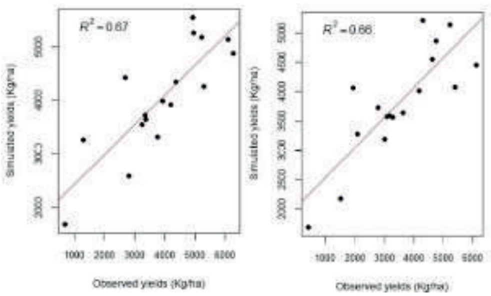

Figure 2. Reported yields and estimated yields simulated by default runs for the reference period (1997 – 2013) for Călărași County (left) and Ialomița County (right). Each point in the graph represents a year and the model fit is expressed through r squared adjusted (R^2)

However, the model overestimates the simulated yields in the years recognized as drought years of the first decade of the 21st century (Mateescu et al., 2013) (Table 1).

Moreover, aggregated measures can mask the variability in both time and space of yield trends, increasing the uncertainty level in the model outcomes and indicating the necessity for calibration and uncertainty measuring.

Table 1. Annual average of observed yields (Y_{obs}) and simulated yields with model default parameters (Y_{sim_d})

Year	Calarasi County			Ialomita County		
	Y_{obs} kg ha^{-1}	Y_{sim_d} kg ha^{-1}	Y_{dif} %	Y_{obs} kg ha^{-1}	Y_{sim_d} kg ha^{-1}	Y_{dif} %
1997	5218	5182	-0.7	5216	5140	-1.5
1998	3379	3645	7.9	3084	3575	15.9
1999	4373	4338	-0.8	4182	4020	-3.9
2000	1307	3259	149.3	2078	3280	57.8
2001	2818	2593	-8.0	1514	2177	43.8
2002	2698	4424	64.0	1930	4065	110.6
2003	3349	3721	11.1	2783	3727	33.9
2004	6111	5142	-15.9	4742	4866	2.6
2005	4941	5540	12.1	4288	5211	21.5
2006	4189	3924	-6.3	3619	3645	0.7
2007	696	1682	141.6	425	1683	295.9
2008	3763	3321	-11.7	2991	3185	6.5
2009	3923	3980	1.4	3152	3590	13.9
2010	4957	5263	6.2	4623	4550	-1.6
2011	5290	4264	-19.4	5382	4075	-24.3
2012	3258	3547	8.9	3265	3561	9.1
2013	6274	4879	-22.2	6113	4452	-27.2

In the process of calibration we adjusted the following crop parameters: PHU, plant density, N and P fertilization rates and irrigation volume applied. This led to an increase of the R2 of the county-base time series yields from 0.67 to 0.70 for Calarasi County and from 0.66 to 0.69 for Ialomita County, respectively.

Nevertheless, we could not identify a distinct pattern of reduction of the difference between the estimated yields and the reported yields in the drought years, suggesting that the model is very much sensitive to water stress conditions and that thorough investigations on model behaviour in water limited environments are highly recommended. Moreover, the sensitivity analysis indicates that the estimated yields are considerably constrained by nutrient stress factors, especially by N fertilization, temperature, as shown by increased PHU values, and water supply (Table 2).

Table 2. Sensitivity analysis on several influential parameters of crop growth and yield development

Crop growth influential parameters	Initial parameters (first iteration)	Adjusted parameters (three number of runs)	Parameter variation unit (from initial value)
PHU			
(accumulated number of heat units)	1640	2017	+0.23
Plant density			
(plants / m^2)	5	4	-0.23
Applied irrigation volume			
(mm / year)	1000	1200	+0.29
Nitrogen application amount			
(mean annual amount in kg / ha)	85 / 82[#]	101 /104[#]	+0.23
Phosphorous application amount			
(mean annual amount in kg / ha)	63 / 57[#]	63 / 57[#]	–

[#]The values represent the multiannual mean of N and P application rates on maize cultivation areas in Călărași and, Ialomița county, respectively.

The spatial distribution of the estimated yields under the calibrated crop growth parameters (i.e. baseline scenario) highlights the strong effects of drought conditions on crop production (Figure 4), showing, for instance, that yields in 2007 were below 2 t / ha in many parts of the study area.

Parameter adjustment at locality level further underpins the need to perform the calibration process on both crop and model structure parameters. In this respect, we considered 4 localities in the study area where we knew from our field work experience that irrigation had not been applied particularly since agricultural privatization reforms have been enforced (Figure 3).

Hence, all cropland in the calibration at locality level analysis was treated as rain-fed in order to reduce any possible bias by additional water supply given irrigation application.

Previous studies found that simulated maize yield was sensitive to several input and model parameters, such as: planting date, PHU, HI (harvest index, i.e. the ratio of grain to total crop biomass under ideal growing conditions), WSYF (the lower limit of HI due to water stress), PARM03 (the fraction of maturity when water stress starts reducing the harvest index) and PARM42 (indicator that affects runoff and thus soil water and evapotranspiration) (Liu, 2009; Wang et al., 2012; Folberth et al., 2012).

Figure 3. Selected sites for validation (LAU level) in the Bărăgan Plain

Therefore, our approach for calibration at locality level consisted in a systematic calibration implemented in a step-wise manner on management input data (fertilization and planting density), crop phenology and cultivar properties (PHU, planting date, HI and WSYF) and model parameters (PARM03 and PARM42).

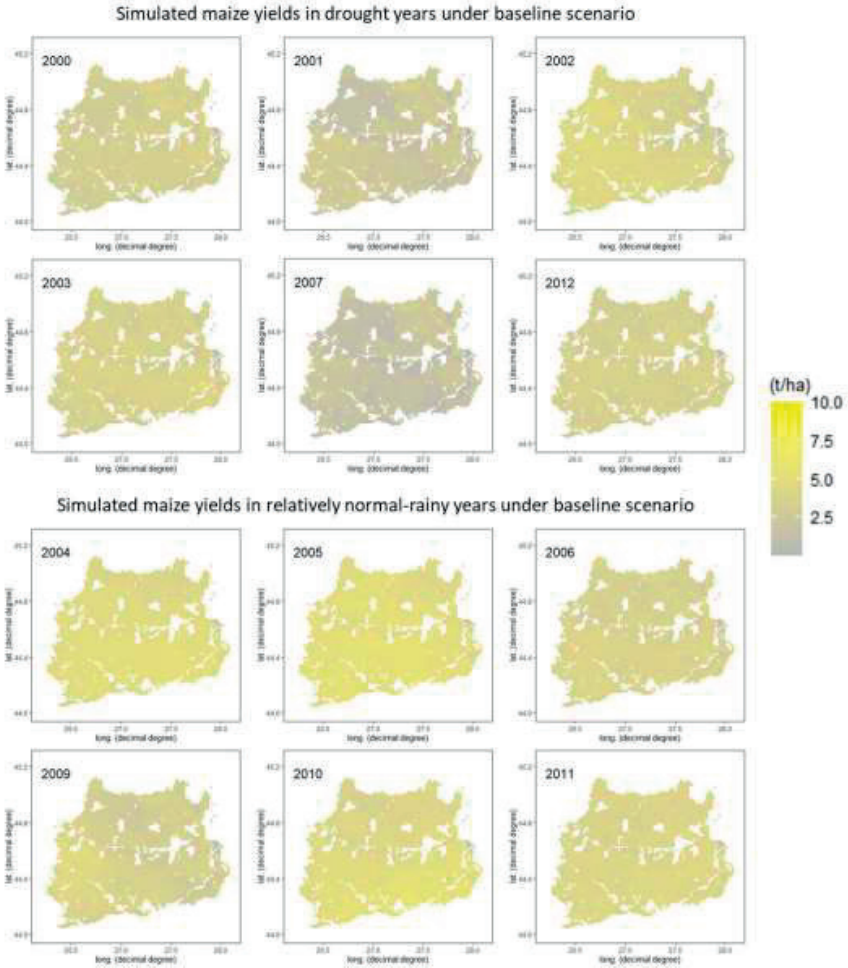

Figure 4. Simulated maize yields in the Bărăgan Plain during the drought and relatively normal or rainy years of the 2000-2013 interval, based on model parameterization for actual crop management conditions (baseline scenario)

The results showed a realistic improvement in model performance statistics, especially in the case of Budesti and Stelnica localities, with considerable decrease in the percentage of bias (PBIAS) between simulated and observed yields in all 4 cases during the calibration process (Figure 5). Enforcing parameter calibration first on 50 (first step), then on 25 (second step) and lastly on 10 (third step) simulation iterations, it clearly led to a reduction in the differences between the simulated and reported yields by producing smaller objective functions and improving model performance statistics (e.g. adjusted R^2 reached in the end values between 72% and 96%) (Figure 5).

The improved crop management scenario shows, as expected, that higher nutrient and water application rates resulted in consistently higher simulated crop yields (Figure 6). The averaged crop yield values for drought and relatively normal-rainy years reach to 4 t / ha and 7 t / ha, respectively, while highest maize yields are closer to 10 t / ha. Moreover, minimizing water stress through increased irrigation it reduces the yield variation throughout the study area, especially in area equipped for irrigation (i.e. the coefficient of variation is much lower for the yields estimated during the normal-rainy years, 0.2, as compared to those estimated during drought years, 0.6).

The results obtained in this scenario support future analyses for examining which crops and agricultural areas could benefit more from improved fertilization levels and additional blue water (irrigation water), as well as where blue water would account more of a larger share of the total water demand. Such approaches are relevant for analyses regards water use efficiencies in the context of drought intensification and water demand increases in all economic sectors.

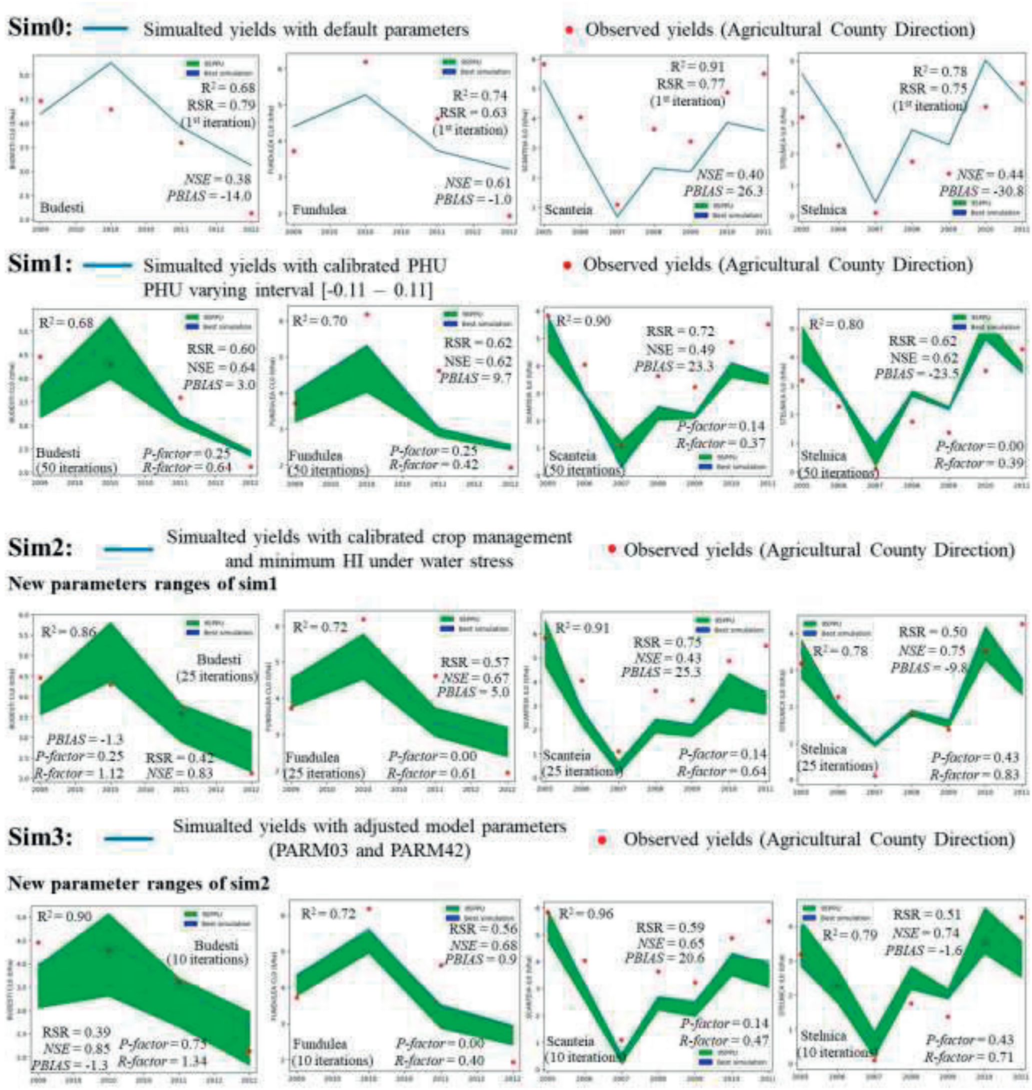

Figure 5. Comparison between the reported yields and the simulated yields expressed by the 95PPU prediction uncertainty band and the best simulation obtained after a number of iterations evaluated through RSR criteria in four localities in the Bărăgan Plain. Two statistics, i.e. r-factor and p-factor, show the goodness-of-fit and the model uncertainty. Top row displays the yields simulated with the first model iteration, while the bottom row shows the calibrated model results. The model performance was evaluated in each case by several statistical criteria, like Nash-Sutcliffe efficiency (NSE), R^2 (coefficient of determination) and percent bias (PBIAS). The reported yields used in this calibration exercise were available for 2009-2012 / 2005-2011 periods.

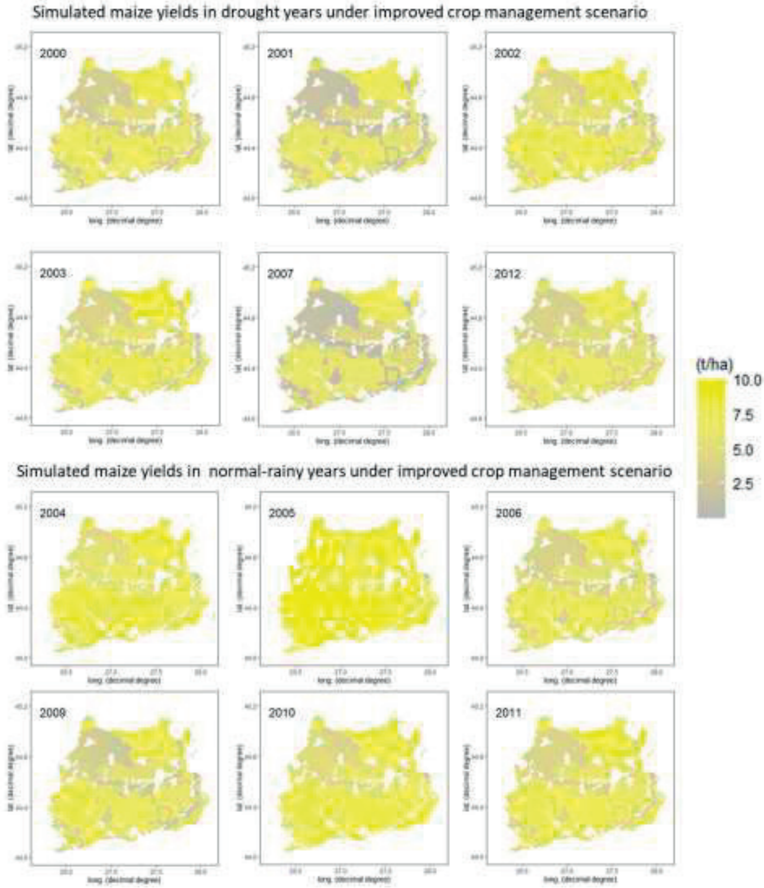

Figure 6. Simulated maize yields in the Bărăgan Plain during the drought and relatively normal or rainy years of the 2000-2013 interval, based on model parameterization for improved crop management conditions (improved crop management scenario)

CONCLUSIONS

EPIC$^+$ proved to be a promising tool for calibration of the EPIC model and thus for future assessments on various topics ranging from agricultural resources use in various environments to soil quality and impacts on agricultural production. Similarly, the model can be used at different spatial and time scales and offers the possibility to implement different crop management strategies. However, the model reliability greatly depends of fine scale input data as well as on long-term available yield records for validation (Wang, et al., 2012; Abbaspour, 2015; Kamali et al., 2018).

The sensitivity analysis on crop model parameters shows that PHU largely influences maize yields, suggesting a plus 23% change in the parameter´s space for model optimization process. This is particularly true because PHU sets the time scale (expressed in temperature rather than days), within which short PHU values give rapid early growth but less time to convert energy to biomass, thus highlighting the weather variables´ importance for crop yield (Liu, 2009). However, future studies could benefit from better estimation of PHU values using optimal crop calendars, more homogeneous units of simulation and longer climatic data ranges (Folberth et al., 2012; Flach et al., 2020). In this way it is possible to reduce the sources of uncertainty, especially in what regards model parameterization and to achieve higher consistency between PHU values and crop planting / harvesting dates.

In the calibration process, it was shown that with the adjustments of sensitive crop parameters and agricultural practices in the model setup, crop growth is, in many parts, mainly constrained by nutrient, temperature and water stress factors. Nevertheless, sensitivity analyses on parameters that influence, for instance, harvest index under water stress conditions or soil water are necessary. It is also recommended to consider soil input parameters into model calibration and validation procedures, given the influence of soil physical and chemical properties, especially through their water and nutrient storage capacities, on crop yield model simulations (Folberth et al., 2016).
Moreover, whenever detailed information is available, particularly in terms of observed data on yields and other crop growth parameters (e.g. actual evapotranspiration), automatic calibration procedures offer possibilities to simultaneously adjust EPIC input parameters on cropland management, phenology, yield and cultivar properties, as well as on model influential parameters for the respective cropping system. In this study we used SUFI2 module of $EPIC^+$ (Kamali et al., 2018) to perform a systematic calibration of EPIC crop model at locality level, choosing four localities as exemplary showcases. The results were expressed by objective function, uncertainty quantification and model performance criteria. Automatic calibration procedures applied at locality level led to considerable reduction of biases between the simulated and reported yields by producing smaller objective functions and improving model accuracy.
The application of the model requires suitable infrastructure given the large computational time needed for simulation runs and calibration, as well as collaborative research frameworks for its implementation.

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RESULTS REGARDING THE VALORIZATION OF WASTEWATER IN IRRIGATION OF GRAIN SORGHUM CULTIVATED ON SANDY SOILS

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Abstract

Studies conducted on sorghum cultivation, located in the conditions of sandy soils in southern Oltenia, followed the influence of fertigation with filtered waste water, with a content of 221 mg total nitrogen/liter compared to classical fertilization and irrigation with normal water from surface water sources. The results of the biometric determinations highlight differences depending on the fertilizer wastewater doses applied, on the plant height, stem diameter and leaf area. The production results obtained showed yields between 4167-7667 kg/ha, noting the variant fertilized with $N_{80}P_{80}K_{80}$, applied at preparation of the germination bed + N70, in vegetation, provided by fertigation with filtered waste water, applied fractionally in doses of 25% + 25% + 25 % + 25% (7667 kg/ha). The grain production obtained from sorghum grains in the different fertigation variants was positively correlated significantly with the weight of 1000 grains.

Key words: *fertigation-sorghum-treated water-stress resistance-productivity.*

INTRODUCTION

Climate change, unpredictable weather conditions and drought contribute significantly to the availability of freshwater resources. The growing demand for water and the discharge of a large amount of untreated wastewater is a major challenge for the management of water resources in an integrated manner.

Agriculture is a sector that consumes large amounts of water and can use lower quality water compared to the industrial sector or drinking water for human consumption (Ayer and Wescot, 1989). Direct reuses of untreated wastewater, as well as the use of freshwater resources polluted with agricultural wastewater, are very common in urban and peri-urban areas. Therefore, wastewater and biosolids are important resources that can help in the fight against water, food and energy crises (Lazarova and Bahri, 2004). Kretschmer et al., (2003) describe the advantages of using wastewater by: conserving freshwater resources, recharging aquifers, reducing the use of fertilizers.

The use of wastewater for irrigation depends largely on climatic conditions, physical properties of the soil, salt tolerance of the cultivated plant and management practices (Pescod, 1992). It is suggested to study and design the use of treated wastewater in agriculture, as part of the approach of a water circuit, which allows connecting different problems, different areas of some disciplines, in a logical and gradual framework (Huibers and Van Lier, 2005). The results obtained by Wang et al., 2002 showed that the use of sewage sludge increased the content of nitrogen, phosphorus and organic matter in the soil, thus being advantageous for the growth and development of crops. Wastewater contains the main plant nutrients (N, P, K) and also trace elements (Ahmed, 2009).

The results of the study conducted by Vijayasatya et al., (2019), showed that treated wastewater can be successfully used for the cultivation of bioenergetic sorghum in arid region. Sorghum is one of the crops with a high adaptability to unfavorable ecological conditions (poor soils, arid climate), due to the high capacity to efficiently capitalize on natural resources and increased drought tolerance (Matei, 2018, 2016, Draghici, 2019).

In the current climate arid conditions, the identification of agricultural practices with the potential to mitigate the impact of climate

change on the security of agricultural production is of increasing interest.
In this context, the orientation towards drought-resistant crops and finding an alternative to water scarcity was the objective of the present study.

MATERIALS AND METHODS

The study was carried out in the conditions of sandy soils in southern Oltenia, aiming at the influence of fertigation with an effluent obtained by wastewater treatment (treated water), compared to classical fertilization and irrigation with normal water from surface water sources. The treated water was obtained by the National Research-Development Institute for Environmental Protection, Bucharest, with the help of a wastewater treatment plant, within the complex project 27PCCDI / 2018, in order to use them for fertigation of energy crops. The experiment was performed in vegetation vessels, filled with sand with low natural fertility, with a low total nitrogen content (0.044%-0.085%) and a medium supply of extractable phosphorus (27-35 ppm) and exchangeable potassium (45-80 ppm). When filling the vessels with sand, it was fertilized with complex fertilizers type $N_{15}P_{15}K_{15}$, in a dose of $N_{80}P_{80}K_{80}$/ha (3.2 g for the vessel with an area of 0.06154 m^2).
The experiment was placed according to the method of randomized blocks, taking into account 6 experimental variants on irrigation of sorghum for grains with an effluent obtained by wastewater treatment, compared to irrigation with water from the street network, quality parameters being given in Table 1.
Irrigation with water from the street network was experimented on two variants of sorghum fertilization: $N_{80}P_{80}K_{80}$, $N_{150}P_{80}K_{80}$, the dose of N_{70} being applied in vegetation, in the phenophase of 5-7 leaves of the plant, in the form of ammonium nitrate, in a dose of 1.3 g vessel, representing 0.43078 g nitrogen active substance / vessel. Irrigation with effluent obtained by wastewater treatment, which had a total nitrogen content of 221 mg / l, was tested in 4 variants of application in vegetation of the dose of N_{70} (0.43078 g N / vessel), on the agrofound of $N_{80}P_{80}K_{80}$, used in the preparation of the germination bed.

Table 1. Water quality used to irrigate the sorghum crop

No.	Water quality indicators	Value of Quality Indicators	
		Treated water	Normal water
1	$N-NH_4$	189 mg/l	0.06 mg/l
2	$N-NO_2$	0.05 mg/l	0
3	$N-NO_3$	8.2 mg/l	0.61 mg/l
4	P total	0.9 mg/l	0
5	Al	3 mg/l	0
6	Ca	19.6 mg/l	73.4 mg/l
7	Mg	31.1 mg/l	44.5 mg/l
8	Na	226.1 mg/l	22 mg/l
9	HCO_3	1156 mg/l	400 mg/l
10	CO_3	72 mg/l	0
11	Chlorides	264 mg/l	25.5 mg/l
12	Conductivity	3.5 mS/cm	0.57 mS/cm
13	K	150 mg/l	0
14	CBO_5	100 mg/l	0
15	Sulphates	176 mg/l	30

There were 4 variants of achieving the dose of N_{70}, by using treated water, as follows: in 4 stages of 25% of the nitrogen dose, in 3 stages of 33% of the nitrogen dose, in 2 stages of 50 % of the nitrogen dose and in a single step, in a dose of 100%. The dose of 100% was applied in the phase of 5-7 leaves of the plant, and the other doses were applied weekly, until the flowering of the plant.
During the vegetation period of the plant, were performed: morphological, biometric, physiological and productivity determinations. From a physiological point of view, in the flowering phase of the plant, the following were analyzed: diurnal variation of photosynthesis and foliar perspiration, using the LC Pro+ device. Also determined were: water forms and dry matter from the leaves by gravimetric method, using the Venticell oven for drying the samples. At harvest, the following were determined: biomass production, grain yield in panicle, grain production and a thousand grains weight (TGW).
The obtained results were analyzed and interpreted by analysis of variance (ANOVA) and with the help of mathematical functions.

RESULTS AND DISCUSSIONS

The obtained results showed a differentiation of the growth rate of sorghum plants, depending on the fertigation variants applied. Thus, in the first two weeks after emergence, when the plants have developed up to 5-7 leaves, there was a uniform growth, with slightly higher values (1.1-1.2 cm / day) in the variant fertilized with $N_{80}P_{80}K_{80}$, when preparing the germination bed. In the period between the phenophase of 5-7 leaves / plant and the bellows phase of the panicle, the growth rate of the plant intensified, reaching values of 2.2-3.6 cm / day, with the maximum value in the variant fertilized with $N_{80}P_{80}K_{80}$, at preparation of the germination bed + N_{70}, applied fractionally in vegetation, by fertigation with treated water, in doses of 25% + 25% + 25% + 25%. In the period between the phenophase of the panicle bellows and the phenophase of the appearance of the panicle, the vegetative growth rate was reduced to 1.6-3.1 cm / day, during this period being more intense the process of generative development of the sorghum plant. The highest values of the growth rate were registered in the period between the exit of the panicle and the flowering phase of the panicle, the increases being between 5.2-6.4 cm / day, with the maximum value for the variant fertilized with $N_{80}P_{80}K_{80}$, at the preparation germination bed + N_{70} in vegetation, by fertigation with treated water, applied fractionally in doses of 25% + 25% + 25% + 25% (Figure 1).

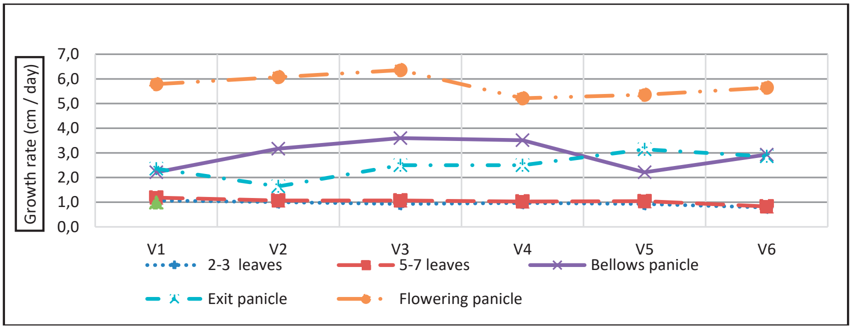

Figure 1. Influence of wastewater fertigation on sorghum plant growth rate

Biometric determinations on the growth and development of sorghum plants showed a plant height between 98.5 - 110 cm, the highest value being recorded for fertilization with $N_{80}P_{80}K_{80}$, for the preparation of the germination bed + N_{70}, in vegetation, by fertigation with treated water, applied fractionally in doses of 25% + 25% + 25% + 25%.

The diameter of the stem showed high values (15.99 mm) for the variants fertilized with $N_{80}P_{80}K_{80}$, for the preparation of the germination bed + N_{70}, in vegetation, in vegetation, by fertigation with treated water, applied fractionally in doses of 25% + 25% + 25% + 25%.

Good results of the stem diameter (15.84 mm) were also recorded for fertigation with treated water, applied fractionally in doses of 33% + 33% + 33%, on the same NPK agrofond.

The index of the leaf surface varied between 4.7-7.8, with the highest value (7.8) achieved when applying a fertilization with $N_{80}P_{80}K_{80}$, for the preparation of the germination bed + N_{70}, in vegetation, by fertigation with treated water, applied fractionally in doses of 25% + 25% + 25% + 25% (Table 2).

Table 2. Influence of wastewater fertigation on the development of grain sorghum plants

No.	Fertilization / Fertigation variant	Plant height (cm)	Panic length (cm)	Stem diameter (mm)	Leaf area index
V1	$N_{80}P_{80}K_{80}$	98.5	24.25	13.55	4.7
V2	$N_{80}P_{80}K_{80}$ + N_{70} from ammonium nitrate	104	25	15.54	6.5
V3	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 25% + 25% + 25% + 25%	110	29.5	15.99	7.8
V4	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 33% + 33% + 33%	106.5	23	15.84	6.8
V5	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 50% + 50%	103	26.5	13.76	7.3
V6	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 100%	103.5	27	14.79	5.9

The water content of the leaves is a measure of the plant's fight against stress conditions, and severe decreases can contribute to structural disruptions of important biological functions in plants that lead to tissue damage or death (Devnarain, 2016). The results on dry matter and water forms in the leaves showed that they are influenced by fertigation with wastewater. The percentage of dry matter in the leaves of sorghum plants was between 20.17–24.22% with the maximum value (24.22%) in plants fertilized with $N_{80}P_{80}K_{80}$, in the preparation of the germination bed + N_{70}, in vegetation, by treated water fertigation, applied in a dose of 100%. The free water showed values between 71.65 - 76.07%, higher values being registered for the variant fertilized with $N_{80}P_{80}K_{80}$, when preparing the germination bed. The plants from the version fertilized with $N_{80}P_{80}K_{80}$, when preparing the germination bed + N_{70}, in vegetation, ensured by a phase fertilization with ammonium nitrate, registered the highest content of bound water 4.63%. The concentration of vacuolar juice, which is a reaction of plants to defend themselves under stress conditions, had values in the range of 9.9-14%, the maximum value being recorded in the variant fertilized with $N_{80}P_{80}K_{80}$, in the preparation of the germination bed + N_{70}, in vegetation, by fertigation with treated water, applied fractionally in doses of 33% + 33% + 33% (Table 3). Sorghum [Sorghum bicolor (L.) Moench], a C4 photosynthetic plant, is the fifth most important cereal plant in the world in terms of world production and one of the most drought tolerant and efficient in terms of of water consumption, grown in semi-arid environments (Blum, 2004, Rooney 2004).

Table 3. Influence of fertirigării with treated water on the physiological indices grain sorghum

No.	Fertilization / Fertigation variant	Dry matter %	Total water %	Free water %	Bound water %	Vacuolar juice concentration %
V1	$N_{80}P_{80}K_{80}$	20.17	79.83	76.07	3.76	11.4
V2	$N_{80}P_{80}K_{80}$ + N_{70} from ammonium nitrate	22.06	77.94	73.31	4.63	10.6
V3	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 25% + 25% + 25% + 25%	22.24	77.76	73.44	4.32	9.9
V4	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 33% + 33% + 33%	22.77	77.23	72.77	4.46	14
V5	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 50% + 50%	20.70	79.30	75.23	4.07	10.7
V6	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 100%	24.22	75.78	71.65	4.13	11.6

The analysis of the values registered by the active radiation in photosynthesis and the temperature at the level of the foliar apparatus, underlines significant differences during the day, which had a decisive role in the development of the physiological processes of the plant (Table 4).

Table 4. Climatic conditions recorded with the LC Pro + device at the time of physiological determinations

Time of day	Active solar radiation in photosynthesis ($\mu mol/m^2/s$)	Temperature (°C)	Atmospheric pressure (hPa)
9 o'clock	800-870	24-26	1011
12 o'clock	1444 – 1689	38.7 – 39.7	1011
15:30 o'clock	100 – 111	31.1 – 32.2	1010

In grain sorghum, the diurnal variation of photosynthesis presented a unimodal curve, with the maximum values recorded at noon, in all variants analyzed (Figure 2).

The highest average daily accumulation rate of CO_2 in the process of photosynthesis was recorded at fertilization with $N_{80}P_{80}K_{80}$, at the preparation of the germination bed + N70, in vegetation, applied by fertigation with treated water, divided into 3-4 doses (18, 24-18,61 (μmol CO_2 / m^2 / s).

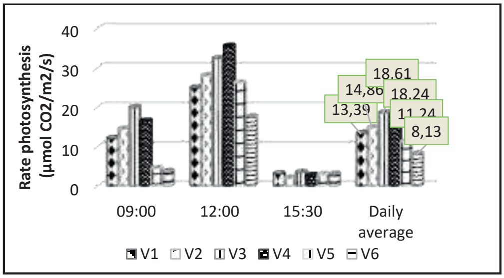

Figure 2. Diurnal variation of the photosynthesis process in grain sorghum

Between the process of foliar perspiration,, with average daily values between 1.90-3.33 mmol H_2O / m^2 / s and the photosynthesis process, with values in the range of 8.13-18.61 μmol CO_2 / m^2 / s, it was established a positive correlation, distinctly significant, which emphasizes the efficient recovery of water lost through foliar perspiration. The values of foliar perspiration increased in direct proportion to the intensity with which the photosynthesis process took place, the best results registering the variants fertilized with $N_{80}P_{80}K_{80}$, at the preparation of the germination bed + N_{70}, in vegetation, by fertigation with treated water, applied fractionally 3-4 doses (Figure 3).

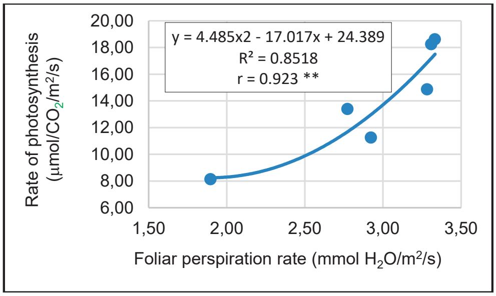

Figure 3. Correlation between foliar perspiration and photosynthesis in grain sorghum

Improving the efficiency of perspiration, especially in dry environments, can have a major impact on improving sorghum yield because high efficiency of perspiration will allow plants to either delay the symptoms of water stress or produce more biomass from the same amount of soil moisture available. or a combination of both (Xin et al., 2008, Thapa et al., 2017).

The production results, recorded for grain sorghum, showed an increase in productivity compared to the control variant, depending on the application of different doses of fertigation with treated water (Table 5). The best production results (6823-7667 kg / ha) and the weight of one thousand grains (33.2-33.6 g) were noted, the variants in which the dose of 70 kg N / ha, required in vegetation, was ensured by irrigating the sorghum with treated water divided into 3 doses of 33% or 4 doses of 25%, starting with the phase of 5-7 leaves, on an agrofund of $N_{80}P_{80}K_{80}$. Similar results were obtained on the sandy soils from Tâmburești, where grain sorghum achieved maximum yields at fertilization with a dose of $N_{150}P_{80}K_{80}$ (Matei Gh., 2011).

Table 5. Production results obtained from sorghum grains in different fertilization / fertigation variants

No. Var..	Fertilization / Fertigation variant	Biomass production (t/ha)	Grain yield / panicle %	Grain yield (kg/ha)	Significance	(TGW) (g)
V1	$N_{80}P_{80}K_{80}$	24	72.5	4147	Mt.	31.3
V2	$N_{80}P_{80}K_{80}$ + N_{70} from ammonium nitrate	24.5	89.3	5708	-	32.8
V3	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 25% + 25% + 25% + 25%	32	83.5	7667	**	33.6
V4	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 33% + 33% + 33%	35	85.1	6823	*	33.2
V5	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 50% + 50%	26.6	76.8	5613	-	33.2
V6	$N_{80}P_{80}K_{80}$, + N_{70} from treated water, in doses of 100%	27.1	75	5519	-	31.9
LSD 5% = 2238 kg/ha ; LSD 1% = 3510 kg/ha; LSD 0,1% = 5976 kg/ha						

The biomass production registered values between 24-35 t / ha, highlighting with the highest values the fertilization with $N_{80}P_{80}K_{80}$, at the preparation of the germination bed + N_{70}, in vegetation, ensured by fertigation with treated water, applied fractionally in doses of 33 % + 33% + 33%. The results of the surveys performed by Jerbi et al. (2020) and Fakhroddin et al. (2020), showed increases in production of various irrigated crops with wastewater. The grain production obtained from sorghum grain in the different fertigation variants was positively correlated, insignificantly with the grain yield on the panicle and positively significant with the weight of one thousand grains (Figure 4).

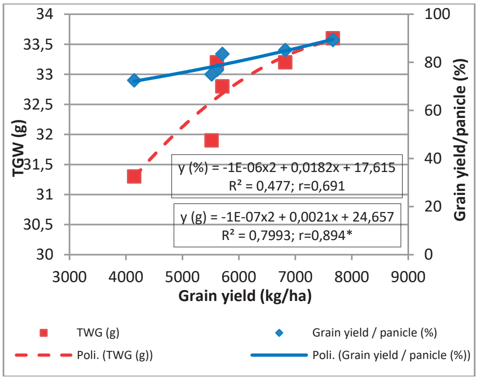

Figure 4. Correlation between grain yield, one thousand grain weight and grain yield / panicle

CONCLUSIONS

The obtained results highlighted the role of fertigation with an effluent obtained by wastewater treatment on the results obtained from grain sorghum grown in sandy soil conditions.

The index of the leaf surface varied between 4.7-7.8, with the highest value achieved when applying a fertilization with $N_{80}P_{80}K_{80}$, when preparing the germination bed + N_{70}, in vegetation, by fertigation with treated water, applied fractionally in doses of 25% + 25% + 25% + 25%.

The concentration of vacuolar juice, which is a reaction of plants to defend themselves under stress conditions, had values in the range of 9.9-14%, the maximum value being recorded in the variant fertilized with $N_{80}P_{80}K_{80}$, in the preparation of the germination bed + N_{70}, in vegetation, by fertigation with treated water, applied fractionally in doses of 33% + 33% + 33%.

The highest average daily rate of CO_2 accumulation in the process of photosynthesis was the fertilization with $N_{80}P_{80}K_{80}$, at the preparation of the germination bed + N_{70}, in vegetation, applied by fertigation with purified water, divided into 3-4 doses (18,24-18, 61 (μmol CO_2 / m^2 / s).

The best results regarding the production (6823-7667 kg / ha) and the weight of one thousand grains (33.2-33.6 g) were noted, the variants in which the dose of 70 kg N / ha, required in vegetation, was ensured by irrigating the sorghum with purified water divided into 3 doses of 33% or 4 doses of 25%, starting with the phase of 5-7 leaves

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A NEW METHOD FOR PREPARATION OF PESTICIDAL SOAPS VIA MACERATION OF ORGANIC SCRAPS

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Abstract

Exctraction is a method for separation of biologically active substances from plant's (and animal's) cells by using of different solvents. The oldest variation of this method is a preparation of tea and coffee drinks via hot water. Exactly water was the oldest and the most common used solvent especially in the area of food preparation and folk medicine. In the present day inductriual extractions can be achieved by various technologies include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet) and so on. The soap is one of the oldest chemical products in the human mankind dates back from the times of ancient Babylon. The soap has enormous influence on human society as major biocidal product against harmful microorganisms but also as one of the oldest pesticide in the world. During the resent years, there is a renew interest for the soaps as a natural friendly pesticides. Traditionally soaps are made by mixing organic acids (from petroleum, animal or plant origin) with sodium or potassium hydroxide, with or without heating. The study in this paper shows that there is yet another effective and cheap method for this, by maceration of different organic scraps with low concentrated water solutions of potassium or sodium hydroxide. The soaps produced by this method show excellent aphicidal activity against different aphid species.

Key words*: soap, maceration, aphids, pesticide.*

INTRODUCTION

Extraction is a method for separation of biologically active substances from plant's (and animal's) cells by using of different solvents (Gupta et al., 2012). The oldest variation of this method is a preparation of tea and coffee drinks via hot water. Exactly water was the oldest and the most common used solvent especially in the area of food preparation and folk medicine (Altemimi et al., 2017). In the present day inductriual extractions can be achieved by various technologies include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet) and so on (Azmir et al., 2013). In this paper a new method for preparation of pesticidal soaps via maceration of organic scraps is described. The soap has enormous influence on human society as major biocidal product against harmful microorganisms but also as one of the oldest pesticide in the world (Weinzierl, 2000). During the resent years, there is a renew interest for the soaps as a natural friendly pesticides. Traditionally soaps are made by mixing organic acids (from petroleum, animal or plant origin) with sodium or potassium hydroxide, with or without heating. There are two major drawbacks in this methods: the requirement for heating from one side and the using of highly concentrated solutions of potassium hydroxide from the other. Plus the facts that for this manufacture a pure oils or fatty acids are also required. Organic scraps are pretty big problem in nowadays however. There are a lot of wastes from many industrial, agricultural and plublic manufactures and services. In the most cases this scraps are used for animal feed, heating sources or organic manures (Odlare et al., 2015). However such wastes can be full with harmful micro and macro organisms which even the heat generated from the composting process can not kill. From the other side during recent years the pesticidal soaps became more and more popular especially in the area of organic agriculture and integrated pest management (Curkovic, 2016). The crisis with COVID-19 additionally boosts enormously the needs and uses of disinfectants (Rubio-Romero et al., 2020)

MATERIALS AND METHODS

Different organic scraps as olives and apricot pits, dry fallen tree leaves, wheat straw and seeds husks, tobacco wastes from cigars manufacture ("furda") were used.

The scraps were soaked in distilled water solution of potassium hydroxide at different concentrations for different amount of time. After that the solutions were filtered and were used for preparation of 1 % (v/v) water solutions also with distilled water. Via standard tensionmeter Kruss Force Tensiometer – K6 (Ali et al., 2019) was measured the surface tensions of this 1 % (v/v) water solutions for determination of the best variant for making soaps via this method i.e. – determination of the most appropriate concentration of potassium hydroxide and time for maceration.

The standard in vivo trials insecticidal activity towards different aphid species (*Aphis pomi*, *Aphis roase, Aphis gossypii* and *Aphis nerri*) were conducted (Ganchev & Atanasova, 2015). The percent effectiveness was calculated via formulae of Abbot (Fleming & Retnakaran, 1985). The phytoxocity in vivo test for establishing the eventual harmful action various towards cultural and non-cultural crops ware also performed (Ganchev et. al, 2012).

RESULTS AND DISCUSSIONS

The results show that all used organic wastes can be sources for soaps. However the amount of potassium hydroxide solution needed for saponification of the different organic scraps is different. The indicator in this case was to be produced as concentrated as possible solutions i.e. – the amount of potassium hydroxide solution to be as minimal as possible but the thinness of the received liquid soaps to allow filtration. If the amount of potassium hydroxide solution is too low, the filtration was completely impossible.

The Figure 1 show the concentration of the used organic scraps in the hydroxide solution in the maceration process i.e saponification

The results clearly show how small is the ratio between organic scraps and potassium hydrate solution. Even in the case of dry fallen tree leaves it is 2.5 %. The pits (olive and apricot) have the highest percent but this is due the highest density and presence of lignocellulosic materials

The results show also that the 1 % (v/v) water solutions from the liquid soaps produced by this method from different organic scraps can have different surface tension. And even more, the lowest possible surface tension can be achieved at different concentration of potassium hydroxide for the different type of wastes.

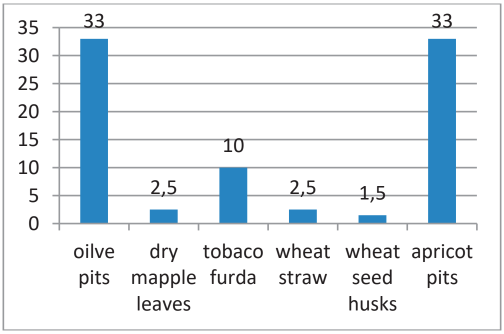

Figure 1. Percent concentration of organic scraps in KOH solution

However if the concentration of solution is too low (0.1 - 0.5 % (m/v)) this can be also not appropriate for making soaps by this way, due to the spoiling of solutions. The maceration period by this way was almost the same for all soaps approximately 20-21 days for optimal results, minimum 24-48 hours period for saponification. On the graphics bellow (Figures 2-7) is shown the surface tension of 1 % water solutions of created soaps:

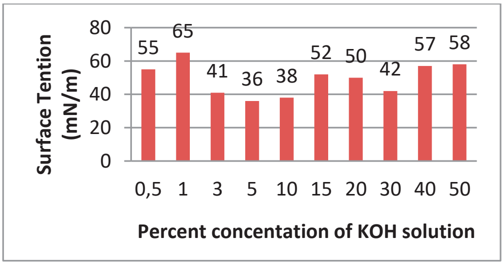

Figure 2. Surface tension (mN/m) of soaps made by maceration of olive pits

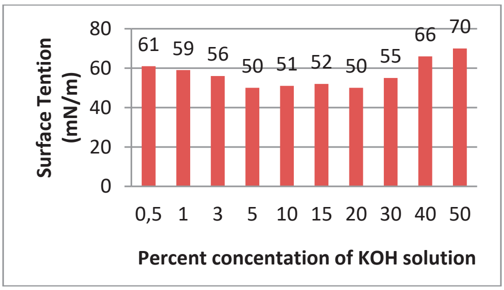

Figure 3. Surface tension (mN/m) of soaps made by maceration of dry mapple leaves

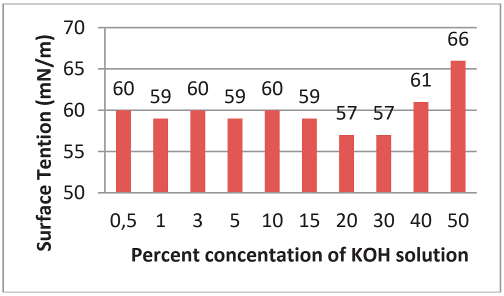

Figure 4. Surface tension (mN/m) of soaps made by maceration of tobaco furda

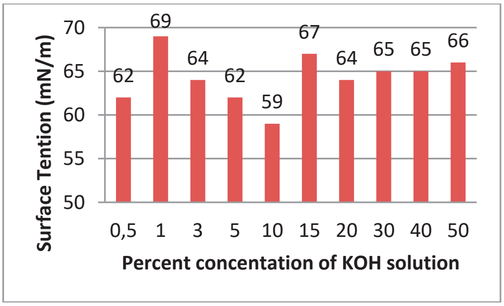

Figure 5. Surface tension (mN/m) of soaps made by maceration of wheat straw

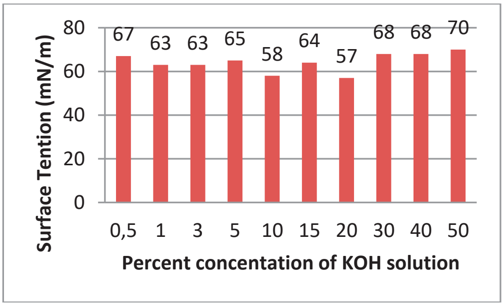

Figure 6. Surface tension (mN/m) of soaps made by maceration of wheat husks

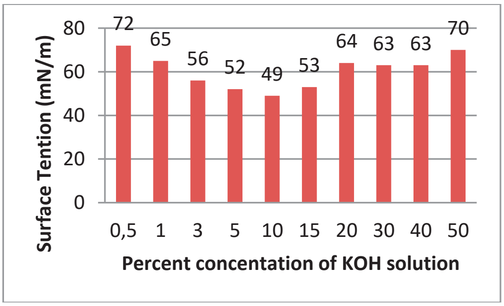

Figure7. Surface tension (mN/m) of soaps made by maceration of apricoat pits

The results show that for the different organic scraps, the concentration of potassium hydroxide of solution which can produce soaps with highest ability to low the surface tension is between 5 and 10 %. Exception is for the tobacco furda (tobacco organic scraps from manufacture of cigars). Soaps made by wastes from fruda, wheat straw, husks and tree dry leaves had lower ability for decreasing of surface tension but this is due to the lowest lipids content from the one hand (compared with the same of pits, especially oilves) and lowest concentrations of the scraps into potassium hydroxide solutions from the other.

In the Figure 8 is shown the surface tension of 1 % water solutions (prepared with distilled water) of popular trade mark various cleaning products in Bulgaria plus one of the most popular surfactant for agricultural plant protection products – Silwet L77. The measurements were made at 25°C:

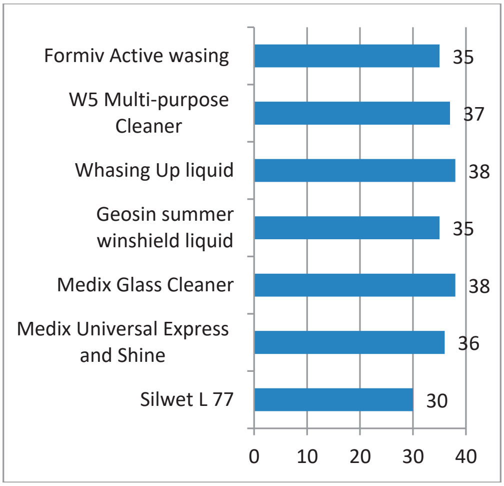

Figure 8. Surface tension (mN/m) of cleaning products

As can be see, the 1 % water solution of soap made by maceration from olive pits (33 % solution at 5 % KOH) can achieve the same (and even better) lowering of the surface tension compared with commercial cleaning products. The conducted test reveal that the same olive pits can be reuse for this yet 2 times. The same is valid for apricot pits. However the other tested organic scraps like dry leaves, furda, straw and husks were almost or at high degree disassembled during the maceration (saponify-cation) process to be reused again.

The conducted trials for insecticidal activity reveal the strong insecticidal action of all tested soaps towards different aphid species. On the graphics below (Figures 9-11) are listed some of the Dose – Responses Curves (Models):

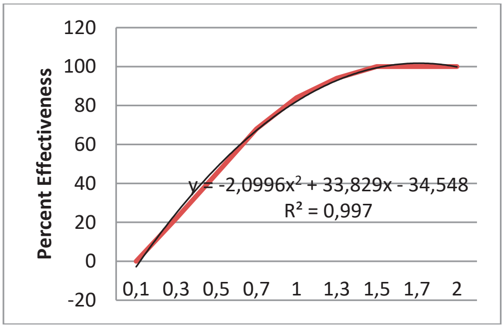

Figure 9. Olive Pits Soap, Aphis pomi: Dose - Response Curve

- LD05= 0.34 % (v/v)
- LD25=0.46 % (v/v)
- LD50=0.6 % (v/v)
- LD90=0.78 % (v/v)

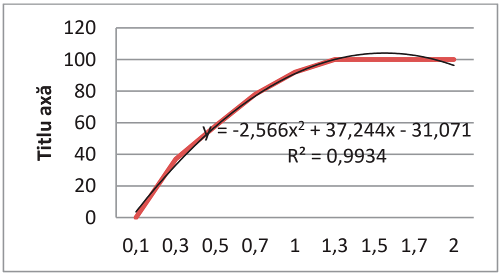

Figure 10. Apricot Pits Soap, Aphis rosae : Dose - Response Curve

- LD05=0.104 % (v/v)
- LD25=0.25 % (v/v)
- LD50=0.66 % (v/v)
- LD90=0.91 % (v/v)

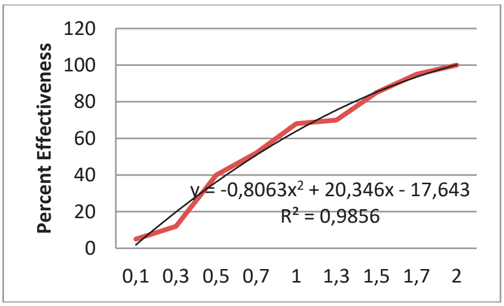

Figure 11. Dry Maple Leaves, Aphis pomi Dose - Response Curve

- LD05=0.16 % (v/v)
- LD25=0.34 % (v/v)
- LD50=0.94 % (v/v)
- LD90=1.55 % (v/v)

The soaps on the base of pits (olive and apricot) were able to kill aphids at 0.7-0.8-0.9 % concentrations, while the rest of the soaps – at 1.5-1.7-1.8 % concentrations - the difference is due to the lower percent of organic wastes in potassium hydroxide solution. There were no statistically significant differences ($p>0.05$) in the effectiveness of the given soap type towards different aphid species.

However there is the phytotoxicity of the soaps in some cases towards different plants. The trials show that plants like oil – yielding roses, wheat, apples, cherries and sour cherries and walnut are pretty resistant to the action of soaps and react with no or minimal phytotoxic symptoms (necrosis, chlorosis, deformations, whitening), with chemotherapeutic coefficient = 3.5-4. Other plants like cucumber, pumpkin, water melons, peach, potatoes, peppers can be sensitive and soaps can cause significant damages on them, with chemotherapeutic coefficient = 0.2-0.5

CONCUSIONS AND DICUSSIONS

The conducted trials reveal that maceration of organic scraps in potassium hydroxide solution can be viable method for production of soaps and utilization of such kind wastes. Even garbage organic materials like dry fallen tree leaves can be used for production of soaps. The produced soaps from one hand can be used as a standard disinfectants and for naturally friendly plant protection product – from the other. The soaps of course can undergo additional chemical procedures as pH correction, addition of other active organic or non organic substances fragrances addition and other. In this process concentration of potassium hydroxide is much more lower than in the standard soap manufacture, plus the fact that percent ratio between organic wastes and potassium hydroxide solution is also low.

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HOW EFFECTIVE IS FOLIAR TREATMENT FOR CONTROLLING THE MAIZE LEAF WEEVIL (*Tanymecus dilaticollis* Gyll) IN ROMANIA?

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Abstract

Maize leaf weevil (Tanymecus dilaticollis Gyll) is the main pest of the maize crops, mainly in south and south-east of the Romania. Each year, around one million hectares cultivated with maize is attacked by this pest, with different level of the attack intensities. This paper presents the results of researches concerning the effectiveness of the maize foliar treatment with acetamiprid active ingredient, for controlling of the maize leaf weevil, in absence of the seeds treatment with systemic insecticides. The experience were carried out in south-east of the Romania, in favorable area for T. dilaticollis, at NARDI Fundulea, ARDS Marculesti and ARDS Braila, in 2019 and 2020. In case of moderate weevils attack, registered in spring of 2019, the percentage of plants that escaping of the attack ranged from 76.25 to 86.36 % in case of untreated variant, while in case of treated variant, with acetamipird active ingredient, saved plants percent ranged from 77.73 % to 88.12 %. In spring of 2020, the weevils attack at maize plans was low at NARDI Fundulea and ARDS Marculesti, where percentage of the saved plants was higher then 90 %, and moderate at ARDS Braila, when saved plants percent was higher then 77 %. Results from the field trials show that foliar treatment with acetamiprid, don't provide effective protection of the maize plants, in first vegetation stages (BBCH 10-BBCH 14), against maize leaf weevil attack.

Key words*: maize, weevil, foliar treatment, control.*

INTRODUCTION

In last years, Romania has more than 2.4 million hectares cultivated with maize which represents the highest area within the EU27 (Eurostat, 2019; MADR data, 2021). Maize leaf weevil (*Tanymecus dilaticollis* Gyll) is the main pest of maize crops, mainly in south and south-east of the Romania (Paulian et al., 1972; Voinescu et al., 1985; Barbulescu et al., 1991, 2001a; Popov, 2002; Popov et al., 2004; Georgescu et al., 2014, 2018; Toader et al., 2020). Each year, around one million hectares cultivated with maize is attacked by this pest, with different level of the attack intensities (Barbulescu et al., 2001a; Popov, 2002, 2003; Popov et al., 2005, 2006a). The pest is dangerous when maize plants crops are in early vegetation stages, from the emergence until the four leaves stage (Popov et Barbulescu, 2007). After four leaves (BBCH 14) stage, weevils have feeding only with leaf margins and damages are less economically important (Rosca et Istrate, 2009). In some cases, weevils attack can occur before plants emergence above soil surface, causing high yield losses, sometimes compromising not only maize crops, but also sunflower and sugar beet (Barbulescu et al., 2001b). Data from the literature make in evidence that in south-east of the Romania, pest density ranged between 15 and 80 weevils/m^2 (Barbulescu, 1995, 1996, 1997, 2001; Popov et al., 2007a). In some extreme cases, it has reported a pest density of 160 weevils/m^2, in the Dobrogea area (Voinescu, 1987 cited by Rosca and Istrate, 2009). Researches made at NARDI Fundulea revealing that higher biological reserve of the maize leaf weevil (*T. dilaticollis*) it has registered in case of maize monoculture and sunflower cultivated after maize (Paulian, 1972; Barbulescu et Voinescu, 1998; Voinescu et Barbulescu, 1998). In some cases, even if the maize crop was sowed in plots with low pest reserve, the weevils can migrate from neighborhood plots that were sowed with maize in previous years (Barbulescu, 2001b; Popov et Barbuescu, 2007). As a result of both, the increasing area cultivated with maize and sunflower and decreasing number of economically effective crops, in favorable pest area from south and south-east of Romania, farmers couldn't make a proper rotation (Dachim, 2016; Lup et al., 2017). Maize leaf weevil is a thermo and xerophilous insect specie, being spreading especially in arid and semi-arid areas from Romania (Paulian, 1972). According to Popov et al. (2006b), weevils are very active at high air temperatures and low humidity, registered in the period when maize plants are in early vegetation stages during low air temperatures and high rainfall amount represent unfavorable conditions for pest activity at ground surface. Long term studies make evidence that climate changes will increase the prevalence of insect pests in maize agroecosystems from Central and South-East of Europe, including Romania Diffenbaugh et al., 2008; Olesen, et al., 2011; Bebber et al., 2014; Choudhary et al., 2019). Researches from Romania effectuated in last decades, at I.C.C.P.T. Fundulea (actual N.A.R.D.I. Fundulea), demonstrate that the highest effective method for controlling the maize leaf weevil attack, when maize plants are in early vegetation stages (BBCH 10-BBCH 14) is seeds treatment with systemic insecticides with a high solubility degree and rapid translocation of the active ingredient to the young plants (Voinescu, 1985; Barbulescu et al., 1993, 1994, 1995; 2001b; Popov, 2002, 2003; Popov et al., 2006b, 2007b; Popov et Barbulescu, 2007; Vasilescu, 2005; Georgescu et al., 2014, 2018; Trotus et al., 2018). After European Commission Regulations 218/783, 218/784, and 218/785, the use of imidacloprid, clothianidin and thiamethoxam active ingredients for all field crops, both like seeds treatment and foliar application will be totally banned in UE, from 2019 (Official Journal of the European Union, 2018a,b,c). As result no insecticides will remain available for sunflower seed treatment against *T. dilaticollis* in Romania. According Ionel (2014), lack of the seeds treatment alternatives for spring crops, including maize, can have negative impact in Romanian agriculture in following years. Kathage (2018) mentioned that after ban of seeds treatment with neonicotinoids of the maize, sunflower and oilseed rape in EU, farmers use foliar and soil treatments that are more expensive comparative with seeds treatment. In this paper, it has presented some results of the researches concerning the effectiveness of the foliar treatment of the maize crops with the acetamiprid active ingredient, in three locations from south-east of Romania for controlling the maize leaf weevil (*T. dilaticollis*), in the absence of the seed treatment with systemic insecticides.

MATERIALS AND METHODS

The field trials were carried out between 2019 and 2020, at the experimental field of the Plants and Environment Collective, from National Agricultural Research Development Institute (NARDI), Fundulea, Calarasi County (lat. N: 44.46; long. E: 26.32; altitude: 68 m), experimental field of Agricultural Research Development Station (ARDS) Marculesti (lat. N: 44.25, long. E: 27.30, alt. 39.30 m), Calarasi County and experimental field of the Agricultural Research Development Station (ARDS), Braila (lat. N: 45.12, long. E: 27.55, alt. 14.52 m), Braila County. These three locations are placed in the favorable area of *T. dilaticollis* in south-east of the Romania.

Table 1. Active ingredients used in this research

Variant no.	**Active ingredient** (concentration)	**Rate** (kg. c. p./ha)*
1	control (untreated)	—
2	acetamiprid (20 %)	0.1

*kilogram commercial product per hectare

The active ingredient used in this study was acetamiprid (20 %), a wide broad-spectrum insecticide authorized for foliar treatment of the maize crops in Romania (table 1). Experimental plots were arranged according to the randomized blocks scheme. Each plot has 10 m in length, and 4.2 m wide as a result plot area was 42 m^2. For this experience, it has used F423 maize hybrid (FAO 420). Distance between rows was 0.7 m.

For this experience it has used lower plants density. On each plot it has sowed 180 maize seeds, that correspond on a density of 42857 seeds/ha. The purpose for using of lower plants density in this experience is to assure better conditions for weevils attack. The field trial was carried out in the plots cropped previous years with maize, thus favoring a high pest biological reserve. To avoid migration of the weevils from one plot to another, the plots were laterally isolated with a 2 m wide strip sowed with pea, a plant repellent for this insect (Paulian, 1972).

- In 2019, at NARDI Fundulea, maize plants were sowed on 12 May, full plants emergence was recorded on 20 May while BBCH 14 stage was recorded on 27 May;
- In 2019, at ARDS Marculesti, maize plants were sowed on 22 April, full plants emergence was recorded on 4 May while BBCH 14 stage was recorded on 14 May;
- In 2019, at ARDS Braila, maize plants were sowed on 4 May, full plants emergence was recorded on 16 May while BBCH 14 stage was recorded on 26 May;
- In 2020, at NARDI Fundulea, maize plants were sowed on 15 April, full plants emergence was recorded on 26 April while BBCH 14 stage was recorded on 8 May;
- In 2020, at ARDS Marculesti, maize plants were sowed on 24 April, full plants emergence was recorded on 4 May while BBCH 14 stage was recorded on 14 May;
- In 2020, at ARDS Braila, maize plants were sowed on 19 May, full plants emergence was recorded on 2 June while BBCH 14 stage was recorded on 13 June.

Foliar treatment with acetamiprid active ingredient was applied, after plants emergence, when maize plants were in BBCH 11-12 stages.
Evaluation of the effectiveness of the foliar treatment for controlling the maize leaf weevil was made after a methodology elaborated at NARDI Fundulea, by Paulian (1972) and improved by Bărbulescu (1995, 2001b).
Attack intensity of weevils was assessed when sunflower plants arrive in four leaves stage (BBCH 14). At each plot it has marked 20 plants, from four central rows (5 plant/row). Before assessment, the plants were marked with sticks, in stair system. Weevils attack was rated on a scale from 1 to 9, as follows:

• Note 1: plant not attacked;
• Note 2: plant with 2-3 simple bites on the leaf edge;
• Note 3: plants with bites or clips on all leafs edge;
• Note 4: plants with leafs chafed in proportion of 25 %;
• Note 5: plants with leafs chafed in proportion of 50 %;
• Note 6: plants with leafs chafed in proportion of 75 %;
• Note 7: plants with leafs chafed almost at the level of the stem;
• Note 8: plants with leafs completely chafed and beginning of the stem destroyed;
• Note 9: plants destroyed, with stem chafed close to soil level.

Saved plant percent was rated at 30 days from sunflower emergence, by counting all the emerged plants from a plot and comparing them with the number of sowed sunflower seeds/plot.
Meteorological data were collected from automatic weather stations, placed in the field site from the three locations were it was carried out this study. It has registered average air temperature and daily rainfalls amount.
The data were **statistical analyzed** using Student–Newman–Keuls (SNK) test for multiple comparisons used to identify sample means that are significantly different from each other (Student, 1927; Neuman, 1939; Keuls, 1952).

RESULTS AND DISCUSSIONS

Temperatures and rainfalls from the spring period influenced weevils activity on the ground, such as the feeding process and mating. During this field trial, in three locations from the south-east of Romania it has monitoring weather conditions. Rosca et Istrate (2009) mentioned that the maize leaf weevil is very active when the temperature recorded on the ground level is higher than 18 °C and daily air temperature is higher than 20 °C. Popov et al. (2006b) refer to high weevils activity in case of lack of rainfalls. The same authors concluded that if weather conditions from the early stages of the maize (BBCH 10-BBCH 14) are favorable for weevils activity, then attack intensity of this pest at maize plants can be higher. Analyzing the daily temperatures and rainfalls, recorded between maize plants emergence and four leaves stage, it has ascertained that in 2019, at NARDI Fundulea, the maximum temperature was higher than 20 °C on all days (Figure 1). Rains occurred 24 hours after the emergence and at 4 days from the emergence favored plants development, as a result, the weevils attack intensity was moderate. In the spring of 2020, at NARDI Fundulea, plants emergence was recorded on 26 April. In the first four days after plants' emergence, maximum air temperatures were higher than 20 °C. However, it has recorded high-temperature differences between minimum and maximum air temperature (higher than 20 °C on 29 April) in the same interval.

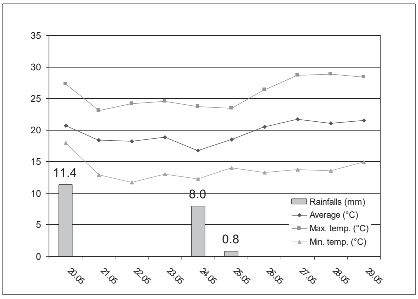

Figure 1. Daily temperatures and rainfalls recorded between maize plants emergence and four leaves stage in the spring of the year 2019 at NARDI Fundulea

Between 3 and 6 May it has recorded 30.8 mm of rains while temperatures decreasing (Figure 2). As result of the high differences between minimum and maximum air temperature recorded in first 96 hours after the plants emergence, the weevils activity of the ground was low. Decreasing of the air temperature after 30 April and high rainfalls amount recorded in next days favored maize plants growing and development and represent unfavorable conditions for pest activity.

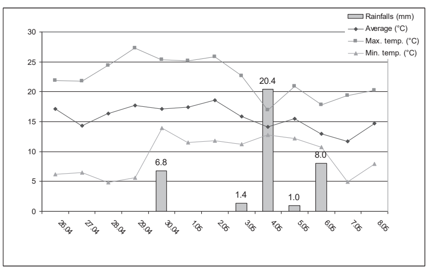

Figure 2. Daily temperatures and rainfalls recorded between maize plants emergence and four leaves stage in the spring of the year 2020 at NARDI Fundulea

As a result, because of unfavorable weather conditions recorded in early vegetation stages of the maize plants, the weevils the attack was lower than the previous year. The situation was quite similar at ARDS Marculesti experimental site. In the spring of 2019, weather conditions recorded between maize emergence and four leaves stage was less favorable for weevils activity on the ground. After 48 hours from the emergence, it has recorded 18.2 mm of rains and air temperatures decreasing below 15 °C (Figure 3).

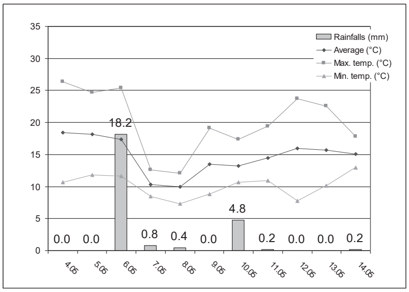

Figure 3. Daily temperatures and rainfalls recorded between maize plants emergence and four leaves stage in the spring of the year 2019 at ARDS Marculesti

In the next five days, air temperatures were lower then 20 °C, as results weevils activity was lower and maize plants escape of the attack. The situation was quite similar in the spring of 2020. At 24 hours after the plant's emergence, it has recorded 15.8 mm of rains and maximum air temperature decreasing below 15 °C (Figure 4).

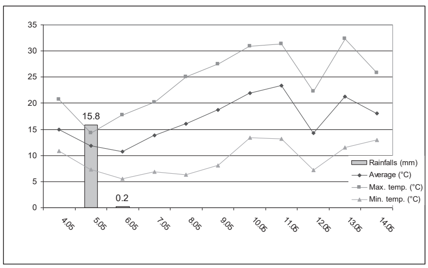

Figure 4. Daily temperatures and rainfalls recorded between maize plants emergence and four leaves stage in the spring of the year 2020 at ARDS Marculesti

Even if the temperatures increasing gradually in next days, as a result of the high rainfalls, amount recorded at 24 hours after emergence maize plants have a normal growing and development in first days after the emergence, as a result, weevils attack intensity was lower, and most of the plants escape the attack.

Weather conditions recorded at ARDS Braila in the spring of 2019, between maize emergence and four leaves stage, were favorable for the weevils attack. The maximum air temperature was higher than 20 °C in all days between plants emergence and four leaves stage (Figure 5). However, due to the rains on 24 and 25 May, maize plants have rapid growth, and the weevil attack intensity was moderate.

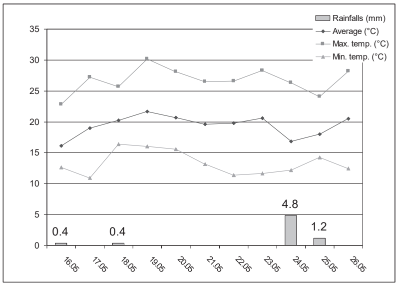

Figure 5. Daily temperatures and rainfalls recorded between maize plants emergence and four leaves stage in the spring of the year 2019 at ARDS Braila

In 2020, at ARDS Braila, maize plants were sowed on 19 May. However, as a result of the drought, plants emergence occurred on 2 June. Weather conditions recorded between maize emergence and the four leaves stage were favorable for weevils activity on the ground (Figure 6). However, the pest attack was moderate because plant emergence occurred in June. Paulian (1972) mentioned that the highest weevils attack occurred in plants that emerged in the last 10 days of April and the first 10 days of May.

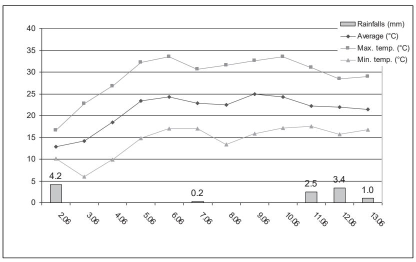

Figure 6. Daily temperatures and rainfalls recorded between maize plants emergence and four leaves stage in the spring of the year 2020 at ARDS Braila

Recent researches made at NARDI Fundulea make in evidence that there weren't high statistical differences on weevils attack at maize plants sowed in April and May (Georgescu et al., 2019).

In this field trial, in spring of 2019, the attack intensity of the maize leaf weevils (*T. dilaticollis*) at maize untreated plants, on a scale from 1 to 9, was moderate at NARDI Fundulea and ARDS Braila (5.24 and 5.25) and lower at ARDS Marculesti (Table 2).

Table 2. Attack intensity of *T. dilaticollis* at maize plants, in field conditions, (2019)

Active ingredient (concentration)	**Rate** (kg. c. p./ha)	**Attack intensity (1-9)**		
		1*	**2***	**3***
control (untreated)	—	**5.24a**	**4.00a**	**5.25a**
acetamiprid (20 %)	0.1	**4.80a**	**3.81a**	**4.73a**
LSD P=0.05		1.214	0.982	0.568
Standard deviation (SD)		0.540	0.436	0.252
Variation coefficient (C.V.)		10.750	11.170	5.060
Replicate F		0.107	0.729	0.346
Replicate Prob(F)		0.9505	0.5996	0.7965
Treatment F		1.315	0.369	8.646
Treatment Prob(F)		0.3347	0.5862	0.0605

*1-NARDI Fundulea; 2-ARDS Marculesti; 3-ARDS Braila
**Means followed by same letter or symbol do not significantly differ ($p<0.05$, Student-Newman-Keuls test)

At the same time, attack of the weevils at maize plants treated with acetamiprid active ingredient was slightly lower compared with untreated variants in all three locations. However, in all three locations there weren't recorded significant statistical differences between weevils attack at treated plants and attack at untreated plants ($p<0.05$).

Table 3. Influence of the foliar treatment, concerning saved plats percent, at 30 days from maize emergence (2019)

Active ingredient (concentration)	**Rate** (kg. c. p./ha)	**Saved plants percent (%)**		
		1*	**2***	**3***
control (untreated)	—	**76.25a**	**86.38a**	**82.67a**
acetamiprid (20 %)	0.1	**77.73a**	**88.12a**	**83.60a**
LSD P=0.05		6.431	11.961	4.235
Standard deviation (SD)		2.858	5.316	1.882
Variation coefficient (C.V.)		3.710	6.090	2.260
Replicate F		2.215	1.038	2.727
Replicate Prob(F)		0.2653	0.4881	0.2160
Treatment F		0.539	0.219	0.493
Treatment Prob(F)		0.5160	0.6719	0.5332

*1-NARDI Fundulea; 2-ARDS Marculesti; 3-ARDS Braila
**Means followed by same letter or symbol do not significantly differ ($p<0.05$, Student-Newman-Keuls test)

Concerning saved plants percent, data from table 3 reveal that in spring of 2019, at three experimental locations, in the case of foliar application with the acetamiprid active ingredient, when maize plants were in BBCH

12-13 stage, the percentage of plants that escape of weevil attack was slightly higher in case of treated variant. However, there weren't recorded significant statistical differences compared with untreated variant ($p<0.05$).

In conditions of the lower weevils attack, recorded at NARDI Fundulea and ARDS Marculesti, in spring of 2020, there weren't recorded significant statistical differences between treated and untreated variant ($p<0.05$). The weevils attack was higher at ARDS Braila, compared with the other two locations. However, even if the attack was lower at plants treated with acetamiprid active ingredient compared with untreated plants, there weren't recorded significant statistical differences between the two variants (Table 4).

Table 4. Attack intensity of *T. dilaticollis* at maize plants, in field conditions, (2020)

Active ingredient (concentration)	**Rate** (kg. c. p./ha)	**Attack intensity (1-9)**		
		1*	**2***	**3***
control (untreated)	—	**3.86a**	**3.66a**	**5.38a**
acetamiprid (20 %)	0.1	**3.66a**	**3.47a**	**4.94a**
LSD P=0.05		0.231	0.682	1.336
Standard deviation (SD)		0.103	0.303	0.594
Variation coefficient (C.V.)		2.730	8.500	11.520
Replicate F		7.039	1.031	0.046
Replicate Prob(F)		0.0716	0.4903	0.9847
Treatment F		7.775	0.850	1.099
Treatment Prob(F)		0.0685	0.4246	0.3715

*1-NARDI Fundulea; 2-ARDS Marculesti; 3-ARDS Braila
**Means followed by same letter or symbol do not significantly differ ($p<0.05$, Student-Newman-Keuls test)

As a result of the low weevils attack at the maize plants recorded in the spring of 2020 at NARDI Fundulea and ARDS Marculest, the saved plant percent was higher than 90 $ (Table 5). Contrarily, due to the higher pest attack, the percentage of that maize plant that escapes the attack was below 80 % at ARDS Braila. In all situations that occurred during this two-years field study, in three locations, there weren't recorded significant statistical differences in case of saved plants percent at treated variant compared with control (untreated) variant ($p<0.05$). Foliar spray with the acetamiprid active ingredient, in the absence of the seeds treatment, didn't provide proper protection of the maize plants in early vegetation stages against maize leaf weevil attack. Similar results were obtained in studies effectuated in the conditions of a commercial farm in case of high pest pressure (Georgescu et al., 2018, 2020).

Table 5. Influence of the foliar treatment, concerning saved plats percent, at 30 days from maize emergence (2020)

Active ingredient (concentration)	**Rate** (kg. c. p./ha)	**Saved plants percent (%)**		
		1*	**2***	**3***
control (untreated)	—	**90.59a**	**92.94a**	**77.54a**
acetamiprid (20 %)	0.1	**91.86a**	**93.67a**	**79.06a**
LSD P=0.05		8.999	5.374	2.719
Standard deviation (SD)		4.000	2.389	1.208
Variation coefficient (C.V.)		4.390	2.560	1.540
Replicate F		2.060	1.907	12.871
Replicate Prob(F)		0.2840	0.3046	0.0321
Treatment F		0.220	0.189	3.189
Treatment Prob(F)		0.6709	0.6928	0.1721

*1-NARDI Fundulea; 2-ARDS Marculesti; 3-ARDS Braila
**Means followed by same letter or symbol do not significantly differ ($p<0.05$, Student-Newman-Keuls test)

CONCLUSIONS

In the climatic conditions from the south-east of Romania, at the experimental field from NARDI Fudulea and ARDS Marculesti, *T. dilaticollis* attack at maize plants, in early vegetation stages (BBCH 10-BBCH14) was moderate in the spring of the year 2019 and lower in the spring of the year 2020. At ARDS Braila, weevils attack was moderate in both years of this field study.

In conditions of both moderate and lower attack recorded in three locations from this field trial, in spring of 2019 and 2020, foliar spray with the acetamiprid active ingredient, in the absence of seed treatment, don't provide effective protection of the maize plants, in first vegetation stages (BBCH 10-BBCH 14), against maize leaf weevil attack.

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RESEARCH REGARDING WEEDS CONTROL IN GRAIN LEGUMES CROPS

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Abstract

Although there are quite small areas sown with grain legumes in Romania, in recent years these crops have become more attractive to farmers also due to the fact that it leaves the soil enriched in nitrogen. Dried grain legumes crops have certain peculiar features that farmers must take into account to obtain profitable harvests. One of these features refers to the sensitivity of pea and bean crops to weed infestation, especially in the early stages of vegetation. In this context, the aim of the present study was to evaluate the efficacy and selectivity of the herbicides applied in pre and post-emergence on weed control in peas and beans. To this end, three experimental fields were conducted in Ilfov, Calarasi and Bucharest during spring and summer of 2020. Assessments were made at 14, 28 and 42 days after treatments application and they aimed at the effectiveness in controlling the mono and dicotyledonous weeds and crop safety. The results obtained have shown that the effectiveness of herbicides applied in peas and beans crops depends on weed species, their density on square meter, pedoclimatic and agrophytotechnical indicators.

Key words: *effectiveness, herbicides, phytotoxicity, bean, pea.*

INTRODUCTION

Grain legumes are valuable crops, rich in proteins and essential amino acids, playing an important role in human nutrition (Esmaeilzadeh & Aminpanah 2015; Ivanov, 2019). Alone, they contribute up to 33% of the dietary protein needs of humans (Vance et al., 2002). These crops also leave the soil enriched in nitrogen and they have an important role in crop rotation (Bazitov, 2002). Peas (*Pisum sativum* L.) and beans (*Phaseolus vulgaris* L.) are crops intended for both food and fodder, being appreciated by the Romanian consumer. Romania has the largest number of grain legume farms in the EU, almost 100,000 agricultural holdings (*2017).

A Romanian farm that grows peas or beans measures an average of 1.8 hectares, being 3.5 times smaller than the European average. Pea and bean crops have become more attractive to Romanian farmers since the European Union decided to encourage these crops by providing grants, namely "payment for greening".

Weeds are one of the major biological constraint in crop production and, therefore, their control is an important component of any crop production system.

Bean and pea plants are sensitive to weed competition, mainly during the early vegetative growth stages (Blackshaw, 1991; Pynenburg et al., 2011; Teixeira et al., 2009; Vidal et al., 2010; Fontes et al., 2013), found that seed yield in dry bean was reduced up to 85% result of season-long weed competition. Also, weeds compete vigorously with pea and yield reduction of 20-40 % are common (Blackshaw & O Donovan, 1993; Wall et al., 1991). Wider spacing in peas provides ample opportunities for weed infestation resulting in 18-76% yield losses (Singh et al., 1991; Kundra et al., 1993; Banga et al., 1998). Chemical weed control is still the predominant component of weed management in crop production (Arevalo et al., 1992; Tironi et al., 2012; Aboali & Saeedipour, 2015). The objective of this research was to investigate weed control efficacy and phytotoxicity to the crop of some herbicides applied in peas and beans at different doses, with different application timings.

MATERIALS AND METHODS

For this purpose, 3 experimental fields were placed in Ilfov, Călărași and Bucharest counties during spring and summer 2020 at SC Ghinea Prod. S.R.L., Călăraşi county (N: 44°28'77.6, E: 27°02'69.0), Didactic farm Moara Domnească (N: 44°29'38.6, E: 26°14'02.0) and Research Development Institute for Plant Protection - Bucharest (N: 44°30'82.5, E: 26°04'17.8). The soils in the experimental fields were: brown redish with clay loam texture at Ilfov and Bucharest, and cambic chernozem at Călăraşi, rich in organic matters.

The trials were carried out in accordance with good experimental practices, as concerns soil tillage and seedbed preparation (Bonciarelli & Bonciarelli, 2001; Pannacci et al., 2007).

The main agronomic practices are shown in Table 1. Experimental design was always a randomised block with four replicates and plot size of 30 m². In each trial, some herbicides were used in pre or post-emergence applications in order to assess weed control ability and selectivity to the crop. Herbicides under investigation (Table 2) were: aclonifen (Challenge, 600 g a.i./L), S-metolachlor (Dual Gold, 960 g a.i./L), imazamox+ bentazone (Corum, 22.4 g a.i./L + 480 g a.i./L) and Fluazifop-P-butyl (Fusilade max, 125 g a.i./L). Untreated plots were always added as checks. Post-emergence treatments were always performed with the crop at the 2–5 leaves stage, broadleaved weeds at the 2–4 true leaves stage and grasses at the 3–5 leaves stage.

Weed density was assessed in ground % and in number of plants per square meter. Weed control (efficacy) was assessed at 10, 28, and 42 days after each application in % control in comparison with the untreated plots. In each trial, herbicide phytotoxicity was rated visually at - at each date of the efficacy assessments on a 0–10 scale (0: no visible injury; 10: plant death). Also, there were observations on the weed found in the experimental plots before treatment. Determination of segetal flora was performed on one square meter using a metric frame. Statistical data - processing of the assessments was based on the analysis of ARM-9 software (P=.05, Student-Newman-Keuls), ADC GmbH company.

Table 1. Agronomic practices in the field experiments

Location	**Călărași**		**Ilfov**		**Bucharest**	
Crops	**Bean**	**Pea**	**Bean**	**Pea**	**Bean**	**Pea**
Preceding crop	Maize	Sunflower	Wheat	Wheat	Maize	Maize
Sowing date	25.04.2020	04.03.2020	08.05.2020	13.03.2020	05.05.2020	11.03.2020
Cultivar	Rocco	Salamanca	Diva	Belmondo	Bianca	Trendy
Density (plants/m^2)	40	140	45	120	40	140
Spacing between rows (cm)	70	12.5	70	12.5	70	12.5
Fertilisation (Kg/ha)	NPK 180	NPK 180	NPK 180	NPK 180	NPK 180	NPK 180
Emergence date	06.05.2020	29.03.2020	15.05.2020	01.04.2020	12.05.2020	25.03.2020
Pre-emergence treatments date	29.04.2020	20.03.2020	12.05.2020	25.03.2020	08.05.2020	23.03.2020
Post-emergence treatments date	15.05.2020	15.04.2020	28.05.2020	18.04.2020	25.05.2020	15.04.2020
Fungicide azoxystrobin 2.5 l/ha	18.05.2020	02.05.2020	22.05.2020	01.05.2020	24.05.2020	01.05.2020
Insecticide Deltametrin 0.5 l/ha	19.05.2020	04.05.2020	30.05.2020	05.05.2020	20.05.2020	05.05.2020

Table 2. Herbicide treatments on beans and peas in 2020

Treatment	Active Subst. g/l	Formulation	Rates l/ha	Application time	Code	Water volume l/ha
Aclonifen	600	SC	3.0	Pre-emerg.	A	300
S-metolachlor	960	EC	1.5	Pre-emerg.	A	300
Imazamox +Bentazone	22.4 + 480	SC	1.25+ 0.6 Dash	Post-emerg.	B	300
Aclonifen +Fluazifop-P-butyl	600 + 125	EC	3.0 + 2.0/1.5*	Pre+Post	A+B	300

*1.5 l/ha for pea

RESULTS AND DISCUSSIONS

The segetal flora in the experimental fields was composed mainly of annual dicotyledonous and monocotyledonous weeds. The annual dicotyledonous group was the most numerous, being found: *Ambrosia artemisiifolia* L., *Amaranthus retroflexus* L., *Capsella bursa-pastoris* (L.) Medik., *Chenopodium album* L., *Datura stramonium* L., *Daucus carota* L., *Fallopia convolvulus* (L.) Á.Löve., *Galium aparine* L., *Erigeron annuus* (L.) Pers., *Fumaria officinalis* L., *Lamium purpureum* L., *Tripleurospermum inodorum* (L.) Sch.Bip., *Polygonum aviculare* L., *Portulaca oleracea* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., *Solanum nigrum* L., *Sonchus asper* (L.) Hill., *Sonchus oleraceus* L., *Stellaria media* (L.) Vill., *Tribulus terrestris* L. and *Veronica* species. Of the group of annual monocotyledonous weeds there were found the species *Echinochloa cruss-galli* (L.) Beauv., *Poa annua* L., *Panicum miliaceum* L., and *Setaria* species. Perennial species were also present like *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* (L.) and S*orghum halepense* (L.) Pers., but they had a low density on square meter.

The major, dominant weeds with a % of soil cover higher than 15% at 42 days after treatments were: *Setaria* spp., 23.8%, *L. purpureum* 20%, *E. cruss-galli* 18.8%, *P. oleraceea* 16.8%, *G. aparine* 16.5%, *P. convolvulus* 16.3%, *A. retroflexus* 16.3%, *Veronica* spp., 16% and *E. annuus* 15.8%. Because the peas are sown in early spring (in the melted snow), the experimental fields were infested in the first part of the vegetation period mainly with weeds with early emergence in spring as well as with species that can overwinter: *L. purpureum*, *S. media*, *C. bursa-pastoris, F. officinalis G. aparine*, *Veronica* spp. Weeds infestation reduces the nutritional area of plants and creates conditions for the development of the diseases and pests, difficulties for the harvesting machinery and the result is strong yield reduction (Angelova & Yancheva, 1996; Dimitrova, 2000). In such conditions of weeds infestation, the herbicides provided good result in control of annual dicotyledonous and monocotyledonous weeds species in grain legumes (Tables 3, 4 and 5). The best results were obtained in the sample in which pre-emergence herbicide was performed with aclonifen followed by the post-emergence application of the herbicide Fusilade max based on Fluazifop-P-butyl in a dose of 2.0 l/ ha for beans and 1.5 l/ha for peas. Fluazifop-p-butyl is a selective aryloxyphenoxypropionate herbicide and provides excellent control of annual and perennial weeds. Fluazifop-P-butyl is quickly absorbed into the leaf surface, hydrolysed to fluazifop-P and translocated through the phloem and xylem and it is accumulated in the rhizomes and stolons of perennial weeds and the meristems of annual and perennial weeds. Weeds treated with fluazifop-p-butyl stopped growing within a few hours, showed gradual discoloration on newer growth in 3 to 4 days, and eventually necrosis, desiccation, and plant death occurs within 2 to 3 weeks (Urano, 1982; Erlingson, 1988). Thus, for *Setaria* species at pre-emergence herbicide applicaton with aclonifen and post-emergence application with fluazifop-p-butyl, the control percentage ranged from 81.5% for peas to 100% for beans in the Dâlga experimental field (Table 3). In the experimental field with beans of RDIPP Bucharest in which the *Setaria* species had a % of soil cover of 22% at 42 days after application of the treatments, % of control compared to the untreated sample was of 90.7% (Table 5).

In the experimental samples in which only pre-emergent herbicides were applied, the effect on weeds was lower, especially on species with a high degree of infestation. Thus, in Dâlga in the case of the species *E. annuus*, which had a 42% cover of the soil at 42 days after the treatment, the effectiveness in control was 48.7% in the case of application in pre-emergence of aclonifen and 66.6% for s-metolachlor. In Dâlga peas at *Veronica* species that had a 16% soil cover, the control efficacy was 30.3% for aclonifen and 60% for S-metolachlor (Table 3). Dual Gold herbicide has been shown to be more effective in controlling weeds, especially against grass weeds because it has high solubility, remains active and does not move out, even when small amounts of precipitation occur. Compared to S-metolachlor, aclonifen does not prevent germination but controls germinating weeds.

Table 3. The efficacy of herbicides in crop after 42 days of treatment (Dâlga 2020)

Weeds EPPO CODE*	**Treatment name**											
	Bean						**Pea**					
	Untreated (ground %)	Challenge	Dual Gold	Corum	Challenge + Fusilade	LSD (P=.05)	Untreated (ground %)	Challenge	Dual Gold	Corum	Challenge + Fusilade	LSD (P=.05)
	Dose L/ha											
	-	3.0	2.0	1.25	3.0+2.0	-	-	3.0	2.0	1.25	3.0+1.5	-
	Efficacy - % control in compared to the untreated plots											
ERIAN	15.8d	48.7c	66.6b	96.2a	97.2a	7.9-16	10.0d	49.8c	60.3bc	92.6a	85.7ab	24.7-30
TRBTE	7.3d	53.8c	68.8b	100a	100a	0.3-5.5	8.0c	39.5b	60.5ab	84.4a	85.8a	30.9-34
DATST	6.0d	48.7c	60.1b	100a	100a	0.5-7.2	-	-	-	-	-	-
CHEAL	11.3d	47.0c	67.1b	93.0a	92.1a	10-15	7.0b	50.0a	65.1a	70.7a	80.3a	23.7-24
SINAR	5.0d	38.3c	53.8b	95.4a	94.5a	8.6-15	-	-	-	-	-	-
AMARE	7.0d	49.9c	63.0b	100a	100a	1.6-12	5.0c	60.2b	70.2b	94.8a	90.3a	12.5-18
GALAP	10.0d	44.9c	60.2b	100a	100a	1.3-11	15.0c	39.5b	60.5ab	84.4a	85.8a	30.9-34
ECHCG	18.8c	40.8c	52.6b	89.3a	92.4a	8.5-13	6.0d	60.5c	81.5 b	100a	100a	2.9-15
SETSS	12.0c	53.9b	64.0b	100a	100a	1.0-10	10.0d	38.3c	60.9b	81.5a	80.5a	16.1-18
VERSS	4.5d	65.4c	77.1b	100a	100a	1.3-10	16.0c	40.c	55.0ab	76.3a	76.7a	20.0-21
STEME	-	-	-	-	-	-	12.0c	55.1b	65.3ab	84.4a	80.1ab	19.3-21
FUMOF	-	-	-	-	-	-	8.0c	60.9b	71.4b	100a	100a	4.6-19

*ERIAN = *E. annuus*; TRBTE=*T. terrestris*; DATST= *D. stramonium;* CHEAL = *C. album*; SINAR= *S. arvensis*; AMARE= *A. retroflexus;* GALAP= *G. aparine;* ECHCG= *E. cruss-gall;* SETSS=*Setaria* spp., VERSS= *Veronica* spp., STEME= *S. media;* FUMOF= *F. officinalis.*

Because the velocity of the effect is influenced by the available reserves of weed seeds in the soil, the effectiveness of this herbicide was lower. In such a way, at 42 days after treatments, in pea experimental field of Research Development Institute for Plant Protection - Bucharest, the erbicide Dual Gold provided a good effectiveness in weed control: *A. retroflexus* 60.6%, *C. album* 76.0%, *P. aviculare* 80.5%, *S. oleraceus* 72.3%, *Setaria* spp., 68.0%, *E. cruss-galli* 75.0% (Table 5). At Ilfov, the erbicide Dual Gold had a moderate effectiveness in control of dicotyledonous weeds in peas: *D. carota* – 65.2%, *P. convolvulus* – 51%, *G. aparine* – 50%, *S. media* – 60% and *M. inodora* –51% (Table 4). At the initial stages, due to the slower pace of development, pea and bean are suppressed by the rapidly developing of broadleaf weeds. For example, as far as concerns the invasive species *E. annuus*, the ground coverage was 15.8% in bean and 10% in peas in experimental field of Dâlga. In these conditions of infestation with this species, the herbicides applied in preemergence had a lower efficacy.

During that period especially, herbicide Corum was efficient at 1.25 l/ha, applied at stage of 2-5 leaves of crops together with the adjuvant Dach. Composed of two active substances (bentazone and imazamox), the herbicide Corum had a very good effectiveness in controlling weeds in peas and beans, the results obtained being close to those obtained in the pre-emergent version treated with aclonifen and post-emergent with fluazifop-p-butyl. Thus, at Moara Domnească, the effectiveness of herbicide Corum ranged from 90% to 100%: *D. carota* 100%, *P. convolvulus* 97%, *A. artemisiifolia* 100%, *G. aparine* 90%, *Setaria* spp. 91%, *P. annua* 100% and *P. miliaceum* 100%. Bentazone acts by contact, with an impact on the process of photosynthesis, being absorbed by leaves and other green organs. Imazamox is taken up by plants up to the growth areas where it blocks the synthesis of essential amino acids, responsible for cell growth and division, being absorbed by plants especially through the leaves, but also through the roots.

Herbicide action is enhanced by the light sunny weather, which stimulates active growth, while the cool, dark weather or prolonged drought inhibit the growth of weeds or reduces their turgor, and thus can reduce the effectiveness of the herbicide.

Table 4. The efficacy of herbicides in crop after 42 days of treatment (Moara Domnească 2020)

Weeds EPPO CODE*	Treatment name											
	Bean						Pea					
	Untreated (ground %)	Challenge	Dual Gold	Corum	Challenge + Fusilade	LSD (P=.05)	Untreated (ground %)	Challenge	Dual Gold	Corum	Challenge + Fusilade	LSD (P=.05)
	Dose L/ha											
	-	3.0	2.0	1.25	3.0+2.0	-	-	3.0	2.0	1.25	3.0+1.5	-
DAUCA	10.0d	52.5c	66.6b	100a	99.4a	1.3-11	8.0d	50.0c	65.2b	80.6a	80.2a	11.3-13
POLCO	16.3d	47.5c	61.7b	97.4a	94.5a	5.6-13	10.0d	35.0c	51.1b	70.3a	75.2a	11.1-12
AMBEL	7.0d	50.0c	63.0b	100a	100a	1.4-11	-	-	-	-	-	-
GALAP	16.3d	37.0c	58.0b	89.7a	91.9a	10-15	15.0d	29.8c	50.1b	75.4a	70.3a	12.-12.7
SETSS	23.8d	39.5c	60.5b	91.7a	92.2a	12-18	-	-	-	-	-	-
POAAN	10.0d	58.8c	71.4b	100a	100a	0.3-5.6	-	-	-	-	-	-
PANMI	12.5d	48.7c	63.0b	100a	100a	1.5-12	6.0d	60.1c	80.6b	100a	00a	0.8-8.8
STEME	-	-	-	-	-	-	12.0d	44.9c	60.1b	85.5a	80.1a	7.6-9.9
MATIN	-	-	-	-	-	-	10.0d	39.9c	51.1b	75.3a	72.2a	11.1-12
SONAS	-	-	-	-	-	-	6.0c	50.0b	65.2b	88.5a	93.0a	1.3-24.8

*DAUCA= *D. carota,* POLCO= *F. convolvulus*, AMBEL=*A. artenisiifolia.* POAAN= *P. annua*, PANMI =*P. miliaceum*, MATIN= *T. inodorum,* SONAS= *S. asper*.

The application of adjuvant results in better weed coverage with a thicker wax layer on the leaf surface and prevents the possible reduction of herbicidal action at low atmospheric humidity and high temperatures.
The combination between the herbicide Corum and the adjuvant Dach has no impact on the next crop in crop rotation, but it is not recommended to mix them with foliar fertilizers and organophosphorus insecticides (Tibets & Saskevich, 2006). No phytotoxicity symptoms have been shown in the experimental plot. No symptoms of chlorosis, necrosis, leaf deformation, height reduction, distortion and delay at flowering in plots treated with clopyralid were seen (*, 2014).
In the experimental fields were also present 4 invasive weed species: *A. artemisiifolia*, *E. annuus*, *V. persica* and *S. halepense* which were carefully monitored given the growing invasion of alien species, allogeneic in natural ecosystems and anthropogenic in our country.

Table 5. The efficacy of herbicides in crop after 42 days of treatment (ICDPP-Bucharest, 2020)

Weeds EPPO CODE*	Treatment name											
	Bean						Pea					
	Untreated (ground %)	Challenge	Dual Gold	Corum	Challenge + Fusilade	LSD (P=.05)	Untreated (ground %)	Challenge	Dual Gold	Corum	Challenge+ Fusilade	LSD (P=.05)
	Dose L/ha											
	-	3.0	2.0	1.25	3.0+2.0	-	-	3.0	2.0	1.25	3.0+2.0	-
AMARE	16.3d	45.0c	63.9b	95.4a	99.4a	4-13.8	2.0c	47.5b	60.6b	89.4a	91.a	22.2-27
CHEAL	12.0c	48.7b	58.9b	99.4a	100a	1.4-12	10.0b	56.8a	76.0a	90.6a	92.6a	27-30.8
POLAV	12.0e	42.4d	56.4c	92.5b	98.1a	5.5-14	7.0c	62.1b	80.5b	100a	100a	7.5-23.8
POROL	16.8c	44.9b	56.3b	97.1a	93.7a	6.8-14	-	-	-	-	-	-
SONOL	6.5d	50.0c	60.2b	100a	100a	0.9-9.8	5.0b	59.6a	72.5a	88.8a	89.3a	23.5-25
SETSS	22.5d	46.2c	63.9b	93.4a	90.7a	7.3-12	15.0c	53.8b	68.0ab	88.7a	82.7a	16.4-20
ECHCG	13.0d	53.8c	63.9b	100a	100a	0.6-8.0	10.0d	6.3b	75.0ab	100a	100a	23-15
LAMPU	-	-	-	-	-	-	20.0c	32.1b	51.3ab	81.2a	80.4a	32.2-34
CAPBP	-	-	-	-	-	-	8.0c	51.3b	64.4b	96.2a	96.8a	15-25.5
RAPRA	-	-	-	-	-	-	6.0c	43.7b	57.8 b	88.8a	82.4a	1.9-23.6

*POLAV=*P. aviculare*, *POROL* = *P. oleracea*, SONOL=*S. oleraceus.* LAMPU=*L. purpureum,* CAPBP= *C. bursa-pastoris,* RAPRA= *R. raphanistrum*

CONCLUSIONS

Bean and pea crops are very sensitive to weed infestation, especially at the early stages of vegetation. The annual mono and dicotyledonous weeds were dominant in the experimental fields. Herbicides applied provided good results in control of weeds in grain legumes. The post-emergence Corum herbicide applied together with the Dash adjuvant had a good efficacy in controlling the segetal flora that suppressed the experimental fields. The obtained results demonstrate that the application of pre-emergence treatments before the emergence of crops and post-emergence in vegetation is the best strategy to control weeds in pea and bean crops. No phytotoxicity symptoms have been shown in experimental plots. The results obtained have shown that the effectiveness of herbicides applied in peas and beans crops depends on weed species, their density, pedoclimatic and agrotechnical indicators.

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PRODUCTIVITY AND QUALITY OF SPRING RAPESEED VARIETIES IN THE FOREST STEPPE OF THE MIDDLE VOLGA REGION

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Abstract

With the advent of non-erucic low-glucosinolate varieties, rapeseed has developed into a crop of great potential. In this regard, one of the most important tasks is to identify varieties of spring rape that are capable of forming a stable yield of seeds of good quality in the forest-steppe conditions of the Middle Volga region. The research was carried out at OOO Telegino-Agro of the Penza region on leached heavy loamy chernozem. In terms of meteorological conditions, 2013 was the most favorable. The Ratnik cultivar was distinguished by the maximum field germination (80.1%) and the best preservation (85.2%). The varieties Ratnik, Fregat, Geros had the highest resistance to shattering. On average, over three years, the number of pods per plant and seeds in them by varieties was in the range of 22.8-24.4 and 14.4-15.9 pieces, respectively. The weight of 1000 seeds was practically at the same level (2.55-2.67 g), their weight per plant varied from 0.85 to 0.99 g, and an increase in these indicators was observed in the Ratnik variety. Depending on the variety and hydrothermal conditions, rapeseed is able to form the yield of oilseeds in the range of 0.99-2.19 t/ha. The Ratnik variety in 2013 provided a maximum yield of 2.19 t/ha, and on average over three years 1.68 t/ha, which is 0.34 t/ha higher than the standard. It has been established that the more oil the seeds contain, the lower their protein content. The highest oil content of 41.00% was observed in seeds of the Ratnik variety, with the maximum yield of pure vegetable oil and protein (0.71 and 0.44 t/ha).

***Key words**: variety, hydrothermal coefficient, field germination, safety, productivity, oil, protein.*

INTRODUCTION

In Russia, over the past 20 years, against the background of a total reduction in acreage, one can observe the disappearance of some oilseeds and the dominance of others. This situation threatens to lead to a reduction in biodiversity, disruption of the agroecological balance and deterioration of the phytosanitary situation (Smirnov, 2011).
Oilseeds can occupy their ecological niches in addition to traditional sunflower, which will increase the stability of oilseed production (Gavrilova et al., 2007; Volovik et al., 2017).
Rapeseed for Russia is not a new culture, it was still cultivated in the 19th century.
Russian farmers called it "turnip seed" (Gushchina et al., 2015). With the advent of new non-erucic, low-glucosinolate varieties and hybrids, rapeseed has turned into a crop of high potential (Lupova et al., 2020).
Rapeseed oil is used for food and technical purposes. Rapeseed contains 40.1-48.0% oil, 21.0-32.4% protein substances, 6.1-8.9% fiber, 4.3-5.2% minerals. Rapeseed oil containing polyunsaturated fatty acids (linoleic acid (ω-6) - 22.4%, linolenic acid (ω-3) about 8%) meets the requirements of the Institute of Nutrition of the Russian Academy of Medical Sciences to a greater extent than sunflower oil, which is one of the most consumed vegetable oils in the Russian Federation (Gebauer et al., 2006; Haar et al., 2014; Hossain et al., 2012).
Secondary products of oil production (cakes and meal) are used to obtain plant proteins for feed and food purposes, as well as for compound feed (Mustafaev et al., 2012; Gushchina et al., 2018; Sharafi et al., 2015). In fodder production, green mass of rapeseed and silage prepared from it is used (Volovik et al., 2020).
The steady growth of the gross yield of rapeseed oilseeds is the result of the active demand for raw materials from the oil-extracting enterprises. In addition, producers of biodiesel fuel stimulate the production of rapeseed (Gorlov et al., 2006), the value of which lies not only in its reproducibility, but

also in its lower toxicity. During the combustion of rapeseed oil, less hydrocarbons and nitrogen oxides are formed, and the exhaust gases do not contain sulfur and heavy metals (Graf, 2008; Johnson et al. 2007; Matthaus, 2006; Morgan et al., 2000). According to the EU directive, the share of biodiesel fuel should be 20% by the end of 2020.

The use of rapeseed as a green manure crop is promising, which makes it possible to reduce costs by 1.5-2.0 times compared to the introduction of manure (Volovik et al., 2020).

For the successful cultivation of agricultural plants, it is necessary to assess the compliance of the hydrothermal conditions of the cultivation zone with the biological requirements of the crop, and the study of varieties is necessary in order for them to meet the high requirements of modern agricultural producers (Koshelyaev et al., 2012).

MATERIALS AND METHODS

The study of varieties of spring rape was carried out in 2013-2015 in the conditions of the Bessonovsky branch of LLC Telegino-Agro of the Penza region on leached heavy loamy chernozem, the humus content in the arable horizon is 6.9% (GOST 26213-91), mobile phosphorus is 86-89 and exchangeable potassium - 127-140 mg/kg of soil (GOST 26204-91), pH(KCL), 5.3 (GOST 26483), the amount of absorbed bases - 43.0 mg-eq. per 100 g of soil (GOST 27821-88).

The predecessor of the crop was winter wheat, after harvesting of which, stubble plowing and plowing to a depth of 8-10 and 20-22 cm, respectively, were carried out. Presowing soil preparation consisted in early spring harrowing, carrying out two cultivations, from deeper to shallower, and rolling. The method of sowing is an ordinary one with a row spacing of 15 cm. The seeding depth is 2-3 cm. The seeding rate is 2.5 million viable seeds per hectare. All seeds for sowing are treated with an insecticidal disinfectant of systemic action Kruiser, SK (350 g/l thiamethoxam). To combat pests and weeds in the leaf rosette phase, a tank mixture was treated with a contact action insecticide Qi-Alpha, EC (100g / l Alpha-cypermethrin) and herbicide Tatrel-300, BP (300 g/l clopyralid) for control of annual and perennial dicotyledonous weeds... During budding, an insecticide against rape flower beetle and flea beetle was used (List of pesticides and agrochemicals permitted for use in the Russian Federation, 2013). All analyzes and counts were carried out according to the methodology of the State variety testing of agricultural crops (Methodology of state variety testing, 1989).

Four varieties of spring rape (Brassica narus oleifera annua, Metzger), which included in the State Register for the Middle Volga region (7): Radical (st), Ratnik, Frigate and Geros.

RESULTS AND DISCUSSIONS

One of the main factors that are of decisive importance for the productivity and quality of spring rapeseed in the conditions of the Middle Volga region is the amount of precipitation and their distribution over the growing season. The years of research were characterrized by different hydrothermal conditions, which developed quite favorably in the first year of sowing. In total, during the growing season, 251.0 mm of precipitation fall the sum of active temperatures was 2130.1 ° C.

During the sowing period, the soil was well warmed up and moistened, although precipitation fell within the normal range (13.0 mm). Seed germination was influenced by autumn-winter moisture reserves, and seedlings appeared on the sixth day, the hydrothermal coefficient (HC) according to G.T. Selyaninov was 0.9. In the leaf rosette phase, when the harvest of spring rapeseed was laid, precipitation fall only 10.0 mm at a rate of 22.0 mm, the average temperature was 18.0°C, and the HC was 0.5. The stalking phase was also characterized by a moisture deficit (HC - 0.4). Budding proceeded with an abundant amount of precipitation (75.0 mm) and an average temperature of 19.9°C, HC - 2.7. The flowering period was distinguished by a high temperature regime (19.1-21.2°C) and sufficient humidity (21.0 mm), it was at this time that the quality indicators of spring rape seeds were formed.

A significant role in the accumulation of the yield was played by precipitation in late July-early August, since they fall on the period of plant development, corresponding to the ripening phase. Abundant precipitation

(124.0 mm) during the green pod-ripening period prolonged the growing season.

In 2014, during the growing season of rapeseed, precipitation fall 2.3 times less than in the same period in 2013, with a sum of positive temperatures of 2104.1°C. By the time of sowing, the amount of precipitation was 10.0 mm at an average air temperature of 20.3ºC, which is 6.5ºC more than the average annual, HC - 0.7.

The development of a rosette of rapeseed leaves took place with a sufficient amount of moisture (24.5 mm) and heat. During the steming phase, the increased temperature regime (21.5°C) and the deficit of precipitation (2.2 mm) were extremely unfavorable for the growth and development of plants. Lack of precipitation and high temperatures (higher than the average annual by 4.2°C) led to weak branching of plants, HC - 0.1. During the period of flowering and ripening of seeds, there was a deficit of precipitation and hot weather (HC - 0.04-0.30), which had a negative effect on fruit formation and seed formation.

In 2015, during the growing season of rapeseed, 271.6 mm of precipitation fall with a sum of positive temperatures of 2388.0°C, and in May the average daily air temperature was at the level of 13.7°C, and precipitation fell 5.2 times less than the norm, which extended the process of rapeseed germination to 30 days (HC - 0.3).

The periods of rosette leaves - stemming, stemming - budding are characterized as excessively wet (HC - 3.0; 2.8), budding - flowering - acutely arid (HC - 0.2). Dry and hot weather contributed to the rapid and friendly ripening of seeds (HC - 0.2).

Therefore, the study of productivity issues of various varieties of spring rapeseed is impossible without taking into account the prevailing conditions. Analysis of the dynamics of precipitation and thermal regime shows that the modern agro-ecological situation in the forest-steppe conditions of the Middle Volga region is characterized by an aggravation of drought and extreme moisture in certain periods. Under these conditions, the ecological plasticity and drought resistance of the crop are important factors in increasing productivity. In this regard, it is necessary to give more substantiated recommendations for varieties of spring rape, which do not allow a sharp deterioration in the quality of oilseeds.

The germination phase is decisive in the formation of the number of plants per unit area, since not all sown seeds give viable seedlings. The field germination of rapeseed during the years of research was greatly influenced by the conditions in which the sowing was carried out. In 2013, seed germination was 3.9-12.4% higher than in 2014 and 2015 and for the studied varieties was 83.8-85.0%.

The number of plants during the germination period in 2014-2015 is 10.0-31.0 pieces/m^2 less than in the first year and varied according to the experimental options from 179.5 to 185.5 pieces/m^2. A decrease in germination to 71.8-74.2% is associated with dry and hot weather during the sowing-germination period. On average, over three years, the maximum field germination (80.1%) was observed in the Ratnik variety.

The safety of plants for harvesting depends on weather conditions, moisture availability and, to a large extent, on the quality of the care techniques carried out, since during the growing season, partial death of plants occurs as a result of self-thinning.

The highest plant safety was noted in 2013 at 81.0-85.2%, i.e. 171.7-181.1 of plants remained for harvesting per 1 m^2, the Ratnik variety was distinguished by the best preservation.

The number of plants preserved in 2013 exceeded their number in 2014 by 1.4 times, since in the first year of research, more friendly shoots were obtained, which compete well with weeds. The number of plants to be harvested in 2015 was 157.5-166.7 pc/m^2.

Productivity - is an integral indicator of the variety, depending on the degree of development of the elements of the structure of the crop, is associated with a variety of properties reflecting its response to resistance to unfavorable environmental factors, adaptability to soil conditions, resistance to lodging and yield losses, as well as immunity to diseases (Boroevich, 1984; Ashaeva et al., 2016).

Moisture deficiency during the steming period contributed to the weak branching of the plant in 2014. Number of branches of the first order decreased by 29.0-34.8% compared to the previous year, when the number of productive

branches was 4.2-5.0 pcs. Growth processes were more intensive in the Ratnik variety.
In 2013, an increase in relative humidity of air and temperature during the critical period of plant growth and development contributed to an improvement in the elements of its productivity, especially in the varieties Fregat and Geros, which allowed them to form the largest number of pods per plant - 26.7-26.9 pieces versus 21.8 -23.6 pieces in 2014-2015. Lack of precipitation during flowering in these years led to the "physiological wilting" of flowers and their fall. As a result, the number of pods on plants was 8.0-19.0% less than in the first year.
One of the most important features of spring rapeseed varieties is their resistance to pod cracking, which leads to a decrease in yield losses. The varieties Ratnik, Fregat, Geros had the highest resistance to shedding, Radical (st) was distinguished by medium resistance, which to a certain extent influenced the yield (Figure 1).

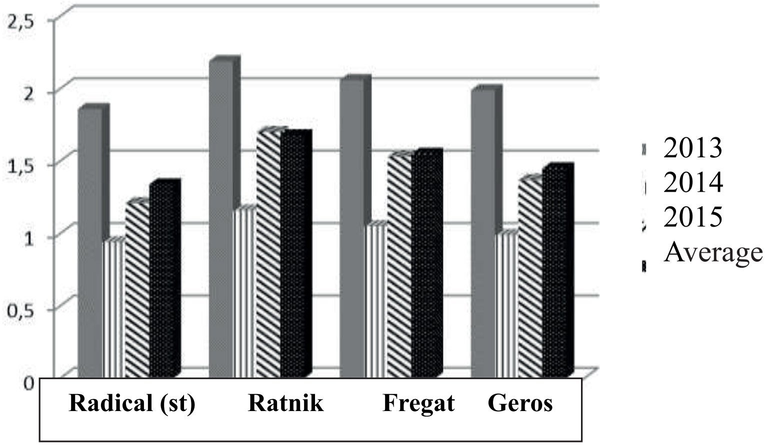

Figure 1. Yield of varieties of spring rape, t/ha

The most significant influence on the formation of productivity of spring rapeseed plants is exerted by the number of seeds in a pod and the weight of 1000 seeds. The maximum number of seeds in the pod was formed in 2013 - 15.9-17.4 pieces, which is 13.0-18.0% higher than in 2014, and 7.0-12.0% than in 2015 year. On average, over three years of research, the number of pods per plant changed insignificantly, as well as seeds in a pod, and varied by cultivars within the range of 22.8-24.4 and 14.4-15.9 pieces, respectively.
Drought during flowering and seed filling in 2014-2015 led to a decrease in the mass of 1000 seeds by 0.11-0.24 g in relation to the first year of research. The mass of seeds per plant was the highest (1.08-1.21 g) in 2013, which is 1.2-1.6 times higher than in 2014-2015.
On average, over three years, the weight of 1000 seeds was practically at the same level (2.55-2.67 g), the weight of seeds per plant varied from 0.85 to 0.99 g, and an increase in these indicators was observed in the Ratnik variety.
According to the years of research, the yield of seeds of spring rape is significantly varied. In 2013, favorable for hydrothermal conditions, it was almost 2.0 times higher than in 2014 and by 0.49-0.65 t/ha more than in 2015 and amounted to 1.86-2.19 t/ha, depending on the variety, and the maximum yield was provided by the Ratnik variety, which exceeded the standard by 0.34 t/ha, and Fregat - by 0.13 t/ha.
Radical (st) Ratnik Fregat Geros
The value of spring rapeseed is determined mainly by the content of fats and proteins, which are of great nutritional and feed value (Schierholt et al., 2001). The chemical composition of oilseeds varies significantly depending on weather conditions and agricultural practices (Gushchina et al., 2015). The even distribution of atmospheric precipitation and moderate average daily air temperatures during the growing season of

2013 contributed to the full passage of all phases and stages of development of spring rapeseed. Later, this had a positive effect on the oil accumulation processes. The variability of seeds of spring rapeseed in 2013 was 46.74-46.93%, depending on the variety, protein content 21.97-22.23%. Increased temperature and insufficient the amount of moisture in 2014 during the growing season of plants contributed to a greater accumulation of protein (33.45-33.78%) and a decrease in fat in seeds to 35.00-35.58%. In 2015, the content of oil and protein in seeds was 39.15-40.51% and 27.06-27.93%, respectively.

On average, over three years, the seeds of the Ratnik variety had the highest oil content of 41.00%, and the grain of the Geros variety accumulated more protein - 27.85%.

Thus, the process of oil accumulation in grain of spring rapeseeds depends on both weather conditions and the variety. Increased rainfall over the flowering period - seed formation increases fat and decreases protein.

Oil content is not a determining factor in productivity. An important indicator is the yield of oil per hectare. The maximum amount of oil (1.03 t/ha) was obtained with the yield of the Ratnik variety in 2013 with a yieldseed rate 2.19 t/ ha, protein harvest was 0.48 t/ha. With the harvest 1.86 t/ha variety Radical provided 0.89 t / ha of oil, 0.41 t/ha of protein.

The lowest oil yield (0.33-0.41 t/ha) was obtained in dry 2014 due to low yield (0.94-1.16 t/ha) and oil content of seeds (35.00-35.58%). However, the maximum (0.41 t/ha) was reached by the Ratnik variety. The highest protein harvest of 0.39 t/ha was provided by the same variety. The protein yield by varieties varied within 0.32-0.39 t/ha. In 2015, the amount of oil was 1.5-1.9 times less than in 2013 and 1.2-1.7 times more than in the previous year.

On average, over three years of research, 0.60-0.71 tons of rapeseed oil and 0.35-0.44 tons of protein were obtained from one hectare.

CONCLUSIONS

Thus, the field germination capacity of spring rapeseed varieties largely depended on weather conditions. In 2013, it was more favorable in terms of moisture supply, it was higher by 11.8-12.4%, the Ratnik variety was distinguished by the best preservation - 80.1%. The process of forming the elements of the structure of the yield of spring rape was greatly influenced by the weather conditions of the growing season. An increase in air temperature with sufficient moisture supply during the critical period of plant growth and development in 2013 contributed to the improvement of the elements of its productivity. The maximum seed yield of 1.68 t/ha was provided by the Ratnik variety. The yield increase in relation to the standard was 0.28 t/ha. The seeds of the Ratnik variety had the highest oil content of 41.00%, and the seeds of the Geros variety accumulated more protein - 27.85%. Depending on the variety, 0.60-0 was obtained per hectare.

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SYNTHETIC AMPHIDIPLOID WHEAT LINES WITH LARGER GRAIN SIZE

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Abstract

Modern genetic methods of breeding leaded to the obtaining of new wheat germplasm, which can be an important source for higher yield potential. The present study describes the data obtained by 10 synthetic amphidiploid lines for some morphological and quantitative characters and their correlation. The analysis of variance for analyzed characters showed highly significant differences among the genotypes under study, indicating the presence of considerable amount of variability in the lines. E6-A line combined well yield, protein and starch content while the lines with highest length and width of grains presented the lowest yield.

Key words*: synthetic amphidiploid wheat lines, grains, size, quantitative characters.*

INTRODUCTION

Global increased demand for continuous wheat production to keep up with the world's increasing population needs a significant yield jump like in the Green Revolution, but global warming is becoming a worldwide threat, with more severe drought and heat occurring at higher frequencies.

In wheat, yield capacity is a complex quantitative character, determined by the components of production and resistance to the unfavourable action of external factors. In turn, they have multi-genic determinism. However, it is know that polygenes do not have the exclusivity in the genetic control of production and its components.

The creation of varieties with high yield potential requires the optimization of morphogenesis processes. As crop formation is a sequential process, possibilities for genetic manipulation in order to increase production potential exist in each phase.

The size of the grains depends on the size of the ovaries and floral coverings, the filling time of the grains, the size of the assimilating surface and its photosynthetic efficiency, the longevity of the leaves correlated with the longevity of the root system, the competition between plants for light, water and nutrients.

Most of the successes obtained in improving yield capacity were attributed to the selection for some characters with simple heredity, including the grain size, which proved to be correlated with the production potential.

Grain size is a major selection and breeding target in modern tetraploid wheat (Gegas et al., 2010) because it is in association with other important characteristics. Research has to find new methods to increase yield potential of plants. Advances in new technologies have made possible to identify genes which control wheat grains size that enabling an increase for width and length. So, characterisation of a gene (GW2) which is responsible for control of grain width and construction of a triple mutant indicate an increase in seed size of 20% (https://www.jic.ac.uk).

Grain size is an important yield component and new technologies use the ability of gene-based on SNP markers to follow specific alleles for bolder, bigger grains could help to improve milling yields, increase the specific weight of varieties and may also help improve yield stability. Introduction in modern agriculture of new material such as amphidiploid species represent a diversification of existing high yield varieties.

Amphidiploids are plant organisms made by biotechnological methods based on wide hybridization and combining the genomes of the parental species involved in the cross (Stoyanov, 2013a). A large number of amphidiploids were created into the *Triticae* tribus and cultivated forms are synthetic hexaploid wheat (2n=6x=42, AABBDD), because of the identity of their genomic constitution with that of bread wheat *Triticum aestivum* (2n=6x=42, AABBDD), allows for the introduction of various genes from wild species (Stoyanov, 2014).

Grains size (its dimensions), as the degree of filling, are important characteristics depending upon the weight of the grain and the yield of the flour respectively the quality of the milling (Ciulca et al., 2020). Also, wheat embryo and the lining are more plastic than the endosperm and the humidity contained in whole wheat is distributed differently which significantly influences the milling process (Matei and Dodocioiu, 2016).

The purpose of this research was to experiment some new amphidiploid wheat in order to identify high-yield forms which could take part in future breeding programs leaving from larger grain size characteristics.

MATERIAL AND METHOD

Biological material and its genealogy is presented in Table 1.

The mentioned lines above were sowed in the field from Agricultural Research and Development Station Caracal of University of Craiova (440 06' N, 240 21' E and 98 m altitude) in 2016- 2019. Sow was made in last decade of October and the harvest was made in the first 10 days of July. A completely randomized block design was used in three repetitions and standard agronomic practices were followed.

Water conditions of the three years during wheat vegetation period and especially during the grain filling period were different (Table 2).

Table 1. Biological material and its genealogy

No.	Code	Genealogy		Origin country
		T. durum genotype	*Ae. squarrossa* biotype	
1	E 1-A	Pandur	Ae. squarrosa typica - 2472	Iran
2	E 5-A	Agedur	Ae. squarrosa strangulata - 2475	Iran
3	E 6-A	Agedur	Ae. squarrosa meyeri - 2530	Iran
4	E 7-A	Elidur	Ae. squarrosa meyeri - 2386	Pakistan
5	E 17-A	Amadur	Ae. squarrosa typica - 2472	Iran
6	E 19-A	Grandur	Ae. squarrosa strangulata - 2377	Iran
7	E 24-A	Condur	Ae. squarrosa strangulata – 2464	Iran
8	E 25-A	Condur	Ae. squarrosa typical - 2472	Iran
9	E 32-A	DDU 297	Ae. squarrosa strangulata - 2569	Kazahstan
10	E 55-A	Grandur	Ae. squarrosa strangulata - 2569	Kazahstan

Source: ADER 116/2015 project

Table 2. The variation of precipitation recorded during the years of experimentation and the calculation of deviations from the multiannual values

Year		Oct.	Nov.	Dec.	Jan.	Feb.	March	Apr.	May	June	July	Sum
2016-2017	Value	46	63.8	103	38.8	56.4	86.4	104.6	55.6	10.2	39.6	604.40
	Deviation	5.6	11.4	56.3	0.7	18.5	45.6	52.7	-8.1	7.3	-14.9	175.10
2017-2018	Value	56	48	14	6.8	12.4	53	54	84.8	17.6	101.4	448.00
	Deviation	15.6	-4.4	-32.7	-31.3	-25.5	12.2	2.1	21.1	14.7	46.9	18.70
2018-2019	Value	7.4	46.8	53.4	38.6	14.2	25.2	44.4	69	28.5	60	387.50
	Deviation	-33	-5.6	6.7	0.5	-23.7	-15.6	-7.5	5.3	25.6	5.5	-41.80
Multianual		40.4	52.4	46.7	38.1	37.9	40.8	51.9	63.7	2.9	54.5	429.30

RESULTS AND DISCUSSIONS

Umesh et al., 2019, sustain that synthetic hexaploid wheat (AABBD'D') is developed by artificially generating a fertile hybrid between tetraploid durum wheat (*Triticum turgidum*, AABB) and diploid wild goat grass (*Aegilops tauschii*, D'D'). It is known that *Aegilops* species contributed to wheat breeding despite the difficulties involved in the handling of wild species, such as crossability and incompatibility.

Grain size in wheat is the most stable component with favourable effect on flour yield (Giura and Saulescu, 1996) and is characterized by grain weight and area, whereas shape means a relative proportion of the main growth axes of the grain and also estimated by length, width, vertical perimeter, sphericity and horizontal axes proportion (Breseghello and Sorrells, 2007).

Average data and variations coefficients for the studied characteristics are presented in table 3 where it can be seen smaller or larger variations among the analysed lines for the studied parameters. So, the highest grains (both for length and width) were measured in E32-A line (8.64/3.37 mm), while the shortest in E6-A line (7.78/3.26 mm) and variation amplitude of 0.87/0.32 mm. Values below 7 mm for grains length were reported by Mandea et al., 2016 in some important cultivars experimented in yield trials performed also in South Romania.

Spike length varied from 10.20 cm (E19-A line) to 13.30 cm (E25-A line) with variation amplitude of 3.10 cm and a coefficient variation of 8.19. No. of spikelets/spike varied from 18.20 (E25-A line) to 22.00 (E1-A line) and 6.38 variation coefficient. No. of grains/spike presented higher variation, from 38.80 (E32-A line) to 56.60 (E7-A line) which means a variation amplitude of 17.80 spikelets. Grains weight/spike was the most variable character presenting a variation coefficient of 25.96% and a minimum value of 1.87g (E32-A line) and a maximum of 4.79g (E5-A line). TGW varied from 45.58g (E5-A line) to 61.66g (E25-A line) comparative to an average of 53.87g.

The argic chernozem from ARDS Caracal is a very fertile soil and water as vegetation factor has a high influence for the capacity of production of the varieties (Matei et al., 2017).

Yield varied in larger limits from 3640.00 kg/ha (E32-A line) to 7560.00 kg/ha (E1-A line) which means 3920.00 kg/ha variation amplitude and 24.52% variation coefficient. It can be noticed that the most productive line do not have the highest grain length or grain width, but over average number of fertile tillers, spike length, no. of spikelets/spike, no. of grains/spike and grains weight/spike. TGW, protein content and starch content were under the average of the lines.

Protein content varied from 10.70% (E5-A line) to 15.60% (E35-A line) while starch content varied from 69.60% (E1-A line) to 79.60% (E19-A line). E6-A line combined a good yield with protein and starch content.

Stem length presented variation amplitude of 27.60 cm varying from 98.40 cm (E6-A line) to 126 cm (E24-A line) and number of fertile tillers varied from 9.40 (E6-A line) to 13.80 (E7-A line). Giura, 2021 stated that longer stem can provide larger assimilation area and allows storing more assimilates that could be available for grain filling.

Analyses of the obtained data revealed that grain length and width are largely independent traits. This can be the result of artificial selection during breeding since these characters are independent variables in parental forms.

Accurate characterization of grain size and shape remains a big challenge due to complex nature of wheat grain shape (Patil et al., 2013).

Highly significant and positive correlations were identified between some of the experimented characters (table 4): grain width and protein content (0.860), grain length and protein content (0.720), no. of spikelets/spike and no. of grains/spike (0.735), no. of spikelets/spike and grains weight/spike (0.769), no. of spikelets/spike and yield (0.883), no. of grains/spike and yield (0.842), grains weight/spike and yield (0.880) and TGW and yield (0.920).

Grain characteristics, particularly grain weight, grain size and shape and grain protein, are important components of grain yield and quality in wheat due to their significant effect on grain yield, milling yield, end-use quality and market price (Abdipour et al., 2016).

Grain length is significantly increased by chromosomes 4A, 4B, 2B, 3A and 1B while grain width by chromosomes 1A and 1B (Giura and Saulescu, 1996). Larger grains is not only directly relate to grain yield, but also have favorable effects on seedling vigor and early growth, thereby promoting and stabilizing yielding ability (Cristina et al., 2016).

Table 3. Variation limits for analyzed characters (2016-2019)

	Grain width (mm)	Grain length (mm)	Stem length (cm)	No. of fertile tillers	Spike length (cm)	No. of spikelets/ spike	No. of grains/ spike	Grains weight/spike (g)	TGW (g)	Yield (Kg/ha)	Protein content (%)	Starch content (%)
E1-A	3.16	7.92	121.40	11.00	12.50	22.00	56.40	4.22	51.85	7560.00	12.80	69.60
E5-A	3.04	7.97	103.40	10.80	10.50	21.80	51.80	4.79	45.58	7020.00	10.70	71.30
E6-A	3.26	7.78	98.40	9.40	10.80	21.60	47.40	3.39	52.61	6980.00	13.10	71.60
E7-A	3.31	8.14	99.20	13.80	12.40	20.80	56.60	2.96	48.71	5800.00	13.00	72.30
E17-A	3.27	8.13	102.00	9.20	11.70	21.40	42.40	3.21	53.48	5080.00	13.90	72.40
E19-A	3.34	8.46	105.60	10.40	10.20	20.40	47.20	2.72	52.84	5280.00	13.50	79.60
E24-A	3.34	8.27	126.00	13.40	12.10	20.20	44.40	2.87	55.29	4740.00	13.80	72.80
E25-A	3.36	8.42	121.60	10.00	13.30	18.20	40.40	2.62	61.66	4060.00	14.80	70.40
E32-A	3.37	8.64	105.80	9.80	12.70	19.40	38.80	1.87	57.46	3640.00	14.60	74.10
E35-A	3.34	8.54	103.00	9.40	12.20	18.40	39.00	2.49	59.20	3920.00	15.60	71.50
Average	**3.28**	**8.23**	**108.64**	**10.72**	**11.84**	**20.42**	**46.44**	**3.11**	**53.87**	**5408.00**	**13.58**	**72.56**
Standard deviation	0.10	0.27	9.73	1.55	0,97	1.30	6.35	0.81	4.54	1325.96	1.27	2.63
Min.	3.07	7.78	98.40	9.20	10.20	18.20	38.80	1.87	45.58	3640.00	10.70	69.60
Max.	3.37	8.64	126.00	13.80	13.30	22.00	56.60	4.79	61.66	7560.00	15.60	79.60
Variation amplitude	0.32	0.87	27.60	4.60	3.10	3.80	17.80	2.92	16.08	3920.00	4.90	10.00
Variation coefficient	2.99	3.33	8.96	14.46	8.19	6.38	13.68	25.96	8.43	24.52	9.38	3.62

Table 4. Correlation coefficient value for analyzed characters (2016-2019)

Specification	Grain width	Grain length	Stem length	No. of fertile tillers	Spike length	No. of spikelets/spike	No. of grains/spike	Grains weight/spike	TGW	Yield	Protein content
Grain length	**0.716**										
Stem length	0.088	0.115									
No. of fertile tillers	0.006	-0.127	0.304								
Spike length	0.425	0.362	0.484	0.153							
No. of spikelets/spike	-0.732	-0.843	-0.217	0.163	-0.540						
No. of grains/spike	-0.606	-0.664	-0.052	0.558	-0.247	**0.735**					
Grains weight/spike	-0.964	-0.817	0.012	0.100	-0.425	**0.769**	**0.694**				
TGW	**0.745**	**0.670**	0.386	-0.379	0.606	-0.853	-0.813	-0.744			
Yield	-0.808	-0.915	-0.108	0.154	-0.474	**0.883**	**0.842**	**0.880**	-0.784		
Protein content	**0.860**	**0.720**	0.153	-0.302	0.570	-0.816	-0.761	-0.860	**0.920**	-0.829	
Starch content	0.389	0.451	-0.240	-0.007	-0.513	-0.077	-0.150	-0.407	-0.040	-0.265	0.083

CONCLUSIONS

Experimented synthetic hexaploid wheat provides a valuable diversity for different traits, such as larger grains size or higher protein content.

Across the 10 amphidiploid lines, individual grain length varied between 7.78 (E6-A) and 8.64 cm (E32-A). Larger grain size is negative correlated with yield, but positive with protein content.

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PRODUCTIVITY AND BOTANICAL COMPOSITION OF NATURAL GRASSLAND (*Chrysopogon gryllus*) IN PASTURE AND HAY-MAKING MODE OF USE

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Abstract

The experiment was conducted in the region of Central Balkan Mountain, on natural grassland of Chrysopogon gryllus type and covered a five-year study period. The grasslands in the studied variants were a pasture harvesting (PH1 - 31.05.-09.06.; PH2 - 10.06.-19.06. and PH3 - 20.06.-29.06.) and as hay-making harvesting (HH1 - 30.06.-09.07; HH2 - 10.07.-19.07. and HH3 - 20.07.-31.07.) in three periods. The grasslands harvested at pasture maturity stage in the third decade of June had the highest yield of fresh (833.3 kg/da) and dry (280.6 kg/da) mass. The values of the traits in the studied variants significantly exceeded (P <0.001) the control by 55.5% and 64.8%, respectively. At hay-making mode of use with the highest productivity of fresh (988.2 kg/da) and dry (130.5 kg/da) mass, the grasslands were harvested in the second decade of July. The amount of formed biomass exceeded (P <0.001) the control by 39.6% and 30.5%, respectively. The long-term pasture and hay-making harvest led to the elimination of Chrysopogon gryllus from the botanical composition in the observed grasslands, and Agrostis capillaris established itself as an edificator with a dominant impact in the formed aboveground mass. The studied modes of use favored an increase in the share of leguminous meadow grasses (Lotus corniculatus, Trifolium pratense, Trifolium repens, Medicago falcata, Vicia cracca, Vicia sativa) which presumed better quality and higher nutritional value of pasture and hay.

Key words: *natural meadows and pastures, productivity, botanical composition.*

INTRODUCTION

Meadows and pastures in the mountainous regions of Bulgaria are a major source for animal feeding. The species diversity in the composition of the above-ground mass is directly dependent on environmental factors, the type of use and the applied environmentally friendly measures (Mitev et al., 2010 a, b; Kulakov & Sedova, 2013; Georgiev et al., 2017). The balanced approach between them is the basis for a better distribution of components in plant communities and production of feed with high economic value (Fattahi & Ildoromi, 2011; Zziwa et al., 2012; Vasileva & Enchev, 2018). Unsystematic management of natural and semi-natural grasslands, as well as human activity lead to the emergence of aggressive and invasive grass species, shrubs and trees and the loss of valuable species affecting the yield and quality of grass mass (Suding et al., 2008; Gross et al., 2009). The optimal cutting period and the technology for utilization of the above-ground mass have a positive impact on the botanical composition, productivity and longevity of the meadow and pasture vegetation (Stevanovıć et al., 2008; Pellegrini et al., 2010).

Climate conditions in the mountain regions of Bulgaria allow the development of perrenial grass-forage species (*Festuca pseudovina* Hask., *Festuca rubra* L., *Chrysopogon gryllus* L., *Agrostis capilaris* L., *Poa bulbosa* L., *Poa silvicola* Guss., *Poa pratensis* L., *Deschampsia flexuosa* L. Trin., *Poa alpine* L., *Phleum boehmery* Wit., *Phleum pretense* L. etc.) with high resistance to the environmental factors and a high biological potential (Kirilov & Mihovski, 2014; Popescu & Churkova, 2015). In the pasture phase, they provide easily digestible biomass, which is readily accepted by ruminants, which is a prerequisite for improving the quality of animal production (Kulakov & Sedova, 2013; Dimitrova-Hristova, 2019). The selective and sharp reduction of the number and types of perennial meadow grasses requires a purposeful and regulated selection of agro-technical measures for maintenance and application of systems for the use of grass stands (Tanova et al., 2008). In pasture and hay-making, pasture and mowing times affect yield and variability in floristic composition of the grassland (Kemp & Michalk, 2005). Knowledge of these patterns aims to create favourable conditions for better recovery and development of valuable species in different botanical groups (Bovolenta et al., 2008) and obtaining feed with higher nutritional value. Grass communities respond to changes in the environment both through species turnover and through intraspecific biological and morphological changes (Volf et al., 2016). Local species (legumes and cereals) form highly productive and long-lasting grasslands (Naydenova & Mitev, 2008; Mitev et al., 2011). The mode of use can be associated with their limited distribution or create conditions for their more stable participation in the grassland. Pasture use favours low-growing species (Li et al., 2013), and hay use of grass cover stimulates high-growing grasses.

The aim of the present study was to determine the impact of the harvest period on the bioproductive indicators of fodder from natural meadows (*Chrysopogon gryllus* type) in the region of Central Balkan Mountain.

MATERIAL AND METHODS

The experiment was conducted on natural grasslands, a meadow of bunch grasses (*Chrysopogon gryllus* L. type), at the foot of Central Balkan Mountain (latitude_N - 42° 54' 37", longitude_E - 24° 41' 31" and 515 m above sea level) and covered a period of five years (2013-2017). The pasture and hay-making productivity of the grassland in the first regrowth was monitored. Two modes of use were applied for three harvest periods (early, medium-early and late), with respective dates of implementation, as indicated in Table 1.

Table 1. Variants according to the mode and dates of use of natural grassland of *Chrysopogon gryllus* L. type

Variants	Pasture harvesting (PH)	Variants	II. Hay-making harvesting (HH)
PH1	Pasture (Control) – from 31. May to 09. June	HH1	Mowing (Контрола) from 30. June to 09. July
PH2	Pasture from 10. June to 19. June	HH2	Mowing from 10. July to 19. July
PH3	Pasture from 20. June to 29. June	HH3	Mowing from 20. Юли to 31. July

The early terms of pasture harvesting in tasseling phenophase (PH1) as well as hay harvesting in flowering phenophase of bunch grasses (HH1) were used as control variants. The medium-early and late harvesting periods in both modes were determined after a 10-day and a 20-day period, respectively, compared to the control harvesting period.

The grass cover was used without management before the experiment. During the first three years (2013 and 2014) in the experimental variants, we applied stockpile fertilization with N6P6. Fertilization with triple superphosphate (containing 44–48% P2O5) was applied once in the autumn (September-October). Nitrogen fertilization (NH4NO3) was also used once in spring (April). Experimental data were statistically processed by analysis of variance (ANOVA).

Research indicators

Yield of fresh and dry mass (kg/da) - the yield of fresh and dry mass was determined by mowing the area of each harvest plot (in replications) followed by drying the plant samples (0.5 kg) in a laboratory dryer at 105°C and recalculating for an area of 1 da, based on dry mass content.

Botanical analysis of grassland (%) - determined by weight analysis of grass green mass samples taken at each mowing of each variation. The percentage share of each species per year from the group of grasses and legumes, of motley grasses (total) in both modes of use and their total ratio in the main botanical groups (grasses, legumes and motley grasses) was established.

Agroclimatic characteristics in the experimental area

Climate conditions during the experimental years affect the development of plants (by species and groups), productivity and quality of grass cover (Andreeva et al., 2015).

In 2014, autumn-winter precipitation shown the highest amount (1164. 9 mm) compared to the other experimental years the vegetation and autumn-winter precipitation shown the highest amount (1164.9 mm) compared to the other experimental years (Table 2).

The relative difference in the precipitation amount in 2015 (922.7 mm) and 2017 (983.2 mm), as well as in 2013 (807.3 mm) and 2016 (837.0 mm) compared to the maximum value of the feature varied respectively from 18.5 to 26.2% and from 39.2 to 44.3%.

For the experimental period, the highest average air temperature (11.9°C) and the highest average temperature for the months of July, August and September (22.4°C) were measured in 2015.

The relative difference in the average air temperatures for 2013 (11.4°C), 2014 (11.0°C) and 2016 (11.1°C) year varied from 0.9 to 3.6%. The data on the temperature level show that the lowest average annual temperature (10.5°C) was measured in 2017. The interesting thing in this case is that in the fifth harvest year, the active vegetation of the plants in the grassland began at the highest average temperature in March (8.1°C) compared to the other experimental years.

Table 2. Average monthly temperatures (T°C) and monthly precipitation amounts (mm) of 2013-2017

Year / Month	2013	2014	2015	2016	2017
	Precipitation				
I-III	155.6	145.6	179.5	207.2	151.9
IV-VI	457.0	322.3	312.2	333.6	336.7
VII-IX	98.8	498.1	282.0	151.2	238.7
X-XII	95.9	198.9	149.0	145.0	255.9
Total	**807.3**	**1164.9**	**922.7**	**837.0**	**983.2**
	Temperature				
I-III	3.4	4.6	2.9	4.6	1.6
IV-VI	16.1	14.6	14.7	15.8	14.8
VII-IX	19.2	18.7	22.4	19.1	19.1
X-XII	6.9	6.0	7.3	4.7	6.5
Average	**11.4**	**11.0**	**11.9**	**11.1**	**10.5**

RESULTS AND DISCUSSIONS

Productivity of natural grassland depending on the harvest period

On average for the five-year study period, the highest yield of fresh (833.3 kg/da) and dry mass (280.6 kg/da) was gathered from grasslands harvested at **pasture maturity** stage during the third decade of June (20.06.-29.06.) - Table 3. The values exceeded the control (31.05.-09.06) by 55.5% (fresh mass) and 64.8% (dry mass), respectively, with a high degree of proof (P <0.001). This trend was also observed in the period 10.06.-19.06., where the amount of fresh and dry biomass was 47.4 and 49.9% (P <0.01) higher than that of the control. The obtained data give grounds to assume that the most favorable period for pasture harvesting of grasslands of *Chrysopogon gryllus* type in the conditions of the Central Balkan Mountain was the second half of June.

Table 3. Yield of fresh and dry mass in kg/da at natural grassland of bunch grasses depending on the terms of harvesting, average for the period 2013-2017

Variants	Fresh mass		Dry mass	
	kg/da	% compared to PH1	kg/da	% compared to HH1
Pasture harvesting				
PH1	536.0	100.0	170.3	100.0 -
PH2	789.8	147.4	255.3	149.9
PH3	**833.3**	**155.5**	**280.6**	**164.8**
$LSD_{0.05}$	119.4	22.3	40.5	23.8
$LSD_{0.01}$	180.8	33.8	61.3	36.1
$LSD_{0.001}$	290.5	54.3	98.5	57.9

Hay-making harvesting				
HH1	707.8	100.0	259.1	100.0
HH2	**988.2**	**139.6**	**337.9**	**130.5**
HH3	756.2	106.8	268.6	103.7
$LSD_{0.05}$	100.8	14.2	30.8	11.9
$LSD_{0.01}$	152.7	21.5	46.7	18.0
$LSD_{0.001}$	245.3	34.5	75.0	29.0

In **hay-making mode**, the mowing period had a significant impact on plant development. The grasses harvested in the second ten days of July (10.07.-19.07.) ave the highest productivity. The amount of biomass exceeded (P <0.001) the control by 39.6% (fresh mass) and 30.5% (dry mass), respectively. With advancing age and development phase (insemination, flowering) of the grassland, the yield of aboveground biomass decreased. Statistical data processing shows an unproven and insignificant difference in the values of dry and fresh mass between the observed variants and the regulated controls.

Botanical composition of natural grassland of *Chrysopogon gryllus*

The sustainable development of phytocenoses is related to a system that includes the specific relationships between the vegetation factors of the environment and the requirements of the plants in the pasture-meadow biomass. In pasture and hay-making harvesting, only *Agrostis capillaris* is the species with relatively constant participation in all variants by years (Figures 1, 2, 3, 4 and 5).

In 2013, with pasture mode, the share of *Agrostis capillaris* at a later harvest date decreased from 8.8 (PH2) to 50.0% (PH3) compared to the control.

Festuca pseudovina participated in the composition of the grassland only during the first vegetation year, as in the period 10.06-19.06 (PH2), the presence of *Festuca pseudovina* in grass biomass was over 140% lower than in the control period. The participation of the species in the second variant was completely opposite, where it dominated by more than 120% compared to the control.

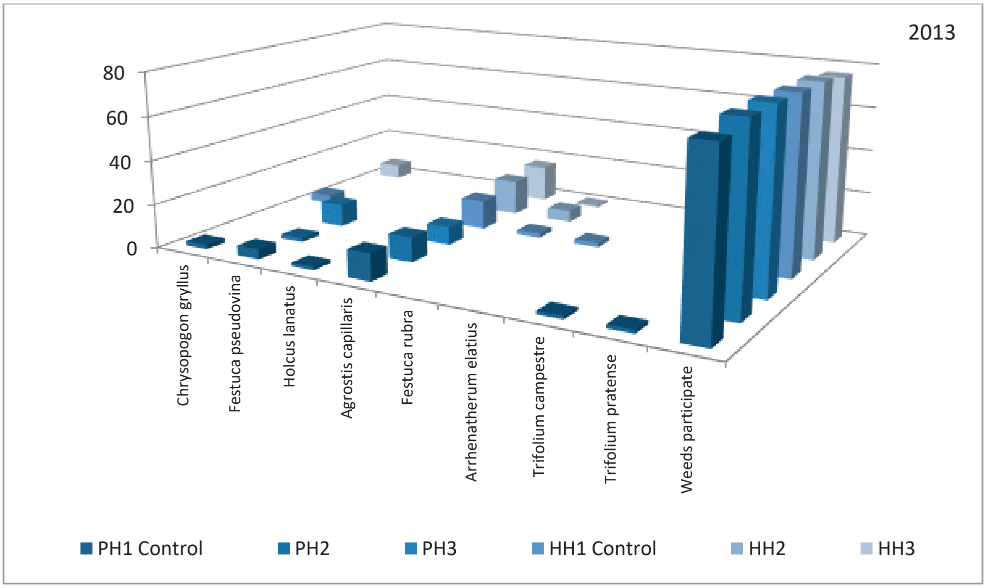

Figure 1. Botanical composition (%) of natural grassland of *Chrysopogon gryllus* L. type in 2013

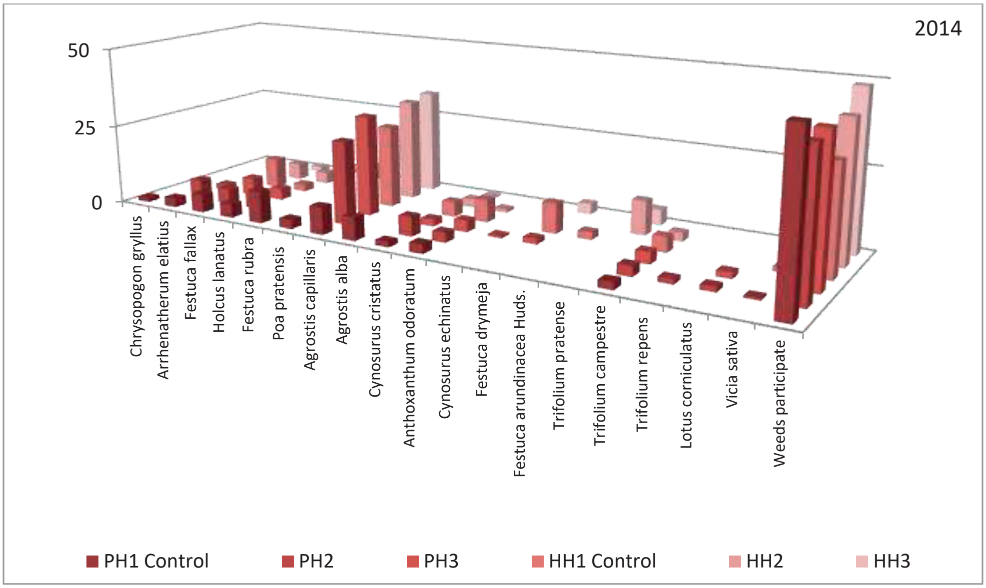

Figure 2. Botanical composition (%) of natural grassland of *Chrysopogon gryllus* L. type in 2014

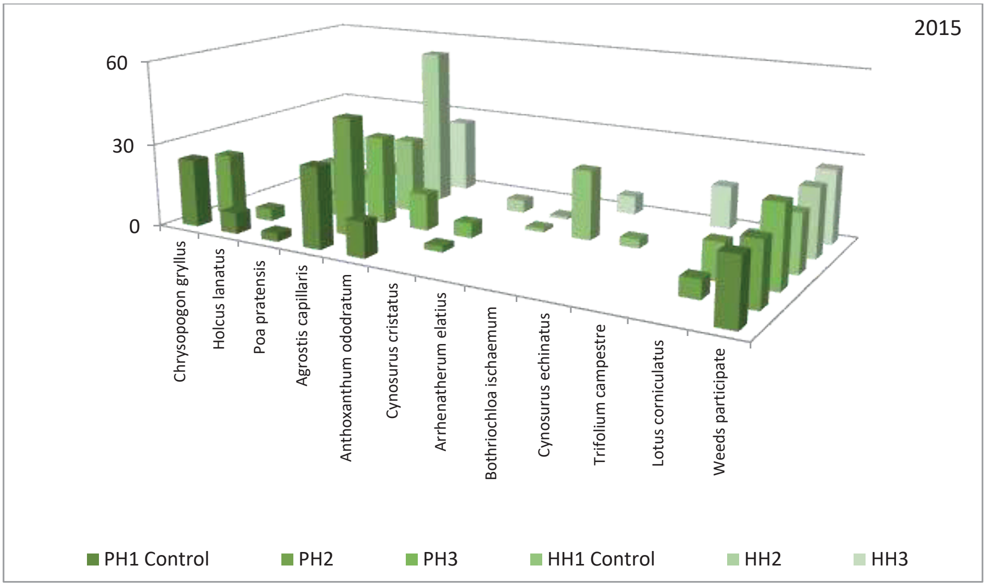

Figure 3. Botanical composition (%) of natural grassland of *Chrysopogon gryllus* L. type in 2015

The grasslands formed in the year, with the highest soil and air humidity (2014), are characterized by the richest floristic composition in pasture harvesting. Of the grass components, *Agrostis capillaris* occupied the largest share in the grassland in the variants in both modes of use. In percentage terms, the representatives of the species in the second and third variant occupied 26.0 and 31.4% respectively at 8.6% in the control variant in pasture mode, and 31.7 and 32.6% in 25.8% in the control variant in hay-making mode, followed by those of:

- *Festuca fallax*: 4.6 and 5.5% compared to 5.7% in the control variant in pasture mode, and 5.3 and 1.6% with 9.4% in the control variant in hay-making mode;

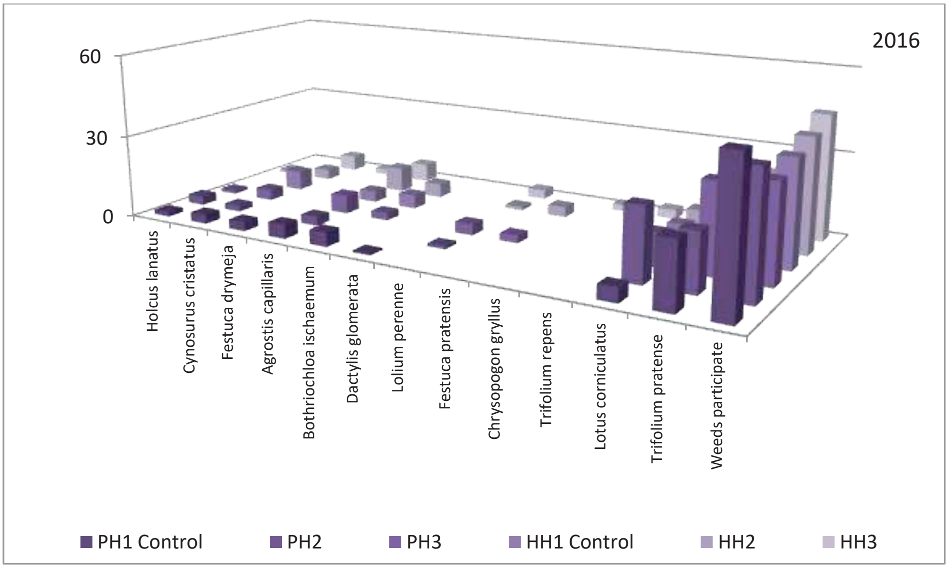

Figure 4. Botanical composition (%) of natural grassland of *Chrysopogon gryllus* L. type in 2016

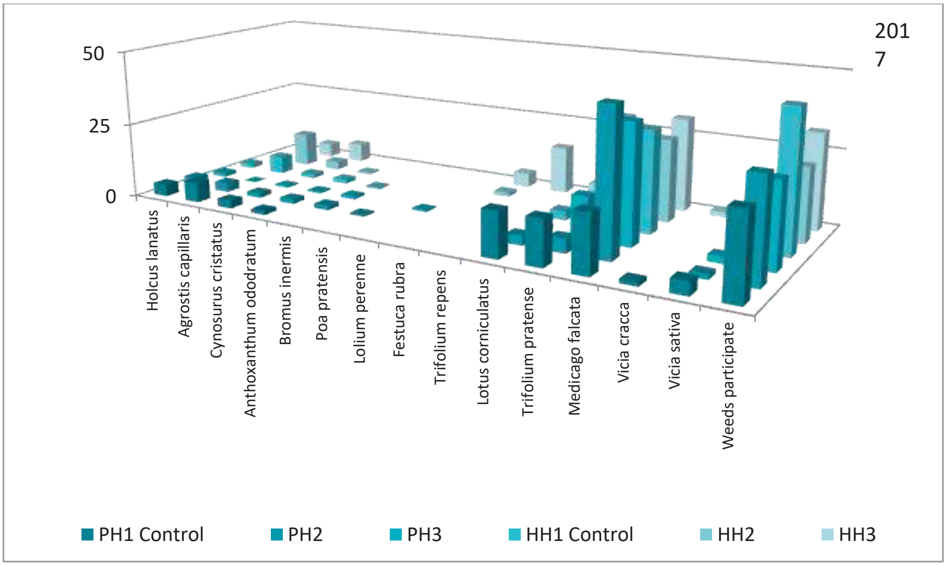

Figure 5. Botanical composition (%) of natural grassland of *Chrysopogon gryllus* L. type in 2017

- *Holcus lanatus*: 4.6 and 3.6% compared to 4.3% in the control variant in pasture mode, and 3.7 and 3.3% compared to 2.3% in the control variant in hay-making mode;
- *Cynosurus cristatus*: 6.1 and 2.2% compared to 1.7% in the control variant in pasture mode, and 2.6 and 0.7% compared to 4.7% in the control variant in hay-making mode.

In 2014, French ryegrass registered a share from 1.8 to 6.1% in pasture options alone.

Anthoxanthum odoratum is one of the grass species with an increased share during the spring and summer growth of the researched grassland in the second experimental year. Pasture harvest increased by 6.9-24.1% the share of *Anthoxanthum odoratum* in the second and third variants of the control. The hay-making use mode points to a declining trend (1.1% at 7.0% in control variant) regarding the share of this species in above-ground biomass at a later harvest date.

In pasture mode variants, *Festuca rubra* and *Poa pratensis* participated in the composition of observed grasslands only in the period 31.05.-09.06. (control variant) and in the last ten days of July (hay-making mode). Single plants of the species *Cynosurus echinatus* and *Festuca drymeja* were also found in later harvest dates.

The share of the representatives of the Family *Fabaceae* (*Trifolium campestre*, *Lotus corniculatus*, *Trifolium repens* and *Vicia sativa*) was 0.6 to 3.6% (in pasture mode) and from 1.4 to 4.7% (in the case of a hay-making mode). In 2014, the white clover and bird's-foot-trefoil did not have a share in the formed above-ground mass in July.

In the year with the highest average air temperature (2015), the percentage share of *Agrostis capillaris* followed a growing trend in grasslands of pasture and hay-making mode. In variants with an earlier (10.06.-19.06. and 10.07.-19.07.) and later harvest date (20.06.-29.06 and 20.07.-31.07), the species prevailed by 45.0-112.4% and 1.0-8.6% respectively relative to the controls. *Holcus lanatus* recorded the highest share in the grass cover formed in the period 31.05-09.06. (control - 7.6%) and 30.06-09.07. (control - 13.8%) in both types of harvesting. The species is also involved in the composition of the grass mass in PH2 variants (4.4%), HH2 (5.2%) and HH3 (4.4%). The annual share of *Anthoxanthum ododratum* ranged from 12.8% (PH1) to 13.2% (PH3). It was found only within the range of the marked parts of grassland in mode/period of pasture use. A similar trend was observed in *Poa pratensis*, except in control variants (PH1 – 3.1% and HH1 – 9.2%), the species was mainly present (7.9%) in the grass cover formed in the 20-29th June *Cynosurus cristatus* is a tuft-like perennial grass common in high-mountain pastures, spread mainly on more moist to dry soils (Velev et al., 2011). High summer temperatures and low soil and air humidity during the year limited the share (PH2 – 2.2% and PH3 – 5.3%) of the species to the pasture harvesting modes in the grassland and its absence in the variants with hay harvesting.

In 2015, *Arrhenatherum elatius* (HH1 – 1.4% and HH2 – 1.0%), *Bothriochloa ischaemum* (HH1 – 24.9% and HH3 – 6.7%) and *Cynosurus echinatus* (HH1 – 3.2%) participated only in the marked hay-making parts of the grassland, the above-ground mass for the purpose of hay-making.

Of the legume representatives, *Lotus corniculatus* exhibited high plasticity in pasture (6.7-13.2%) and hay-making (5.2-6.7%) grasslands. *Trifolium campestre* participated by 15.6%, only in the grassland formed at the end of July (HH3).

In the fourth experimental year (2016), *Cynosurus cristatus* and *Agrostis capillaris* were continuously involved in the grasslands of all variants in both modes of use.

Grasses include the following species: *Holcus lanatus* (1.3-3.3%), *Festuca drymeja* (2.2-3.4%), *Bothriochloa ischaemum* (2.7-5.2%), *Dactylis glomerata* (0.7%), *Lolium perenne* (1.1-4.0) %) and *Festuca pratensis* (2.7-3.8%).

Compared to previous experimental years (2013, 2014 and 2015), the share of legume meadow grasses in the grassland during the vegatation period in the fourth attempt was higher. The share of *Lotus corniculatus* (PH1 – 5.2%, PH2 – 26.1%, PH3 – 12.0% and HH1 – 10.0%, HH2 – 10.3%, HH3 – 9.9%), *Trifolium pratense* (PH1 – 23.4%, PH2 – 20.7%, PH3 – 32.0% and HH1 – 36.0%, HH2 – 24.4% and HH3 – 22.0%) and *Trifolium repens* (HH3 – 3.3%) implies the formation of a grass mass of better nutritional value.

With an increase in the period of mode use, the quantitative and species composition of the representatives of the family *Fabaceae* increased. In the last experimental year (2017), during the vegetation period, the above-ground grass mass of the pasture and hay-making harvesting variants had the highest share of the following species:

- *Medicago sativa subsp. falcata* - (PH1 – 18.9%, PH2 – 46.6%, PH3 – 38.6% and HH1 – 33.1%, HH2 – 27.0%, HH3 – 30.8%);
- *Trifolium pratense* - (PH1 – 14.9%, PH2 – 4.7%, PH3 – 13.8% and HH1 – 7.5%, HH2 – 32.7%);
- *Lotus corniculatus* - (PH1 – 14.9%, PH2 – 3.1%, PH3 – 1.4% and HH1 – 3.0%, HH3 – 4.7%);
- *Vicia sativa* - (PH1 – 4.4%, PH2 – 1.6%, PH3 – 2.2%);
- *Vicia cracca* - (PH1 – 1.4%)
- *Trifolium repens* - (HH3 – 15.7%).

Cynosurus cristatus and *Agrostis capillaris* are typical representatives of grasses which participate in the grasslands of all variants in both modes of use. *Holcus lanatus* occupied 4.1% in the biomass of the control variant in pasture harvesting. In variants with later harvesting, the quantity of the species decreased to 1.4%. The composition of the natural grassland is also enriched by species such as:

- *Anthoxanthum ododratum* - (PH1 – 1.4%, PH2 – 1.6%, PH3 – 0.6% and HH1 – 1.5%, HH2 – 0.6%),
- *Bromus inermis* - (PH1 – 4.4%, PH2 – 1.6%, PH3 – 1.1% and HH1 – 0.6%),
- *Poa pratensis* - (PH2 - 0.6%),
- *Lolium perenne* - (PH3 – 0.6%)
- *Festuca rubra* - (HH2 – 1.4%, HH3 – 4.7%).

Impact of the period and mode of use on the botanical composition of natural grasslands

The interaction between environmental conditions and applied management practices (Pacurar & Rotar, 2004) have an impact on diversity in the composition of natural grasslands. In this experiment, **pasture harvesting** resulted in the gradual dropping of *Chrysopogon gryllus* from the botanical composition of the grasslands in the fourth and fifth experimental year. Earlier pasture harvesting decreased the percentage share of the valuable rhizome rare tufted plants such as: *Poa pratensis, Festuca pratensis, Festuca fallax, Cynosurus cristatus*, *Cynosurus echinatus* and *Agrostis alba*. On the other hand, the pasture use mode increased the share of species with creeping and low stems (genus *Trifolium*). Genotypic factor successfully determines abiotic restriction and specific adaptation of legumes to environmental conditions (Naydenova & Vasileva, 2019 a, b). The pasture of legumes in a later phase allowed the formation of generative organs and supported their natural reproduction. Between 20.06.-29.06, the percentage share of the dominant during the year - *Trifolium pratense* and *Lotus corniculatus* was increased by 36.8% and 8% and 130.0% (2016) compared to that in the control date in grassland in pasture mode of use (31.05.-09.06.). The mode of use supports better development of species such as: *Medicago falcata* and *Vicia sativa.* In contrast, survey data show that the grazing (pasture mode) of grasslands in the early, medium and late phases limits the development and spread of *Trifolium repens* and *Trifolium campestre*.

Mowing in an earlier phase inhibits the development of the so-called indicator plants and significantly affects the botanical composition and productivity of natural grasslands (Duru et al., 2010; Duru et al., 2014). Earlier mowing did not allow insemination and self-sowing of certain grasses (*Festuca pseudovina, Arrhenatherum elatius, Poa pratensis, Cynosurus echinatus, Dactylis glomerata* and *Lolium perenne*) and led to their removal from the grass cover. The use mode allowed the validation of rhizomes (of genus *Trifolium*) and long-lasting grasses (*Agrostis capillaris*, *Holcus lanatus*, *Anthoxanthum ododratum*) to improve the floristic composition, meadow and pasture grasslands in the conditions of the Central Balkan Mountains. The motley grasses group is a constant presence factor in the above-ground grass mass of the variants under both harvest modes. In later mowing (in a period with high atmospheric temperatures), soil drought and compaction inhibits the growth and development of motley grass species, as a result of which the share of economically significant grasses and legumes has increased.

CONCLUSIONS

In the conditions of the Central Balkan Mountains, the most favourable period for pasture harvesting of grassland of *Chrysopogon gryllus* type was the second half of June, when the yield of fresh (833.3 kg/da) and dry mass (280.6 kg/da) exceeded the control by 55.5 % and 64.8 %, respectively. At hay-making mode of use with the highest productivity of fresh (988.2 kg/da) and dry (130.5 kg/da) mass, the grasslands were harvested in the second decade of July. The excess over control was 39.6% and 30.5%, respectively. Pasture and hay-making harvesting enriched the spectrum of the typical grasses for the region (*Agrostis capillaris*, *Holcus lanatus*, *Festuca rubra*, *Lolium perenne*, *Cynosurus cristatus*, *Bromus inermis*, *Festuca fallax*) and legumes (*Trifolium pratense*, *Lotus corniculatus* and *Medicago sativa subsp. falcata*). The long-term pasture and hay-making harvesting led to the elimination of

Chrysopogon gryllus from the botanical composition in the observed grasslands, and *Agrostis capillaris* established itself as an edificator with a dominant impact in the formed aboveground mass.

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INTRODUCTION OF MUSHROOM WASTE IMPACT ON SOIL FERTILITY AND YIELD OF SPRING WHEAT

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Abstract

The paper discusses the possibilities of the practical use of organic substrates after cultivation of fungi - basidiomycetes on them. The waste resulting from the cultivation of mushrooms is rich in nutrients and can be recycled as soil fertilizer and can also contribute to the remediation of polluted soils. The composition of the mycelial-substrate complex of the oyster fungus (Pleurotus ostreatus), in addition to the enzymatically active mycelium that absorbs pollutants, includes the mass of straw, which also contains nutrients and is able to improve the structure of the soil and increase its fertility. Straw material contains about 95% of organic matter. The dynamics of the humus content in soil samples was studied as a result of the introduction of P. ostreatus fungus production waste as a fertilizer. The use of fungus waste allows maintaining and slightly increasing (by 0.08–0.14%) the humus content in the soil. It also increases the yield of spring wheat to 29.5%.

***Key words**: mushroom waste, soil fertility, soil bioremediant.*

INTRODUCTION

Soil is the most important natural resource that ensures the existence of the biosphere and human beings as a part of it. As a result of anthropogenic activities, the soil is destroyed, its fertility is reduced and pollution is becoming global. The sources of pollution of agricultural land are mineral fertilizers, pesticides, some of which contain phenolic, organochlorine compounds, mercury and other heavy metals that accumulate in the soil, affecting its quality. Therefore, special attention is paid to restoring the productivity of disturbed soils and improving environmental conditions (Kachmazov, 2017).

Xylotrophic basidiomycetes, possessing a rich complex of nonspecific enzymes - polyphenolokidases that are able of destroying phenolic pollutants, including their chlorinated derivatives, were a topical object of research for a significant period of time (Muller et al, 1988; Álvarez-Martín, 2016; Sun et al, 2018). During the cultivation of fungi in the frame of biotechnological researches, the waste results from the cultivation of mushrooms, made up of the growing substrate and mycelium, have been actively discussed (Grimm et al, 2018).

The sorption properties of spent mushroom substrates also make them promising for the purification of media (Marín-Benito, et al, 2016; Menk, et al, 2019).

The aim of this work was to assess the possibility of using the waste of mushroom production - the spent mycelium of xylotrophic oyster mushrooms (*Pleurotus ostreatus*) as a bioremediant and a factor that increases soil fertility and plant productivity.

at increases soil fertility and plant productivity.

MATERIALS AND METHODS

The research was carried out on the basis of the Penza State Agrarian University. A marker compound (2,4 dichlorophenoxyacetic acid) was used to study the destruction of halogenated polyphenols by the enzymes of *Pleurotus ostreatus* mycelium. This substance is used as a herbicide. The experiment studied the dynamics of the content of this substance in model substrates under the influence of enzymes of the *Pleurotus ostreatus* mycelium. For this, 2.4 D were added to sterile nutrient substrates of fungi in an amount of 10 mg/kg of dry substrate, calculated on the active ingredient. Then, monthly, as the mycelium developed, the dynamics of the content of the model toxicant in

the substrate was noted. The control was a sterile substrate to which no fungal culture was added, but a similar concentration of the model compound was added.
To assess the change in the integral toxicity of extracts from the samples of the studied model substrates, biotesting techniques used in environmental studies were used. Biotesting is a technique of conducting analyzes to determine toxicity using living organisms. Toxicity is the degree of manifestation of the harmful effects of various chemical compounds and their mixtures. This is one of the important factors that determine the quality of the environment, quite informative, giving an idea of the degree of danger or safety of objects. Toxicity criterion is a reliable quantitative value of the test parameter, on the basis of which a conclusion is made about the toxicity of the object under study. Among the test parameters, the most often used are survival, fertility, suppression of the enzymatic and metabolic activity of organisms. Toxic effects recorded by biotesting methods include complex, synergistic, antagonistic and additional effects of all chemical, physical and biological components present in the test object, adversely affecting the physiological, biochemical and genetic functions of test organisms. In our studies, we used techniques based on the reactions of *Escherichia coli* and *Daphnia magna* test objects (Terekhova et al., 2014; Olkova et al., 2015).
Using the waste of the mushroom production of LLC "Botanik", located in the village of Lunino in the Penza region (Russia), which are straw cutting, partially fermented by the mycelium of the *Pleurotus ostreatus* fungus (Photo 1). A field experiment was carried out according to the following scheme:
1. No fertilizers (control);
2. Waste of mushroom production 4 t/ha (equivalent to 12 t/ha of manure in terms of carbon);
3. Waste of mushroom production 5 t/ha (equivalent to 15 t/ha of manure in terms of carbon);
4. Waste of mushroom production 6 t/ha (equivalent to 18 t/ha of manure in terms of carbon). The plot area was 5 m^2, the experiment was organized in four replicates, and the variants in the experiment were placed by the method of randomized repetitions. Spring wheat "Tulaykovskaya 10" was grown on the plots.

The soil in the area of the experiment is gray forest loamy, the humus content does not exceed 2.6%, which corresponds to the characteristics of soils of this type.
Statistical data processing was performed using the Excel functions of the Microsoft Office package.

RESULTS AND DISCUSSIONS

Through long-term cultivation of mycelium *Pleurotus ostreatus* on substrates using a marker compound (2,4 dichlorophenoxyacetic acid), it was found that the development of mycelium significantly decreases the content of the marker compound in the substrate (Figure 1).

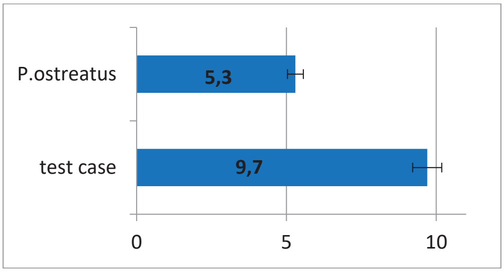

Figure 1. Content of 2,4 dichlorophenoxyacetic acid in the model substrate (mg / kg) under the influence of *Pleurotus ostreatus* mycelium after 12 months of cultivation ($p < 0.05$, error bars - mean error)

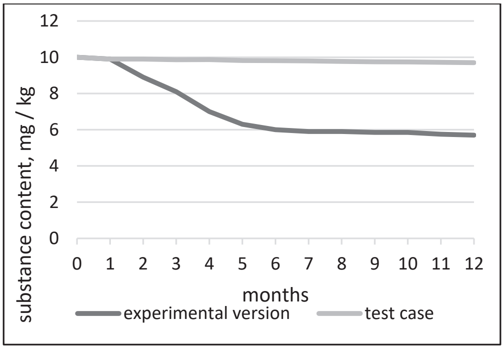

Figure 2. Dynamics of the loss of 2,4 dichlorophenoxyacetic acid in the model substrate (mg / kg) under the influence of *Pleurotus ostreatus* mycelium during the cultivation period ($p < 0.05$)

The results of studying the dynamics of the decrease in the content of the model pollutant are also indicative. It was found that the destruction of the substance by mycelium enzymes occurs unevenly, and the maximum

rate of loss falls on 3-5 months of development (Figure 2).
Such results could be explained by the most significant volumes of the formed mycelium biomass during this period. This, in turn, potentially provides the volumes of synthesized enzymes, as well as the area of the material that absorbs the pollutant molecules.
Considering the large volumes of waste oyster mushroom substrate resulting in mushroom growing, it should be noted that the mycelium of the studied fungus (*P. ostreatus*) can be considered as a bioremediant of soils contaminated with phenolic compounds.
The composition of the mycelial-substrate complex of *Pleurotus ostreatus* mycelium that is enzymatically active and absorbing pollutants, includes the mass of straw, which also contains nutrients that could improve the structure of the soil and increase its fertility. Since the straw contains about 95% of organic matter valuable for increasing fertility, and with five tons of straw, 20 ... 25 kg of nitrogen, 5 ... 7 kg of phosphorus, 60 ... 90 kg are returned (Shvetsova, 1988).
Studying the dynamics of the humus content, a mycelial-substrate complex was introduced into the experimental soil samples as a bioremediate - a waste after growing oyster mushroom. The reproduction of such a remediation technique can only be ensured by the volumes of waste of the fungus, which is widely cultivated in industrial conditions.
The results of a three-year field experiment shown that the humus content in soils without fertilization gradually decreases, while the use of mushroom waste as fertilizers allows maintaining and slightly increasing the humus content in the soil by 0.08 ... 0.14% (Table 1).

Table 1. The influence of wastes from mushroom production on the humus content in the soil (%, $p > 0.05$)

Experience Option	Humus content			
	original value	First year	second year	third year
The control	2.60	2.60	2.59	2.58
Waste from *P. ostreatus* production (4 t / ha)	2.62	2.64	2.73	2.70
Waste from *P. ostreatus* production (5 t / ha)	2.59	2.70	2.72	2.69
Waste from *P. ostreatus* production (6 t / ha)	2.61	2.72	2.75	2.73

Table 2. The influence of wastes from mushroom production on the spring wheat yield "Tulaykovskaya 10" ($p > 0.05$)

Experience Option	Productivity t / ha	Deviation from control	
		t / ha	%
No fertilizer (control)	2.34 ± 0.21	-	-
Waste from *P. ostreatus* production (4 t / ha)	2.59 ± 0.18 *	0.25 ± 0.02 *	10.7
Waste from *P. ostreatus* production (5 t / ha)	2.87 ± 0.23	0.53 ± 0.03	22.6
Waste from *P. ostreatus* production (6 t / ha)	3.03 ± 0.19	0.69 ± 0.07	29.5

* *differences are unreliable*

The influence of wastes from *P. ostreatus* production on the spring wheat yield is shown in Table 2. The maximum yield was observed in the variant with the rate of 6 t/ha, where it was 3.03 t/ha before, which is higher than in the control, by 0.69 t/ha or 29.5%.
On average, over three years, field germination of spring wheat compared to control, according to the options relative to the control, increased by 1.3-10.8%. The most significant indicators, exceeding the control values, were obtained in the variant with the introduction of 6.0 t/ha of mushroom waste. The application of mushroom waste as soil fertilizer contributed to a significant increase in the spring wheat yield (Table 3).
Fertilization with waste from mushroom production has contributed to a significant increase in spring wheat yield (Table 2). The maximum yield was observed in the variant with the rate of 6 t/ha, where it was 3.03 t/ha compared to control, which is higher than in the control by 0.69 t/ha or 29.5%.
Mixing the waste substrates was carried out using mechanization means (Photo 2).

Table 3. The influence of wastes from mushroom production on the spring wheat yield "Tulaykovskaya 10" (p> 0.05)

Evperience option	Productivity t/ha	Deviation from control	
		t/ha	%
No fertilizer (control)	2,34±0,21	-	-
Waste from P. ostreatus production (4 t / ha)	*2,59±0,18**	*0,25± 0,02**	*10,7*
Waste from *P. ostreatus* production (5 t / ha)	2,87±0,23	0,53± 0,03	22,6
Waste from *P. ostreatus* production (6 t / ha)	3,03±0,19	0,69± 0,07	29,5

* *differences are unreliable*

Photo 1. Mycelial-substrate complex, which is a waste of mushroom production - straw cutting, partially fermented by the mycelium of the fungus

Photo 2. Mixing of volumes of introduced mushroom production wastes using mechanization mean

CONCLUSIONS

Spent mycelium on an organic carrier - waste from the production of oyster mushroom, possessing active enzyme complexes, are promising for bioremediation of soils contaminated with phenols.

In addition, the use of organic waste of mushroom production allows to improve the structure of the soil, having a positive effect on the humus content in the topsoil.

A significant increase in the yield of spring wheat "Tulaykovskaya 10" was established with the introduction of mushroom waste as fertilizers.

The greatest effect was obtained on the variant with the rate of waste application of 6 t / ha. Thus, the use of mushroom production wastes provides both an environmental effect in terms of bioremediation of contaminated agricultural soils and an agronomic effect in terms of maintaining soil fertility and increasing crop yields.

Waste from the production of oyster mushroom possessing an active enzyme complexe are important for bioremediation of soils contaminated with phenols.

In addition, the use of organic waste from mushroom production allows improving the structure of the soil, having a positive effect on the humus content in the arable soil layer.

A significant increase in the yield of spring wheat "Tulaykovskaya 10" was recorded with the introduction of mushroom waste as fertilizers.

The greatest effect was obtained on the variant with the rate of 6 t/ha waste application. Thus, the use of mushroom waste provides both an environmental effect in terms of bioremediation of contaminated agricultural soils and an agronomic effect in terms of maintaining soil fertility and increasing crop yields.

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FIRST WINTER WHEAT VARIETY VARIABILITY BY PLANTS MORPHOLOGY FROM WHITE LUVIC SOIL CONDITIONS

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Abstract

The development of research to improve wheat began by creating local varieties with best possible yields, but also with increased adaptability) to the various crop conditions from us. The sufficiently wide genetic dowry obtained demonstrated that even in the conditions of white luvicsoil in the South, varieties with tolerance to aluminium ions can be obtained. The Albota variety obtained in 70th period and adapted under conditions have had proved new plant characteristics, required by the new intensification crop conditions. Compared to the Bezostaia 1 variety, some gains of morphological components were found, which contributed to the development over time of this new wheat breeding center. The study of morphological characters in newly created varieties), along with those of genetics and physiology, could be useful for the constant progress of wheat improvement. From the obtained data it was found that if the straw length of the two varieties was similar, its thickness at the base was higher for the Albota variety. The length of the spike/ear increased in Albota by 1.3 cm, the weight of the spike was by 0.4 g, and the number of spikelets in a spike was by 1.8. Instead, the Bezostaia 1 variety had an external glume and a slightly longer lower palea, and the number of grains in a spike was one unit longer (1 g). The grain sizes showed longer lengths and smaller thicknesses at Bezostaia 1. While the mass of one thousand grains (MTW) was larger at Albota. Positive correlations were obtained between the morphological characters, somewhat more obvious in the Albota variety. The data obtained demonstrate a progress of improving the morphological characteristics of the Albota variety, due to the gain of tolerance to the concentration of aluminium ions.

Key words: *Bezostaia 1 & Albota, grains, ear, variability, wheat.*

INTRODUCTION

Wheat [*Triticum aestivum* (L.) Thell ssp. *vulgare* (Will.) MK], (pro syn. *Triticum hybernum* L., *T. macha* Dekap. & Menab., *T. sativum* Lam., *T. sphaerococcum* Percival, *T. vulgare* Will., common wheat, bread wheat) is one of the most important crop (Balaj, 1990; Hancock, 2004; Olaf, 2009; Slafer et al., 1996). The name *Triticum* expresses the adaptation of the words *threshing* or *bruising* which means mechanical threshing, and *aestivum*, in summer, indicates the maturation of plants this season. The wheat that is threashed is also called *spelta*. By crossing with the species *Aegilops tauschii* were added to the wheat the resistance to cold, very important for cultivation in temperate climates and not only. The two researched varieties also contain the modern RHtB1b gene, which induces the short stalk/ straw, very important for more sustained fertilization and mechanized harvesting, elements necessary for their cultivation in intense conditions (Mohamed et al.,, 1990; Hoisington et al., 1999). In general, wheat has a terminal spike inflorescence (Balaj, 1990; Shewry, 2009), distich, 4-18 cm long, with sterile spikelets, caught solitary on the spike, zig-zagged. The spikelet is 10-25 mm long, being compressed laterally, it has two glume and flowers. The glume has the tip like a short, blunt tooth, but also a slightly smaller edge. Each flower has a palea and *lemma*. Depending on the variety, the lemma extends in the form of an edge, or as a hood. When the palea and the *lemma* adhere to the grain, it thus becomes dressed. The caryopsis- type bean has an ellipsoidal shape, with a central channel on one side. The grain is 4-11-14 mm long and 1.5-4.5 mm thick. The mass of one thousand grains (MTG) is between a minimum of 15 g and a maximum of 60-70 g. The plant generally forms stems with heights between 50(60) and 140(150) cm. Lower values are manifested in dwarf varieties. The researches carried out to observe the variation of some characters of wheat plants from the two varieties included: i) the stem by the total length of the straw and the thickness of the basal internode, ii) the length and weight of the spike, iii) the number of spikelets/ spike, the length of the outer glume, the length of the lower glume (lemma) and the length of the awns, iv) the number of grains/ ear, their weight, the mass of the thousand grains (MTG) and the dimensions of the grains: length and thickness.

MATERIALS AND METHODS

In the last two years, the two varieties have been cultivated: *Bezostaia 1* (Moon, 2008) and *Albota* using the technology recommended by the resort. The experiments were set up according to the block method, with variants of 25 m^2 each in 4 replicates. At full maturity, 25 plants (stems) from each repetition were randomly selected (zig-zag method), which meant a total of 100. These stems were cut from the ground and brought to the laboratory. The following were measured and determined for the 100 stems: straw length, basal internode diameter, spike length and weight, number of spikelets in spike, length of glume and lemma, number of grains in an ear and their weight, mass of one thousand grains (MTG), as well as grain sizes: length and thickness. These morphological characters (Slafer et al., 1996; Slafer & Satorre, 1999) of the wheat plants obtained were then analyzed by histogram method. Both class intervals and absolute values were used in their expression. Each histogram was established according to the specific sequence of values obtained. Following the establishment of the respective intervals, the modal values (with the highest frequencies), the limits of the variability intervals of the studied characters and the specificity of each character of the two cultivated wheat varieties resulted. The correlations between the analyzed characters were also established, with the help of which their tendencies within the studied ecotypes could be observed. Excel was used to express values. The significance of the correlation coefficients was obtained by comparing with the r_{max} values for the levels of 5%, 1% and 0.1% of the transgression probabilities. The statistical calculation was also performed, which was based on the analysis of variance (Anova test), namely on the variation strings. Statistical indices were obtained using the formulas: $\bar{a} = \frac{\Sigma x}{n}$, where $\bar{a}$ = mean of determinations, and x = the values, S^2 (variance) $= \frac{1}{n-1}\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]$, S (standard error) = $\sqrt{S^2}$, and S % (variation coefficient) = $\frac{S}{\bar{a}}100$.

RESULTS AND DISCUSSIONS

Variability in wheat straw size. The wheat stalk or straw is segmented by several internodes (5-7) with lengths towards the ear. Straw length is between 40-50(60) cm for intensive varieties and up to 130-150 cm for extensive varieties. At the harvest maturity, the straw has a vertical position, being suitable for mechanized harvesting. The measurements showed that the straw of the two varieties of wheat was between 43 and 76 cm. A relative similarity was found between the varieties (Figure 1). The straw frequency was 58-61 cm (22-23%), followed by those with 64 cm (16%). The diameter (thickness) of the straw at the base was differentiated between the two varieties. In the *Bezostaia 1* variety, the straw was between 2.0 and 3.4 mm, and in *Albota* in the range of 2.2-4.0 mm (Figure 2). They dominated the segments with 2.6 mm in the first variety (29%) and respectively with 3.0-3.2 in the second variety (30-35%). From these data it appears that the Albota variety, having a slightly thicker straw, could represent a gain in increasing the plant’s favorability (Bonjean & Angus, 2001; Evans, 1993; Willcox & Willcox, 2006) to the degree of crop intensification (Săulescu et al., 2006).

Variability of wheat ears. The appearance and size of the ear of the two varieties had different characteristics. Thus, the length of the ear ranged between 6 and 10 cm for the *Bezostaia 1* wheat variety and between 6 and 11.5 cm for the *Albota* variety. The lengths of 8 cm (28%) for the first variety of wheat and those of 9 cm (20%) for the second variety (Figure 3) dominated. And in the case of the length of the ears, there is an increase in the length of the ear in the *Albota* variety.

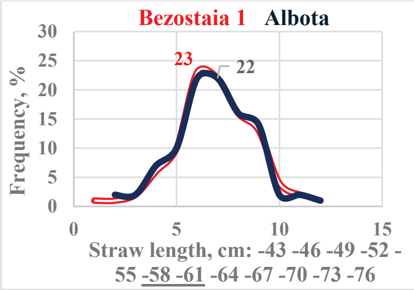

Figure 1. Frequencies of straw length

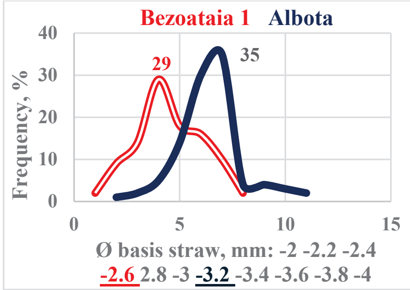

Figure 2. Frequencies of straw thickness

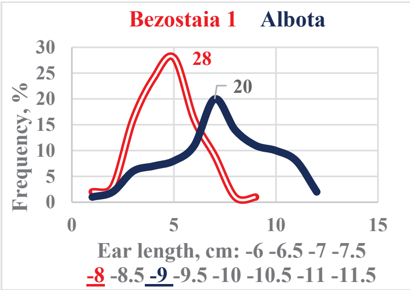

Figure 3. Frequencies of ear length

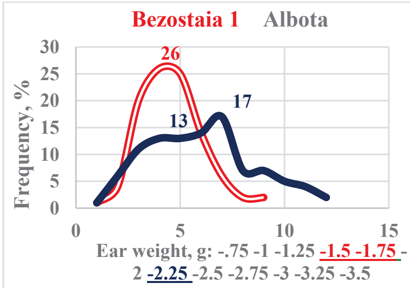

Figure 4. Frequencies of ear weight

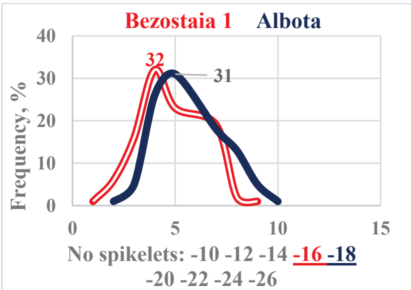

Figure 5. Frequencies of no. spikelets/ear

The weight of the ears was between 0.75 g and 2.75 g for the first variety and between 0.75 g and 3.5 g for the second variety (Figure 4). They dominated th ears whose weights were between 1.5-1.75 g in the *Bezostaia 1* variety (25-26%) and 2.25 g in *Albota* (17%). The most obviuos tendency to improve the morphology of the ear was found in the case of the total weight of the ear, which in *Albota* was increasing. The number of spikelets in one spike ranged from 10 to 28 for both varieties (Figure 5). They dominated the ears with 15-16 spikelets (32%) in the first variety (the reference one) and those with17-18 spikelets in the second variety (31%). The glume of the spikelets had different lengths. These were between 6 and 11 mm (Figure 6). In the case of *Bezostaia 1* wheat, 8 mm long (56%) glumes from the 7-11 mm range dominated, while in the *Albota* variety, 8 mm (38%) glumes from the 6-10 mm range also dominated. In general, the *Bezostaia 1* variety had noticeably longer membranes than *Albota*. The lower palea (lemma) was generally between 7 and 12 mm long. Both varieties has sizes between 7 and 12 mm, with a frequency of 40% for the first and 562% for the second wheat variety, respectively (Figure 7). And in this case the *Bezostaia 1* variety had grouped values at significantly larger dimensions than the *Albota* variety.

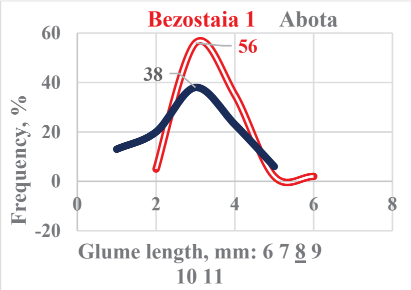

Figure 6. Frequencies of glume length

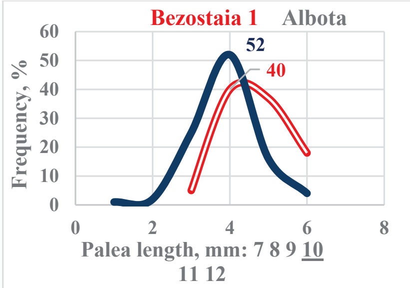

Figure 7. Frequencies of palea length

Wheat grain variability. Research has shown that each variety has characteristic aspects related to grains. In the case of the number of ears in an ear, the values were between 15 and 51. In both varieties, the ears with 28-31 grains had the highest frequency (29% in *Bezostaia 1* and 24% in the *Albota* variety), without finds obvious differences (Figure 8). The weight of the grains formed in an ear had corresponding values between 0.3 g and 2.3 g. The highest frequency was obtained at weights of 1.3 g (26%) for the *Bezostaia 1* variety and at the same weights, 1.3 g (19%) at *Albota* variety (Figure 9). The size of the grains also had some characteristics. Thus, the grain length was in the range of 5.5-8 mm, with a maximum of 7 mm (57%) in *Bezostaia 1* and in the range of 4-8 mm, with a maximum of 6.5 mm (29%) in *Albota* (Figure 10). The grain thickness was between 2.4 and 3.4 mm, with the modal value at 3 mm (45%) in the case of the first variety and between 2-3.8 mm with the dominant range of 2.9-3 mm (37%) in the case of the *Albota* variety (Figure 11).

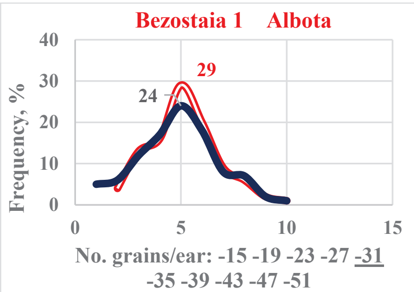

Figure 8. Frequencies of no. grains/ear

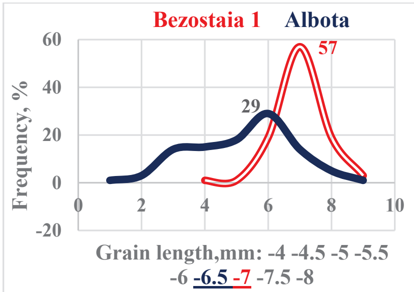

Figure 10. Frequencies of grain length

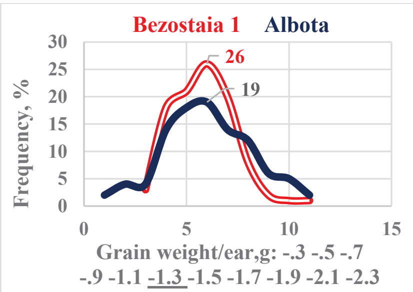

Figure 9. Frequencies of grain weight/ear

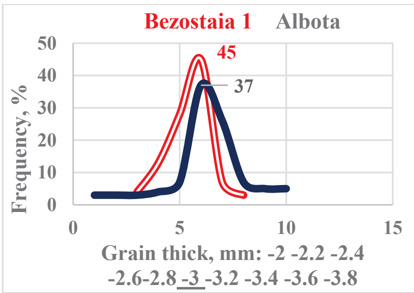

Figure 11. Frequencies of grain thick

The mass of a thousand grains (MTG) demonstrated several differences. The extremes of this character were between under 29 and 56 g. The range of variability in the first variety was 32-50 g, with the modal value at 39-41 g (38%). For the second variety, the range was between 29 and 56 g with the modal value also at 39-41 g (22%) (Figure 12). The characteristics of the

Albota variety plants are presented in figure 13. The aspects of the grains of Bezostaia 1 and Albota varioeties are presented in Figures 14 and 15.

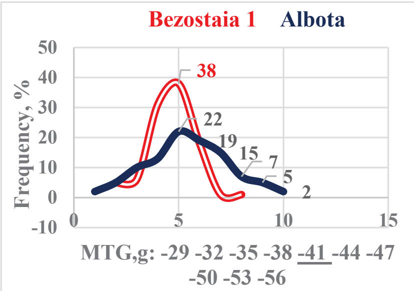

Figure 12. Frequencies of grains MTG

Figure 13. Wheat *Albota* variety

Figure 14. Wheat *Bezostaia 1* grains

Figure 15. Wheat *Albota* grains

Correlations between the main morphological characteristics of wheat plants. If we analyze the whole set of correlations between all the characters analyzed for the two varieties of winter wheat, we find specific situations. Thus, in wheat *Bezostaia 1* variety were obtained statistically assured correlations in most cases. Of these, the positive correlations betwen the weight of the ear and the other characters were noted. The grain length correlated insignificantly with some of the other characters. The mass of a thousand grains correlated very significantly positively with the thickness of the grains. A positive correlation was found between the number of grains in the spike with the grain length and an insignificant correlation between the number of spikelets in an ear with MTG (Table 1). In the *Albota* wheat variety, most correlations were statistically ensured at tighter levels than the first variety. One explanation would be that this variety has a more obvious adaptation to the ecology of white luvicsoils.

Statistical analysis of the variability of morphological characters in wheat. The results obtained in the morphological analysis of some characters for the two varieties of winter wheat, showed specific aspects. Thus, by comparing the *Bezostaia 1* and *Albota* varieties, the straw length measured on average 59.6 cm compared to 59.4 cm. The diameter of the straw at the base was a few millimeters more în *Albota*. The length of the ears was 7.7 cm in *Bezostaia 1* compared to 9.00 cm at the *Albota* variety. The weight of spikes was in the same comparison of 1.54 g compared to 1.90 g. The number of spikelets/ spike was on the one hand 16.2 compared to 18.0 (Table 2). The weight of the ear had greater variability in both varieties. Between the two varieties of wheat, the length of the glume was 8.4 mm compared to 7.6 mm, and that of the palea 10.7 mm compared to 9.7 mm. In the same order, the number of grains formed in an ear was 30.1 to 29.2. the weight of the grains in an ear was 1.18 g to 1.21 g. Tha grains had average dimensions of 6.9/2.8 mm compared to 6.0/3.0 mm. The mass of one thousand grains was 38.9 g in *Bezostaia 1* compared to 40.2 g in the *Albota* variety (Table 3). Greater variability was found in the number of grains in an ear and in the weight of the grains in both varieties.

Table 1. Correlations between the main morphological characters of the two varieties analyzed

Indices	Ø stem. mm	Ear length. cm	Ear weight, g	No. spikelets	Glume mm	Palea mm	Awns cm	No. grains/ ear	Grains weight, g	Grain length, mm	Grain thick, mm	MTG, g
Winter wheat, Bezostaia 1 variety												
Straw, cm	.332	.099	.396	.171	.166	.192	.060	.293	.393	.175	.208	.586
Ø stem mm	1	.501	.427	.403	.158	.046	.024	.471	.459	.165	.224	.181
Spike, cm		1	.603	.756	.324	.124	.044	.703	.592	.244	.067	-.030
Spike weight, g			1	.789	.361	.336	.091	.905	.977	.357	.233	.415
No. spikelets				1	.302	.217	.149	.838	.775	.322	.084	.077
Glume, mm					1	.233	-.156	.379	.341	.157	-.030	.072
Palea, mm						1	-.009	.345	.320	.095	.024	.080
Awn, cm							1	.057	.057	-.032	-.130	.024
No. grains								1	.898	.297	.091	.117
Grain weight, g									1	.355	.250	.437
Grain length, mm										1	.182	.164
Grain thick, mm											1	.377
MTG, g												1
Winter wheat, Albota variety												
Straw, cm	.094	.260	.375	.045	.134	.069	.303	.291	.365	-.316	.077	.343
Ø stem mm	1	.311	.279	.244	.309	.277	.237	.288	.246	.319	.379	.109
Spike, cm		1	.691	.752	.206	.299	.528	.618	.549	.078	.114	.255
Spike weight, g			1	.600	.160	.379	.648	.873	.943	.020	.253	.705
No. spikelets				1	.194	.190	.518	.587	.492	.036	-.032	.182
Glume, mm					1	.426	.213	.179	.127	.529	.217	-.024
Palea, mm						1	.246	.333	.376	.409	.272	.313
Awn, cm							1	.494	.547	.059	.166	.385
No. grains								1	.909	.064	.147	.497
Grain weight, g									1	.047	.267	.792
Grain length, mm										1	.585	.002
Grain thick, mm											1	.370
MTG, g												1
LSD 5 % = 0.19 DL 1 % + 0.25 DL 0.1 % = 0.32												

Table 2. Statistical indices of winter wheat straw and spike

Indices	High , cm	Internode 3, cm	Internode 2, cm	Internode 1, cm	Basis diameter, mm	Ear length cm	Ear weight g	No. spikelets
Winter wheat Bezostaia 1 variety								
Mean, ā	59.57	11.63	16.46	20.78	2.668	7.652	1.5351	16.21
Variance, s^2	29.025	1.533	3.867	17.769	.0982	.592	.163	6.612
Std. deviation, s	5.387	1.238	1.966	4.215	.313	.727	.404	2.571
Var. coef,. s%	9.04	10.65	11.95	20.29	11.73	9.51	26.32	15.86
Winter wheat Albota variety								
Mean, ā	59.40	8.94	14.92	22.78	3.046	8.953	1.895	17.97
Variance, s^2	31.07	2.208	5.216	15.042	0.096	1.104	0.430	7.464
Std. deviation, s	5.574	1.486	2.284	3.878	0.310	1.0505	0.6559	2.732
Var. coef,. s%	9.38	16.62	15.31	17.03	10.16	11.73	34.61	15.20

Table 3. Statistical indices winter wheat ear components

Indicii	Glume length, mm	Palea length, mm	Awn length, cm	No. grains/ ear	Grains weight g	Grain length mm	Grain thick mm	MTG, g
Winter wheat Bezostaia 1 variety								
Mean, ā	8.40	10.68	1.89	30.11	1.1775	6.859	2.846	38.893
Variance, s^2	5.50	.684	.483	47.28	.106	.1527	.0564	10.914
Std. deviation, s	2.357	.827	.695	6.876	.3258	.391	.2376	3.304
Var. coef,. s%	28.06	7.74	36.77	22.84	27.67	5.70	8.35	8.49
Winter wheat Albota variety								
Mean, ā	7.60	9.67	6.51	29.15	1.211	5.961	3.007	40.218
Variance, s^2	0.919	0.772	1.750	57.40	0.196	0.780	0.144	87.96
Std. deviation, s	0.9585	0.879	1.323	7.576	0.443	0.883	0.380	9.379
Var. coef,. s%	12.61	9.09	20.32	25.99	36.60	14.82	12.63	23.32

CONCLUSIONS

The morphological characteristics of winter wheat, studied in comparison to the two varieties, had specific aspects. The choice of the two wheat varieties was made due to the relatively close morphological similarity. The *Albota* variety has benefited from some new genetic improvements since the first period of breeding activity. It has a clear tolerance to soil acidity induced by aluminium ions, but also high production capacity, different tolerances.

By comparison between the two varieties, the stem/ straw had average lengths of both at 59 cm. The values obtained show the existence of low waist in both plants, a condition increasingly induced in the improvement of winter wheat to maximize crop yields (Acevedo et al., 1991).
The spike was 7.7 cm long in the first variety and 9.0 cm in the second variety, and weighted 1.5 to 1.9 g at Bezostaia 1. The number of spikelets was 16 to 18 in the same order. The spikelet pieces: glumes and paleas were quite sensible long for *Bezostaia 1*.
The number of grains in an ear was 30 to 29, weighting 1.18 g to 1.21 g, the mass of one thousand grains was from 39 g în *Bezostaia 1*, compared to 40 g in *Albota*. The grains were 7 to 6 mm in length and 2.9 to 3.0 mm in thickness. Through these results, the Albota variety demonstrated improved characteristics by the thickness at the base of the straw, the larger and heavier spike, but with low dimensions of the spikelet membranes (outer glume and lower glume) and relatively smaller grains.
Simple correlations were established between all the studied characters, with some small defferentiations. Thus, both between characters of the straw and those between the elements of productivity, mostly positive and significant correlations were obtained, closer to *Albota*. Very close positive links have been established between the components of the ear, which demonstrates the great productive possibilities that the two varieties of wheat have in the analyzed area.

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THE DYNAMICS OF WHEAT DISEASES IN THE PERIOD 2015-2019 IN THE MOARA DOMNEASCA LOCATION, ILFOV COUNTY

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Abstract

The aim of the research was to evaluate the presence and attack of wheat micromycetes in the period 2015-2019 within the Moara Domneasca Didactic Resort, Ilfov County. There was monitored the attack of micromycetes Blumeria graminis f sp. tritici, Zimo septoria tritici (FA Septoria tritici) Septoria spp. (FA) and Fusarium spp. and Puccinia recondita to the Glosa and Boema varieties in the period 2015-2017 and to the Katou, Pitbul and Jaguar varieties in the period 2017-2019. In the experimenting years of 2015-2017 for the Glosa variety, the lowest values of the attack on the detected pathogens were registered. In 2017-2018, the frequency of the Fusarium attack was noted, which was 4% for the Glosa variety and 65 for the Boema variety. In the conditions of 2018-2019 year, the Pitbul variety was noticed, on which the attack of Septoria spp. was observed in a percentage of 5.85%.

***Key words**: wheat, micromycetes, variety, degree of attack.*

INTRODUCTION

Wheat is an important crop in Romania and wherever this plant is the main source in bread preparation (Muntean et al., 2003). This is why the health of wheat crops is a permanent concern for fundamental and applied research. The importance of knowing the diseases and pathogens of wheat, the dynamics of their evolution are relevant in the elaboration of intervention schemes in stopping their attack. The application of the most effective and efficient control measures, their integration ensures superior productions from a quantitative and qualitative point of view (Weber et al., 2001). Wheat is attacked every year by fungal infections such as powdery mildew, caused by the micromycete *Blumeria graminis* f. sp. *tritici*, septoria, caused by the pathogens *Zymoseptoria tritici* and *Parastagonospora nodorum*, rust, the most widespread of which is the brown rust produced by the micromycete *Puccinia recondita*, fusariosis caused by *Fusarium* spp. If they are not subject to phytosanitary control, the attack of these pathogens can have major consequences for the grower, both in terms of placement in time and space of crops and harvest. Also, a considerable number of micromycetes are associated with wheat caryopsis, involved in the black point phenomenon (Cristea et al., 2008), which could have consequences for seed quality (Tamba-Berehoiu et al., 2010). Research on micromycetes associated with wheat seeds (Cristea et al., 2015; Cristea and Berca, 2013; Khanzada et al., 2002; Singh et al., 2011) showed that some of these micromycetes were also present on the seeds of some species of plants with which wheat usually enters in rotation and crop rotation in our agriculture (Dudoiu et al, 2016; Cristea et al., 2015, Cristea et al., Manole et al.2015, Mardare et al.,2015; Pană et al., 2014; Berca et al., 2014).

MATERIALS AND METHODS

The research took into account the wheat crop from the Didactic Resort from MoaraDomnească within USAMV from Bucharest, in the period 2015-2019. Observations were made on the attack of the present diseases and the pathogens responsible for their appearance in the period 2015-2019 were identified. The frequency (F%) and intensity (I%) of the attack were calculated. Frequency (F%) = nx100 / N, where N = number of plants observed (%), n = number of plants specific symptoms (%). The intensity was noted in percentages and calculated according to the formula: Intensity (I%) = Σ (ixf) / n (%) where, i = percentage given, f = number of plants (organs) with the respective percentage, n = total number of attacked plants (organs). The biological material was represented by the varieties Glosa and Boema (2015/2016, 2016/2017) and Katou and Pitbul (2017/2018) Jaguar and Pitbul (2018/2019). Observations were made in the plots before the treatments were performed. The seed was treated with specific products. Based on the obtained data, the degree of attack (GA%) was calculated. Degree of attack (GA%) = (FxI) / 100, where F (%) represents the frequency of the attack and I (%) the intensity of the attack. Regarding the attack of *Fusarium* spp. its frequency was noted considering the intensity as having maximum value, by extending the attack on the monitored ears.

RESULTS AND DISCUSSIONS

The research focused on the phytosanitary condition of wheat in the research location during 2015-2019. The research period was characterized by specific weather conditions that influenced the presence of wheat-specific pathogens and their dynamics (table 1 and table 2). The data from table 1 regarding the temperature values in the period 2015/2017 show higher values compared to the multiannual average values and the fluctuating precipitation values in their annual distribution compared to the multiannual average. In 2017/2018 the accentuated deficit of precipitations is noticed and in 2018/2019 a large amount of precipitations was found in May and June, compared to the multiannual average (table 2). In the climatic conditions of the experimental period for wheat cultivation in the localized research area, the following foliar and ear diseases were identified: powdery mildew, produced by the micromycete *Blumeria graminis* f. sp. *tritici*, leaf septoria caused by micromycete *Zimoseptoria tritici* (2015/2016). In 2016/2017, the observations regarding wheat diseases highlighted the presence of pathogens: *Blumeria graminis* f.sp. *tritici, Septoria* spp. *Fusarium* spp. and in 2017/2018 the attack of *Septoria* spp., *Fusarium* spp. was noted. In the agricultural year 2018/2019 in wheat crop there was an attack of *Septoria* spp. And *Puccinia recondita* f. sp. *tritici,* micromycete responsible for the appearace of brown rust. Observations and notations of the attack were made on wheat leaves in the case of pathogens *Blumeria graminis* f.sp. *tritici*, *Sept*oria spp. and *Puccinia recondita* f. sp. *tritici* and on ears and caryopsis in the case of *Fusarium* spp. attack.

Table 1. Climatic conditions in the agricultural year 2015-2017, Moara Domnească, Ilfov county

Month	Temperature (°C)		Rainfall (mm)		Temperature (°C)		Rainfall (mm)	
	2015-2016	Multiannual average	2015-2016	Multiannual average	2016-2017	Multiannual average	2016-2017	Multiannual average
October	10.7	11.0	81.7	35.8	11.2	11.0	70.0	35.8
November	7.7	5.3	17.6	40.6	6.5	5.3	32.5	40.6
December	3.1	0.4	1.2	36.7	2.0	0.4	25.5	36.7
January	-3.45	-3.0	33.2	30.0	-2.5	-3.0	41.0	30.0
February	1.79	-0.9	7.6	32.1	2.2	-0.9	17.5	32.1
March	7.58	4.4	37.3	31.6	15.0	4.4	44.5	31.6
April	14.34	11.2	116	48.1	16.5	11.2	90.0	48.1
May	15.86	16.5	88.0	67.7	18.6	16.5	47.3	67.7
June	22.41	20.2	113.0	86.3	22.4	20.2	46.8	86.3
July	24.18	22.1	38.0	63.1	26.4	22.1	105.2	63.1
August	23.08	21.1	26.2	50.5	24.2	21.1	37.1	50.5
September	18.9	17.5	60.6	33.6	19.1	17.5	37.0	33.6

Source: Afumaţi weather station, Ilfovcounty

Table 2. Climatic conditions in the agricultural year 2017/2019, Moara Domnească, Ilfov county

Month	Temperature (°C)		Rainfall (mm)		Temperature (°C)		Rainfall (mm)	
	2017-2018	Multiannual average	2017-2018	Multiannual average	2018-2019	Multiannual average	2018-2019	Multiannual average
October	11.5	11.0	70.9	35.8	13.8	11.0	10.4	35.8
November	7.2	5.3	55.1	40.6	5.0	5.3	53.7	40.6
December	2.4	0.4	30.0	36.7	-0.1	0.4	25.1	36.7
January	-1.3	-3.0	0.0	30.0	-1.2	-3.0	32.0	30.0
February	1.3	-0.9	0.0	32.1	3.4	-0.9	14.6	32.1
March	3.7	4.4	0.2	31.6	9.5	4.4	31.2	31.6
April	16.4	11.2	0.0	48.1	11.1	11.2	78.4	48.1
May	19.7	16.5	0.0	67.7	16.7	16.5	148.2	67.7
June	22.5	20.2	53.2	86.3	20.4	20.2	109.4	86.3
July	23.0	22.1	107.6	63.1	22.6	22.1	76.0	63.1
August	24.1	21.1	2.0	50.5	24.3	21.1	2.4	50.5
September	19.2	17.5	28.9	33.6	-19.1	17.5	4.8	33.6

Source: Afumaţi weather station, Ilfov county

The data from table 3 show that in 2015/2016 in wheat, the pathogens *Blumeria graminis* f. sp. *tritici* and *Zimoseptoria tritici* anamorphic form (*Septoria tritici*), were detected in both analyzed varieties. It can be seen that in the Glosa variety the value of the frequency of the powdery mildew attack was higher, of 72% and in the Boema variety of 65% but in terms of the intensity of the attack the higher value was noted in the Boema variety (I = 20%). The value of the degree of attack in case of powdery mildew was higher for the Boema variety. The degree of powdery mildew attack (*Blumeria graminis* f. sp. *tritici)* was 13% compared to 10.8% Glosa variety. As a result, the level of attack degree of fungus *Blumeria graminis* f. sp. *tritici* was determined by the frequency of attack, evaluated quite high in both varieties. As for the attack of the leaves, it can be seen that the frequency of the attack was 75% for the Glosa variety and 70% for the Boema variety. The intensity of the *Septoria* spp. attack was 20% for the Glosa variety and 25% for the Boema variety, respectively. This shows that although the values are close, in the Glosa variety the pathogen was more aggressive, and in the Boema variety more virulent. The values of the attack frequency were high for both varieties. Thus, the values of the degree of attack were 15% (Glosa) and 17.5% (Boema) (Table 3).

Table 3. Phytosanitary condition of wheat, M. Domnească, Ilfov county, 2015-2017

Variety	The pathogen / 2015-2016						The pathogen/ 2016-2017						
	Blumeria graminis f. sp. *tritici*			*Zimoseptoria tritici* (FA *Septoriatritici*)			*Blumeria graminis* f. sp. *tritici*			*Septoria* spp. (FA)			*Fusarium* spp.
	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)	F (%)
Glosa	72	15	10,8	75	20	15	58	12	6.96	56	15	8.4	4
Boema	65	20	13	70	25	17.5	45	14	6.3	75	17	12.75	6

In the conditions of 2016/2017 for the same varieties, the attack of powdery mildew, septoria (FA *Septoria* spp.) and the frequency of attack of fusarium wilt on ears were noted. It was found that the attack of the pathogen *Blumeria graminis* f. sp. *tritici* had values close to the two varieties of 6.3% (Boema) and 6.96% (Glosa). Under this year's conditions, the values of the frequency of the powdery mildew attack were lower, of 58% for the Glosa variety and 48% for the Boema variety. Regarding the intensity of the attack, it had values of 12% for the gloss variety and 14% for the Boema variety. As a result, the values of the powdery mildew attack were lower compared to the previous year. Regarding the attack of the *Septoria* spp. fungus, it was found that in the Boema variety the value of the attack degree was 12.75% compared to 8.4% in the Glosa variety. In the case of the attack of *Septoria* spp. micromycetes, the data in table 3 show that the Boema variety had a high level of attack frequency of 75%, even higher than the attack of the previous year. In contrast to the Glosa variety, the intensity level was lower by 56% compared to 75% in the previous year. The intensity of the attack was lower, of 15% for the Glosa variety and 17% for the Boema variety. In both varieties the intensity values were lower than in 2015/2016. So, in the conditions of 2016/2017, a low value of the attack of septoria on leaves was noted. In 2016/2017, the frequency of the attack of fusariosis on the ears was also observed and noted. The micromycete *Fusarium* spp. had an incidence of 6% in the Boema variety and 4% in the Glosa variety, the micromycete being known for the attack on ears and wheat caryopsis (Ittu et al., 2010). As a result, in the conditions of the period 2015-2017, at the Glosa variety, lower levels of the detected micromycete attack were determined compared to the Boema variety (Table 3). The presence of *Fusarium* spp. fungus on caryopsis implies a special attention on the treatment of wheat seeds and their quality in conditions similar to the experimental year. *Septoria* spp. micromycetes are common year after year in wheat (Cioneag et al., 2015) and the conditions at the monitored location also favored the attack of *Blumeria graminis* f. sp. *tritici* and *Fusarium* spp.

Table 4. Phytosanitary condition of wheat, M. Domnească, Ilfov county, 2017-2018

Variety	The pathogen/ 2017-2018					
	Blumeriagraminis f. sp. *tritici*			*Septoria* spp.(FA)		
	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)
Katou	80	20	16	85	20	17
Pitbul	45	15	6,75	65	15	9.75

In 2017/2018, the varieties Katou and Pitbul varieties were cultivated. The attack of powdery mildew and septoria on wheat leaves was observed and noted. Also, the frequency and intensity of the attack of the two micromycetes were evaluated and the degree of attack was calculated. Regarding the powdery mildew attack, it was observed in the Katou and Pitbul varieties, with frequency values of 80% in the Katou variety and 45% in the Pitbul variety. Intensity of pathogen attack *Blumeria graminis* f. sp. *tritici* was 20% in the Katou variety and 15% in the Pitbul variety. As a result, an attack value of 16% was calculated for the Katou variety and 6.75% for the Pitbul variety. As it results from the data of table 4 for the Katou variety, the highest values of the frequency and intensity of the leaf powdery mildew attack were registered. As a result, we consider that the high value of the attack frequency determined a high level of attack degree. The septoria attack recorded the highest frequency value, 85% for the Katou variety. Also, the value of the intensity was quite high, namely 20%, which led to a level of attack of 17%, the highest in the analyzed period. In the Pitbul variety, the values of frequency and intensity of septoria attack were lower, of 65% and 15%, respectively. The level of attack by *Septoria* spp. reached 9.75%. It was found that in the Katou variety the attack values were higher than in the Pitbul variety in both monitored pathogens (Table 4). In 2017/2018, the Pitbul and Jaguar varieties were cultivated and analyzed. In 2017/2018, the Pitbul and Jaguar varieties were cultivated and analysed (Table 5).

Table 5. Phytosanitary condition of wheat, M. Domnească, Ilfov county, 2018-2019

Variety	The pathogen/ 2018-2019								
	Blumeria graminis f. sp. *tritici*			*Septoria* spp. (FA)			*Puccinia recondita*		
	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)
Pitbul	-	-	-	45	13	5.85	-	-	-
Jaguar	48	11	5.3	65	20	13	35	13	4.55

In 2018/2019, the varieties analyzed, Pitbul and Jaguar, were attacked by *Blumeria graminis* f. sp. *tritici,* and *Puccinia recondita* (Jaguar) and *Septoria* spp (Jaguar and Pitbul). In the case of the Jaguar variety, the powdery mildew attack had values of 5.3% on the leaves, the *Septoria* spp. attack was 13% and the brown rust attack of 4.55% was also calculated. The intensity of the septoria attack registered values of 20%, one of the highest values in the monitored period for the analyzed varieties The Pitbul variety was also noticed this year, on which only a reduced attack of septoria of 5.85% was observed. No attack of powdery mildew and brown rust was detected on wheat leaves of the Pitbul variety (table 5). Both the environmental conditions and the genotype contributed to the onset and evolution of the attack of pathogens specific to agricultural crops (Cristea, 2005; Iacob et al., 1998; Cioneag 2015).

CONCLUSIONS

During the analyzed period in wheat culture, the micromycetes *Blumeria graminis* f.sp. *tritici, and Septoria spp*. the attack of *Fusarium* spp. was presented in the conditions of 2016/2017 for the varieties Boema and Glosa. In 2018-2019 the Pitbul variety presented an attack of *Septoria* spp. with reduced values of 5, 85 %. In the conditions of 2018-2019, the Pitbul variety did not show an attack of powdery mildew and brown rust

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RESEARCH ON THE IDENTIFICATION OF HIGH PRODUCTIVITY WINTER WHEAT VARIETIES AND LINES, TESTED ON LUVISOL FROM ȘIMNIC IN THE PERIOD 2004-2018

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Abstract

Over the period 2004-2018, on the luvisol from Șimnic were tested 394 varieties and lines of winter wheat of different origins, at least 3 years each, in order to identify those with high productive potential and a high degree of adaptability to the pedoclimatic conditions in the area. The analysis of the results was made in terms of average yields obtained from the varieties and lines of wheat tested. The registered values had extreme limits: 267 kg / ha for the Bancal variety in 2015 and 8420 kg/ha for the Gruia variety in 2004. The average preponderant classes were 4001-5000 kg/ha and 5001-6000 kg/ha, with 25% of the results placed in each of them. The fact that the Glosa variety registered the highest average yield over a period of 15 years (5078 kg/ha) certifies the results of this research, knowing that this variety is the most cultivated and the most stable variety in Romania.

Key words*: wheat, luvisol, yield, correlation, stability.*

INTRODUCTION

Bread wheat is one of the most widely grown and most consumed food crops all over the world. The breeding programs need to produce germplasm capable of maximizing the agricultural potential of specific areas and of minimizing the occurrence of crop failures or very low yields in unfavorable years. The cultivars well adapted confer stability and diminish the risks (Bunta, 2020).

The use of genetic diversity at the territorial level, by cultivating in each area several different varieties, is the simplest and most accessible way to reduce the fluctuation of wheat crops (Săulescu et al., 1980). Also, the cultivation of varieties with wide adaptability to contrasting environmental conditions can reduce the risks of declining yield in unfavorable years (Mustăţea et al., 2008).

Previous research mainly focused on accurate and real-time monitoring of the occurrence and development droughts (Aghakouchack et al., 2015), but very few reports address the impacts of droughts on growing crops and related crop yield (Yu et al., 2018). Droughts with different intensities that occur during different growth stages of crop can have distinctly different impacts on crop yield (Cakir, 2004).

The incipient droughts that occurred during the wintering period of the winter wheat growth helped to increase the winter wheat yield, while the mild droughts that occurred during the maturity stage of winter wheat resulted in a reduction yield. The mild droughts that occurred during the filling and maturity period had a significant effect on the yield of winter wheat (Yu et al., 2018). Along with climate impact a range of regional and global political and economic factors intensify food insecurity and long term vulnerability in certain regions (Paraschivu et al., 2017, 2019).

A study comparing 14 wheat varieties was carried out in Secuieni during 2007-2010. The highest yields were obtained for the varieties: Glosa (8991 kg/ha), Boema 1 (8224 kg/ha), Delabrad 2 (8084 kg/ha), Faur F (8062 kg/ha) and Gruia (7932 kg/ha). Of the three years of experimentation, one year was characterized as a normal year in terms of rainfall and with a uniform distribution during the wheat vegetation period, namely, the year 2007-2008, the year in which the largest wheat yields were also made (Pochișcanu et al., 2011).

All over the world, multi-annual studies have been carried out on the adaptability and stability of wheat varieties. In general, the relatively small yields of wheat can be explained by the negative influence of the lower water supply of the soil in the unfavorable years and the high number of weeds in the case of monoculture and 2-year rotation (Partal and Paraschivu, 2020).

Using data from 1973-2004, Stone and Schlegel (2006) reported that grain yield was correlated with soil water available for the plant at germination but also with rainfall during the growing season. Studies conducted by Xiao et al. (2008) between 1981 and 2005, in the semi-arid area of China, showed that once there were changes in temperature and precipitation, there are also significant changes in the phenology of the plant whose yield is higher at high altitude. In the face of climate change, wheat yield in this region is higher at high altitudes than at low altitudes. By 2030, wheat yield is expected to increase by 3.1% at low altitude and by 4% at high altitude.

For the period 1978-1995, in the southern part of China, Li et al. (2010) found that precipitation variability explains 23-60% of yield variability, while temperature variability justifies 37-41% of yield variability.

The climatic impact on wheat in Picardy (region in northern France) and Rostov (region in southern Russia) was studied by Licker et al. (2015) for a period of 37 years (1973-2010). During this period, the summer precipitation in Rostov decreased by 61% while the summer temperatures increased by 4ºC. In Picardy the total precipitations decreased by 9% while the maximum spring temperatures increased by 2.4ºC. Wheat yield was strongly correlated with the number of climate variables. The average temperatures in May and June explained 49%, respectively 16% of the variability of yield in Rostov, while the precipitation in November and the minimum summer temperature explain 26%, respectively 23% of the yield.

MATERIALS AND METHODS

Over the period 2004-2018, on the luvisol from Șimnic were tested 394 varieties and lines of winter wheat of different origins, at least 3 years each, in order to identify those with high productive potential and a high degree of adaptability to the pedoclimatic conditions in the area.

The analysis of the results was done with the help of yield.

The applied technology did not include foliar treatment, only seed and vegetation treatment for pests.

To present the distribution of values from a series of data, the boxplot method was used, which marked the variants with large deviations from the average. Highlighted were those that were clearly detached from the outliers values (exceeded by more than 1.5 to 3 times the interval in which 50% of the values found themselves) and extreme ones (exceeded 3 times or over, same range) (Hawkins, 2009).

The study was performed for each year and on average for cultivars tested for at least 3 years.

The minimum values, the maximum values, the amplitude, the distribution of values and the cultivars that registered yields over 2000 kg / ha regardless of the climatic conditions were highlighted.

The correlations between the average yields and those obtained in the driest year (2007), on the one hand, and in the rainiest year (2018), on the other hand, were calculated.

RESULTS AND DISCUSSIONS

The number of cultivars tested for 15 years varied greatly depending on the year - from 27 in 2004 to 469 in 2016 (Figure 1). The results underlying this study came from cultivars tested for at least 3 years but there were varieties that were tested throughout. These include the Glosa variety.Depending on the number of years of testing, when taking into consideration the yield, the following varieties are leading: Unitar - 5440 kg / ha on average for 3 years; Falado - 6304 kg/ha on average for 4 years; Gabrio - 6442 kg/ha on average for 5 years; Basmati - 5077 kg/ha on average for 6 years; Mv Martina - 5056 kg/ha on average for 7 years; Pajura - 4562 kg/ha on average for 8 years; Bitop - 4058 kg/ha on average for 9 years; Brakes - 4265 kg/ha on average for 10 years; Orion - 4656 kg/ha on average for 11 years; Gruia - 4666 kg/ha on average for 12 years; Cezanne - 4906 kg/ha on average for 13 years; Exotic - 5213 kg/ha on average for 14 years and Glosa - 5078 kg/ha on average for 15 years.

The fact that the Glosa variety recorded the highest average yield over a period of 15 years, certifies the results of this research, knowing that this variety is the most cultivated and the most stable variety in Romania. The study provides valuable information for the wheat breeding program on identifying genetic sources with a high degree of adaptability.

Figure 1. Number of cultivars tested each year

The distribution of yield according to the year of experimentation was much differentiated. In the years 2004 (Figure 2), 2007 (Figure 6), 2008 (Figure 7), 2009 (Figure 8), 2011 (Figure 10), 2012 (Figure 11), 2013 (Figure 12), 2015 (Figure 14), 2017 (Figure 16) and 2018 (Figure 17), most yields were grouped mainly in a single class. In 2005 (Figure 4), 2006 (Figure 5), 2010 (Figure 9), 2014 (Figure 13), 2016 (Figure 15), most yields were grouped into several classes, which indicates that the favorable climatic conditions were their characteristic.

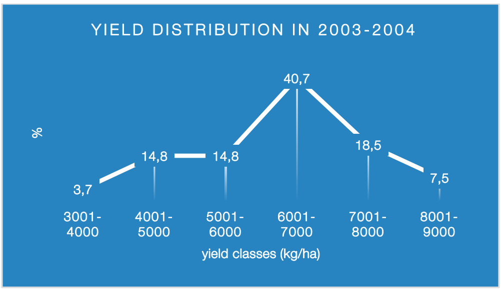

Figure 2. Yield distribution in the agricultural year 2003-2004

The values of the registered yields had extreme limits between 267 kg/ha for the Bancal variety in 2015 and 8420 kg/ha for the Gruia variety in 2004 (Figure 3). The limits (minimum and maximum yield) of each year were totally different from each other. The only variety that appeared twice was Apache but as a lower limit in 2012 (3156 kg/ha) and as an upper limit in 2006 (5450 kg/ha).

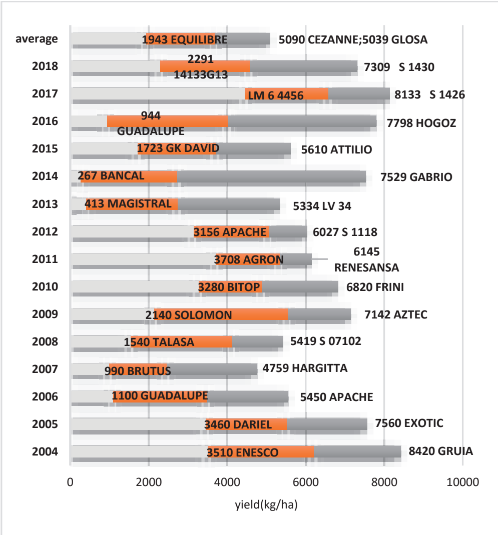

Figure 3. Extreme limits of yield obtained in each year of testing

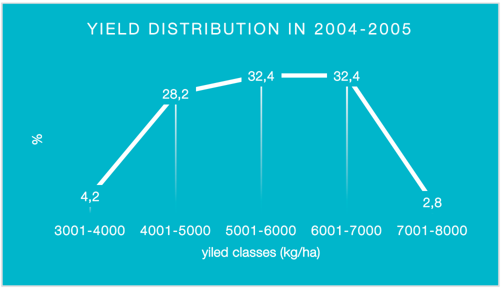

Figure 4. Yield distribution in the agricultural year 2004-2005

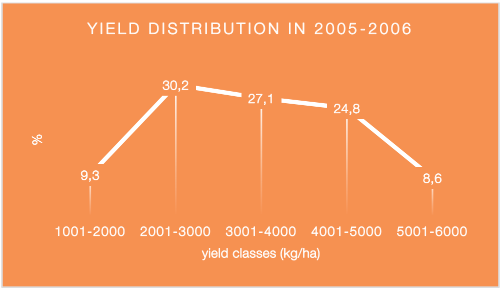

Figure 5. Yield distribution in the agricultural year 2005-2006

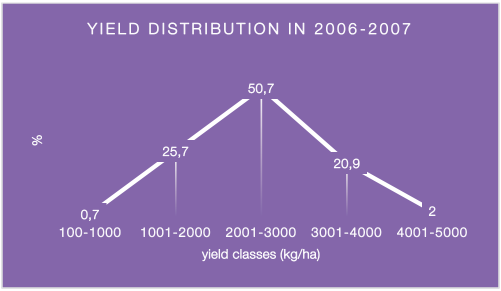

Figure 6. Yield distribution in the agricultural year 2006-2007

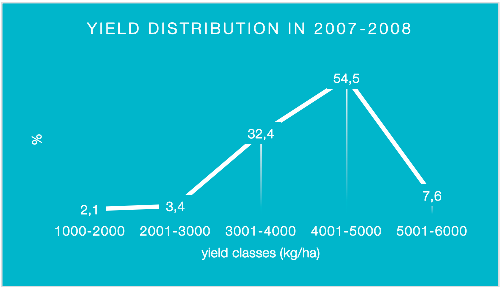

Figura 7. Yield distribution in the agricultural year 2007-2008

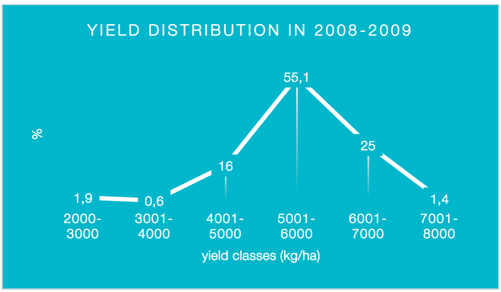

Figure 8. Yield distribution in the agricultural year 2008-2009

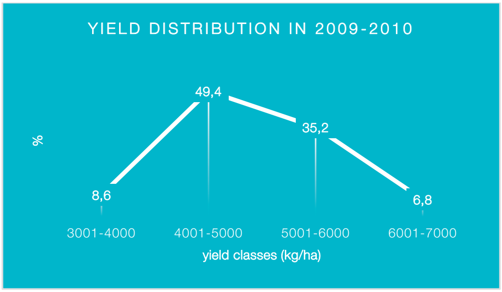

Figure 9. Yield distribution in the agricultural year 2009-2010

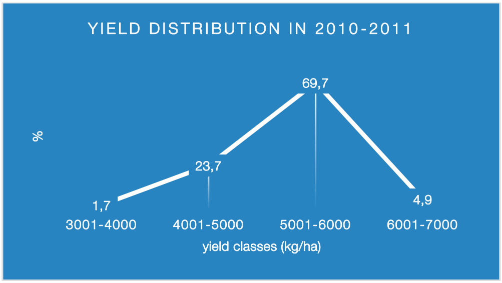

Figure 10. Yield distribution in the agricultural year 2010-2011

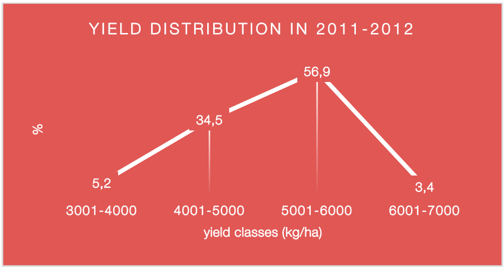

Figure 11. Yield distribution in the agricultural year 2011-2012

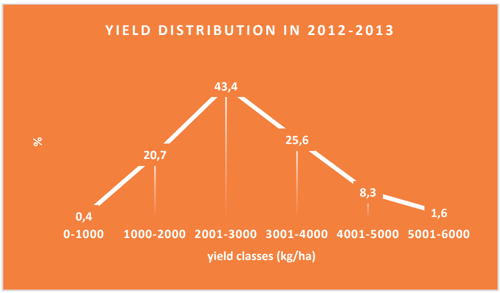

Figure 12. Yield distribution in the agricultural year 2012-2013

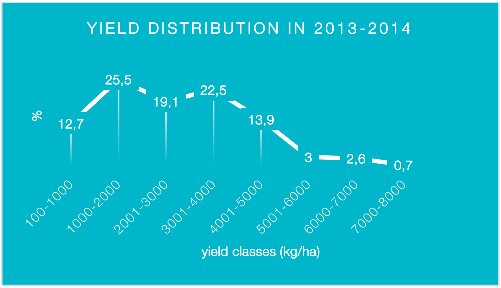

Figure 13. Yield distribution in the agricultural year 2013-2014

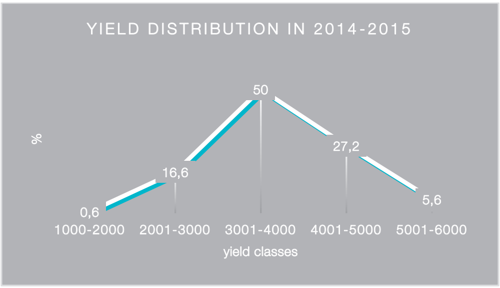

Figure 14. Yield distribution in the agricultural year 2014-2015

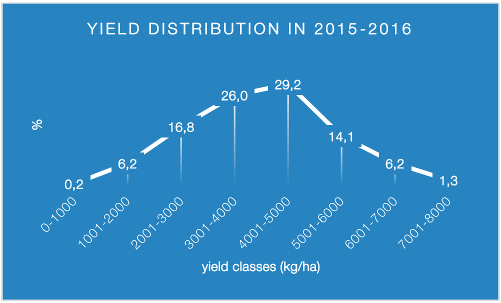

Figure 15. Yield distribution in the agricultural year 2015-2016

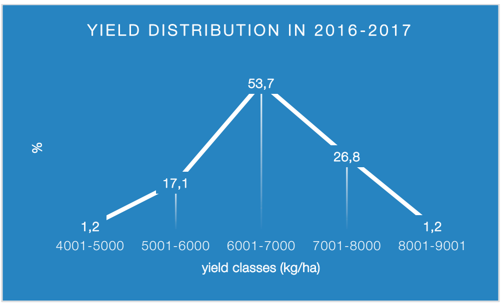

Figure 16. Yield distribution in the agricultural year 2016-2017

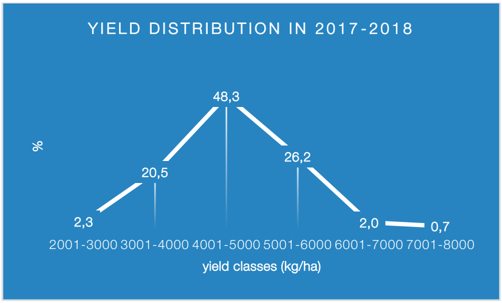

Figure 17. Yield distribution in the agricultural year 2017-2018

The average preponderant classes for the studied period were 4001-5000 kg/ha and 5001-6000 kg/ha, with 25% of the results placed in each of them (Figure 18).

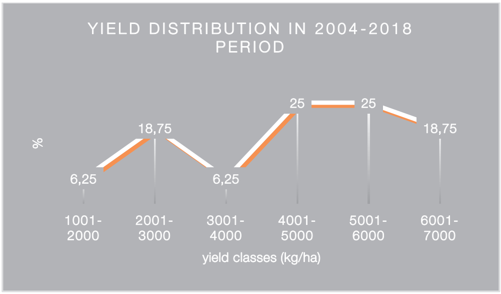

Figure 18. Yield distribution in the agricultural year 2004-2018

The average yield for the years of experiment-tation ranged from 2461 kg/ha in 2007 and 6588 kg/ha in 2017. The relationship between the rainfall and the predominant yield class (the yield interval in which most of the tested cultivars were grouped) showed that the latter increases with increasing rainfall but decreases when over 575 mm were recorded.

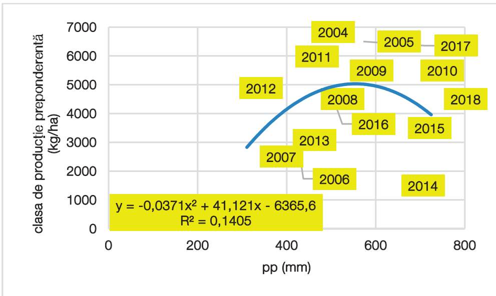

Figure 19. Correlation between rainfall and the predominant yield class in each year of testing

The variability of rainfall explains 14% of the variability of the grouping of yields in the preponderant class (Figure 19).
The correlation between average yield and yield in a dry year showed that the variability of average yield explains 22% of the variability of yield under drought conditions. A variety whose average yield increases by 100 kg / ha, in a dry year registers an increase of only 60 kg / ha, for the studied interval (Figure 20).
The varieties Exotic, Glosa, Mv Martina and Orion stood out as varieties with high average yield (5213 kg/ha, 5078 kg/ha, 5056 kg/ha, respectively 4656 kg/ha) and high yields under drought conditions (4421 kg/ha, 3330 kg/ha, 3380 kg/ha, respectively 3670 kg/ha).

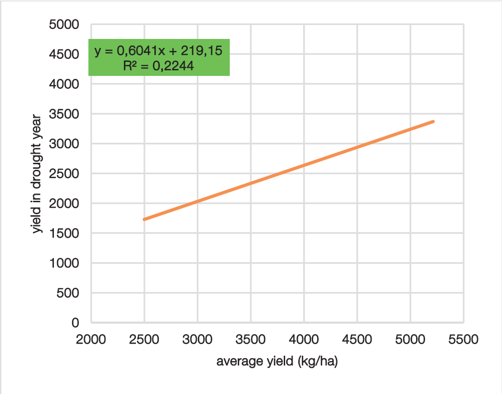

Figure 20. The correlation between average yield and yield in a dry year

The correlation between average yield and yield in a rainy year highlighted the fact that the variability of average yield explains 35% of the variability of yield under water supply conditions. A variety whose average yield increases by 100 kg / ha, in a rainy year registers an increase of only 55 kg/ha (Figure 21). Therefore, a large amount of rainfall does not increase yield.
The basis for the selection of adapted and stable varieties consists of varieties that fall into the group of cultivars with high average yield (at least 4000 kg/ha) and minimum yield of at least 2500 kg/ha.

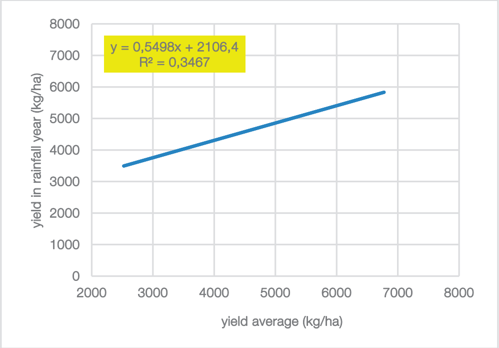

Figure 21. The correlation between average yield and yield in a rainy year

Regarding our testing, cultivars that showed average yields above 4000 kg/ha but minimum yields of at least 2500 kg/ha are shown in the figure below (Figure 22). Of these, as expected, because they are more adapted to the conditions, most of them are local varieties - Glosa, Izvor, Voroneț, Șimnic and Fundulea lines. Among the foreign varieties, the following ones stood out: Nathan (average yields for 6 years) and Cezanne (average yields for 13 years). Since the two variables studied are not correlated (coefficient of determination of only 3.5%), it is very important to make a selection among the large values in their projection.

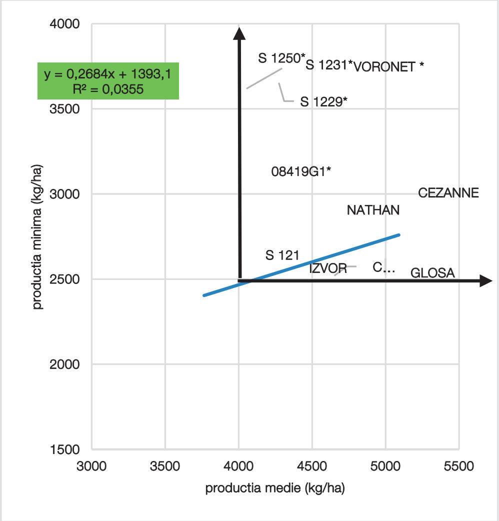

Figure 22. The correlation between average yield and minimum yield with the highlighting of the optimal quadrant (average yields over 4000 kg / ha and minimum yields over 2500 kg / ha).

The results analyzed by the boxplot method are presented in table no.1. Among the varieties tested, the Esquisit, Pegassos, Bhash, Solomom varieties frequently appear with values of deviant or extreme yield.

Varieties recorded as negative outliers or extremes are not recommended for the area because they are inferior to any other variety tested. The probability that those climatic conditions will be repeated exists and it is good that the varieties marked on these positions (outliers or extreme negative) are eliminated from the start so as not to record low yields.

The outliers foreign varieties that stood out and can be successfully cultivated in the area were: Exotic, Hogoz, Gabrio, Hargitta, GK Hattyu, Frini, Orion, GK Petur, Mandolin, Solehio and Falado.

CONCLUSIONS

Depending on the number of years of testing and regarding the yield, the varieties that stood out were the following: Unitary - 5440 kg/ha on average for 3 years; Falado - 6304 kg/ha on average for 4 years; Gabrio - 6442 kg/ha on average for 5 years; Basmati - 5077 kg/ha on average for 6 years; Mv Martina - 5056 kg/ha on average for 7 years; Pajura - 4562 kg/ha on average for 8 years; Bitop - 4058 kg/ha on average for 9 years; Brakes - 4265 kg/ha on average for 10 years; Orion - 4656 kg/ha on average for 11 years; Gruia - 4666 kg/ha on average for 12 years; Cezanne - 4906 kg/ha on average for 13 years; Exotic - 5213 kg/ha on average for 14 years and Glosa - 5078 kg/ha on average for 15 years.

The fact that the Glosa variety recorded the highest average yield over a period of 15 years, certifies the results of this research, knowing that this variety is the most cultivated and the most stable variety in Romania.

The relationship between the rainfall and the predominant yield class (the yield range in which most of the tested cultivars were grouped) showed that the latter increases with the increasing amount of precipitation but decreases when over 575 mm were recorded. The variability of precipitation explains 14% of the variability of the grouping of yields in the preponderant class.

The varieties Exotic, Glosa, Mv Martina and Orion stood out as varieties with high average yield and high yields under drought conditions.

The correlations showed that 22% of the variability of the average yield of a variety is associated with the variability of the variety's yield in a dry year and 35% in a rainy year. The varieties with a minimum yield of over 2500 kg / ha regardless of the year of experimentation and average yields over 4000 kg/ha were: Glosa, Cezanne, Izvor, Nathan.

The foreign varieties that stood out and can be successfully cultivated in the area were: Exotic, Hogoz, Gabrio, Hargitta, GK Hattyu, Frini, Orion, GK Petur, Mandolin, Solehio and Falado.

The study provides valuable information for the wheat improvement program regarding the identification of genetic sources with a high degree of adaptability and stability.

Table 1. The results of the yield analysis by the box-plot method

YEAR	THE LIMITS OF 50% OF THE VALUES	INTER QUARTIL	1,5*INTER QUARTIL	3*INTER QUARTIL	MAXIMUM	MINIMUM	OUTLIERS	EXTREMES
2004	5270-7110	1840	2760	5520	8420	3510	-	-
2005	4960-6280	1320	1980	3960	7560	3460	-	-
2006	2460-4380	1920	2880	5760	5450	1100	-	-
2007	2910-1940	970	1455	2910	3896	990	HARGITTA GK HATTYU EXOTIC	-
2008	3857-4605	748	1112	2244	5419	3000	ESQUISIT Mv SUGEVES PEGASSOS SOLOMON BHASH	TALASA
2009	5247-6029	782	1173	2346	7142	4180	CORNELIUS DEFENCE	BHASH SOLOMON

							LADA ESQUISIT MASSON RHEIA PEGASSOS	
2010	**4440-5270**	**830**	**1245**	**2490**	**6510**	**3280**	**FRINI ORION**	**-**
2011	**4981-5754**	**773**	**1160**	**2319**	**6145**	**4429**	**-**	**-**
2012	**4767-5498**	**731**	**1097**	**2193**	**6027**	**4009**	**AGRON KISKUN G. APACHE**	**-**
2013	**2046-3289**	**1243**	**1865**	**3729**	**4991**	**413**	**LOVRIN 34 MANDOLIN GK PETUR**	**-**
2014	**1495-3819**	**2324**	**3486**	**6972**	**6925**	**267**	**GABRIO SOLEHIO**	**-**
2015	**1723-4172**	**1003**	**1505**	**3009**	**5610**	**1723**	**-**	**-**
2016	**3098-7553**	**1782**	**2673**	**5345**	**7798**	**944**	**HOGOZ**	**-**
2017	**6161-7044**	**883**	**1324**	**2648**	**8133**	**4456**	**AS 5 LM 6**	**-**
2018	**4082-5080**	**1012**	**1518**	**3036**	**7309**	**2291**	**S 1430 S 1431 S 1426 S 1429 FALADO**	**-**
							FRUMENTO 14133G13	**-**

Legend

(yellow)	**Positive variants**
(red)	**Negative variants**

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SCIENTIFIC RESULTS ON TECHNOLOGICAL PROCESS OF VARIOUS LEVEL FERTILIZERS APPLICATION AND SOWING SEEDS WITH A COMBINED SEEDER

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Abstract

The design-technological scheme and design of the combined opener for laying and embedding of seeds of grain crops and granules of mineral fertilizers at their multilevel application have been substantiated and developed. It has been substantiated that the most urgent is the sowing of grain crops with simultaneous multilevel fertilization below the sowing bed with the formation of a soil layer, which ensures uniform distribution of seeds and fertilizers over the area of application and allows the most rational use of mineral fertilizers and reduce labor and production costs for production. The technique of researching the technological process of multilevel fertilization and sowing of seeds is presented. The results are determined based on theoretical research. The values of design parameters have been determined: the width of the bed seal (7 ... 25 mm), the distance between the planes of the outlet of the guide and the seed tube (20 ... 80 mm), the distance from the base of the outlet of the guide to the U-shaped harrow (40 ... 50 mm).

Key words: *opener, soil, seeder, ecology, furrow former.*

INTRODUCTION

According to the results of an analytical review of modern designs of openers for laying and embedding seeds of grain crops and granules of mineral fertilizers with their multilevel application, double-disc openers are most suitable for high-quality sowing. However, they have a number of disadvantages, which include: loosening of the walls and bottom of the furrow, shattering of the furrow during the placement of seeds along its bottom, the absence of a stable soil layer between the seeds and fertilizers, as well as the uneven flow of seeds and the ingress of seeds onto the rotating discs of the opener. All this reduces the quality of sowing, which leads to a decrease in crop yields and an increase in production costs.

Currently, we have made a combined opener with a U-shaped harrow (Figure 1) for laying and embedding seeds of grain crops and granules of mineral beads at their multilevel application. Since this combined opener was used for the first time, the task of theoretical research includes the determination of the necessary design parameters.

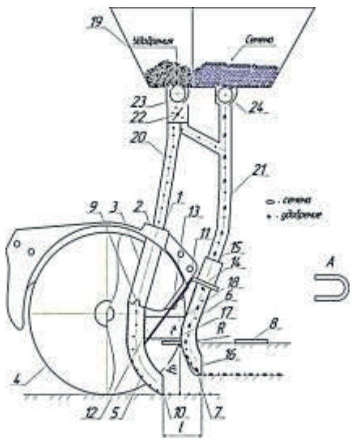

Figure 1. Structural and technological diagram of a combined opener with a U-shaped harrow for laying and embedding seeds of grain crops and granules of mineral fertilizers with their multilevel application: 1 - rack; 2 - neck; 3 - funnel; 4 - discs; 5 - directional note; 6, 21 - seed tube; 7 - bed sealant; 8 - closing working body; 9 - eyelet; 10 - outlet; 11 - bracket; 12 - stiffening rib; 13 - scrapers; 14 - shelf; 15 - neck; 16 - outlet; 17 - U-shaped harrow; 18 - bar; 19 - bunker; 20 - pipeline; 22 - fertilizer flow divider; 23 - fertilizer dispenser; 24 - sowing device; l is the distance between the planes of the outlet openings of the guide and the seed tube; h1 - distance from the base of the outlet of the directional guide to the U-shaped harrow;

MATERIALS AND METHODS

The basic laws of classical mechanics, mathematics and the working processes of seeding and planting machines are taken as the basis for theoretical research. Experimental studies were carried out on the basis of comparative laboratory-field studies of the estimated indicators of laying and embedding of seeds of grain crops and granular mineral fertilizers with their multilevel application by a seeder equipped with combined openers with a U-shaped harrow.

Experimental studies were carried out using standard techniques (GOST 31345-2007, STO AIST 5.6-2010). Analysis and processing of research results were carried out using the programs Statistica 6 RUS, Microsoft Office, etc.

RESULTS AND DISCUSSIONS

The covering of the granules of mineral fertilizers with the soil occurs due to the soil layer flowing around the fertilizer guide and naturally shedding it from the walls of the furrow.

During the operation of the combined coulter with a U-shaped harrow, the soil is deformed and rises to a certain height. After passing through the opener, the gap formed by the fertilizer guide manages to close, and some lower part of the soil lies at a certain distance from it, having time to cover the bottom of the furrow with fertilizers. Thus, a technologically important indicator of the process under consideration is the distance at which the particle is deposited after the soil has descended.

reliability The range of falling soil particles is determined by the dependence:

$$L = \mu\left(\frac{v^2 cos^2\alpha}{2g} tg\alpha + \right.$$
$$\left. + \sqrt{vtg^2\alpha + \frac{2gB_{л} sin\alpha}{v_0^2 \sin(2\gamma_{п}) cos^2\alpha}}\right), \quad (1)$$

where v_0 – is the rate of descent of soil particles, m/s;

α –angle of setting the directional guide to the bottom of the furrow, degrees;

$2\gamma_{п}$ – disc opening angle, degrees;

B_A – width of the furrow formed, m

For the mathematical calculation of the sowing process, we make the following assumptions:

1. The soil is represented by particles of certain sizes in a volume close to a spherical shape.
2. Particles of soil when interacting with the coulter move, obeying general laws.
3. Soil particles have small dimensions d = 1 to 3.47 mm and mass m = 0.1 to 2 g.
4. Moisture content of the soil is 18-24% in accordance with the norm.
5. The structure of the soil is fine crumbly, stickiness does not appear.
6. Speed of movement of the unit v = 1-3 m/s.
7. Values of parameters α = 20o,, = 0.015m.$2\gamma_{п} = 32^{o} B_A$

The graph of the change in the distance of laying a soil particle after the seam vanished is given L from its descent speed v_0 . The calculation results show that when changing from 1 m/s to 3 m/s, the values vary from 0.015 m to 0.078 m.

Then the point of installation of the seed tube is determined from the expression:

$$l \geq L , \quad (2)$$

The L value is determined at the maximum value v.

Based on the obtained values of L, following expression 2, we take l from 0.02 m to 0.08 m.

The width of the bed compactor determines the width of the bottom of the furrow for which the seeds will be placed.

The width of the bottom of the furrow should be sufficient for the placement of seeds from the sowing unit and good contact with the compacted soil. For this, it is necessary that the width of the compactor is greater than the maximum length of the placed seed, but less than the width of the furrow formed by the discs, i.e.:

$$c > b > l_{max} , \quad (3)$$

where

b is the width of the bed seal, m;

l_{max} - maximum length of the laid seed, m (0.007 m);

c - width of the furrow formed by the coulter discs, m (0.025 m).

The soil is deposited at an angle of self-deposition α, also called the angle of repose and also depends on the location of the U-shaped doses of the harvester. Particles located in adjoining layers will crumble first. Their fall can be taken as a free fall with an initial vertical velocity equal to zero. Then, almost at the same time, the particles will begin to move, which are at an angle of self-falling.

Let us take the direction of the Ox axis in the direction of the velocity vector, and the direction of the Oy axis vertically downward, the coordinates will be counted from the point O located in the center of the particle located on the outer surface of the adjoining layers (Figure 2).

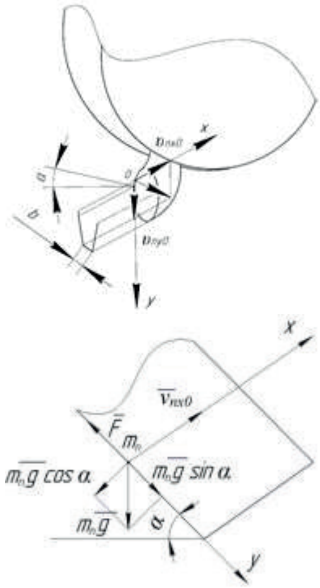

Figure 2. Falling soil: a - into the furrow; b - along lines located at an angle of slope of soil particles; α - angle of incidence; b - the width of the bed seal

At the moment the soil particle passes the cut of the fertilizer guide, it begins to fall into the furrow under the action of its weight mg and the force of air resistance.

Taking into account the absence of the initial vertical component of the particle velocity during the descent from the directional fuel and the small distance between the compaction to the furrow surface, the air resistance is not taken into account.

Consider the obtained law of motion of a soil particle

$$\begin{cases} m\dfrac{d^2x}{dt^2}=0 \\ m\dfrac{d^2y}{dt^2}=mg \end{cases}, \quad (4)$$

where m is the mass of a soil particle, kg;

Suppose that $\dfrac{d^2x}{dt^2}=\dfrac{d\upsilon_x}{dt}$, and $\dfrac{d^2y}{dt^2}=\dfrac{d\,\upsilon_y}{dt}$ and canceling all refer to m we get

$$\begin{cases} \dfrac{d\upsilon_x}{dt}=0 \\ \dfrac{d\upsilon_y}{dt}=g \end{cases} \quad (5)$$

Multiplying both sides of expressions (5) by dt and integrating them, we find

$$\upsilon_x = C_1, \text{ m / s}, \quad \upsilon_y = gt + C_2, \text{ m/s}. \quad (6)$$

From the initial conditions, we have that for t = 0 $\upsilon_x = \upsilon_{x0}$, $\upsilon_y = 0$.

Then $C_1 = \upsilon_{x0}$, $C_2 = 0$.

After substituting the integration constants into expression (6), we obtain

$$\frac{dx}{dt}=\upsilon_x=\upsilon_{x0}; \quad \frac{dy}{dt}=\upsilon_y=gt;$$

where will we find

$$x=\upsilon_{x0}t+C_3; \quad y=\frac{gt^2}{2}+C_4.$$

From the initial conditions, we define $C_3 = C_4 = 0$. Then

$$x=\upsilon_{x0}t; \quad y=\frac{gt^2}{2},$$

from where

$$y=\frac{gx^2}{2\upsilon_{x0}^2}. \quad (7)$$

Substituting the value $\upsilon_x = \upsilon_{x0}$ from expression (5) to equation (7) we obtain

$$y=\frac{x\,tg\alpha}{(tg\varphi - tg\varphi_1)+(tg\varphi - tg\beta)\sin\beta}. \quad (8)$$

Speed can now be found

$$\upsilon_y=\frac{gx}{\upsilon_{x0}}= \quad (9)$$

$$=\sqrt{\frac{gx\cdot tg\alpha}{(tg\varphi - tg\varphi_1)+(tg\varphi - tg\beta)\sin\beta}}, \text{м / с}.$$

Resultant falling velocity of the particle

$$\upsilon_n=\sqrt{\upsilon_{x0}^2+\upsilon_y^2}, \text{м / с}, \quad (10)$$

and the angle of incidence

$$\alpha = arctg\frac{\upsilon_y}{\upsilon_{x0}} \quad (11)$$

running Soil particles, far from the boundary layer, slide along the inclined ones, making up the angle of laying or natural slope α.

The particle is affected by weight, the component of which mg sin α (Figure 2, b) makes the particle move along an inclined surface. This movement is counteracted by the friction force F = mg cos α tan φ1. Particle motion equation:

$$m\frac{d^2x}{dt^2}=0$$

$$m\frac{d^2y}{dt^2}=mg\sin\alpha-mg\cos\alpha\cdot tg\varphi_1. \quad (12)$$

depreciation After transformations we get

$$\frac{d\upsilon_x}{dt}=0,\ \frac{d\upsilon_y}{dt}=g\cos\alpha(tg\alpha-tg\varphi_1),$$

mixing or

$$\upsilon_x=C_1, м/c$$

$$\upsilon_y=gt\cos\alpha(tg\alpha-tg\varphi_1)+C_2, м/c \quad ... \quad (13)$$

segments Since at t = 0 $\upsilon_x=\upsilon_{x0}$, $\upsilon_y=0$, we find $C_1=\upsilon_{x0}$, $C_2=0$.

After substitution of constants in the expression for υx and υy we get:

$$\upsilon_x=\upsilon_{x0}, м/c.$$

$$\upsilon_y=gt\cos\alpha(tg\alpha-tg\varphi_1), м/c.$$

or

$$\frac{dx}{dt}=\upsilon_{x0},\ \frac{dy}{dt}=gt\cos\alpha(tg\alpha-tg\varphi_1), \quad (14)$$

from where

$$x=\upsilon_{x0}t+C_3,$$

$$y=\frac{1}{2}gt^2\cos\alpha(tg\alpha-tg\varphi_1)+C_4. \quad (15)$$

According to the initial conditions, we define $C_3 = C_4 = 0$. Finally, we obtain

$$x=\upsilon_{x0}t;$$

$$y=\frac{1}{2}gt^2\cos\alpha(tg\alpha-tg\varphi_1)= =\frac{1}{2\upsilon_{x0}^2}gx^2(tg\alpha-tg\varphi_1). \quad (16)$$

Looking for the vertically directed part of the speed of a soil particle

$$\upsilon_y=\frac{gx\cos\alpha}{\upsilon_{x0}}(tg\alpha-tg\varphi_1), м/c \quad (17)$$

Resulting speed of falling soil particle

$$\upsilon=\sqrt{\upsilon_{x0}^2+\upsilon_y^2}, м/c \quad (18)$$

Soil particle incidence angle

$$\alpha=arctg\frac{\upsilon_y}{\upsilon_{x0}},. \quad (19)$$

The time of falling of particles of the boundary layer and particles at the angle of self-precipitation is, respectively,

$$t_1=\sqrt{\frac{2y}{g}}, c.\ t_2=\sqrt{\frac{2y}{g\cos\alpha(tg\alpha-tg\varphi_1)}}, c \quad (20)$$

During this time period, the fertilizer bed is open. Depending on the speed of the seeder, the length of the open part of the furrow L, the path measured from the outlet of the fertilizer guide to the crumbling soil ridge, i.e. a2a3, will be different (Figures 3,4).

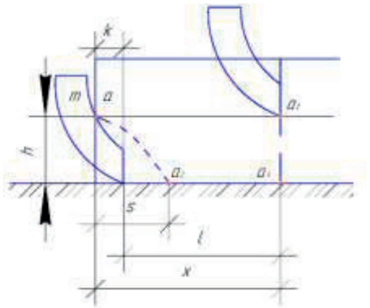

Figure 3. Scheme for calculating the shedding time of the furrow: L - the length of the open part of the furrow; s - the path of the traversed soil during the shedding; H is the height of the fall of the soil particle; k - distance of the beginning of particle motion to the exit hole

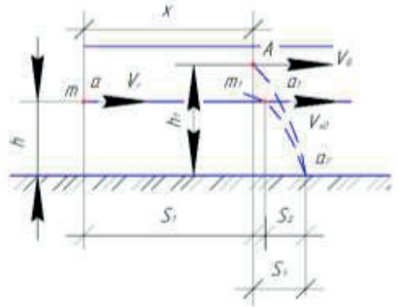

Figure 4. Fixation of fertilizers at the moment of falling to the bottom of the furrow during self-filling of the soil: H - height to the U-shaped harrow; h is the height of the fall of the soil particle; m is a particle of soil; S1, S2, S3 - the path traveled by the soil

During the time of shedding, a soil particle passes the following path s = $\upsilon_{x0}t$, and the path traversed by the seeding unit during this time period will be equal to $\upsilon_m t$... Then

$$L = l - m + k, м,$$

$$L = l - m + k = (\upsilon - \upsilon_{x0})\sqrt{\frac{2H}{g}}, м \quad (21)$$

type where υ - seeder speed, m / s.

$$l \geq (\upsilon - \upsilon_{x0})\sqrt{\frac{2H}{g}}, м$$

From here

With an increase in the speed of the seeding unit and the depth of the coulter, the opening of the furrow increases. Until the furrow closes, the fertilizers ejected by the guide must fall to its bottom, and in addition, the geometrical location of the ejection relative to the outlet of the guide and the ejection speed must be such that the fertilizers have time to settle down at the bottom until the furrow closes. In this case, there will be a minimum unevenness of the response of the soil layer.

Conferences $$\sqrt{\frac{2H}{g}} = \frac{s_1}{\upsilon_c} + \sqrt{\frac{2h}{g}}, \quad (22)$$

Where

$$s_1 = \upsilon_c\sqrt{\frac{2(H-h)}{g}}. \quad (23)$$

On the other hand, the quantity x can be determined from the equality

$$x + s_3 = s_1 + s_2, \quad (24)$$

or

$$\upsilon_B\sqrt{\frac{2h_1}{g}} = \frac{l+k}{\upsilon} + \upsilon_{x0}\sqrt{\frac{2h}{g}}, \quad (25)$$

specific From this condition at known speeds of the seeder υ_c, fertilizer ejection υ_B and soil crumbling υ_{x0}, were processed by specifying the height of the fall of soil particles h and the distance between the planes of the outlet openings of the seed tube and the guide l, it is possible to determine the height to the U-shaped harrow N.

$$h_1 = \frac{\left(\frac{l+k}{\upsilon} + \upsilon_{x0}\sqrt{\frac{2h}{g}}\right)^2}{2\upsilon_B^{\,2}}$$

Substituting the previously obtained values of the distance between the planes of the outlet openings of the seed tube and the guide from 0.02 m to 0.08 m, the height to the U-shaped harrow will be 0.04 ... 0.05 m.

Due to the fact that the falling out of soil particles behind the opener has a certain sequence, they are mixed. As noted above, the particles that are in direct contact with the plane of the U-shaped harrow first fall out, the lower particles fall off faster than the upper ones. Therefore, the bottom of the fertilizer furrow will be covered with a very thin bottom layer and then the top one. Following this, the bulk of the soil will crumble.

The pressure exerted by the bed compactor on the bottom of the furrow is determined by the formula:

$$p = \frac{R_1}{b_1 l_o}, \quad (26)$$

where l0 is the length of the platform providing crushing, m;

b1 - revealed the width of the crumple area, m;

R1 is the resulting reaction;

$$l_o = 2r_1 sin\delta, \quad (27)$$

a

$$p = \frac{R_1}{2b_1 r_1 sin\delta}... \quad (28)$$

The R1 value is determined by the technical formula:

$$R_1 = \frac{2}{3} q b_1 \sqrt{2r_1}\, h_o... \quad (29)$$

Substituting expression (29) into (28) we get:

$$p = \frac{\sqrt{2qh_o}}{3\sqrt{r_1} sin\delta}, \quad (30)$$

Because

надлежащая $$\sin\delta = \frac{R_{1x}}{R_1} = \frac{3h_o}{4\sqrt{2r_1}}, \quad (31)$$

we get:

$$p = \frac{8qh_o}{9}... \quad (32)$$

The density of the soil at the bottom of the furrow can be determined by the coefficient of porosity, which is defined as:

$$\epsilon = \frac{\gamma}{\rho} - 1, \quad (33)$$

where γ is the density of soil particles, g / cm^3;

ρ - soil density, g/cm^3

and the density of the soil:

$$\rho = \frac{\gamma}{\epsilon - 1}... \quad (34)$$

To determine the coefficient of porosity at pressure p V.F. Babkov proposed dependence:

$$\epsilon = \epsilon_o - \frac{1}{B_1} \text{разных} \ln\frac{\rho}{9{,}8\ 10^4} \quad (35)$$

where p is pressure, Pa,

ϵ_o - coefficient of porosity under load;

B_1 - the degree of change in the porosity coefficient under load.

Substituting the obtained values into formula (34), we get:

$$p = \frac{\gamma \boldsymbol{B}_1}{B_1(1+\epsilon_o) - \ln(\frac{qh_o}{1{,}1\ 10^5})} \quad (36)$$

For highly compressible chernozem soils, the following values are recommended: = 0.75 ... 0.85; B = 5 ... 10. The specific mass of the solid phase of the soil is 2.4 g/cm^3 for ordinary chernozems at a depth of 0 ... 20 cm. With the coefficient of volumetric crushing of the soil q = 2 · 106 N/m^3 and the depth of the bed compactor stroke h0 = 0.06 m, the density of the furrow bottom will be:ϵ_o

$$p = \frac{2{,}4 \cdot\ 10^7 \cdot 7}{7(1+0{,}8) - \text{концом}\ \ln(\frac{2 \cdot 10^6 \cdot 0{,}06}{1{,}1\ 10^5})} \approx 1{,}34 \cdot 10^3,, \text{кг/м}^3$$

CONCLUSIONS

We have substantiated and developed the structural and technological scheme and design of a combined opener with a U-shaped harrow for laying and embedding seeds of grain crops and granules of mineral fertilizers with their multilevel application (patent series for a mass invention applied by RF No. 2671704). A distinctive feature of this design is the use of a closed-type seed tube and feed guide, a U-shaped harrow, a bed compactor designed to seal the walls and bottom of the furrow, without shedding the furrow during the placement of seeds along the bottom of the furrow, preventing the opener from clogging with soil. According to the results of theoretical calculations, it can be noted that the distance from the base of the outlet of the guide to the U-shaped harrow will be equal to 0.04 ... 0.05 m; the width of the bed seal is 0.007… 0.025 m; the distance between the planes of the outlet openings of the seed tube and the guide is equal to 0.02 ... 0,

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BIOLOGICAL EFFICACY OF SEGADOR FOR WEED CONTROL IN NON-CROPPED AREAS

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Abstract

The efficacy of Segador (organic fertilizer with a contact herbicide effect), applied alone or in combination with the adjuvant Melamyel for controlling of the invasive weed species Sorghum halepense (L.) Pers., Cirsium arvense (L.) Scop. and Convolvulus arvensis L. in non-cropped areas was studied. Segador at concentration 5%, 8% and 12% indicated high efficiency from 95 to 100%. It proved to be a successful element in a strategy for controlling of invasive weed species (Cirsium arvense (L.) Scop. and Convolvulus arvensis L.) and greatly important for reducing to population density of perennial dicotyledonous weeds. The resistance of Segador was demonstrated to Sorghum halepense (L.) Pers. at all tested rates with or without Melamyel. Segador successfully controlled perennial dicotyledonous weeds, but it was not toxic to Sorghum halepense (L.) Pers.

Key words*: non-cropped areas (stubbles), invasive weeds, organic herbicide, Segador, efficacy.*

INTRODUCTION

Weeds are undesirable plants impeding the use of land and water resources and in this way they affect human life indirectly (Nasim and Shabbir, 2012). According to Oerke (2005), Dimitrova (2009) and Bentsen and Felby (2012) limited acreage for crop cultivation, a need for more special nutrition, increased demand for bioenergy crops and negative consequences of global warming will necessitate greater production in the future, while at the same time reducing to a minimum the damage of the environment. All this increases the need for study for weed control, especially due to the fact weeds reduce yield of agriculture crops more than other pests. The great diversity of species and their high biological plasticity remain a major problem associated with weeds, allowing their rapid spread and adaptation. Due to the large regenerative capacity of their underground organs and their high seed productivity, some of the perennial weed species such as perennial monocotyledonous (*Sorghum halepense* (L.) Pers.) and perennial dicotyledonous (*Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L.) spray quickly in arable land (Dimitrova, 2009; Nasim and Shabbir, 2012; Schwinning et al., 2017).

Summary studies of Tonev (2000), Schwinning et al. (2017) and Tiley (2010) determine *Sorghum halepense* (L.) Pers., *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L. like some of the one hundred most economically important weeds characterized by high biological and ecological plasticity, and hight allelopathic potential (Golubinova and Ilieva, 2014).

According to studies of (Gyenes et al., 2005; Dimitrova and Serafimov, 2007a; 2007b; Stoimenova et al., 2008; Johnson et al., 2014; Tavaziva et al., 2019) in a non-cropped area (stubble) of conventional agriculture *Sorghum halepense* (L.) Pers., *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L. can be controlled by selective herbicides.

At organic farming, however, the use of herbicides for weed control against invasive weed species been banned and the may be used only biological or mechanical methods for weed control (Kropff et al., 2000 and Webber et al., 2012).

The importance of the successful control of the species *Sorghum halepense* (L.) Pers., *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L. by using of herbicides attracted the attention of many researchers (Boutin et al., 2012; Schwinning et al., 2017 and Tavaziva et al., 2019). The reports of use of organic products with the herbicide contact action which can be used for weed control in farms in the period of conversion to biological (organic) agriculture production are sporadic and extremely limited (Marinov-Serafimov and Golubinova, 2015; Georgieva et al., 2018).

Many methods were being developed to reduce the use of herbicides and notably organic herbicides were developed to have the same herbicidal effect but without the side effects from the organic herbicides (Cheng, 2014).

The objective of this work was to conduct a biological study of the effectiveness of organic product Segador (organic fertilizer with a contact herbicide effect) in controlling invasive weed species *Sorghum halepense* (L.) Pers., *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L. in non-cropped areas (stubbles).

MATERIALS AND METHODS

The study was conducted in a non-cropped area (stubble) after harvest of winter forage pea (*Pisum sativum* L.) grown in conventional production conditions under natural weed infestation pressured at the Institute of Forage Crops in Pleven in the 2014-2016 period. The experiment was set up using the block design method at four replications and the size of the experimental plot of 10 m^2 under non-irrigating conditions. The soil type was leached chernozem of pH 7.3-7.6. Treatments of the trial are shown in Table 1.

The herbide products used in this study (Basta 15 CL and Reglone Forte) are registred for weed control in Bulgaria. Segador is not registered in Bulgaria. Segador is organic fertilizer with a non-selective contact herbicide action against weeds. Segador is a complex natural hydroxyphosphate in the form of an emulsion, and natural surfactant depressor of water activity that and thus causing desiccation of the plant cell. Contains phosphorus (P_2O_5) 25.5% water-soluble and zinc (Zn) water-soluble 0.20%.

Melamyel is organic product consists of an emulsion of ethoxylated castor oil and used as a non-ionic for surface moisturizer. Melamyel is not registered in Bulgaria.

The applied product and standard rates were as recommended by their manufacturers and were applied with a working solution quantity of 500 l/ha.

Table 1. Variants of the study

Treatments	Herbicides	Active Ingredient (s)	Chemical family	Dose (concentration) of commercial product
T_1	Control (untreated) K_u			-
T_2	Basta 15 SL	150 g/l glufosinate-ammonium	bipyridylium	5.0 l/ha
T_3	Reglone Forte (Standart)	150 g/l diquat and 150 g/l humidifier		3.0 l/ha
T_4	Segador	phosphorus (P_2O_5) 25.5% water-soluble and zinc (Zn) water- soluble 0.20%	natural hydroxyphos-phate	5.0%
T_5	Segador			8.0%
T_6	Segador			12.0%
T_7	Segador	phosphorus (P_2O_5) 25.5% water-soluble and zinc (Zn) water- soluble 0.20%	natural hydroxyphos-phate	5.0%
	Melamyel	emulsion of ethoxylated castor oil	non-ionic surface moisturizer	0.4 l/ha
T_8	Segador	phosphorus (P_2O_5) 25.5% water-soluble and zinc (Zn) water- soluble 0.20%	natural hydroxyphos-phate	8.0%
	Melamyel	emulsion of ethoxylated castor oil	non-ionic surface moisturizer	0.4 l/ha
T_9	Segador	phosphorus (P_2O_5) 25.5% water-soluble and zinc (Zn) water- soluble 0.20%	natural hydroxyphos-phate	12.0%
	Melamyel	emulsion of ethoxylated castor oil	non-ionic surface moisturizer	0.4 l/ha

Treatment was conducted at the at the growth stage for perennial dicotyledonous (*Cirsium arvense* L.) Scop. (BBCH-12-14) and *Convolvulus arvensis* L. (BBCH–39-42) and perennial monocotyledonous (*Sorghum halepense* (L.) Pers.) (BBCH–12-14) (Hess et al., (1997). The efficacy of the herbicides and the organic products were reported at 7th, 14th

and 21th days after treatment (recommended time for contact herbicides) using the 1-9 scale of the EWRS (European Weed Research Society) (0-100% killed weeds - score from 1 to 9). The experimental data is presented as average values for the period of study because the tendency was the same in the different years.

Ground cover of the weeds was determined visually and was recorded on a scale of 0-100% (0- no plants, 100% very healthy plants - covering the entire surface area) (Fontenot et al., 2015). The growing weeds after treatment with herbicides and the organic product was recorded on the 14th and 21th day after treatment which is recommended time for herbicides or organic products with herbicide effect with contact action. For the characterization of the arid/humid characteristic during the study period was used index De Martonne's (I_{DM}). To determine arid/humid characteristic of a specific month, was calculated by Equation (1) (Coscarelli et al., 2004).

$$I_{DM} = \frac{12P_i}{T_{ai}+10} \quad (1)$$

where P_i is the monthly amount of precipitation (in millimeters) and T_{ai} is the mean monthly air temperature (in degree Celsius) recorded in the considered month, 12 and 10 are constants.

All data were analysed using the Statistica version 10 software. Means separated using Fisher's protected least significant difference when F tests were significant at α=0.05.

RESULTS AND DISCUSSIONS

Analysis of meteorological factors shows that during the study period the monthly amount of precipitation has slight deviations from -7.4% to +27.3% and relatively stronger changes in temperature from 0.6^{0}C to 1.9 ^{0}C compared to multi-annual period from 1961to 2011.

Assessing the complex impact of meteorological factors (rainfall and air temperature) according the De Martonne airdity index *(Iar-DM*) during the study years can be classified conventionally for 2015 and for 2016 are moderately airdity respectively I_{ar}-DM_{2014} - 27.0 and I_{ar}-DM_{2015} – semi – humid and 2016 is semi-dray (*Iar-DM* – 19.5) (Table 2).

Table 2. Rainfall amount, air temperature and index of aridity (*Iar*-DM) for the period from March to September

Year	Monthly rainfall, mm		Average monthly temperature of the air, ^{0}C		Index of airdity (*Iar*-DM) March – September
	For the 1964-2014 period				
	March – September 403.3 mm	Deviation, %	March – September 16.8 ^{0}C	Deviation, ^{0}C	
2014	484.8	120.2	18.1	1.3	25.9
2015	512.9	127.3	17.4	0.6	28.1
2016	373.6	92.6	18.7	1.9	19.5

Species composition within the weed association of experimental area was represented by invasive weed species from different biological groups: perennial monocotyledonous (*Sorghum halepense* (L.) Pers.) and perennial dicotyledonous (*Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L.) (Table 3a and 3b).

Before treatment, the degree of weed infestation was high during the study years. For *Sorghum halepense* (L.) Pers. From 46.2 stems/m^2 to 64.5 stems/m^2, average for the period 53.8 stems/m^2 For *Cirsium arvense* (L.) Scop. from 41.6 stems/m^2 to 59.3 stems/m^2, average for the period 52. stems/m^2 and for *Convolvulus arvensis* L. from 13.2 stems/m^2 to 18.8 stems/m^2, average for the period 16.5 stems/m^2.

The total weed density varied in the period of the study from 101.0 plants/m^2 to 142.6 plants/m^2, average 123.0 plants/m^2 in the treatment area which is a prerequisite for a realistic assessment to determine the efficacy of the tested herbicides (Table 3a, 3b and Figure 1).

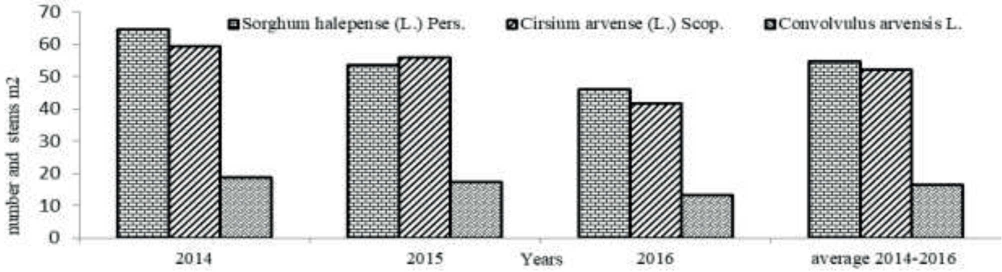

Figure 1. Species composition and density of weeds in a non-cropped area (stubble) after harvest of winter forage pea (*Pisum sativum* L.) during the period of the study

Table 3a. Efficacy of Segador againts weeds in non-cropped area (stubble) average for the period 2014-2016

Treatments	Weed	BBCH	Ground cover of the weed	BBCH	Efficacy, %	EWRS *	Ground cover of the weed	Regrowing, %
		0 DBT		7 DAT				
T_1	*Cirsium arvense*	12-14	25	14-16	0^{a}	9	45	-
	S. halepense	12-14	10	12-16	0^{a}	9	15	-
	C. arvensis	39-42	20	42-49	0a	9	30	-
T_2	*Cirsium arvense*	12-14	25	14-16	95^{d}	2	25	0.0
	S. halepense	12-14	10	14-16	90^{d}	2	10	0.0
	C. arvensis	39-42	20	47-59	95^{d}	2	15	0.0
T_3	*Cirsium arvense*	12-14	25	14-16	95^{d}	2	25	0.0
	S. halepense	12-14	10	14-16	95^{d}	2	10	0.0
	C. arvensis	39-42	20	47-59	95^{d}	2	15	0.0
T_4	*Cirsium arvense*	12-14	25	14-16	85^{cd}	3.5	30	0.0
	S. halepense	12-14	10	14-16	45^{b}	7	15	0.0
	C. arvensis	39-42	20	47-59	85^{cd}	3	15	0.0
T_5	*Cirsium arvense*	12-14	25	14-16	95^{d}	2	25	0.0
	S. halepense	12-14	10	14-16	55^{b}	6	15	0.0
	C. arvensis	39-42	20	47-59	95^{d}	2	15	0.0
T_6	*Cirsium arvense*	12-14	25	14-16	95^{d}	2	20	0.0
	S. halepense	12-14	10	14-16	58^{b}	6	15	0.0
	C. arvensis	39-42	20	47-59	95^{d}	2	15	0.0
T_7	*Cirsium arvense*	12-14	25	14-16	95^{d}	2	20	0.0
	S. halepense	12-14	10	14-16	50^{b}	7	15	0.0
	C. arvensis	39-42	20	47-59	95^{d}	2	15	0.0
T_8	*Cirsium arvense*	12-14	25	14-16	95^{d}	2	20	0.0
	S. halepense	12-14	10	14-16	55^{b}	6	15	0.0
	C. arvensis	39-42	20	47-59	95^{d}	2	15	0.0
T_9	*Cirsium arvense*	12-14	25	14-16	95d	2	20	0.0
	S. halepense	12-14	10	14-16	$65b^{c}$	4	15	0.0
	C. arvensis	39-42	20	47-59	95^{d}	2	15	0.0

*Legend: ** Treatments- see Table 1; DBT - day before treatments; DAT - days after treatment; BBCH scale—general for the descriptions of the growth stages of monocotyledonous and dicotyledonous weeds, Hess et al. (1997); Means with different a ,b, c and d in P=0.05 level of probability by LSD test;* EWRS (European Weed Research System) scale for weed control efficacy (100% (score 1) = total control and 0% (score 9)= no effect on the weeds); Ground cover of the weed were recorded on a scale of 0-100% (0- no plants. 100% very healthy plants - covering the entire surface area); Regrowing weeds on a scale of 0-100% (0- no plants, 100% very healthy plants - covering the entire surface area).*

The studied product Segador is organic fertilizer with a non-selective contact herbicide effect against weeds is total, foliar-applied product with a contact action. Phytotoxicity symptoms on the weeds were typical for this group of herbicides and organic product with contact total actions. As a result of the biological process of contact products for weed control, the active ingredient of the tested organic product exerts a phytotoxic effect on the green leaf mass of all plant parts of the aboveground surface of the weeds species. In the first-second week after treatment, there was chlorosis of the aboveground parts, which changed into necrosis later.

Table 3b. Efficacy of Segador againts weeds in non-cropped area (stubble) average for the period 2014-2016

**Treatments	weeds	BBCH	Efficacy, %	EWRS *	Ground cover of the weed	Regrowing %	BBCH	Efficacy, %	EWRS *	Ground cover of the weed	Regrowing %
		14 DAT					21 DAT				
T_1	*Cirsium arvense*	16-17	0^{a}	9	60	-	17-18	0^{a}	9	70	-
	S. halepense	16-17	0^{a}	9	15	-	21-23	0^{a}	9	20	-
	C. arvensis	59	0^{a}	9	40	-	63	0^{a}	9	65	-
T_2	*Cirsium arvense*	14-16	100^{d}	1	10	6.5	14-16	100^{d}	1	10	15.0
	S. halepense	14-16	98^{d}	1.5	5	5.2	14-16	100^{d}	1	5	10.8
	C. arvensis	47-59	100^{d}	1	5	7.4	47-59	100^{d}	1	5	14.9
T_3	*Cirsium arvense*	14-16	100^{d}	1	10	5.2	14-16	100^{d}	1	10	10.7
	S. halepense	14-16	98^{d}	1.5	5	8.6	14-16	100^{d}	1	5	21.3
	C. arvensis	47-59	100^{d}	1	5	7.8	47-59	100^{d}	1	5	16.9
T_4	*Cirsium arvense*	14-16	89^{cd}	1.5	15	11.3	14-16	90^{d}	2	15	26.5
	S. halepense	14-16	55^{b}	6	15	18.5	14-16	70^{bc}	4	15	58.2
	C. arvensis	47-59	90^{d}	2	10	10.4	47-59	90^{d}	2	10	34.3
T_5	*Cirsium arvense*	14-16	98^{d}	1.5	10	10.9	14-16	100^{d}	1	10	25.8
	S. halepense	14-16	65^{b}	4	10	19.5	14-16	75^{bc}	3.5	10	46.9
	C. arvensis	47-59	96^{d}	1.5	5	16.8	47-59	100^{d}	1	5	33.4
T_6	*Cirsium arvense*	14-16	98^{d}	1.5	10	6.3	14-16	100^{d}	1	10	25.0
	S. halepense	14-16	76^{bc}	3	10	17.4	14-16	75^{bc}	3.5	10	52.3
	C. arvensis	47-59	96^{d}	1.5	5	13.3	47-59	100^{d}	1	5	40.0
T_7	*Cirsium arvense*	14-16	97^{d}	1.5	10	10.6	14-16	100^{d}	1	10	21.9
	S. halepense	14-16	60^{bc}	5	10	32.6	14-16	70^{c}	3	10	64.3
	C. arvensis	47-59	99^{d}	1	5	12.8	47-59	100^{d}	1	5	37.5
T_8	*Cirsium arvense*	14-16	98^{d}	1.5	10	9.4	14-16	100^{d}	1	10	18.9
	S. halepense	14-16	65^{bc}	4	10	28.0	14-16	70^{b}	2	10	62.0
	C. arvensis	47-59	100^{d}	1	5	16.0	47-59	100^{d}	1	5	36.1
T_9	*Cirsium arvense*	14-16	98^{d}	1.5	10	8.6	14-16	100^{d}	1	10	19.1
	S. halepense	14-16	72^{cd}	4	10	26.9	14-16	75^{b}	3.5	10	59.6
	C. arvensis	47-59	100^{d}	1	5	18.6	47-59	100^{d}	1	5	43.0

*Legend: ** Treatments - see Table 1; DBT - day before treatments; DAT - days after treatment; BBCH scale—general for the descriptions of the growth stages of monocotyledonous and dicotyledonous weeds, Hess et al. (1997); Means with different a ,b, c and d in P=0.05 level of probability by LSD test;* EWRS (European Weed Research System) scale for weed control efficacy (100% (score 1) = total control and 0% (score 9)= no effect on the weeds); Ground cover of the weed were recorded on a scale of 0-100% (0- no plants. 100% very healthy plants - covering the entire surface area); Regrowing weeds on a scale of 0-100% (0- no plants, 100% very healthy plants - covering the entire surface area).*

The organic product Segador at concentration 5.0%, applied alone or with the adjuvant Melamyel in a dose 0.4 l/ha causes a rapid initial effect of desiccation of the above-ground biomass of the perennial dicotyledonous weeds *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L. several hours after treatment, regardless of the change meteorological conditions (Table 2 and 3).

Regarding perennial dicotyledonous weeds (*Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L.). 7 days after treatment (DAT) was established a fast initial effect of the herbicide on the weeds. 95% of them were killed (score 1.0÷1.5) at the lower concentration of 5.0% of Segador were observed without deviations from the standards Reglon Forte in a dose 3.0 l/ha and Basta 15 CL in a dose 5.0 l/ha. In the perennial monocotyledonous (*Sorghum halepense* (L.) Pers.), the efficacy of the same herbicide rate on organic product Segador was slighter to insignificant, the killed weeds accounting for 45-65% (score 4÷7).

With increasing the growing period to 14 and 21 days after treatment (DAT), the organic product Segador was studied again with Reglon Forte (150 g/l diquat and 150 g/l humidifier) as the standard and Basta 15 CL (150 g/l glufosinate-ammonium) herbicide (Table 3a and 3b). The low rate of the Segador (5.0% solution) successfully killed all perennial dicotyledonous weeds from 90 to 100% efficiency (score - 1÷2), but from *Sorghum halepense* (L.) Pers. was not sufficiently efficient from 55.0 to 70.0% solutions). The high rate (12.0 % solution) achieved 100% efficacy against *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L., while in *Sorghum halepense* (L.) Pers. it remains relatively low from 75.0 to 76.0% (score - 4÷6).

With regard to the total efficacy of Segador, expressed as a percentage, applied alone or with an adjuvant Melamyel in a dose 0.4 l/ha at all studied concentrates 5.0%, 8.0% and 12.0% against perennial dicotyledonous weeds it ranges from 95 to 100% (score 1). Under the the conditions of the study the efficiency of Segador is almost the same like the efficiency of Reglon Forte in a dose 3.0 l/ha used as a standard. The insignificant differences in the values of this indicator between the single application of the organic product Segador and that with the addition of adjuvant (Melamyel - 0.4 l/ha) justifies the assumption that it is self-application (Table 3a and 3b). In this case, the additive of the adjuvant increases the initial effect of Segador, but has little effect on the final herbicidal effect.

As a result of the phytotoxic effect of Reglon Forte in a dose 4.0 l/ha accepted for the standard and Basta 15 CL in a dose 4.0 l/ha 14 days after treatment, the lower regrowing of the perennial dicotyledonous species is in a range from 5.6% to 7.6% while for monotyledonous *Sorghum halepense* (L.) Pers. it varied from 5.2% to 8.6%. After treatment with the organic product Segador, the regrowing varied in the range from 6.3 to 13.3 for *Cirsium arvense* (L.) Scop. and for *Convolvulus arvensis* L. and for *Sorghum halepense* (L.) Pers. the range is from 17.4 to 19.5%.

21 days after treatment (DAT), the weed species regrowing capacity increased from 0.94 times for *Sorghum halepense* (L.) Pers. up to 2.68 times for *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L.. It is more pronounced after treated with the organical product Segador with or without the adjuvant Melamyel from 2.06 times to 3.27 times compared to the standard (Reglon Forte in in a dose 4.0 l/ha) and Basta 15 SL in a dose 5.0 l (Table 3a, 3b and Figure 2).

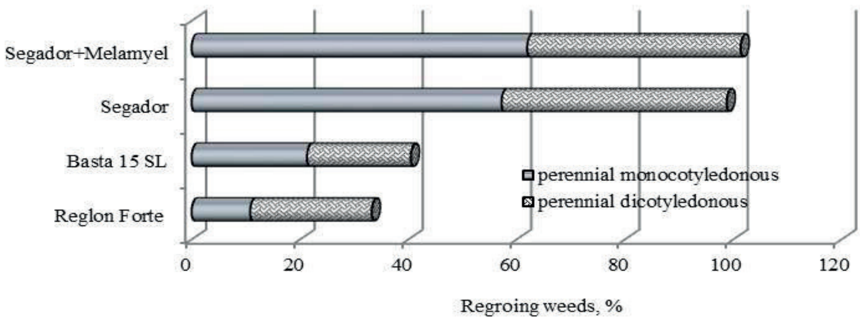

Figure 2. Regrowing of perennial monocotyledonous and dicotyledonous weeds after treatment with herbicides or Segador

The results obtained with respect to the weed species' recovery after treatment of the test areas with Segador in the applied concentrates (5.0%, 8.0% and 12.0%) with additive adjuvant (Melamyel - 0.4 l/ha) are similar. At the end of the three-week period after treatment of non-cropped areas (stubbles), which is sufficient for translocation of total herbicides and organic product Segador, preparation of the areas for next crops could begin. In order to achieve a long-term effect of the investment made in controlling perennial weeds in non-cropped areas, due to an abundance of weed seeds present in the soil, it is necessary to continue their control also during the growing season with crop cultivation by including selective herbicides (Dimitrova, 1995; Dimitrova, 2001; Schwinning et al., 2017 and Tiley, 2010).

CONCLUSIONS

Treatments of non-cropped areas (stubbles) with Segador at concentrations 5.0%, 8.0% and 12.0% indicated high efficiency from 95% to 100%. It is proved to be a successful element in the strategy for controlling of invasive weed species (*Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L.). and greatly important for reducing to population density of perennial dicotyledonous weeds.

Resistance of the organic product Segador are demonstrated for *Sorghum halepense* (L.) Pers. at all tested rates. Segador with or without ajuvant Melamyel in a dose 0.4 l/ha has efficiency only from 50% to 70% and strong growing capacity from 58.2% to 64.3%.

Segador has high efficiency against perennial dicotyledonous weeds, but it is not toxic for monocotyledonous *Sorghum halepense* (L.) Pers.

Segador like organic fertilizer with contact herbicidal effect can be used successfully for control against *Cirsium arvense* (L.) Scop. and *Convolvulus arvensis* L. in non-cropped areas (stubbles) during the conversion period from conventional agriculture to organic (biological) one.

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THE VARIETY - MAIN FACTOR FOR INCREASING YIELD AND QUALITY OF DURUM WHEAT GRAIN

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Abstract

During the period 2013-2016 a field experiment was conducted in the Experimental and Implementation Base of the Agricultural University of Plovdiv, Bulgaria in order to establish the influence of the variety on the productivity and chemical-technological properties of durum wheat grain in the ecological conditions of Plovdiv region. The object of the study were the Bulgarian varieties of durum wheat: Progress, Saturn, Victoria, Beloslava, Vazhod, Deyana, Predel, Zvezditsa and Zagorka and the foreign varieties of different origin: Auradur (Austria), Karur and Pescadou (France) Selyendur (Hungary), Grecale and Levante (Italy), Janejro (Spain). Variety Progress was used as standard for productivity and variety Saturn 1 was used as standard for quality. The experiment was based on the block method in 4 replications with a harvest plot size of 15 m2 after predecessor rapeseed. As a result of the field experiment it was found:
On average for the three-year study period most productive are the Bulgarian varieties of durum wheat Deyana, Predel and Zvezditsa, for which the grain yield increased respectively by 44.0 kg/da (12.8%); 38.1 kg/da (11.1%) and 33.9 kg/da (9.9%) compared to the Progress variety. Of the foreign varieties with higher grain yield on average for the study period, the varieties Karur and Pescadou are distinguished, for them the increase was by 22.3 kg/da (6.5%) and 20.6 kg/da (6%) more than the standard. The group of Bulgarian varieties is characterized by a higher thousand-kernel weight (TKW) and hectolitre weight (HW). The group of foreign varieties is characterized by a higher content of yellow pigments and volume of SDS-sedimentation number. The values in terms of crude protein and yield of wet and dry gluten in all durum wheat varieties are close but foreign varieties have better quality protein, respectively gluten. The Austrian variety Auradur is with highest quality, and Predel is with highest quality among the Bulgarian varieties.

***Key words**: durum wheat, varieties, productivity, grain quality.*

INTRODUCTION

The data presented in the scientific literature show the great opportunities for regulating the yields and quality of durum wheat based on a good study of the biological characteristics of the new varieties, as well as the role of environmental factors for their manifestation. All this is a prerequisite for crop management in accordance with the specific soil and climatic conditions and varietal composition of durum wheat.
A study conducted at the Field Crops Institute - Chirpan on the genetic distance of Bulgarian and European varieties of durum wheat found that Bulgarian varieties are among the best in yield and show better stability by years (Dechev, 2008). The author finds that according to the studied economic indicators, durum wheat varieties are divided into three groups that are genetically distant. Most Bulgarian varieties are in one group. The group of the most remote varieties is dominated by French ones.

When testing the Bulgarian durum wheat varieties Zagorka and Progress and the Italian Simeto, Balsamo and Rasioso (Delchev et al., 2000) it was reported that the Italian varieties in years with more severe and prolonged winters are risky in terms of yield and are not suitable for growing in our country, but due to the good quality of gluten they can serve as a starting material in the selection of high quality. For the same purpose or for direct implementation in the production, 15 samples of different origin were assessed (France, Greece, Jordan and Russia) from the collection of durum wheat in the National Gene Bank - Institute of Plant Genetic Resources, Sadovo (Mangova, 2007; Popova, 2001). The authors reported that the accessions Agathe, Mauragani iraclion, № 97102050, and Kubanka were distinguished by a high content of protein, yellow pigments and strong gluten, and according to a complex of valuable features the accessions No. 97102004, No. 97102025 and No. 97102033 are of interest for the practice.

Comparative studies have been made for the first time on the influence of varietal characteristics on the physical properties, chemical composition, technological properties of the grain of six varieties of common wheat - two Bulgarian and four grown in Northern Greece (Yanchev and Ivanov, 2012). The Bulgarian variety Pobeda (belongs to the group of strong wheat) and the varieties Panifor and Pandas, which belong to the group of wheat with increased strength and can be successfully implemented in grain production in Northern Greece under different agronomic conditions, are characterized by the best physical properties, chemical composition and technological properties of the grain. Outside the varietal characteristics, excessive nitrogen fertilization has an adverse effect on the colour of the grain due to the proven inverse relation of colour-protein content (Autran et al., 1986).
Almaliev et al. (2013) found that in durum wheat the structural elements of yield (wheat-ear length, number of spikelets per wheat-ear, number of grains per wheat-ear, grain weight in 1 wheat-ear), productivity (grain yield and grain protein) and grain quality (grain protein concentration, wet and dry gluten content, total grain vitreousness) are strongly related to the applied nitrogen fertilization within the norms 0-18 kg/da.
On average for a three-year period of research in Pazardzhik region, Progress variety forms the highest grain yield - 3.88 t/ha. Higher yields in the other studied varieties Predel and Victoria are achieved when applying the first term of sowing and double fertilization with nitrogen fertilizer. (Dragov, & Samodova, 2020).
The relevance of this research is related to Bulgaria as a member of the European Union, which is a serious motivation for expanding and consolidating the area of distribution of durum wheat according to the specific climatic conditions of individual regions and countries.
In harmony with the developed strategy for the development of durum wheat and the criteria adopted by the EU under Chapter 7 "Agriculture", the areas of durum wheat in Bulgaria is to reach 218 thousand decares with a single production of 60-70 thousand tons. In this way we will approach the average European level of consumption of pasta per person per year in the range of 7-8 kg. A significant part of this production can be offered on international markets as an export item, from which our country will receive additional revenues.

MATERIALS AND METHODS

The experimental material consisted of nine Bulgarian and seven foreign varieties of durum wheat with a complex of valuable economic characteristics. As a standards are adopted varieties Progress (for productivity) and Saturn 1 (for quality).

Biological indicators

1. Structural elements of yield: wheat-ear length; number of spikelets in the wheat-ear; number of grains in the wheat-ear and mass of grains in the wheat-ear
2. Grain yield, kg/da.

Biochemical and technological indicators

The biochemical and technological quality of grain samples from nine Bulgarian varieties of durum wheat: Progress, Saturn, Victoria, Beloslava, Vazhod, Deyana, Predel, Zvezditsa and Zagorka and seven foreign varieties of different origin were studied: Auradur (Austria), Karur and Pescadou (France), Selyendur (Hungary), Grecale and Levante (Italy), Yaneiro (Spain) grown in the experimental field of the Agricultural University-Plovdiv.
HW (hectolitre weight) and TKW (thousand-kernel weight) are determined according to BDS EN ISO 7971: 2009 and BDS ISO 520: 2010. The glass vitreous content of the grain is determined according to standard EN 15585:20081. The protein content in the grain is determined by the Kjeldahl method (N x 5.7) according to BDS ISO 1871: 2001, and that of wet gluten - according to BDS EN ISO 21415-2: 2008. The functional quality of protein/gluten is determined by the sedimentation volume of ground grain with sodium dodecyl sulphate (SDS) [ICC 151: 1990] and the viscoelastic properties of wet gluten in terms of loosening and compressibility [BDS 13375: 1988]. The yellow pigment content was determined from a standard curve with pure β carotene [ISO 11052: 1994].

Field experiment

Durum wheat is sown after rapeseed as a precursor crop. Before tillage, the experiment was fertilized with P8 kg/da (triple superphosphate) and N4 (ammonium nitrate - 1/3 of the fertilizer rate). Then the soil was disc-processed three times diagonally with disc harrows. The field experiments were conducted using a randomized block design with four replications and a cultivated plot of 15 m^2.

The sowing of sixteen durum wheat varieties was carried out with a Wintershtaiger seeder in the optimal sowing period from 20.10. to 05.11. Early in the spring, the remaining 2/3 of the nitrogen fertilizer norm was fed. After sowing, the soil was compacted by rolling.

Weed, disease and pest control is carried out as needed after inspection of the crop and with appropriate pesticides (Yanev et al., 2008). Harvesting of the tested varieties of durum wheat was carried out in the phase of full maturity with a Wintershtaiger plot harvester.

Precipitation during the growing season of durum wheat is as follows: 2013/2014 - 458.1 mm/m^2; 2014/2015 - 388.5 mm/m^2, 2015/2016 - 396.5 mm/m^2 with a climatic standard of 419.0 mm/m2. In the studied years favourable for the growth and development of durum wheat with good rainfall distribution was the harvest year 2016 (despite less than the climatic standard, but better distributed rainfall during the critical phenophases of plant development) when higher yields were obtained from all durum wheat varieties. The second year 2014/2015 was unfavourable for the development of the plants due to the lower rainfall in April, when the structural elements of the yield are formed. (Figure 1) and (Figure 2).

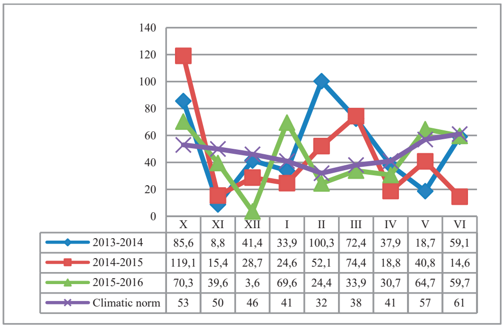

Figure 1. Precipitation by months, sum mm/m^2 (2013-2016)

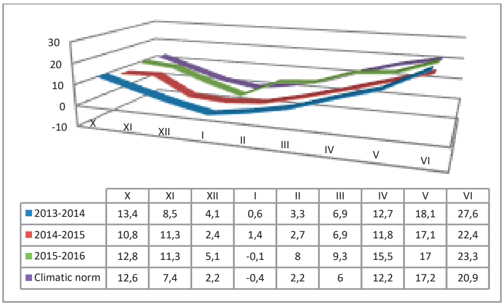

Figure 2. Monthly temperatures (average 2013-2016)

The statistical processing of the data obtained for the studied indicators was performed with BIOSTAT software (Penchev, 1998).

RESULTS AND DISCUSSIONS

Structural elements of yield and productivity of the tested varieties of durum wheat.
For the surveyed period the longest wheat-ears are observed in the plants of Zvezditsa variety 9.14 cm (1.6%) followed by the Karur variety 9.05 cm (0.6%). The length of the wheat-ear of the other tested varieties was shorter than that of the standard Progress variety (Table 1).

Table 1. Structural elements of yield (average 2013-2016)

Varieties	Wheat-ears	Number of spikelets in the wheatear	Number of grains in the wheatear	Mass of grains in the wheatear (g)
1. Progress	9,0	23,0	49,3	2,43
2. Saturn 1	8,36	21,7	47,6	1,96
3. Vuzhod	7,60	23,8	50,6	2,18
4. Victoria	6,90	25,1	51,7	2,35
5. Zvezditsa	9,14	23,6	55,4	2,44
6. Predel	8,12	25,5	52,9	2,38
7. Deyana	8,32	25,7	54,1	2,53
8. Beloslava	8,06	24,2	52,6	2,09
9. Auradur	8,91	22,7	42,3	1,90
10. Levante	8,28	21,6	43,1	2,07
11. Karur	9,05	25,0	52,3	2,21
12. Selyendur	7,72	23,5	49,5	1,71
13. Grecale	7,92	21,8	44,7	1,64
14. Pescadou	8,84	24,0	51,1	2,20
15. Yaneiro	7,93	21,1	44,4	1,47
GD 5 %	0,42	1,48	3,69	0,19

During the three-year period of the experiment it was found that Deyana variety forms the largest number of spikelets in the wheat-ear 25.7 (11.7%) followed by Predel variety with 25.5 pcs. (10.9%), Victoria 25.1 pcs. (9.1%), and Karur 25.0 pcs. (8.7%), which is respectively by 2.7 pcs, 2.5 pcs, 2.1 pcs. and 2.0 pcs. of spikelets more than the standard (Table 1). Of the foreign varieties, more spikelets in the wheat-ear were formed by Karur and Pescadou varieties.

It was found that on average for the study period Zvezditsa variety has the highest number of grains in the wheat-ear of 55.4 pcs. (12.4%). In second place is Deyana variety 54.1 pcs. (9.7%), followed by the variety Predel with 52.9 pcs. (7.3%). Beloslava, Victoria, Karur and Pescadou varieties formed a larger number of grains than the standard. In the varieties Saturn 1, Grecale, Yaneiro, Levante and Auradur, the number of grains in the wheat-ear was lower than of Progress variety (Table 1).

During the three-year period of the experiment it was found that with a higher mass of grains in the wheat-ear compared to the standard variety Progress are distinguished the plants of varieties Deyana 2.53 g (4.1%) and Zvezditsa 2.44 g (0.4%) (Table 1). The mass of the grains in the wheat-ear of the other varieties tested was lower than Progress. The smallest were the grains of the variety Yaneiro 1,47 g.

The highest grain yield (Table 2) on average for the conditions of the studied period was obtained from Deyana variety 387.8 kg/da, which is 44.0 kg/da (12.8%) more than the standard Progress variety. This was followed by Predel variety with 381.9 kg/da (11.1%) and Zvezditsa variety with 377.7 kg/da (9.9).

Table 2. Grain yield, kg/da (average 2013 - 2016)

Varieties	2013-2014	2014-2015	2015-2016	Average	%
1. Progress	340,5	301,5	389,3	343,8	100,0
2. Saturn 1	334,4	297,2	365,1	332,2	96,6
3. Vuzhod	364,7	323,2	401,5	365,1	106,2
4. Victoria	371,8	329,1	416,3	372,4	108,3
5. Zvezditsa	392,3	311,6	429,1	377,7	109,9
6. Predel	383,1	324,3	438,4	381,9	111,1
7. Deyana	387,5	343,8	432,2	387,8	112,8
8. Beloslava	378,6	308,6	413,7	367,0	106,7
9. Auradur	257,8	228,6	315,3	267,2	77,7
10. Levante	266,3	280,9	327,1	291,4	84,8
11. Karur	367,7	323,8	406,8	366,1	106,5
12. Selyendur	359,2	314,3	397,9	357,1	103,9
13. Grecale	272,7	283,5	309,3	321,8	93,6
14. Pescadou	365,7	323,5	404,1	364,4	106,0
15. Yaneiro	294,5	295,2	339,6	309,8	90,1
GD 5 %	26,8	22,5	25,3		

The high productivity is due to the large, and very well-grained wheat-ear with a high grain mass per class. Of the tested foreign varieties Karur, Pescadou and Seliendur are characterized by high productivity, as their yield is by 22.3 kg/da (6.5%); by 20.6 kg/da (6.0%), and by 13.3 kg/da (3.9%) more than the standard.

The obtained grain yield from the varieties Auradur, Levante, Yaneiro, Grecale and Saturn 1 was lower than the standard variety Progress.

Biochemical and technological evaluation of Bulgarian and foreign varieties of durum wheat (*Triticum durum* Desf.)

The grain of the samples was dry, with moisture around the baseline between 13.1% -13.5%.

Depending on the variety, year and origin of the wheat, the TKW and HW of the world collection of durum wheat are in the range of 30-62 g, and 72-87 kg/hl respectively. The studied Bulgarian varieties are characterized by larger and heavier grain. They have the TKW above the standard Saturn 1 and for the variety Zvezditsa the highest value is measured 55.8 g.

Table 3. Physical properties of the grain of Bulgarian and foreign varieties of durum wheat (*Tr. durum* Desf.)

Varieties	Moisture %	TKW G	HW kg/hl	Vitreousness %
1. Progress	13,56	54,5	80,8	98
2. Saturn 1	13,50	49,0	82,0	92
3. Vuzhod	13,31	49,8	82,8	92
4. Victoria	13,04	51,4	82,0	96
5. Zvezditsa	13,09	54,0	82,4	96
6. Predel	13,13	53,6	80,8	98
7. Deyana	13,36	55,8	82,4	72
8. Beloslava	13,25	54,0	81,6	90
9. Auradur	13,33	46,8	80,4	88
10. Levante	13,25	45,6	78,4	90
11. Karur	13,13	42,4	82,4	90
12. Selyendur	13,17	47,0	74,4	92
13. Grecale	13,13	48,0	80,4	90
14. Pescadou	13,10	44,0	81,6	90
15. Yaneiro	13,33	46,6	79,2	86
16. Zagorka	13,18	48,4	83,2	74

Bulgarian varieties are characterized by a higher TKW and HW compared to foreign durum wheat varieties. The HW of the Bulgarian varieties varies from 80.8 kg/hl for the varieties Progress and Predel to 83.2 kg/hl for Zagorka variety. For foreign durum wheat varieties, the HW ranges from 74.4 kg/hl for Selyendur to 82.4 kg/hl for Karur (Table 3).

The relationships between the HW and TKW, as indicators of the endosperm-grain husk ratio, with the milling properties of wheat are quite variable according to the place and year of cultivation. The variability explains the preferences for one or the other indicator as forecast for high yield of semolina and the different strength of their relations with the milling quality. According to Dexter (1996), when the HW decreases, the milling potential of wheat deteriorates due to the combined effect of lower semolina yield, higher ash content, and lower yellow pigment content in semolina. In a field experiment, four varieties of winter wheat were tested, sown on three sowing dates and fertilized with five rates of nitrogen. On average for all sowing dates and fertilization rates, the highest grain mass was reported in the variety Pobeda - 44 g, while the lowest was reported in the variety PKB - Lepoklasa (38 g). The highest TKW was registered for all wheat varieties in 1999, when the meteorological conditions were good for the development of wheat. The date of sowing and the amount of nitrogen fertilizers have a significant effect on the mass of 1000 grains (Protic et al., 2007). Despite the contradictory opinions, HW is a generally accepted standard indicator in the classification of durum wheat as a measure of the health status, as all factors that defect the grain also affect the HW (frost, immaturity, shrinking, quick sprouting, disease).

Table 4. Biochemical and technological evaluation of Bulgarian and foreign varieties of durum wheat (Triticum durum Desf.)

Varietys	Crude protein %	Wet gluten production %	Dry gluten %	Yellow pigment ppm d. m.	SDS - sedimentation number cm^3
Progress	16,76	35,7	13,1	7,05	25
Saturn 1	11,17	31,1	11,0	6,75	46
Vuzhod	14,42	26,8	9,7	7,96	20
Victoria	12,77	33,8	12,2	5,13	25
Zvezditsa	16,82	39,0	13,7	6,46	38
Predel	15,16	33,7	12,5	6,82	30
Deyana	13,57	27,1	9,9	6,22	30
Beloslava	15,96	33,8	12,3	8,98	43
Auradur	16,76	37,0	13,4	10,55	54
Levante	14,36	36,0	12,8	9,90	65
Karur	11,17	36,8	13,0	9,64	84
Selyendur	15,16	44,5	15,3	9,03	71
Grecale	15,96	40,1	13,8	10,49	46
Pescadou	13,57	33,2	11,8	10,33	73
Yaneiro	11,97	34,8	12,0	10,82	35
Zagorka	11,97	31,4	10,9	6,49	30

Vitreousness as a standard indicator reflects the structure of the endosperm, due to its proven relation with yield and size of semolina, ash content, protein and yellow pigments in semolina. In dry years such as the 2015 crop, vitreousness is high, with an average of 90% for the tested varieties, while in wet years, vitreousness decreases significantly, especially if there is precipitation during the grain harvest period, such as the previous 2014 with an average value of 50.5%.

The presence of more flour grains leads to a reduction in the amount of semolina during grinding and its quality.

The colour potential depends on the amount of natural pigments in the grain. The pigments have a yellow and brown colour. Carriers of the yellow component are carotene and xanthophyll and water-soluble flavones. Brown pigments are the products present in the grain from the decomposition of chlorophyll, products from the action of basic proteins, and the isomerization of carotenoids. Durum wheat contains 2 to 5 times more yellow pigments than bread wheat. The better colour potential of the studied foreign varieties included in the project is genetically determined. They have an average of 10.11 ppm d.m. against 6.87 ppm d.m. for the Bulgarians. The grain of the Spanish variety Yaneiro demonstrates the highest content of yellow pigments 10 ppm d.m. Excessive nitrogen fertilization can have an adverse effect on the colour, due to the inverse relation of colour-protein content, or improper storage of wheat in granaries.

Grain crude protein ranges from 11.17% to 16.82% depending on the variety and environmental conditions. Modern pasta production requires semolina with over 14% protein, which corresponds to over 15% in wheat. The highest protein content for the study period was determined for the varieties Vazhod 16.82%, Predel 15.96%, the Austrian variety Auradur 16.76%, the French Pescadou 15.96%, and the Italian Grecale 15.16%.

According to the standard method of ICC (International Cereal Chemists) twice as high SDS - sedimentation number was determined for foreign varieties - 61 cm^3, while the Bulgarian varieties significantly lag with an average value of 32 cm^3 on average for the study period.

The quantity and quality of gluten are key indicators in wheat selection and variety testing programs worldwide. They depend on the variety and are strongly influenced by the growing conditions. During the three-year experiment, the foreign varieties have an average value of 37.5% and are superior to our varieties having an average value 32.5 %.

CONCLUSIONS

On average for the three-year study period the most productive are the Bulgarian varieties of durum wheat Deyana 387.8 kg/da; Predel and Zvezditsa, where the increase in grain yield is higher by 44.0 kg/da (12.8%), 38.1 kg/da (11.1%), and 33.9 kg/da (9.9%) respectively, compared to Progress variety.

Of the foreign varieties with higher grain yield on average for the study period are distinguished the varieties Karur 366.1 kg/da and Pescadou 364.4 kg/da, which is by 22.3 kg/da (6.5%) and 20.6 kg/da (6.0%) more than the standard Progress variety.

The high productivity is a result of the large and very well-grained class with a high grain mass in the wheat-ear of these varieties.

The studied varieties included in the experiment show their potential for quality in the dry, with favourable climatic conditions year of 2015. The wet 2014 and especially the rainfall during the harvest period, strongly negatively affected the indicators TKW and vitreousness of the grain, yield of wet, and dry gluten. The content of crude protein, yellow pigments and the volume of the SDS-sedimentation number were also negatively affected, though to a weaker extent.

Bulgarian varieties are characterized by a higher TKW and HW compared to foreign durum wheat varieties.

The group of foreign varieties of different origin is characterized by a higher content of yellow pigments and volume of SDS-sedimentation number.

The values of crude protein and wet and dry gluten yield are close for all durum wheat varieties studied, but the foreign varieties have better quality protein, respectively gluten.

Of the highest quality is the Austrian variety Auradur, and of the Bulgarian varieties - Predel variety.

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EVALUATION OF ALLELOPATHIC ACTIVITY OF EXTRACTS OF PLANT ORGANS OF VARIOUS VARIETIES OF WINTER WHEAT

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Abstract

The article presents the results of scientific studies devoted to the study of the effect of water extracts of the roots, stems, leaves and grain of winter wheat plants on seed germination and root growth of single-day watercress seedlings. Samples of plants of the studied winter wheat varieties were taken in the phase of full grain ripeness. Water extracts of plant organs at a concentration of 1:100 were prepared for the Biotest. According to the results of research, the stimulating and inhibitory effect of water extracts from the organs of winter wheat plants on seed germination and growth of single-day watercress seedlings was established, depending on varietal characteristics. Water extracts from the roots of plants of all studied varieties reduced the germination rate of test culture seeds by 4.0 up to 40.4% compared to the control, and grain extracts had a slight stimulating effect. It is established that the variety Schedrivka Kyivs'ka has a high allelopathic activity. Water extracts of organs of soft winter wheat varieties Vidrada and MIP Assol were determined to be neutral to the germination of watercress seeds.

***Key words**: allelopathic action, winter wheat, varieties, watercress, seed germination.*

INTRODUCTION

The world's organic economy is constantly developing and its area is growing every year. As of 2018 yr, 1.5% of the world's agricultural land, or 71.5 million hectares, is allocated to organic agriculture, including by region: 36.0 million hectares in Oceania and 15.6 million hectares in Europe (Willer et al., 2020).

Most of the organic arable land (4.8 million hectares) is occupied by cereals, 33.9% of which is wheat. Organic agriculture in Ukraine is dominated by grain production, which is allocated 43.2% of organic land (133,440 thousand hectares). But this is only 0.9% of the total area allocated for these crops in the country, while in Austria it is allocated 15.6%, in Italy it is allocated 10.4%, in Germany it is allocated 4.8%, in Belgium it is allocated 3.8% (Willer et al., 2020). The main reason for the slow expansion of organic acreage is low yields, which is due to the cessation of the use of mineral fertilizers and chemical plant protection products.

The search for biological regulators of plant growth has become one of the promising ways to develop environmentally sustainable methods for regulating the number of weeds in agrophytocenoses (Tahir et al., 2019; França Teixeir et el., 2018; Scavo et al., 2019; Orel et al., 2005; Kyrychenko 2014).

As a process, allelopathy is of great ecological importance for phytocenoses in the context of plant growth regulator, their productivity, and the species composition of natural and cultivated coenoses (Moskalyk & Leheta, 2019; Inayat et al., 2019; Rany Das, & Kato-Noguchi, 2018).

It is known that allelopatically active substances, depending on their concentration and environmental conditions, can have a stimulating or inhibitory effect on growth and development, as well as on various metabolic processes in plants and thus can be natural regulators of plant growth (Fidelis Giancotti et. al., 2020; Salman et. al., 2017; Ozkan. et al., 2019).

Research taken by scientists has established that winter wheat has allelopathic properties for controlling weeds, pests and diseases, which can be used in plant protection and will contribute to its cultivation using organic technologies (Wu et. al., 2001; Aslam et. al., 2016; Lam et al., 2012; Amist 2019). But when studying these properties, varietal characterristics of the culture and origin are not always taken into account.

According to Derevyanko V. A. (2003) in the characteristics of the wheat variety, it is also necessary to take into account allelopathic parameters as an integral component of the variety passport, since plants show different tolerance to allelopathically active substances. Therefore, one of the promising directions for the development of allelopathy is a screening test of various varieties and varieties of cultivated plants in order to study their allelopathic potential or resistance to allelopathically active substances (Derevyanko, 2007; Wu & Pratley, 2000; Kabir, 2010).

Therefore, the aim of our work was to study the allelopathic properties of 10 varieties of soft wheat and 1 variety of spelt winter wheat varieties of domestic and foreign selection.

MATERIALS AND METHODS

We studied the allelopathic activity of vegetative and generative organs of plants of various winter wheat varieties.

To establish the allelopathic activity of various winter wheat varieties in 2019-2020 yrs, the field experiment was conducted in the experimental field of the Educational, Scientific and Practical Center of the Mykolaiv National Agrarian University, Mykolaiv, Ukraine. The technology of winter wheat growing was generally accepted for the southern steppe zone of Ukraine. Its predecessor was sown peas. The sowing period is 1st of October. The seeding rate of winter wheat seeds was 5 million germinating seeds / ha. The material for the study was 9 winter wheat varieties bred in different soil and climate zones of Ukraine (Table 1)

Table 1. Characteristics of winter wheat varieties

Variety denomination	variety	height	disease resistance	grain quality
Vidrada	*erithrospermum*	medium-sized	average	strong
Koshova	*erithrospermum*	medium-sized	above average	strong
Schedrivka kyivs'ka	*lutescense*	medium-sized	above average	valuable
Krasa laniv	*erithrospermum*	medium-sized	above average	strong
Kvitka poliv	*lutescense*	medium-sized	above average	valuable
MIP Assol'	*lutescense*	medium-sized	resistant to diseases	filler
Harantiia odes'ka	*erithrospermum*	medium-sized	persistent	strong
Schedrist' odes'ka	*erithrospermum*	half-dwarf	persistent	valuable
Centurion	*erithrospermum*	half-dwarf	persistent	filler

Variants in the experiment were placed by the split section method, the repetition was four times. The area of the accounting plot was 25 m². Soil of experimental sites was southern black soil low-humus, light loamy on the loess's of wide weakly drained water-dividing plateaus, which is typical for the Southern Steppe zone of Ukraine.

Humus content in the arable soil layer was 2.4%, easily hydrolyzed nitrogen content was 16 mg/kg, mobile phosphorus content was 160 mg/кг and exchangeable potassium content was 187 mg/kg of soil.

To establish the allelopathic properties of winter wheat varieties suitable for distribution in the steppe zone of Ukraine, the method of bioassays of Grodzinsky (1973). Watercress was selected as the test object (*Lepidium sativum* L.) in accordance to it has a high sensitivity to allelopathic substances (Islam and Kato-Noguchi, 2013). The growth of the roots of the test object in distilled water was taken as control (100%).The research was conducted in 2020 at the Department of crop production and gardening of the Mykolaiv National Agrarian University (Mykolaiv, Ukraine).

The effect of water extracts of roots, leaves, stems and seeds of winter wheat plants on root growth and germination of watercress seeds was evaluated.

The greatest number of growth inhibitors accumulates in this phase.

Since the greatest number of growth inhibitors accumulates in the phase of full ripeness of winter wheat grain, sheaf samples of each of the studied varieties were selected during this period. Roots, stems, leaves, and grains were separated in the laboratory. The selected samples of vegetative organs of soft wheat and grain plants were dried at a temperature of

+65°C to a constant mass and they were crushed. An average weight of 1 g was selected, which was extracted in 100 ml of distilled water in a thermostat during the day at a temperature of +25 °C. To remove the solid fraction of the extract, the extract was filtered through filter paper and immediately used for the experiment laying.
1-day-old watercress seed sprouts were placed in sterile Petri dishes with a diameter of 9 cm on sterile filter paper soaked in 3 ml of filtrate (seeds were considered sprouted if the root length was 1 mm. Petri dishes were placed in a thermostat at +25 °C for one day. The control variant was treated with distilled water. The root length was measured using digital caliper DIGITAL CALIPER 391110 with an accuracy of 0.02 mm.
The experiment was completely randomized. A fourfold repetition of each studies of 100 seeds was used.
The germination rate of watercress seeds was determined on the Fifth Day.

RESULTS AND DISCUSSIONS

According to Ivanytska B. O. (2008), plant leaves and their secretions are more toxic; roots and root extracts are less toxic; stems occupy an intermediate position.
Other scientists (Krumsri et. al., 2020) claim that leaf and stem extracts have a higher inhibitory effect on rice seed germination than root extracts, as they contain higher amounts of phenolic compounds. Our research only partially confirms this.
Table 1 shows the effect of water extracts of four parts of plants of different varieties of winter wheat on the germination of watercress seed (Table 2).

Table 2. Effect of various extracts of winter wheat varieties on the germination of watercress seeds, (%)

Variety	Parts							
	root	% to control	stem	% to control	leaf	% to control	seed	% to control
Distilled water (control)	92.0	100.0	92.0	100.0	92.0	100.0	92.0	100.0
Krasa Laniv	88.3	96.0	74.3	80.8	90.3	98.2	94.0	102.2
Vidrada	86.8	94.3	95.8	104.1	66.0	71.7	97.3	105.8
Kvitka Poliv	67.8	73.7	98.8	107.4	70.3	76.4	93.0	101.1
Koshova	54.8	59.6	88.0	95.7	62.8	68.3	99.3	107.9
Schedrivka Kyivs'ka	85.8	93.3	68.3	74.2	70.3	76.4	98.5	107.1
MIP Assol'	88.8	96.5	83.3	90.5	96.8	105.2	94.0	102.2
Harantiia Odes'ka	71.5	77.7	76.3	82.9	72.0	78.3	98.5	107.1
Centurion	76.0	82.6	94.0	102.2	50.3	54.7	89.5	97.3
Schedrist' Odes'ka	84.3	91.6	82.0	89.1	73.0	79.3	95.0	103.3
Average by variety	**79.6**	**86.5**	**85.3**	**92.7**	**74.4**	**80.9**	**95.1**	**103.4**
The least significant difference (LSD) at $p<0.05$: factor A and B = 2.09%								

It was found that water extract from the leaves of winter wheat plants had a greater negative impact on the germination of watercress seeds, which averaged 74.4% for varieties (80.9% of the control). It was by 5.6% less than in the version with water extract from leaves, and it was by 11.8% less than in the version with water extract from stems and by 22.5% less than in the version with water extract from seeds.
It was determined that different parts of the plants of each studied variety had different inhibitory or stimulating effects on the germination of watercress seeds. At the same time, water extracts from the leaves of Centurion plants had a more negative effect (89.5%) on the seed germination of test crops, while extract from the leaves of MIP Asol' plants had a positive effect (105.2%).
The winter wheat variety Koshova was determined, the water extract from the roots of which had a more inhibitory effect on the germination of watercress seeds down to 54.8%, which was 94.3% before the control. It was less reduced seed germination of the studied test culture varieties: such as Krasa

laniv down to 88.3% (96.0% before the control), MIP Asol' down to 88.8% (96.5%), Vidrada down to 86.8% (94.3%), Schedrivka Kyivs'ka down to by 85.8% (93.3%) and Schedrist' Odes'ka down to 84.3% (91.6%).

It was found that water extracts from the stems of winter wheat plants reduced the germination rate of watercress seeds less compared to extracts from roots and leaves, which was an average of 92.7% for varieties compared to the control. The lowest seed germination rate (68.3%) was when it was treated with an extract from winter wheat stalks of the Schedrivka Kyivs'ka variety down to 74.2% before the control. Water extracts from the stems of plants of Vidrada, Kvitka Poliv and Centurion varieties had a slight stimulating effect on the germination of test culture seeds, which was 104.1%, 107.4%, 102.2%, respectively, according to the control.

It was determined that water extracts from the grain of most of the studied winter wheat varieties increased the germination rate of watercress seeds by 101.1 up to 107.9% before the control, while Centurion varieties had a slight inhibitory effect (down to 97.3% before the control).

Research results (Petcu E. at ell., 2017) show that the degree of inhibition of ryegrass roots depended on the genotype of winter wheat. Our research confirms this fact on as well as on watercress. Inhibition of watercress root growth was observed by the action of water extracts from the roots of plants of all studied winter wheat varieties, except for the MIP Assol variety, whose water extracts had a slight stimulating effect. The most inhibitory effect on the length of watercress roots (2.60±0.43 mm) was exerted by water extract from the roots of plants of the Koshova variety, which was by 69.6% less than in the control variant. The coefficient of variation of the trait was 81.6%

The coefficients of variation range from high (=21-30%) to very high (>40%). The lowest coefficient of variation in the growth of watercress roots is characteristic in the variant of action of extract from winter wheat seeds of the Harantiia Odes'ka variety as 29.4%, the largest one (97.6%) is in the variant with water extract from winter wheat roots of the Schedrivka Kyivs'ka variety (Table. 3).

Table 3. Growth of watercress Roots depending on seed treatment with water extract from various plant organs and varieties of winter wheat

Variety	Parts							
	root		stem		leaf		seed	
	M±m min-max	V, %	M±m min-max	V, %	M±m min-max	V, %	M±m min-max	V, %
Control	8,55±0,59	55,75	8,55±0,43	38,25	8,55±0,30	50,81	8,55±0,28	31,73
Krasa laniv	4,55±0,41	67,70	7,30±0,44	48,16	7,43±0,22	32,41	7,65±0,25	32,41
Vidrada	7,21±0,56	68,91	8,47±0,50	56,67	8,01±0,31	32,83	6,76±0,25	33,83
Kvitka Poliv	4,45±0,60	83,41	7,43±0,43	43,62	7,71±0,29	35,84	6,61±0,29	35,85
Koshova	2,60±0,43	81,59	10,41±0,65	56,36	6,64±0,29	59,49	7,68±0,69	63,74
Schedrivka Kyivs'ka	4,87±0,64	97,60	3,83±0,37	56,10	7,88±0,28	45,95	6,56±0,59	62,71
MIP Assol'	9,49±0,71	58,72	7,43±0,44	52,93	9,92±0,29	40,68	6,16±0,22	34,77
Harantiia Odes'ka	3,20±0,32	62,53	6,21±0,53	57,02	8,32±0,32	49,00	9,30±0,28	29,40
Schedrist' Odes'ka	3,55±0,43	85,7	7,43±0,43	50,74	8,86±0,32	44,72	7,65±0,3	37,77
Centurion	4,29±0,43	67,1	11,01±0,47	40,56	6,71±0,28	47,84	6,13±0,31	47,84

The coefficients of variation range from high (=21-30%) to very high (>40%). The lowest coefficient of variation in the growth of watercress roots is characteristic in the variant of action of extract from winter wheat seeds of the Harantiia Odes'ka variety as 29.4%, the largest one (97.6%) is in the variant with water extract from winter wheat roots of the Schedrivka Kyivs'ka variety.

The increase in the root length of the test culture was less than the effect of water extracts from the organs of winter wheat plants of the Krasa laniv, Vidrada, Kvitka Poliv and Schedrivka Kyivs'ka varieties in all the studied variants. The data are statistically reliable, with the coefficient of variation ranging from 32.4% up to 97.6%.

As can be seen from Table 2, the growth of watercress roots was stimulated by using an aqueous extract from the roots of winter wheat of only one studied variety such as MIP Assol'. The root length in this variant was 9.49±0.71 cm, which was by 11.0% higher than the control. Extract from the stems of winter wheat varieties Koshova and Centurion also had a stimulating effect on the germination of test culture seeds, the root length was 10.41±0.65 mm and 11.01±0.47 mm, respectively, which was by 21.8% and by 28.8%, respectively, more than in the control. Extracts from the leaves of winter wheat plants of MIP Assol' (9.92±0.29 mm) and Schedrist' Odes'ka (8.86±0.32 mm) slightly stimulated the germination of watercress seeds, the coefficient of variation was 40.68% and 44.72%, respectively.

Among the studied winter wheat varieties, only water extract from Harantiia Odes'ka grain stimulated the germination of test culture seeds. The length of the roots was 9.30 ±0.28 mm, which was by 8.77% higher than the control. The data is statistically reliable.

The results of our studies showed that in relation to watercress, extracts of water extracts from the roots of plants of most of the studied varieties of winter wheat (except for the MIP Assol variety) showed allelopathic activity. At the same time, the greatest allelopathic activity was characteristic of the Koshova variety (as 65.10%). A significant inhibitory allelopathic effect of water extracts from the stems of winter wheat plants of the Schedrivka Kyivs'ka variety (-55.2%) on the length of watercress roots was observed (Figure 1.)

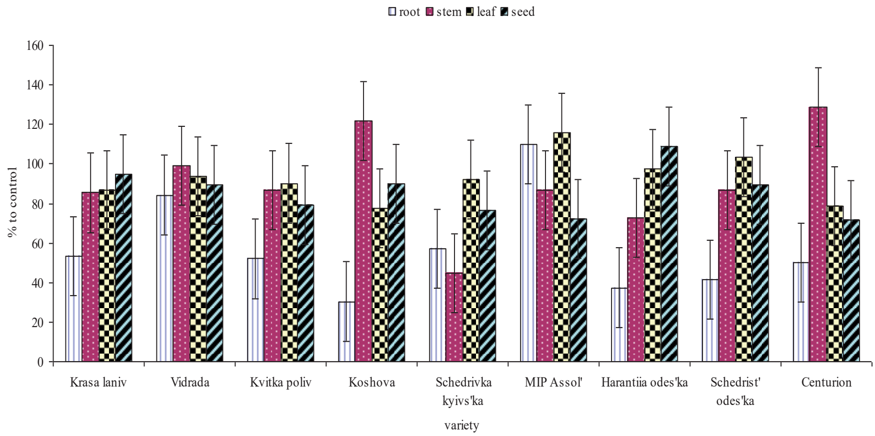

Figure 1. Allelopathic activity of water extracts of P organs of winter wheat plants depending on the variety (% to control)

Studies showed that water extracts from the leaves of winter wheat plants Koshova (-22.34%) and Centurion (-21.52%) had the greatest inhibitory effect, which was 77.7% and 78.5% before the control, respectively .

It was found that the extract from Centurion winter wheat grain had a greater inhibitory effect (-28.3%) on the growth of test crop roots. Varieties of extensive use should have a high allelopathic activity in order to create their own allelopathic regime in the crop and counteract the penetration of weeds. Varieties of intensive use should be characterized by low allelopathic activity (Derevyanko, 2007). We found that the Shchedrivka Kievskaya winter wheat variety had a high allelopathic activity in the phase of full grain ripeness (from -7.84 to -55.2%), and the lowest one were with Vidrada varieties (from -0.94 down to -13.50%) and MIP Assol' (+16.02 down to -27.95%).

The correlation between seed germination and watercress root growth under the influence of water extracts of plant organs of different varieties of winter wheat was determined. A very weak inverse relationship was typical in variants with Krasa Laniv and Schedrist' Odes'ka varieties (-0.03494 and -0.24687, respectively). A weak direct relationship was typical in variants with the varieties Schedrivka Kyivs'ka (0.144954), MIP Assol' (0.221724), Centurion (0.403765) and Kvitka Poliv (0.455515) (Figure 2).

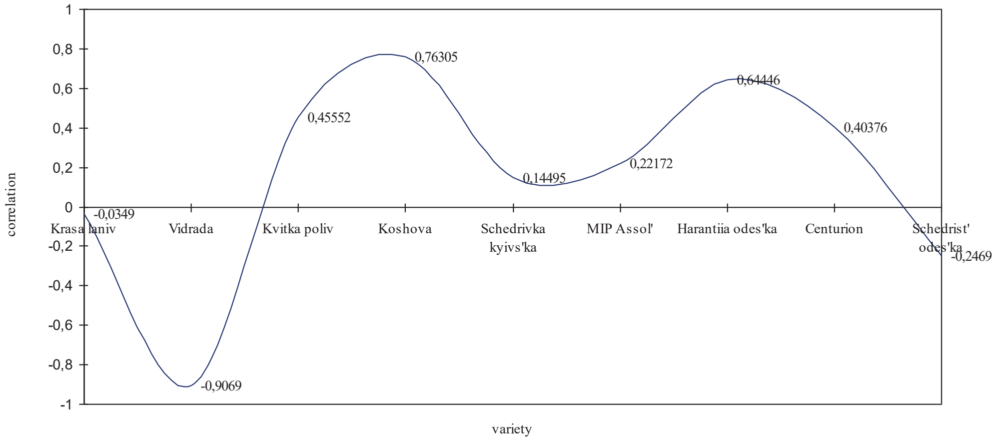

Figure 2. Allelopathic activity of water extracts of organs of winter wheat plants depending on the variety, (% to control)

In variants with water extracts of plants of Harantiia Odes'ka and Koshova varieties, a moderate dependence was observed (0.64446 and 0.763049, respectively). It was determined that only the Vidrada variety was characterized by a high relationship between seed germination and watercress root growth as 0.90691.

CONCLUSIONS

Then, the organs of winter wheat plants of the Koshova variety, in allelopathic terms, are quite active and they contain a large amount of physiologically active substances of both stimulating and inhibitory action.

Water extracts from the organs of winter wheat varieties Centurion, Koshova and Harantiia Odes'ka more suppressed the germination of test culture seeds than other varieties studied.

The most inhibitory effect on the growth of watercress roots was provided by water extract from the roots of plants of the Koshova variety.

It was found that the winter wheat variety Schedrivka Kyivs'ka had a high allelopathic activity, and the varieties Vidrada and MIP Assol were neutral to the germination of watercress seeds. A high correlation (0.90691) between seed germination and watercress root growth was determined.

In the future, the studied varieties will be subjected to more detailed testing both at different stages of development and with other test crops.

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AGROECOLOGICAL EFFICIENCY OF SEED INOCULATION WITH BIOLOGICAL PRODUCTS AND COMPLEX FERTILIZERS WITH MICROELEMENTS IN RESOURCE-SAVING TECHNOLOGY OF CULTIVATION OF CLOVER OF PANNONIAN VARIETY ANIK

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Abstract

The article deals with the influence of biological products and complex fertilizers with microelements in a chelated form on the formation of parameters of photosynthesis, symbiotic activity and productivity of clover of the Pannonian variety ANIK. It was found that inoculation of seeds with biological products of the associative group and complex fertilizers with microelements in a chelated form activated growth, morphogenetic processes, symbiotic and photosynthetic activity of Pannonian clover plants. The number and weight of active nodules in relation to the control increased by 29.2-167.6 million pl./ha and 77.7-564.1 kg/ha, respectively. The maximum parameters of the symbiosis of the agrocenosis of Pannonian clover (active nodules 221.8 million pl./ha with a weight of 893.7 kg/ha) were formed when clover seeds were inoculated with the biological product Gumariz together with the silicon-containing preparation NanoSilicon. The highest indicators of the leaf surface of clover, 53.6 thousand m^2/ha, were observed during the complex seed treatment with Gumariz together with the NanoSilicon preparation, control - 28.7 thousand m^2/ha. The highest productivity of Pannonian clover was obtained when seeds were inoculated with the biological product Gumariz together with the NanoSilicon preparation: dry matter - 12.48 t/ha, feed units - 9.05 t/ha, digestible protein - 1.39 t/ha; the yield of clover seeds was 786.8 kg/ha, which is 2.2 times higher than the control indicators; profitability for cultivation for seeds - 187.3%, for fodder purposes - 149.6%, energy efficiency coefficient - 2.24 and 1.89 units.

Key words: *inoculation, Pannonian clover, biological products, productivity.*

INTRODUCTION

The most important problem in agriculture is to increase the production of feed, improve its quality and energy saturation. As a result of the imperfection of the structure of the sown areas of forage crops, especially the low specific weight of leguminous grasses, the gross harvest and the quality of forage have now decreased.
Despite the richness of natural flora in the field cultivation of the country, the number of adaptive and productive forage legumes is still extremely small. Currently, no more than 25 species of plants are cultivated for fodder purposes, including perennial legumes - oriental goat's rue, red clover, Pannonian clover, alfalfa, sweet clover and others. The actual problem of modern plant growing is the search for the most effective growth regulators and optimal ways of using them.
Preparation of seeds of perennial legumes for sowing includes inoculation. Inoculation consists in treating legume grass seeds with growth bioregulators, which include rhizotorfin, a preparation containing a culture of nodule bacteria. All seeds of perennial legumes, including Pannonian clover, are inoculated without fail, since there are no nodule bacteria in the soils at all that can enter into symbiosis with plants. For each culture, its own rhizotorfin is used, which is part of the growth biostimulant. It is necessary to process seeds with growth biostimulants with preparations of nodule bacteria immediately before sowing.
Of the variety of ways of using biological products, namely, introduction into the soil, pre-sowing seed treatment and spraying of crops, pre-sowing seed treatment turned out to be the most acceptable for production, since it is well-technological, without requiring additional costs, while small doses of biological products are consumed (Kshnikatkina, 2020).
Treatment of seeds before sowing with biological products activates the initial growth

processes, this contributes to a more intensive transition of seedlings from heterotrophic nutrition to autotrophic nutrition. There is an increase in germination energy by 1.2-5.3%, laboratory germination - 1.6-4.2%, sprout length - by 0.2-0.7 cm, embryonic root length - by 0.4-0.8 cm (Kshnikatkina, 2020). In addition, growth regulators have a multifunctional effect, since seeds at the time of germination have high plasticity and susceptibility to changes in environmental conditions.

There are four main forms of biologics: powder, granular, dry and liquid. Powder and dry biostimulants are not always effective, since they have low adhesion and uneven distribution on the seed surface, which affects the quality indicator of inoculation - processing completeness (at least 95%). In order for the particles to better adhere to the surface of the treated seeds, adhesives are added to the aqueous suspension of the preparation, which is not quite technological. Granular biological products are mainly introduced into the furrow during seed placement. With this method, bacteria can die due to high temperatures of the surface layer of the soil, a lack of moisture on the surface of the soil and an increased concentration of chemicals. The best in the method of seed treatment, in the method of application to the surface are liquid biological products, which are mixed with water and applied to the seeds.

According to the Penza Research Institute of Agriculture, an effective method for increasing the productivity of perennial legumes on soils poorly supplied with microelements is the treatment of seeds with liquid complex biological products Mikromak. The increase in dry matter yield of alfalfa variety Camellia of the 1st year of use was 26.2% and exchange energy - 31.8%, meadow clover variety Pelikan - 29.1%, 45.5% and 34, 9%, sweet clover of the hairy variety Solnyshko - 26.2%, 40.9% and 32.4%, respectively (Kshnikatkina et al., 2020).

From the above it follows that the problem of strengthening the fodder base by pre-sowing treatment of seeds of perennial legumes with biological products in order to increase seed germination is relevant and practically significant for the agro-industrial complex of the Russian Federation.

In this regard, it is important to organize adaptive forage production due to highly productive new types of leguminous grasses, which have ecological plasticity, high nitrogen-fixing ability, high fodder values, rationally use the bioclimatic resources of the region, with sustainable seed production (Kshnikatkina, 2015; Kshnikatkina et al., 2018).

One of the main factors in the ecologization and biologization of agriculture is the fixation of nitrogen by the rhizome symbiosis of legumes. The use of biological nitrogen in agriculture provides a reduction in energy consumption, saving material resources, and reduces environmental pollution by products of nitrogen fertilizer degradation (Fedorov, 1952, Mishustin, 1979, Posypanov, 1993).

By optimizing the conditions for the functioning of the legume-rhizome symbiosis, it is possible to increase the efficiency of nitraginization (Trepachev, 1980).

An important element of adaptive, resource-saving technologies of forage crops is the inoculation of seeds with bacterial preparations, the use of complex fertilizers with microelements in a chelated form and growth regulators that provide plants with missing microelements, contribute to an increase in the efficiency of legume-rhizome symbiosis, yield and resistance to stress factors of the external environment and pathogens (Kshnikatkina et al., 2013, Kshnikatkina et al., 2007). One of the directions is the use of nanotechnology, active nanoadditives, in which microelements are used as activators of metabolic processes and growth stimulators (Fedorenko, 2011, Semina et al., 2017).

In this regard, the study of an increase in the nitrogen-fixing activity of agrocenoses of the Pannonian variety ANIK is of scientific and practical importance.

Research goal is to study the effect of seed inoculation with bacterial preparations and complex fertilizers with microelements in a chelated form on the formation of parameters of symbiotic activity and productivity of Pannonian ANIK clover.

MATERIALS AND METHODS

The studies were carried out in LLC Agrofirma "Biokor-S" of the Mokshansk district of the

Penza region on leached medium-humus heavy loamy chernozem, the humus content in the arable layer is 6.5%, mobile phosphorus - 55 mg/kg of soil, exchangeable potassium - 177 mg / kg of soil, availability of mobile forms of micronutrient fertilizers are low, pH(KCL) 5.4.
The object of research is Pannonian clover variety ANIK. The predecessor is winter wheat, the plot area is 15 m^2, the replication is four times, the distribution of plots is systematic. Seed inoculation with bacterial preparations and treatment with micronutrient fertilizers were carried out on the day of sowing. Experimental agrotechnics are generally accepted for perennial legumes in the Penza region. The concentration of the drug was taken according to the established recommendations: Megamix-Semena (2 l/t), Megamiks-Nitrogen (1 l/t). NanoSilicon (1 l/t), Cytovit (1 l/t), Simiplant (1 l/t), Humate K/Na (0.15 l/t), Agriculture + microelements (1 l/t), Rizotorfin 600 g, Gumariz 600 g (hectare norm).
The processing of seeds of clover of the Pannonian variety ANIK was carried out using environmentally friendly biotechnology and a fundamentally new design with an inoculator for pre-sowing treatment of seeds of perennial legumes with biological products. Treatment of seeds of perennial leguminous grasses with an inoculator with a high-pressure fog system with the completeness of seed treatment up to 100% and without damaging them, will increase the germination of seeds up to 98%. The high-pressure fog system (70 to 100 bar) produces a droplet with a diameter of 5-10 microns. Such a drop is kept in the working chamber for a long time, falls on the surface of the seed and easily penetrates to the embryo, thereby provoking the germination of the seed. This biotechnology makes it possible to use for processing any types of seeds of perennial legumes and any types of biological products in liquid form, since there is a system for preparing biostimulants before feeding them into the working chamber. Spray humidification with control and management of the moisture level will prevent excessive waterlogging of seeds during treatment with an inoculator before sowing and immediately sow them into the soil.
The technological scheme of the inoculator is based on the following technical solutions:
- for transporting seeds inside the working chamber in the form of a pipe, an axleless screw auger is used;
- for the treatment of seeds with biological products in the working chamber, a high-pressure fog system with nozzles is used.
The technological process of environmentally friendly biotechnology of inoculation of seeds of perennial legumes includes two devices:
- high pressure installation;
- seed inoculator.
The experiments were set up and the studies were carried out in accordance with the methodological instructions (Dospekhov, 1989, Bogomazov, 2014).

RESULTS AND DISCUSSIONS

Seeds at the moment of germination have high plasticity and susceptibility to changes in environmental conditions, therefore, inoculation of Pannonian clover seeds with bacterial preparations and treatment with micronutrient fertilizers had a multifunctional effect. Field germination on average for two years in relation to the control increased by 5.6-12.4%. The greatest stimulating effect was exerted by the treatment of seeds with the biological product Gumariz together with the microelement fertilizer NanoSilicon, the indicators of field germination increased by 12.6%, the safety of plants at the end of the growing season - 13.2%, after overwintering - 12.6%, the mass of dry roots increased by 2, 4 times.
When analyzing the formation of symbiotic activity of agrocenoses of Pannonian clover of the first year of use, it was found that inoculation of seeds with biological products and microelement fertilizers promoted the activation of symbiotic activity, the number and mass of active nodules in relation to the control according to the variants of the experiment increased by 29.2-167.6 million pcs. and 77.7-564.2 kg/ha. The maximum parameters of symbiosis of agrocenosis of active Pannonian clover nodules of 221.8 million pcs/ha with a weight of 893.7 kg/ha were formed when clover seeds were inoculated with Gumariz together with NanoSilicon preparation. Similar indicators of symbiotic activity differ in agrocenoses of second-year Pannonian clover.

The largest number of active nodules is 275.4 million pcs/ha with a mass of 1105.

Table 1. The number and mass of active nodules of agrocenoses of Pannonian clover (budding phase)

Option	First year (2019-2020)	Second year (2020)
Without processing(k)	54.2 / 329.6	68.2 / 411.6
Rhizotorfin seed treatment	83.4 / 407.3	105.3509.7
Seed treatment with Humariz	167.6 / 598.4	210.4 / 998.6
NanoSilicon	184.7 / 668, "	235.6 / 832.8
Megamix-Seeds	179.6 / 658.3	226.7 / 82.5
Citovit	177.2 / 656.5	220.3 / 818.9
Rizotorfin + NanoSilicon	218.2 / 883.5	275.4 / 1105.8
Rizotorfin_Megamix-Seeds	212.8 / 848.6	264.3 / 1060.3
Rizotorfin + Cytovit	206.8 / 813.7	258.9 / 1018.4
Gumariz + NanoSilicon	221.8 / 893.7	283.4 / 1119.6
Gumariz + Megamix-Seeds	217.6 / 878.9	274.3 / 1098.7
Gumariz + Citovit	212.7 / 844.6	362.7 / 1059.3

A.A. Nichiporovich (1970) claims that in the process of photosynthesis up to 90-95% of dry biomass of plants is formed, therefore, this process plays a leading role in the formation of the yield (Nichiporovich, 1970).

The search for methods that provide favorable conditions for the absorption and maximum use of solar energy is an urgent task. Studies have shown that bacterial preparations and microelement fertilizers provide an increase in the parameters of photosynthesis of Pannonian clover agrocenoses. Thus, the leaf area of agrocenoses of Pannonian clover of the first year of use according to the experimental options, on average for two years, amounted to 37.6-53.6 thousand m^2/ha, in relation to the control increased by 8.9-24.9 thousand m^2/ha or 1.3-1.9 times. The highest indices of the leaf surface of Pannonian clover, 53.6 thousand m^2/ha, are different when seeds are inoculated with Gumariz together with the preparation NanoSilicon, control - 28.7 thousand m^2/ha (Table 2).

Optimization of the conditions of legume-rhizobaric symbiosis, intensive formation of photosynthesis parameters, caused by exogenous treatment of seedlings with biological products and complex microelements, contributed to an increase in the productivity of Pannonian clover.

Table 2. Photosynthetic activity of Pannonian clover agrocenoses

Option	Leaf area, thousand m^2/ha		FP, mln m^2 days/ha		PPF, g/m^2 per day	
	First year p. (2019-2020)	Second year (2020)	First year p. (2019-2020)	Second year (2020)	First year p. (2019-2020)	Second year (2020)
Without seed treatment	28.7	36.2	1.58	1.19	2.91	3.34
Rhizotorfin seed treatment	37.6	43,7	1.62	1.86	2.98	3.39
Seed treatment with Humariz	43.2	48.6	1.79	2.06	3.92	4.58
NanoSilicon	44.6	50.8	1.98	2.23	4.28	4.82
Megamix-Seeds	44.3	49.4	1.91	2.14	4.16	4.65
Citovit	43.9	48.7	1.89	2.11	4.13	4.58
Rizotorfin + NanoSilicon	49.5	55.9	2.18	2.49	4.82	5.35
Rizotorfin + Megamix-Seeds	48.2	54.8	2.05	2.41	4.73	5.17
Rizotorfin + Cytovit	47.5	53.6	2.01	2.36	4.59	5.08
Gumariz + NanoSilicon	53.6	60.5	2.29	2.58	5.06	5.87
Gumariz + Megamix-Seeds	52.1	58.9	2.13	2.43	4.92	5.68
Gumariz + Citovit	51.9	57.2	2.08	2.39	4.81	5.78

On average, over two years, the collection of dry matter of Pannonian clover of the first year of use according to the options of the experiment was 7.29-12.48 t/ha, feed units - 5.25-9.05 t/ha, digestible protein - 0.81 - 1.39 t/ha, exchangeable energy - 58.69-100.47. The highest productivity of Pannonian clover was obtained when seeds were inoculated with a biological product Gumariz together with NanoSilicon: dry matter - 12.48 t/ha, feed units - 9.05 t/ha, digestible protein - 1.39 t/ha. Control, respectively - 5.35, 3.86, 0.60 (Table 3).

With the complex exogenous treatment of seeds with the biological product Gumariz, together with the microelement fertilizers NanoSilicon, Megamiks-Semena and Tsitovit, more favorable conditions were formed for the formation of structural elements and seed productivity of clover of the Pannonian variety ANIK than with mono-treatment with the studied agrochemicals.

Table 3. Productivity of Pannonian clover

Option	First year of use				Second year of use				Seed yield, kg/ha
	SV, t / ha	Feed units, t / ha	PP, t / ha	OE, GJ	SV, t / ha	Feed units, t / ha	PP, t / ha	OE, GJ	
Without seed treatment	5.35	3.86	0.60	42.85	5.84	4.18	0.65	46.6	359.4
Rhizotorfin seed treatment	7.29	5.25	0.81	58.69	7.75	5.58	0.87	62.1	528.6
Seed treatment with Humariz	9.28	6.69	1.03	74.72	10.15	7.27	1.13	81.3	658.9
NanoSilicon	9.76	7.05	1.08	78.59	10.68	7.65	1.19	85.5	667.5
Megamix-Seeds	9.46	6.84	1.05	76.16	10.35	7.42	1.16	82.9	658.3
Citovit	9.39	6.74	1.04	75.61	10.27	7.36	1.15	82.2	650.2
Rizotorfin + NanoSilicon	11.06	7.96	1.23	89.05	12.10	8.67	1.35	96.9	716.8
Rizotorfin + Megamix-Seeds	10.23	7.35	1.14	82.36	11.19	8.02	1.25	89.6	704.5
Rizotorfin + Cytovit	9.76	7.08	1.09	78.58	10.68	7.65	1.19	85.5	698.7
Gumariz + NanoSilicon	12.48	9.05	1.39	100.47	13.65	9.78	1.52	109.3	786.8
Gumariz + Megamix-Seeds	11.89	8.57	1.32	95.72	13.01	9.32	1.04	104.2	774.6
Gumariz + Citovit	11.28	8.14	1.26	90.81	12.34	8.84	0.97	98.8	762.8

At the same time, the number of generative shoots according to the variants of the experiment was 4.18-4.72 million pcs/ha, seeds in the head - 43-49 pieces, seeds per plant - 179-223 pieces, productivity of an individual plant - 0.69 - 0.98 g, weight of 1000 seeds - 3.96-4.25 g.

Yield is an integral indicator characterizing the influence of various factors on growth, morphogenesis and physiological-biochemical processes in plants.

The yield of seeds of Pannonian clover of the first year of use on average for two years varied according to the variants of the experiment from 528.6 to 786.8 kg/ha, the excess in relation to the control was 169.2-437.4 kg/ha. The highest yield of seeds of Pannonian clover 786.8 kg/ha was obtained with complex seed inoculation with the biological product Gumariz together with NanoSilicon, which is 2.2 times higher than that of the control variant (Table 3).

The greatest economic and energy effect was obtained with the binary treatment of Pannonian clover seeds with the bacterial preparation Gumariz together with the microelement fertilizer NanoSilicon, when cultivating for seeds the level of profitability was 187.3%, for fodder purposes - 149.6%, the energy efficiency coefficient, respectively - 2.24 and 1.89 units.

CONCLUSIONS

Inoculation of seeds with bacterial preparations and complex microelement fertilizers contributed to an increase in seed and fodder productivity of clover of the Pannonian variety ANIK.

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INFLUENCE OF PRECURSORS ON BIOMETRIC INDICATORS AND YIELD OF WINTER WHEAT IN DIFFERENT AGROBIOCENOSES

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Abstract

In the structure of sown areas of Ukraine an important place is given to winter wheat. To obtain high and sustainable profits in production, it is necessary to analyze and evaluate the conditions that affect the productivity of crops, in particular winter wheat in different agrophytocenoses. There are many factors on which the yield of this crop depends: soil moisture, agrophysical, agrochemical and microbiological indicators of its fertility. It is known that in modern conditions the choice of precursors based on profitability and market demand, so putting winter crops primarily occurs after such crops which can not provide optimal conditions for its development. Increasing the sown areas of sunflower, corn, soybeans and rapeseed forces farmers to grow winter wheat after these crops. The data obtained to determine the density of productive stems, plant height, ear fullness of grain, the weight of grain from the ear indicate that the precursors affect these indicators throughout the growing season of winter wheat, thus forming its yield. It was found that fallow as a precursor provided the best conditions for the formation of high grain yields of winter wheat. Unsatisfactory precursors were sunflower and corn, which created poor starting conditions for the development of winter wheat in autumn and subsequent stages of development, which in turn led to a decrease of winter wheat yield. Legume precursors: peas, soybeans, and beans significantly improved the starting conditions for wheat plants, which contributed to better formation of vegetative mass and reproductive organs. In terms of yield structure, these options were inferior to fallow, but also had high values and, accordingly, yield.

***Key words**: Winter wheat, precursor, yield structure, yield.*

INTRODUCTION

Predecessors of winter wheat occupy a special place in terms of the impact on its yield. Their agrotechnical significance depends on biological features, namely: the impact on the content of moisture and nutrients in the soil, the phytosanitary condition of the field after harvesting, etc. Under modern conditions, the list of precursors of winter wheat is constantly changing, responding to market demand, and not for the better. After all, now in the economic crisis, agricultural production seeks to increase the area of such highly profitable crops as sunflower, rapeseed, corn, soybeans. In the absence of sustainable livestock development and reduced need to grow perennial grasses and other fodder crops on farms, which were previously used as the best precursors of winter wheat, there is a need to place it after these crops.

According to O. V. Demydenko, P. I. Boyka, M. I. Blashchuk and others. due to the transformation of the structure of sown areas, the structure of the precursors of winter wheat changed. In the period 2017–2018, the correlation between the percentage of industrial crops and the areas of suboptimal predecessors was at the level of strong direct correlation (R = 0.71–0.83 ± 0.02; R = 0.51–0.69). The percentage of areas of industrial crops with the percentage of areas of forage crops and early and optimal predecessors correlated at the level of inverse correlation. The increase in the percentage of areas of industrial crops in the transformed structures of sown areas to 31.0-33.0% radically subordinates the formation of the structure of precursors of winter grain areas of sowing of industrial crops and weakens the relationship with the area of fodder and legumes (Boyko, & Blashchuk, 2019).

As a result, there is a decrease in grain yield and deteriorating soil fertility, as well as the need to create better conditions for plant growth and development. And this is usually the use of high doses of fertilizers, pesticide treatment of plants, which leads

In recent years, the problem of placing winter wheat in crop rotations and selecting the best predecessors for it is quite relevant – it was studied by many scientists: P. I. Boyko, Ye. M. Lebid, A. I. Khorishko, I. S. Hodulyan and others (Boyko & Blashchuk, 2019; Lebid et al., 2005; Khorishko, 1997; Godulyan, 1974)

MATERIALS AND METHODS

Studies to study the impact of different precursors on the productivity of winter wheat were conducted in the period 2017-2020 under the conditions of a stationary experiment of the Department of Agriculture named after O. M. Mozheyka in the research field of Kharkiv National Agrarian University named after V. V. Dokuchaiev. A large part of the territory of Kharkiv region together belongs to the Left Bank Forest-Steppe of Ukraine. The southern and south-eastern parts of the region are located in the Northern Steppe. Kharkiv region in the forest-steppe zone of Ukraine is marked by the largest manifestation of continentality, which noticeably increases from northwest to southeast.

Geomorphologically, the land on which the experiments were conducted is located on the southeastern edge of the forest-steppe zone near the fourth left-bank terrace of the Uda River and crosses it in a narrow strip.

The soil cover on the territory where the research was conducted is represented mainly by chernozem typical leached low-humus heavy loam on forest-like loam. Formed under conditions of well-developed grassy vegetation and moderate moisture on unsalted forest species, typical chernozem is characterized by agronomically valuable granular-lumpy structure, good physical and mechanical properties, large reserves of nutrients available to plants, high content of humus and humus content.

According to the data of soil mapping, a macromorphological description of the profile of agrochernozem (Tykhonenko & Dehtiarov, 2014) of a typical deep low-humus (weakly structural) heavy loam on loess-like loam is given (Tykhonenko & Dehtiarov, 2016).

The arable layer of soil (0-30 cm) contains humus according to Tyurin – 4.9-5.1%, light hydrolysis nitrogen (according to Cornfield) - 8.1 mg per 100 g of soil, mobile phosphorus and exchangeable potassium (according to Chirikov) – 10 and 20 mg per 100 g of soil, respectively. According to the content of mobile forms of phosphorus and potassium, the soil is characterized by increased security. The content of exchangeable cations: calcium – 37.8%, magnesium – 6.6%, sodium – 0.49%, potassium – 0.5%, hydrogen - 21 mg. eq./kg of soil. The soil has a neutral reaction of the soil solution (pH: water – 7.0, salt – 5.2-5.6). Groundwater lies at a depth of about 18 m.

This soil is characterized by agronomically valuable structure, good physical and mechanical properties and intensive biological activity.

According to the amount of precipitation, the territory of the experimental field belongs to the zone of insufficient moisture. The annual amount of precipitation in the area, according to the meteorological station of KhNAU, is on average 529 mm. Depending on the intensity and recurrence of precipitation processes, the amount of precipitation in some years varies significantly from 342 mm (65% of normal) to 767 mm (145% of normal).

A characteristic feature of the climate is aridity, which is due not so much to the total rainfall as to the uneven distribution of them during the year and especially during the growing season. The average annual air temperature is + 7.2ºC with fluctuations in some years from +4.7 to + 9.2ºC (Obraztsova, 2001).

Agricultural techniques for growing crops in the experiment are generally accepted for the conditions of the Kharkiv region (Nezdiur et al., 2016).

Six variants of crop rotations were studied in triplicate. Placement of options is systematic. The area of the sown area is 750 m^2, the accounting area is 100 m^2. The following precursors of winter wheat were studied: pure steam, peas, sunflower, soybeans, beans, corn in short-rotation crop rotations with the following scheme:

1. Precursor of winter wheat.
2. Winter wheat.
3. Row crops.
4. Winter wheat.
5. Sunflower.

RESULTS AND DISCUSSIONS

The productivity of winter wheat is determined by the action of many factors: the conditions of moisture, agrophysical indicators of soil fertility, nutrient regime, the action of biological factors that develop after the cultivation of various predecessors. The combined action of these factors in combination with meteorological conditions affects the formation of elements of the crop structure, and, as a consequence, its yield.

The main elements of the structure of the crop include: the density of productive stems, plant height, the filling of the ear with grain, the mass of grain from the ear. Each of these elements can increase or decrease under the action of the environment. The study of this indicator allows us to trace the relationship between plants and the environment in different periods of the growing season against the background of a predecessor (Kudria, 1999)

One of the elements affected by precursors is the density of productive stems. This indicator depends on the density of plants, the characteristics of the variety, the availability of plants with available moisture, light, nutrients, etc.

In our studies, the number of productive stalks of winter wheat varied significantly depending on the predecessor. On average, in two years of research, the largest number of spike-shaped stems had variants with pure steam – 439 and peas – 449 pcs./m^2 (Table 1). Moreover, the number of plants per 1 m^2 in these variants was almost the same, respectively 192 and 190 pcs./m^2. It was after these predecessors that the most favorable conditions developed in the initial phases of winter development, especially in autumn, when wheat plants formed the most resistant to adverse conditions productive stems and in early spring, during continued tillering. And the variant with peas exceeded steam on 10 pcs./m^2 of productive stalks.

Worse starting conditions for the development of winter wheat were formed after other legume predecessors: beans and soybeans. In these variants at rather high indicator of density of standing of plants of 195 and 193 pcs./m^2 of plants much less productive stalks were formed, according to our data on the average for two years of researches – 388 and 400 pcs./m^2. These two legumes are row crops and are harvested late. The number of leftovers left after them can be much smaller than after peas. In the complex, it affects the soil moisture and the content of nutrients in it, especially nitrogen, and, as a consequence, determines the timeliness of emergence of winter wheat seedlings and their further development

Variants with sunflower and corn had the lowest values of plant density: 165 and 170 pcs./m^2, and the productive bushiness on these variants was respectively: 401 and 411 pcs./m^2.

The tallest plants of winter wheat before harvesting were in the version with pure steam – 93.5 cm.

The height of plants in the variants with leguminous predecessors was inferior to the steam variant and the plants after them were lower by an average of 8.4 cm. It should be noted that this is a significant difference, which indicates some deterioration of development conditions throughout the growing season. The lowest plant height values were obtained in the variants with sunflower and corn – 79.5 and 78.1 cm, respectively.

Table 1. The structure of the winter wheat yield depending on the predecessors

Predecessor	Density, pcs./m^2	Number of productive stems, pcs./m^2	Stem height, cm	Ear length, cm	Number of spikelet's in the ear, pcs.	The number of grains in the ear, pcs.	Weight of grain from the ear, g
Pure steam	193	439	93,5	14,3	16,5	43,5	1,6
Pea	190	449	84,5	14,2	16,0	38,0	1,4
Sunflower	195	388	85,6	12,6	14,0	38,0	1,1
Soybean	193	400	85,8	13,4	15,5	38,5	1,1
Bean	165	401	79,5	14,1	15,5	31,0	1,3
Corn	170	411	78,4	12,9	14,0	31,5	1,1

The yield of winter wheat also depends on the size of the ear and its grain content. Ear formation in plants occurs in early spring and is determined by the conditions that develop during the period of bookmarking and differentiation of the ear and wheat flowering. This process is best done with the optimal amount of nutrients in the soil solution. Insufficient amount of nitrogen leads to delayed ear development and reduced size of its elements (Kudria, 2018).

According to A. I. Nosatovskoho, the presence of sufficient phosphorus in the soil accelerates the process of ear differentiation, as a result of which the number of spikelet's often decreases. According to the scientist, the delay in the formation of the ear, as well as the difference in its structure is due to a violation of the ratio of nutrients, namely: the ratio of nitrogen to phosphorus or nitrogen to potassium (Nosatovskiy, 1963).

As a rule, the amount of moisture in the soil during the spring restoration of winter vegetation is sufficient for its development. It continues to bush and intensively accumulate vegetative mass. It is known that the content of nutrients in the soil depends on the precursors, which accordingly affected the structure of the ear in our studies.

The version with pure steam had a longer ear length – 14.3 cm. Winter wheat placed after peas and beans on average for two years of research had the length of the ear at the level of the option with pure steam. The difference was insignificant and amounted to 0.1; 0.2 cm, respectively. After soybeans, wheat plants formed a slightly shorter ear and compared to the steam version, the difference was 0.9 cm. The lowest indicator of ear length was in the variants with sunflower and corn – 12.6 and 12.9 cm.

A similar trend was observed in determining the number of spikelet's in the ear. Most of them were in the version with pure steam – 16.5 pcs. After legume predecessors, the number of ears in the ear decreased and this figure ranged from 15.5 to 16.0 pcs., but the difference was insignificant. More significant was the difference in options with sunflower and corn. The number of spikelet's in the ear when placing wheat after these crops was the smallest and was in both cases 14.0 pcs., which is less than the steam version by 2.5 pcs.

On average over two years of research, the number of grains in the ear ranged from 31.0 to 43.5 pcs. Most of them were in the ear of wheat placed after pure steam, and the least – after sunflower and corn. Variants with peas, beans and soybeans were equivalent and slightly inferior to the steam variant, the difference averaged 5.3 pcs.

The productivity of an ear is determined not only by the number of grains in it, but also by the weight of the grain. The mass of grain from one ear varied under the influence of predecessors. The heaviest ear was in the version with pure steam, its weight was on average for two years of research – 1.6 g, which exceeded the other options by an average of 0.3 g. The ear weighed the least when placing wheat after sunflower, soybeans and corn, 1.1 g each.

The weight of grain from one ear of winter wheat in the variants with leguminous predecessors: peas and beans were lower than in the steam variant, but exceeded the variants with sunflower and corn by an average of 0.3 g.

Indicators of ear fullness of grain largely depend on the conditions that developed during its formation. Differences between options are due to both meteorological factors and the ability of plants to accumulate plastic substances during grain formation. Winter wheat placed after pure steam, peas, beans and soybeans developed better during the growing season and used moisture and nutrients from deeper soil layers, which contributed to the formation of whole grains. Deterioration of conditions of formation of elements of a crop after sunflower and corn led to decrease in indicators of all components of structure of a crop in these options.

Differences between the options for the formation of elements of the structure of the winter wheat crop determined its yield, which was determined during three years of research (2018-2020). The role of pure steam in crop rotation is known. Many researchers report the highest yields of winter wheat after this predecessor (Lebid, Medved, Kyrchuk, & Pishta, 2005).

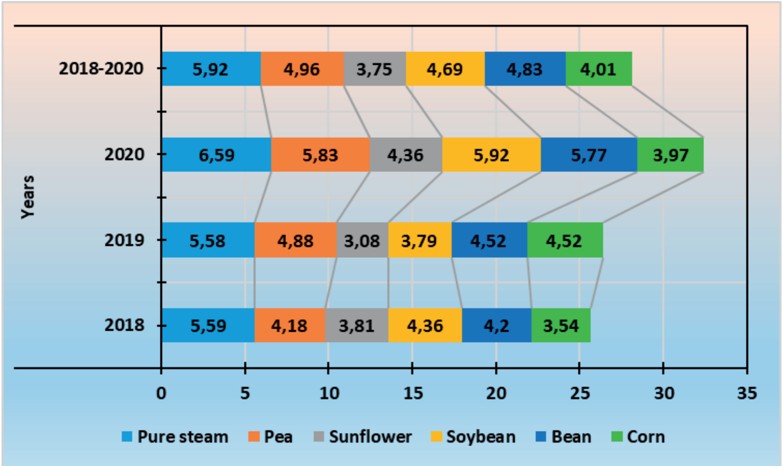

Figure 1. Yield of winter wheat depending on predecessors, t/ha

In our research, the highest yield of winter wheat was formed in the variant with pure steam – 5.92 t/ha, which indicates the positive effect of the steam field and the creation of the necessary conditions for plant growth and development regardless of the weather. According to our data, in each year of research, the yield of wheat in this option was quite high compared to other options.

Studies have shown a significant reduction in grain yield for the placement of winter wheat after legumes predecessors: peas, soybeans and beans.

Compared to the steam version, this figure is lower by an average of 1.09 t/ha. Compared to the steam version, this figure is lower by an average of 1.09 t/ha. This is a significant difference and it indicates the deterioration of plant development conditions after these predecessors (Figure 1).

Peas, as a precursor of winter wheat, have a positive effect on its yield. But this effect is limited by the level of its yield and the amount of crop residues that remain after it. According to V. S. Chumak, O. I. Tsilyurik, I. E. Fedorenko, the amount of crop residues after peas was very low and amounted to 2.15-2.80 t/ha. While after sunflower they remained 66.2–78.9, after corn – 3.49–4.49 t/ha (Chumak, Tsyliuryk & Fedorenko, 2005).

In addition, peas compete poorly with weeds, especially in the early stages of development, which can increase the potential infestation of the field with weed seeds and reduce the yield of the next crop, namely winter wheat.

Soybeans and beans – row crops, harvested relatively late, after which there was much less moisture and crop residues in the soil, which led to a significant decrease in yield compared to pure steam.

Despite the decrease in yield after these predecessors, their agronomic value in crop rotations is very important. A feature of these crops is the fixation of atmospheric nitrogen with the help of nodule bacteria that settle on the roots and its accumulation in the soil. They improve biological processes in the soil due to the favorable chemical composition of root and post-harvest residues. After harvesting, the content of phosphorus and potassium in the soil increases. Accumulated in the roots of legumes and released after their death, calcium cements the soil and improves its structure.

The lowest grain yield was formed by wheat after its placement after sunflower and corn: 3.75 and 4.01 t/ha, which is 2.17 and 1.91 t/ha lower than in the steam version, and on average compared to leguminous predecessors. at 1.07 and 0.81 t/ha. Sunflower and corn are late harvest crops, they remove a large amount of moisture and dissolved nutrients from the soil, which impairs their use by winter wheat at the beginning of the growing season in autumn and during the spring-summer period of development. In addition to the negative aspects, there are also positive factors of action of these crops in agrophytocenoses. First of all, it is a large number of crop residues that remain after harvesting. But in arid weather conditions, which were observed during the years of research, the decomposition of plant residues slowed down and their effect on the development of winter wheat plants was leveled. In the variant where the share of sunflower was 40% and this crop was used as a precursor of winter wheat (sunflower, wheat, row crops, winter rye, sunflower), received the lowest yield – 3.75 t/ha of grain.

CONCLUSIONS

The formation of elements of the structure of the winter wheat harvest was influenced by predecessors. When placing it after pure steam, the best conditions were created, which had a positive effect on the formation of productive bushiness of plants, height and structure of the ear. All these figures were the highest in the steam version.

Bean predecessors: soy peas and beans significantly improved the starting conditions for wheat plants, which contributed to better formation of vegetative mass and reproductive organs of wheat. In terms of the structure of the winter wheat harvest, these options were inferior to steam, but also had high values. The lowest indicators of crop structure elements were after sunflower and corn.

Conditions that developed after different predecessors influenced the development of winter wheat throughout the growing season. The highest yield of winter wheat was obtained in the version with pure steam. Studies have shown a significant reduction in wheat yield after its placement after legume predecessors: peas, soybeans and beans. The lowest yield was in the variants with sunflower and corn. Intensive use of sunflower crops in short-rotation crop rotations (40%) led to a significant decrease in winter wheat yield.

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GENETIC DIVERSITY REGARDING GRAIN SIZE AND SHAPE OF COMMON WINTER WHEAT

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Abstract

Grain characteristics regarding grain size and shape, such as: thousand kernel weigh, grain area, length, width, circularity, and test weight are important components of grain yield and quality in wheat. A set of 22 winter wheat varieties, which included old and new varieties and advanced breeding lines were tested across 2018-2019 and 2019-2020, at NARDI Fundulea, to estimate the historic evolution of these features and the correlation of them with grain yield. Shape grain characteristics were analysed with Marvin seed analyser. ANOVA showed very significant differences both between varieties and between years. The thousand kernel weigh was positively correlated with other parameters of grain shape, such as: grain area, width, length and circularity. The year of release was negatively correlated with some grain parameters, such as length, circularity and positively correlated with yield. It is necessary to pay attention in breeding to increase grain size and weight, but only if this will not be associated with the correlated negative changes in the other components of yield, which might lead to a decrease in grain yield.

***Key words**: grain size and shape, genetic diversity, wheat, yield.*

INTRODUCTION

Grain shape and size are important components of grain yield and quality in wheat. Grain size is an important physical indicator of seed quality that affects early vegetative growth and is frequently related to yield, market grade factors and harvest efficiency.

Wheat kernel size and shape influence flour yield and market price. Seed size has significant impact on seedling emergence percentage.

Grain morphology analysis can play an important role in determining quality of wheat grain especially regarding market value (Kumari et al., 2015).

With increasing world population, it has been estimated that the global demand for wheat will increase by a further 60% by 2050 (Licker et al., 2010). It is a huge challenge to ensure global food security through sustainable wheat production for the projected population, in the context of the increasing adverse impact of climate change (Palm et al., 2010).

In the past four decades, improvement of grain yield has come from increased grains per square meter or larger grain sizes, due to the utilization of *Rht* genes in wheat breeding (Calderini & Reynolds, 2000).

However, TGW is a complex trait, and is largely controlled by several grain traits, including grain size and shape (Zhang et al., 2014). TGW is characterized by a higher heritability than grain yield itself (Deng et al., 2011), and it is positively correlated with agronomic yield (Maccaferri et al., 2011) and flour yield (Williams et al., 2013). In a previous study, the correlation coefficient of grain yield with TKW ranged from -0.4 to +0.6, with most trials showing practically no correlation, and only one trial having significant positive correlation (Mandea et al., 2019). The high heritability values (59% to 96%) in most of the cultivars studied so far proved that this character is phenotypically the most-stable yield component (Giura & Săulescu, 1996; Huang et al., 2006; Sun et al., 2009; Patil et al., 2013). Grain size is mainly characterized by grain weight and area, whereas shape means a relative proportion of the main growth axes of the grain (Gegas et al., 2010). Grain shape is generally estimated by length, width, vertical perimeter, sphericity and horizontal axes proportion (Breseghello & Sorrells, 2007). Many studies have shown that wheat grain size and shape are positively correlated with TGW and they affect flour yield, end-use quality and market price (Evers et al., 1990; Breseghello and Sorrells, 2006; Williams et al., 2013; Rasheed et al., 2014). Theoretical models predict that milling yield could be increased by optimizing grain size and shape with large and spherical grains being the optimum grain morphology (Evers et al., 1990). To gain deeper insights into the genetic basis of grain size and shape variation, Gegas et al. (2010), studied several different populations of recombinant doubled haploids (DH) that capture a broad spectrum of the phenotypic variation present in the elite winter wheat germplasm pool. Grain material from accessions of primitive wheat species and modern elite varieties were measured to determine the phenotypic structure of the traits and assess the extent of variation retained in domesticated wheat. They showed that grain size is largely independent of grain shape both in the DH populations and in the primitive wheat species and that there is a significant reduction of phenotypic variation in grain shape in the breeding germplasm pool probably as a result of relatively recent bottleneck. This phenotypic structure is attributed to a distinct genetic architecture where common genetic components are involved in the control of those traits in different wheat varieties (Gegas et al., 2010). Moreover, the emergence of hexaploid, common or bread wheat, followed by further selection and extensive breeding, led to a crop species of significant financial and nutritional importance since it provides one-fifth of the calories consumed by humans today (Dubcovsky & Dvorak, 2007). Archaeobotanical evidence from around the Fertile Crescent region indicates that the transition from the diploid wild einkorn (*Triticum monococcum* ssp. *aegilopoides*; AmAm) and tetraploid emmer wheat (*Triticum turgidum* ssp. *dicoccoides*; BBAA) to the domesticated forms (*T. monococcum* ssp. *monococcum* and *T. turgidum* ssp. *dicoccum*, respectively) was associated with a trend toward larger grains (Fuller, 2007).

Several other quality criteria used by the industry are influenced by grain morphology. Grain size was also found to be associated with various characteristics of flour, such as protein content and hydrolytic enzymes activity, which in turn determine baking quality and end-use suitability (Evers, 2000). Abdipour et al. (2016), analysed a set of 98 bread wheat landraces from different geographic regions of Iran, across 2013-2014 and 2014-2015 to determine the phenotypic diversity and relations between thousand grain weight (TGW), grain morphology and grain quality. They found that the genotypes were significantly different for all traits, which reflects the high levels of diversity. Significant positive correlations were observed between TGW and grain size (or shape), except for the aspect ratio (AR) and roundness. However, grain quality traits, especially GPC, had significant negative correlation with TGW. The present study was conducted to estimate the historical changes regarding grain characteristics and the association between different grain morphological traits with grain yield.

MATERIALS AND METHODS

A set of 22 winter wheat varieties, which included old and new varieties and advanced breeding lines was tested across 2018-2019 and 2019-2020, at National Agricultural Research and Development Fundulea (44°30’ N, 24°10’ E, 68 m above sea level), as material in this study. The tested cultivars are shown in table below (Table 1):

Table 1. The tested cultivars, the provenience and the year of release

No. crt.	Variety	Provenience	Year of release
1	A15	Romanian cultivar	1939
2	Bezostaia 1	Russian cultivar	1961
3	Dacia	Romanian cultivar	1971
4	Iulia	Romanian cultivar	1974
5	Fundulea 29	Romanian cultivar	1979
6	Arieşan	Romanian cultivar	1985
7	Fundulea 4	Romanian cultivar	1987
8	Dropia	Romanian cultivar	1993
9	Alex	Romanian cultivar	1994
10	Boema 1	Romanian cultivar	2000
11	Glosa	Romanian cultivar	2005
12	Apache	French cultivar	2005
13	Izvor	Romanian cultivar	2008
14	FDL Miranda	Romanian cultivar	2011
15	Otilia	Romanian cultivar	2013
16	Avenue	French cultivar	2013
17	Pitar	Romanian cultivar	2015
18	Voinic	Romanian cultivar	2020
19	Ursita	Romanian cultivar	2021
20	FDL Amurg	Romanian cultivar	advanced breeding line
21	FDL Armura	Romanian cultivar	advanced breeding line
22	FDL Abundent	Romanian cultivar	advanced breeding line

The genotype panel was planted under the open-field conditions, on chernozem soil (pH: 6.3-6.8; humus: 3%), in plots of 6 m^2, using recommended crop management.

Weather conditions during the experiments are summarized in table 2. From the point of view of the grain characteristics, the temperatures during the grain filling are of interest.

Table 2. Weather conditions during the two seasons of research

Season	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Average temperature (ºC)											
2018	19	11.7	6.7	3.6	0.8	1.6	3.3	15.7	19.4	**22.6**	22.8
2019	19.1	13.4	5.2	-0.1	-1.2	3.8	9.3	11.2	17.2	**23.6**	23
2020	19.3	12.8	10.3	4	0.9	5.2	8.3	12.2	17	**21.7**	25.1
Rainfall (mm)											
2018	12.2	111.6	49.2	27.8	36	58.6	40.6	2.4	34	120.6	83
2019	28.6	10.8	23	43	53.8	21.4	21.6	51.4	124	74.6	87.4
2020	6.2	38	33.2	16.2	2	16.6	27.8	14	58	68.4	34.2

The characteristics regarding grain size and shape were analysed with a Marvin seed analyser (high-throughput method).
The analysed parameters were: grain area, length, width, circularity; in addition to these characteristics, we also analysed the test weight and the thousand kernel weight; all of these traits were analysed in three replicates.
ANOVA was used to estimate the significance of differences between wheat genotypes regarding grain characteristics.

RESULTS AND DISCUSSIONS

ANOVA with two factors (genotype and season) calculated for grain width showed very significant differences for both, genotype and year, and also for interaction (Table 3).

Table 3. ANOVA for grain width, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Cultivars	0.57	21	0.027	24.438***	1.677
Seasons	0.10	1	0.104	93.015***	3.949
Interaction	0.17	21	0.008	7.140***	1.677
Within	0.10	88	0.001		
Total	0.94	131			

*** = very significant at P<0.1%.

ANOVA with two factors (genotype and season) calculated for grain length showed very significant differences for both, genotype and year, and also for interaction (Table 4).
The differences between the genotypes, regarding grain width and length, are showed in table below (Table 5).

Table 4. ANOVA for grain length, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Cultivars	12.96	21	0.617	285.50***	1.68
Seasons	2.28	1	2.283	1056.18***	3.95
Interaction	0.27	21	0.013	5.98***	1.68
Within	0.19	88	0.002		
Total	15.70	131			

*** = very significant at P<0.1%.

Table 5. The differences between genotypes, regarding grain width and length and their significance

No. crt.	Variety	Average values and significance	Average values and significance
		Width (mm)	Length (mm)
1	A15	2.90	6.008
2	Bezostaia 1	2.91	6.260***
3	Dacia	3.06***	5.813°°
4	Iulia	2.92	6.113**
5	Fundulea 29	2.79°°°	5.810°°
6	Arieşan	2.96	6.989***
7	Fundulea 4	2.89	6.263***
8	Dropia	2.95	6.048*
9	Alex	2.91	6.211***
10	Boema 1	2.91	5.956
11	Glosa	2.95	6.089**
12	Apache	2.95	5.835°°
13	Izvor	2.84°°	5.664°°°
14	FDL Miranda	2.85°°	6.094**
15	Otilia	2.90	5.608°°°
16	Avenue	2.93	5.726°°°
17	Pitar	2.99*	5.745°°°
18	Voinic	2.91	5.671°°°
19	Ursita	2.90	5.833°°
20	FDL Amurg	3.11***	6.046*
21	FDL Armura	2.88	5.469°°°
22	FDL Abundent	2.95	5.700°°°
		DL5%= 0.054	DL 5%= 0.075

*** = very significant positive at P<0.1%; * = significant positive at P<5%; °°° = very significant negative at P<0.1%; °° = distinct significant negative at P<1%.

The genotypes with higher grain width were: the old cultivar Dacia, the relatively new cultivar Pitar and the breeding line FDL Amurg. The genotypes with smaller grain width were: the old cultivar Fundulea 29, the relatively new cultivars Izvor and FDL Miranda.
Related to grain length, many varieties had a positive significance, namely: the old varieties Bezostaia 1, Iulia, Arieşan, Fundulea 4, Dropia, Alex, the relatively new varieties Glosa and FDL Miranda and the breeding line FDL Amurg. However, many varieties had a negative significance for the grain length, namely: the old varieties Dacia and Fundulea 29, the relatively new varieties Apache, Izvor, Otilia, Avenue, Pitar, Voinic, Ursita and the breeding line FDL Armura and FDL Abundent.
ANOVA with two factors (genotype and season) calculated for grain area showed very significant

differences for both, genotype and year, and for interaction too (Table 6).

Table 6. ANOVA for grain area, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Cultivars	81.53	21	3.88	80.99***	1.68
Seasons	25.71	1	25.71	536.35***	3.95
Interaction	5.29	21	0.25	5.25***	1.68
Within	4.22	88	0.05		
Total	116.75	131			

*** = very significant at P<0.1%.

ANOVA with two factors (genotype and season) calculated for grain circularity showed very significant differences for both, genotype and year, and also for interaction (Table 7).

Table 7. ANOVA for grain circularity, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Cultivars	0.22	21	0.010	104.056***	1.677
Seasons	0.01	1	0.010	98.997***	3.949
Interaction	0.02	21	0.001	7.253***	1.677
Within	0.01	88	0.000		
Total	0.25	131			

*** = very significant at P<0.1%.

The differences between the genotypes, regarding grain area and circularity, are showed in table below (Table 8).

Table 8. The differences between genotypes, regarding grain area and circularity and their significance

No. crt.	Variety	Average values and significance	Average values and significance
		Area (mm²)	Circularity (mm)
1	A15	12.94	1.42
2	Bezostaia 1	13.52***	1.44**
3	Dacia	13.31*	1.35°°
4	Iulia	13.18	1.42*
5	Fundulea 29	11.98°°°	1.42°
6	Arieşan	15.26***	1.53***
7	Fundulea 4	13.44**	1.45***
8	Dropia	13.36*	1.41
9	Alex	13.54***	1.43**
10	Boema 1	12.95	1.41
11	Glosa	13.30*	1.41
12	Apache	12.64	1.39
13	Izvor	11.83°°°	1.40
14	FDL Miranda	12.90	1.44**
15	Otilia	12.07°°°	1.36°°°
16	Avenue	12.40°°	1.38°°
17	Pitar	12.90	1.36°°°
18	Voinic	12.25°°	1.37°°
19	Ursita	12.57	1.39
20	FDL Amurg	13.93***	1.37°°
21	FDL Armura	11.65°°°	1.36°°°
22	FDL Abundent	12.44°°	1.37°°
		DL5%= 0.37	DL 5%= 0.016

*** = very significant positive at P<0.1%; ** = distinct significant positive at P<1%; * = significant positive at P<5%; °°° = very significant negative at P<0.1%; °° = distinct significant negative at P<1%.

The genotypes with higher grain area were: the old genotypes like Bezostaia 1, Dacia, Arieşan, Fundulea 4, Dropia, Alex and two relatively new genotypes also, like Glosa (released in the last two decades) and the advanced breeding line FDL Amurg. The genotypes with smaller grain area were: the old genotype Fundulea 29, the relatively new genotypes (released in the last two decades), Izvor, Otilia, Avenue, Voinic and the breeding lines FDL Armura and FDL Abundent. The varieties with higher values for the index of circularity were: the old varieties Bezostaia 1, Iulia, Arieşan, Fundulea 4, Alex and the relatively new variety FDL Miranda. The varieties with smaller grain circularity were: the old varieties Dacia, Fundulea 29, the relatively new varieties: Otilia, Avenue, Pitar, Voinic, the breeding lines FDL Amurg, FDL Armura, FDL Abundent. The smaller values of the circularity index show a rounder grain, a desirable character in milling industry.

ANOVA with two factors (genotype and season) calculated for thousand kernel weight (TKW) showed very significant differences for both, genotype and year, and also for interaction (Table 9).

Table 9. ANOVA for TKW, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Cultivars	1349.83	21	64.28	71.44***	1.68
Seasons	38.09	1	38.09	42.34***	3.95
Interaction	123.77	21	5.89	6.55***	1.68
Within	79.17	88	0.90		
Total	1590.86	131			

*** = very significant at P<0.1%.

ANOVA with two factors (genotype and season) calculated for test weight (TW) showed very significant differences for both, genotype and year, and for interaction also (Table 10).

Table 10. ANOVA for TKW, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Cultivars	290.93	21	13.85	19.67***	1.68
Seasons	1186.80	1	1186.80	1684.85***	3.95
Interaction	48.39	21	2.30	3.27*	1.68
Within	61.99	88	0.70		
Total	1588.11	131			

*** = very significant at P<0.1%; * = significant at P<5%.

The differences between the genotypes, regarding TKW and TW are shown in table below (Table 11).

Table 11. The differences between genotypes, regarding TKW and TW and their significance

No. crt.	Variety	Average values and significance TGW(g)	Average values and significance TW (Kg/hl)
1	A15	39.65	75.62
2	Bezostaia 1	42.94***	77.17*
3	Dacia	43.44***	73.80°°
4	Iulia	41.63*	76.87
5	Fundulea 29	35.98°°°	77.08*
6	Arieşan	46.91***	75.10
7	Fundulea 4	40.30	76.77
8	Dropia	41.95**	76.67
9	Alex	40.08	75.02
10	Boema 1	40.25	76.73
11	Glosa	40.82	77.47
12	Apache	38.21	74.32
13	Izvor	36.27°°°	76.48
14	FDL Miranda	38.32	74.75
15	Otilia	36.37°°°	77.17*
16	Avenue	36.60°°°	72.75°°°
17	Pitar	40.48	77.77***
18	Voinic	37.78°	78.22***
19	Ursita	38.68	77.52**
20	FDL Amurg	45.61***	78.08***
21	FDL Armura	33.12°	74.07°
22	FDL Abundent	37.78°	75.60
		DL5%= 1.54	DL 5%= 1.36

*** = very significant positive at P<0.1%; ** = distinct significant positive at P<1%; * = significant positive at P<5%; °°° = very significant negative at P<0.1%; ° = significant negative at P<0.1%.

The varieties with higher TKW were: the old varieties, Bezostaia 1, Dacia, Iulia, Arieşan, Dropia and the breeding line FDL Amurg; the varieties with a smaller TKW were: the old variety Fundulea 29, the new varieties Izvor, Otilia, Avenue, Voinic and the breeding lines FDL Armura and FDL Abundent.

The varieties with higher TW were: the old varieties Bezostaia 1 and Fundulea 29, the new varieties Otilia, Pitar, Voinic, Ursita, the breeding line FDL Amurg; the varieties with smaller TW were: the old variety Dacia, the relatively new variety Avenue and the breeding line FDL Armura.

We analysed the correlations between all these parameters with the year of release and yield (Table 12). We obtained significant positive correlation coefficients between TKW and grain area, width, length and circularity.

The grain area was positively correlated with grain width, length, and circularity. The grain length was positively correlated with grain circularity. The test weight was not correlated with any of the other parameters. The year of release was negatively correlated with some grain parameters, such as length, circularity and positively correlated with yield.

Table 12. The correlation coefficients between analysed parameters

	Year of release	area	width	length	circularity	TW	TKW	yield
Year of release	1							
area	-0,35	1						
width	0,11	**0,52**	1					
length	*-0,45*	**0,92**	0,14	1				
circularity	*-0,44*	**0,67**	-0,27	**0,91**	1			
TW	0,09	0,04	0,01	0,02	-0,002	1		
TKW	-0,37	**0,93**	0,67	**0,77**	**0,460**	0,20	1	
yield	**0,75**	-0,19	-0,09	-0,18	-0,111	0,33	-0,27	1

P 5%=0.42.
The bold correlation coefficients are positive significant at P<5%.
The italic correlation coefficients are negative significant at P<5%.

The relation between year of release and some grain characteristics (including TKW and grain length) was negative, but positive with yield (Figures 1, 2 and 3).

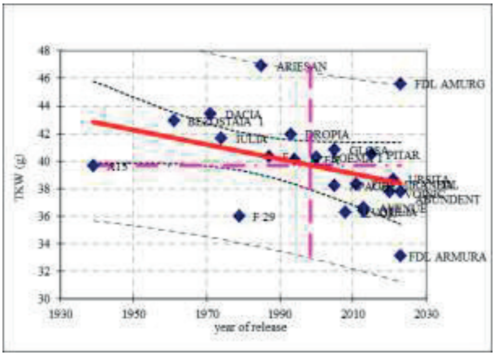

Figure 1. The relationship between year of release and TKW

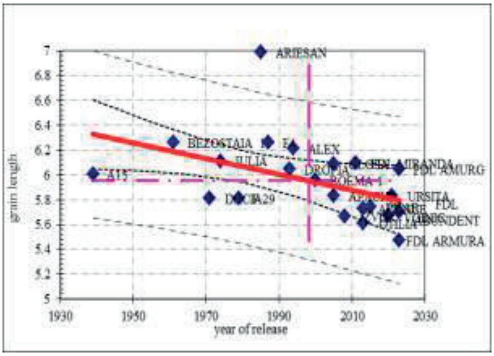

Figure 2. The relationship between year of release and grain length

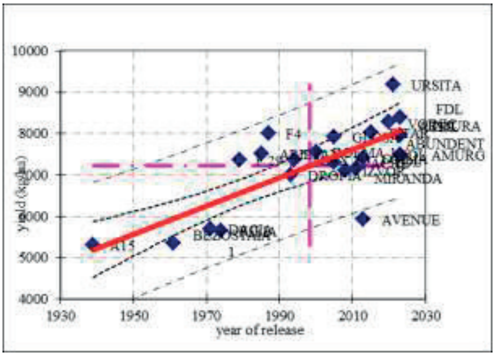

Figure 3. The relationship between year of release and yield

Yield of flour per grain is dependent on grain size and also shape, as this determines the proportion of the grain that is taken up by the endosperm relative to other grain parts. Breeders routinely select new varieties for improvements in grain yield, a component of which is grain size, but have not previously selected specifically for grain shape (https://europepmc.org).

Moreover, the knowledge of morphology of wheat grains is important for designing machines for sowing, handling, milling, cleaning, storing, and conveying purposes. This strategy might be helpful for wheat breeders to develop new varieties with better grain features to improve the milling and baking quality of wheat (https://europepmc.org).

Grain shape (and size), density, and uniformity are important attributes for determining the market value of wheat grain since they influence the milling performance (i.e., flour quality and yield) (Evers et al., 1990). Theoretical models predict that milling yield could be increased by optimizing grain shape and size with large and spherical grains being the optimum grain morphology (Evers et al., 1990). However, accurate characterization of grain size and shape remains a big challenge due to complex nature of wheat grain shape. (Houle et al., 2010; Patil et al., 2013).

Gegas et al. (2010), revealed that grain size and shape are largely independent traits in both primitive wheat and in modern varieties. Moreover, their results showed a significant reduction of phenotypic variation in grain shape in the modern germplasm pool compared with the ancestral wheat species, probably as a result of a relatively recent bottleneck. In our study, the varieties (some old, new and breeding lines) were significantly different for all analysed parameters. We noticed that, over time, some grain characteristics decreased, while grain yield increased.

CONCLUSIONS

The selection for grain yield led, over time, to a decrease (reduction) of grain size and weight. However differences between varieties were noted and the ones that have positive deviations from the regression on year of release deserve attention.

To counteract this trend, it is necessary to pay attention in breeding to increasing the grain size and weight (especially due to the positive effect on milling properties), but only if this will not be associated with the correlated negative changes in the other components of yield, leading to a decrease in grain yield.

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SOWING TIME AT CASTOR BEAN IN SOUTH ROMANIA IN THE CONTEXT OF ACTUAL CLIMATIC CONDITIONS

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Abstract

Castor bean is an important oil plant cultivated mainly for its oil which is a versatile product with a wide range of uses. In Romania, castor bean is cultivated on small areas for scientific, ornamental, industrial and medicinal purposes, but it has a real potential to develop in the future.
In the context of actual climatic conditions which are changing, researches concerning the sowing time are important in view to correlate the plant development and phenology with the evolution of the climatic condition, this being essential for the yield formation in term of quantity and quality. Starting from this necessity, the objective of this paper is to present the results of the researches performed in South Romania in view to establish the temperature threshold for sowing castor bean as the plants to grow and develop well and the first raceme to reach maturity in good conditions. Researches were performed in field conditions at the Agricultural and Development Research Station Teleorman located in South Romania, in the years 2019 and 2020 and on cambic chernozem soil conditions. Four local castor bean varieties (Teleorman, Dragon, Cristian and Rivlas) were sowed at three moments, respectively: sowing time I – temperature of 7°C at 7 o'clock and at 10 cm in the soil; sowing time II – temperature of 10°C at 7 o'clock and at 10 cm in the soil; sowing time III – temperature of 13°C at 7 o'clock and at 10 cm in the soil. The highest yields were obtained at the sowing time I, and the highest yields were obtained at Rivlas variety, which is the latest (mid-late) among the studied varieties.

Key words*: castor bean, variety, sowing time, climatic conditions, South Romania.*

INTRODUCTION

Castor bean (*Ricinus communis* L.) is an oil tropical plant, which has its origin in East Africa. Known since ancient times, the castor bean crop is now in the attention of farmers in countries located in warm and temperate areas of the globe, due to its main product, the most requested, respectively the castor oil, valuable raw material for many industries, such as textile, leather, machine building and aeronautics, paint industry, varnishes, high quality emulsions, printing industry, cosmetics industry, pharmaceutical industry, raw material for the production of sebacic acid, for obtaining synthetic fibres, and chemical industry (Sturzu et al., 2014). Castor oil is a valuable product because of its high content of ricinoleic acid which can reach 90%.
Despite the great importance of the castor oil, it contributes to only 0.15% of the vegetable oil produced in the world (Patel et al., 2016).
In Romania, currently the castor bean is cultivated on small areas for scientific, ornamental, industrial and medicinal purposes.
One possibility to reduce the economic negative effects of the actual climate change would be to introduce into cultivation or to extent the cultivation of the thermophilic plants. One such plant is castor bean. The increase in temperature in the last decades requires a new zoning of crops and the adaptation of cultivation technologies, so as to avoid periods with pedological and atmospheric drought.
The optimal sowing time of spring crops is determined by the achievement of a certain temperature at the sowing depth, depending on the species. New varieties and hybrids have shown earlier sowing requirements, at a thermal threshold 1-2°C lower than old cultivars (Sin, 2007). In most castor bean growing regions, the yield can be rapidly increased through the use of improved

agronomic practices. Key technologies include selecting the right genotype combined with the use of good quality seeds, proper sowing date, irrigation, soil fertilization, weed, pest and disease management, optimized plant density, mechanical harvesting and post-harvest management (Severino et al., 2012; Henderson et al., 2000; Tourino et al., 2002).

In the southern part of Romania, castor bean sowing can be done as early as possible, when in the soil at the sowing depth the temperature reaches 8-10°C and this has a tendency to increase, starting with the first decade of April at the latest on April 25 (Prodan and Prodan, 1993; Prodan et al., 1984).

The reduction of the vegetation period as a result of the breeding works makes possible the sowing of new varieties until May 1, because they reach maturity faster than the old varieties (Negrilă et al., 1993). But, the sowing season greatly influences the yield of the main raceme and therefore the sowing should not be delayed, in order to allow the full maturity of the main raceme before the first frost falls. Also, the date of sowing influences the number of nodes / internodes (Kittock and Williams, 1968). Practicaly, the sowing date is often used to overcome environmental constraints on crop production (Farhadi et al., 2013).

In addition to seed yield, the date of sowing also influences the oil content. Sowing on May 10 led to higher oil content (51.18%), followed by May 25 and June 10. The lowest oil content (43.67%) was observed for June 25. The oil content in the seeds of oilseeds is of enormous importance, because increasing the amount of oil offers a greater economic importance of the crop (Öztürk et al., 2014).

The objective of this paper is to present the results of the researches performed in South Romania in view to establish the temperature threshold for sowing castor bean as the plants to grow and develop well and the first raceme to reach maturity in good conditions.

MATERIALS AND METHODS

Researches were carried out in field experiments at the Agricultural and Development Research Station Teleorman (ADRS Teleorman) located in South Romania (Teleorman county) in the years 2019 and 2020.

The researches were performed under rainfed conditions on a soil of cambic chernozem type, the vertical subtype, having a loam-clay texture on the depth of the ploughed layer (0-25 cm). From the point of view of the physical and chemical properties, the soil is characterized by a clay content of 45%, humus content of 3.1%, weakly acid soil reaction (pH varies between 6.1 and 6.5), total nitrogen content of 0.166%, phosphorus mobile of 40-60 ppm, and mobile potassium of 250 ppm.

The main hydro-physical indices of the soil on the horizon 0-80 cm have the following average values: bulk density of 1.43 t/m^3, field capacity of 27.3% (310.4 mm), and permanent wilting point of 15.0% (171.0 mm).

Experimental design. The field experiments were placed according to the method of subdivided plots with 3 replications, with the following factors:

- Factor A – sowing times, with 3 graduations:
  - a_1 = sowing time I, this being represented by the soil temperature of 7°C at 7 o'clock and at a depth of 10 cm;
  - a_2 = sowing time II, this being represented by the soil temperature of 10ºC at 7 o'clock and at a depth of 10 cm;
  - a_3 = sowing time III, this being represented by the soil temperature of 13ºC at 7 o'clock and at a depth of 10 cm.
- Factor B – castor bean variety, with 4 graduations:
  - b_1 = Teleorman variety;
  - b_2 = Cristian variety;
  - b_3 = Dragon variety;
  - b_4 = Rivlas variety.
- Factor C – year, with 2 graduations:
  - c_1 = 2019;
  - c_2 = 2020.

The biological material consisted of four Romanian varieties of castor bean with different precocities but also with different degree of branching. All the four castor bean varieties (Teleorman – early variety; Cristian and Dragon - mid-early varieties; Rivlas mid-late variety) were created at ARDS Teleorman.

The ideal arrangement of plants in the planting area depends on the intrinsic characteristics of the cultivar, such as size, growth habits and architecture of the plants (Bezerra et al., 2009), as well as on the pedo-climatic conditions and the management system of the castor bean crop (Severino et al., 2006.a, 2006.d; Bizinoto et al., 2010). In our field experiments, the area of the experimental plot was of 14 m^2 (L = 5 m; l = 2.8 m), the plant density of 60,000 plants/ha, the distance between rows of 70 cm, the number of plants/plot of 84, the number of plants/row of 21, the distance between plants/row of 23.8 cm.

Crop management. The preceding crop was common winter wheat. After the preceding crop was harvested, there was performed a harrowing work, and after that the ploughing was performed at a depth of 30 cm. In the autumn, 100 kg of nitrocalcar (27% nitrogen) were applied, being incorporated with the disc harrow work. In the spring, complex chemical fertilizers of 15:15:15 type were applied, in a dose of 200 kg commercial product on ha. For the preparation of the germination bed, two perpendicular works made with a combinator were performed. After preparing the germination bed, the sowing rows were marked with the SPC-8 seed drill. The sowing was done manually with the planter at a depth of 6 cm. To ensure the number of plants on the plot, 2-3 seeds were sown in the nest. After plant emergence, the plants were thinned, leaving only one plant in the nest.

The control of the weeds was performed by the application immediately after sowing of the herbicides Dual Gold 960 EC (S-metolachlor 960 g/l) at a rate of 1.5 l/ha and Roundup (glyphosate 360 g/l) at a rate of 2.0 l/ha. For controlling in the vegetation period of the monocotyledonous weeds, the herbicide Leopard 5 EC (quizalofop-P-ethyl 50 g/l) was applied in a rate of 0.75 l/ha in the growth stage of 5-6 leaves. Unfortunately, for the control of dicotyledonous weeds there is not yet a herbicide for the castor bean crop with application in the vegetation period. As a consequence, in our field experiments the control of dicotyledonous weeds in the vegetation period was done by a mechanical weeding followed by a manual correction weeding. After the growth stage of appearance of the main raceme, the weeds are no longer a problem for the castor bean crop, because they no longer have favourable conditions for development (light, water and nutrients), the land being covered by the canopy of the castor bean plants.

During the vegetation period, no phytosanitary treatments were performed, being necessary to note the reaction of castor bean varieties to the appearance of specific diseases and pests.

Climatic data. In terms of temperature in the experimental years, castor bean benefited throughout the vegetation period from temperatures higher than the multiannual average specific for the area where the field experiments were implemented (Figure 1).

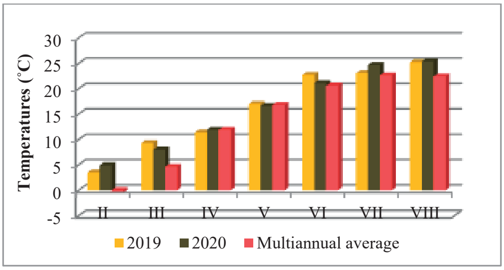

Figure 1. Average monthly temperatures at ARDS Teleorman in 2019 and 2020

In terms of water, in 2019, castor bean benefited of 376.6 mm of rainfall over the entire vegetation period, being 76.6 mm more than the crop requirements for humidity, but their distribution was unfavourable to the castor bean plants. Thus, in the first part of the vegetation period the rainfall was quantitatively higher than the multiannual average by 27.2 mm in April, 48.1 mm in May and 99.3 mm in June. During the period of the plant yield components formation, respectively in July and August, there was an accentuated water deficit of 27.1 mm in July and of 47.2 mm in August, a month in which no rainfall was registered (Figure 2).

In 2020, there were excess rainfall in May (+7.8 mm) and June (+11.6 mm), deficit rainfall in April (-21.8 mm), while in July and August there was registered a cumulative deficit of -92.9 mm, compared to the multiannual averages of the area. In July, it can be said that the drought was installed, when only 2.8 mm of rainfall was recorded, the rainfall being practically absent, and the deficit of the month being of 58.6 mm. In August, the rainfall was of 12.6 mm, of which 12.2 mm in the second decade, the deficit being of 34.4 mm.

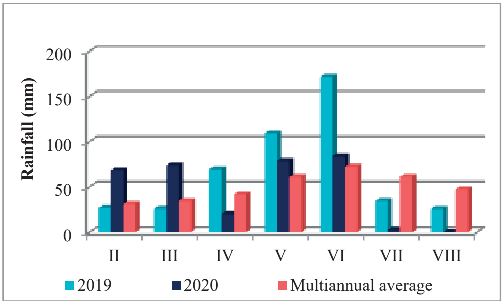

Figure 2. Sum of the monthly rainfall at ARDS Teleorman in 2019 and 2020

In 2020, the high temperatures from the end of February allowed the sowing time I on 28.02.2020. The subsequent evolution of temperatures (snow and blizzard on 8 of March) completely compromised the castor plants, this imposing the resowing later.

The sowing time I was on 20 of March in 2019 and on 28 of March in 2020, the sowing time II was on 2 of April in 2019 respectively on 7 of April in 2020, and the sowing time III was on 26 of April in 2019 respectively on 19 of April in 2020.

Determinations and data analysis. It was calculated the sum of temperature degrees and number of days from sowing to emergency and from sowing to maturity of the main raceme.

In the vegetation period it was determined the percentage of the broken plants and by the end of the vegetation period it was determined the percentage of monoracemal plants, the plant height, and the insertion height of the main raceme.

Harvesting was done manually. After harvesting, the seeds were peeled by hand on each variant and after that they were weighed. Also, it was determined the weight of 1,000 seeds, the number and the mass of seeds on the main raceme, the number of capsules on the main raceme and the length of the main raceme. The percentage of oil in the seeds was determined based on the magnetic resonance phenomenon performed on the Spinlock device.

The calculation and interpretation of the results was made based on the analysis of the variance of the experiments placed in the subdivided plots (Ceapoiu, 1968).

RESULTS AND DISCUSSIONS

On average over the years of experimentation, castor bean plants needed 365.8°C to emerge. Teleorman variety emerged first in all years of experimentation regardless of the time in which it was sown (Figure 3). As number of days, the varieties emerged in 35 days (in average per year) in the sowing time I, in 32 days in the sowing time II, and in 20 days in the sowing time III (Figure 4).

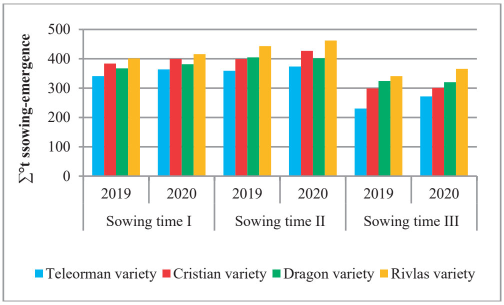

Figure 3. Sum of temperature degrees from sowing to emergence of castor bean plants, at ARDS Teleorman in 2019 and 2020

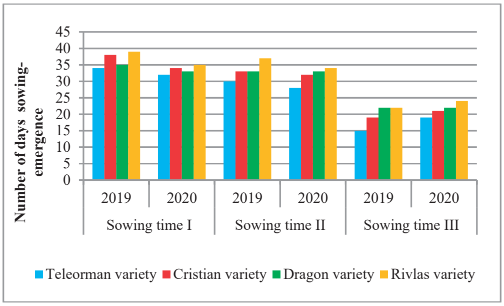

Figure 4. Number of days from sowing to emergence of castor bean plants, at ARDS Teleorman in 2019 and 2020

During the vegetation period, the differences regarding the sum of temperature degrees between sowing times were recovered. Thus, in sowing time I there were registered 2803.95°C, in sowing time II 2815.60°C, and in sowing time III 2743.96°C (Figure 5). As number of days, castor bean plants reached maturity of the main raceme in 147 days in the sowing time I, in 144 days in the sowing time II, and in 128 days in the sowing time III (Figure 6). Of the tested varieties, Teleorman variety is the earliest variety (129 days).

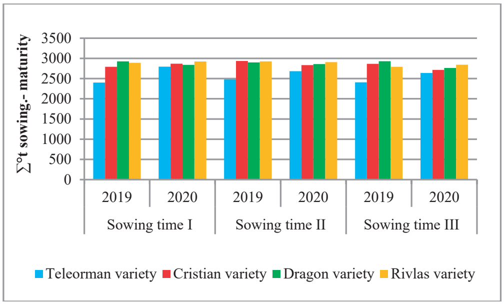

Figure 5. Sum of temperature degrees from sowing to maturity of the main raceme, at ARDS Teleorman in 2019 and 2020

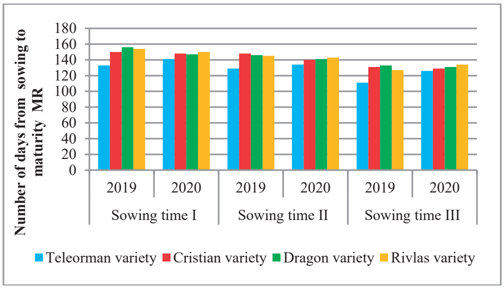

Figure 6. Number of days from sowing to maturity of the main raceme, at ARDS Teleorman in 2019 and 2020

The biomass accumulated by each genotype at the beginning of the reproduction phase is the most important factor for determining the seed yield at castor bean (Carvalho et al., 2010; Severino et al., 2006.c; Soratto et al., 2012). In indeterminate vegetative growth, meristems are very repetitive, reproducing the same or similar structure several times, and their activity can continue indefinitely (Taiz and Zeiger, 2004).
Analyzing the collected data we can say that castor bean plants when they reach the sum of degrees specific to a certain phenophase move to the next phenophase regardless of the number of days and the stage of development. Thus, delaying sowing reduces the period of plant development that directly influence the seed yield.
Due to the architecture of the castor bean plant, the breaking strength is a phenomenon that can cause significant yield losses. In 2019, in the first decade of June, when the plant yield components begin to form and the plants are vigorous in architecture, 114 mm of precipitation fell. The rains were accompanied by strong storms that broke the castor bean plants. This was due to the fistulous stem and the fact that the plant cells were turgid, but also due to the weight of the racemes, which made them prone to the breaking phenomenon. In the sowing time I there was a lower percentage of broken plants because the plants were better developed vegetatively, which brought them better resistance to breakage. On average, during the years of experimentation, the lowest percentage of broken plants had the plants in the sowing time I (9%). The best breaking resistance registered Teleorman variety (12.3% on average/year) (Table 1).

Table 1. The influence of the sowing time and the castor bean variety on the percentage of broken plants, at ARDS Teleorman in 2019 and 2020

Sowing time	Variety	% broken plants		
		2019	2020	Average variety
Sowing time I	Teleorman	15.0	1.2	*8.1*
	Cristian	16.0	2.2	*9.1*
	Dragon	15.7	3.7	*9.7*
	Rivlas	14.7	3.2	*9.0*
Average sowing time I		***15.4***	***2.6***	***9.0***
Sowing time II	Teleorman	25.3	3.4	*14.4*
	Cristian	22.7	3.2	*13.0*
	Dragon	24.7	3.6	*14.2*
	Rivlas	23.7	3.4	*13.6*
Average sowing time II		***24.1***	***3.4***	***13.8***
Sowing time III	Teleorman	25.9	3.1	*14.5*
	Cristian	25.7	4.3	*15.0*
	Dragon	26.7	3.0	*14.9*
	Rivlas	27.3	3.3	*15.3*
Average sowing time III		***26.4***	***3.4***	***14.9***
Average years		**22.0**	**3.1**	**12.6**

As the maturity of secondary racemes is uncertain in the specific growing conditions of Romania, due to their later appearance, a safe production of castor bean is achieved only from the main racemes. The percentage of monoracemal plants was not significantly influenced by the sowing time, the differences of the sowing times being 5.2% (Table 2).
In order to highlight the mode of action of sowing times, varieties and years of experiments as well as the existing interactions between factors, the analysis of variance for morpho-productive characters (Table 3) in castor bean was performed (Table 4).
The mass of 1,000 grains, a partially dominant quantitative genetic character, is not influenced by the sowing time. The tested variety has a significant influence, and the year of experimentation (climatic conditions) has a very significant influence as well as the combination of variety x year.

Table 2. The influence of the sowing time and the castor bean variety on the percentage of monoracemal plants, at ARDS Teleorman in 2019 and 2020

Sowing time	Variety	% monoracal plants		
		2019	2020	Average variety
Sowing time I	Teleorman	65.2	85.1	*75.2*
	Cristian	82.1	89.0	*85.5*
	Dragon	74.0	88.5	*81.2*
	Rivlas	75.3	84.7	*80.0*
Average sowing time I		***74.2***	***86.8***	***80.5***
Sowing time II	Teleorman	84.5	82.1	*83.3*
	Cristian	88.0	88.3	*88.2*
	Dragon	87.6	86.5	*87.0*
	Rivlas	87.0	81.9	*84.5*
Average sowing time II		***86.8***	***84.7***	***85.7***
Sowing time III	Teleorman	85.7	81.5	*83.6*
	Cristian	89.9	87.4	*88.6*
	Dragon	89.3	85.6	*87.5*
	Rivlas	82.3	84.3	*83.3*
Average sowing time III		***86.8***	***84.7***	***85.7***
Average years		**82.6**	**85.4**	**84.0**

The analysis of the variance for the oil content indicates a significant influence of the variety and the year of experimentation. Plant height, main raceme length, number of capsules on the main raceme, number of nodes and height of the first raceme insertion are important agronomic features in the yielding capacity of this species, with the last two directly related to plant precocity, because a main raceme plant with fewer nodes and a shorter vegetation period is harvested earlier and may be more productive in areas with irregular rainfall (Severino et al., 2006.a; Távora, 1982).

On the number of seeds on the main raceme, a very significant influence has the sowing time, the year and the combinations sowing time x year, variety x year, as well as the triple interaction sowing time x variety x year.

Table 3. Morpho-productive characters in the multifactorial experience with castor bean varieties (3 sowing times x 4 varieties x 2 years), at ARDS Teleorman in 2019 and 2020

Sowing time	Variety	Weight of 1000 seed (g)		Oil content (%)		No. seed/MR		Weight seed/MR (g)		No. of capsules / MR		Length of MR (cm)		Plant height (cm)		Insertion height of MR (cm)	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Sowing time I	Teleorman	236	210	47.9	45.9	209	107	49.3	22.4	90	68	46	23	164	104	118	81
	Cristian	286	260	53.8	52.4	243	94	69.4	24.4	94	65	43	21	180	115	137	94
	Dragon	293	315	51.9	50.6	217	95	63.3	29.9	94	68	48	24	188	108	128	84
	Rivlas	322	322	53.4	52.4	188	87	60.3	28.0	86	70	49	21	169	125	120	104
Average sowing I		***284***	***277***	***51.8***	***50.3***	***214***	***96***	***60.6***	***26.2***	***91***	***68***	***47***	***22***	***175***	***113***	***126***	***91***
Sowing time II	Teleorman	230	198	44.9	45.3	212	110	48.9	21.8	88	73	38	23	143	83	105	60
	Cristian	282	240	51.8	52.1	240	97	67.8	23.4	100	65	35	22	162	115	127	89
	Dragon	291	310	50.1	50.1	218	95	63.6	29.5	98	63	44	24	169	110	126	85
	Rivlas	324	318	52.1	52.0	175	91	58.3	28.9	80	64	40	23	171	120	124	96
Average sowing II		***282***	***267***	***49.7***	***49.9***	***211***	***98***	***59.7***	***25.9***	***92***	***66***	***39***	***23***	***161***	***107***	***121***	***83***
Sowing time III	Teleorman	227	200	44.4	46.4	196	106	44.5	21.2	86	71	33	21	139	110	105	60
	Cristian	284	243	51.3	52.8	239	102	68.0	24.8	97	68	44	21	170	117	127	89
	Dragon	295	307	48.3	51.0	211	91	62.4	27.9	97	61	41	21	170	115	126	85
	Rivlas	323	315	50.4	52.8	178	93	57.4	29.3	66	62	40	21	164	120	124	96
Average sowing III		***282***	***266***	***48.6***	***50.8***	***206***	***98***	***58.1***	***25.8***	***87***	***66***	***40***	***21***	***161***	***116***	***121***	***83***
Average years		***283***	***270***	***50.0***	***50.3***	***211***	***97***	***59.4***	***26.0***	***90***	***67***	***42***	***22***	***166***	***112***	***122***	***85***

*MR - main raceme

Table 4. Analysis of variance (ANOVA) for morpho-productive characters in the multifactorial experience with castor bean varieties (3 sowing times x 4 varieties x 2 years), at ARDS Teleorman in 2019 and 2020

Source of variance	Test F							
	Weight of 1000 seed (g)	Oil content (%)	No. seed/MR	Weight seed/MR (g)	No. of capsules/ MR	Length of MR (cm)	Plant height (cm)	Insertion height of MR (cm)
A (Sowing time)	8.0^{NS}	3.3^{NS}	836.7***	3.2^{NS}	4.0^{NS}	38.9**	5.6^{NS}	7.1^{NS}
B (*variety*)	6.0*	5.9*	7.7*	5.9*	4.6^{NS}	14.1**	6.6*	5.5*
AxB	0.007^{NS}	0.013^{NS}	2.7^{NS}	0.035^{NS}	0.981^{NS}	9.82**	0.415^{NS}	0.3^{NS}
C (*year*)	108.5***	0.5^{NS}	9800.6***	5643.3***	621.9***	541.1***	665.8***	562.3***
A X C	4.9^{NS}	6.5*	91.6***	1.9^{NS}	1.6^{NS}	8.3*	5.61*	0.5^{NS}
B X C	97.3***	0.038^{NS}	240.2***	77.7***	30.0***	1.7^{NS}	3.7^{NS}	7.5*
A X B X C	0.8^{NS}	0.1^{NS}	57.3***	0.9^{NS}	3.0^{NS}	1.2^{NS}	2.0^{NS}	0.7^{NS}

*MR - main raceme

The year of experimentation has a very significant influence on the weight of the seeds. The variety has a significant influence and the combination of variety x years has a very significant influence. A similar influence had the experimental factors on the number of capsules on the main racem.

On the morphological characters (the length of the main raceme, the height of the plant and the insertion height of the main raceme) the factor with very significant influence was the year of experimentation. The variety had a significant influence on the height of the plant and the insertion height of the main raceme, and a disctinct significant influence on the length of the main raceme. The sowing time had a distinctly significant influence only in the case of the length of the main raceme.

În both experimental years (2019 and 2020), the sowing time I determined the highest yields (Table 5). Also the highest yields were obtained in the climatic conditions of the year 2019, and among the castor bean varieties, Teleorman (early variety) realised the smallest yields regardless of sowing time and year, while Rivlas variety (mid-late variety) realised the highest yields in both experimental years at the sowing time I and II and the two mid-early varieties (Cristian and Dragon) realised the highest yeilds at the sowing time III.

Table 5. Seed yield obtained at the multifactorial experiment with castor varieties (3 sowing times × 4 varieties x 2 years), at ARDS Teleorman in 2019 and 2020

Sowing time	Variety	Yield kg/ha		
		2019	2020	Average variety
Sowing time 1	Teleorman	1262	890	***1076***
	Cristian	1915	968	***1442***
	Dragon	1873	1028	***1451***
	Rivlas	2067	1097	***1582***
Average sowing time I		***1779***	***996***	***1388***
Sowing time 2	Teleorman	1000	723	***862***
	Cristian	1661	864	***1263***
	Dragon	1380	1126	***1253***
	Rivlas	1758	1129	***1444***
Average sowing time II		***1450***	***961***	***1205***
Sowing time 3	Teleorman	971	785	***878***
	Cristian	1613	908	***1261***
	Dragon	1377	1055	***1216***
	Rivlas	1450	961	***1206***
Average sowing time III		***1353***	***927***	***1140***
Average years		***1527***	***961***	***1244***

Condensed presentation and interpretation of results is generally difficult due to the large number of comparisons (Săulescu and Săulescu, 1967). However, the analysis of variance indicates which of the comparisons represents a higher statistical interest. If we add to this the criterion of practical or scientific importance of some of the possible comparisons, we will be able to establish on this basis, the most convenient form of data condensation. Thus, analyzing the variance table for the multifactorial experience with castor varieties (3 sowing times × 4 varieties x 2 years), we observe the very significant influence of factor A (sowing time) and factor C (year). Also, the interaction A x C (sowing time x year) and B x C (variety x year) are very significant, and the triple interaction of A x B x C factors is distinctly significant (Table 6).

Table 6. Analysis of variance (ANOVA) for seed yield at the multifactorial experiment with castor bean varieties at ARDS Teleorman in 2019 and 2020

Source of variance	Sum of square (SS)	Degree of freedom (DF)	Mean square (s^2)	Test F
TOTAL	**10833723.37**	**71**		
Large plots	**593256.49**	**8**		
Repetition	15100.47	2		
A (Sowing time)	569941.39	2	284970.69	138.8***
Error (a)	8214.63	4	2053.66	
Medium plots	**2901300.67**	**27**		
B (*Variety*)	2840310.71	3	946770.24	7.4^{NS}
A x B	15211.17	6	2535.19	0.020^{NS}
Error (b)	2316148.12	18	128674.90	
Small plots	**7339166.20**	**36**		
C (*Year*)	5935591.56	1	5935591.56	1847.8***
A x C	399180.90	2	199590.45	62.1***
B x C	809852.41	3	269950.80	84.0***
A x B x C	117447.37	6	19574.56	6.1**
Error (c)	77093.96	24	3212.25	

The seed yield differences obtained from the horizontal comparisons between the sowing times from the two years of experimentation were very significant in 2019 of 783 kg/ha at sowing time I, of 489 kg/ha at sowing time II, and of 426 kg/ha at sowing time III (Tables 7 and 8).

Vertically, the differences obtained between the sowing times in the two years of experiment-tation indicate the fact that only in 2019 seed yield increases were obtained between the 3 sowing times, these being of 329 kg/ha for the sowing time I compared to sowing time II, and of 426 kg/ha for the sowing time I compared to sowing time III.

Table 7. The influence of the interaction of the sowing time and the year on the castor bean yield, at ARDS Teleorman in 2019 and 2020

Sowing time (A)	Yield kg/ha		
	Years (C)		Average sowing time
	2019 (c_1)	2020 (c_2)	
Sowing time I (a_1)	1779	996	1388
Sowing time II (a_2)	1450	961	1205
Sowing time III (a_3)	1353	927	1140
Average years	1527	961	1244

Table 8. Limit differences for the influence of sowing time and year interaction on castor bean yield, at ARDS Teleorman in 2019 and 2020

LSD	Yield kg/ha			
	Between average sowing time	Between average years	Interaction	
			horizontal comparisons (a_1c_1-a_1c_2)	vertical comparisons (a_1b_1-a_2b_1)
5%	36.4	27.5	47.6	44.0
1%	60.2	37.4	64.8	65.8
0.1%	112.6	50.1	86.7	106.1

Another very significant interaction resulting from the analysis of variance (ANOVA) for the seed yield in the multifactorial experiment with castor varieties (3 sowing times × 4 varieties x 2 years) is the interaction between variety and year (B x C). Analyzing the average seed yields, during the two years of experimentation, of the castor bean varieties we can say that the Rivlas variety is the most productive with a seed yield increase of 472 kg/ha compared to the Teleorman variety, very statistically assured (Tables 9 and 10).
On average for the years of experimentation, in 2019 the tested varieties had a very significant increase in seed yield, statistically assured of 566 kg/ha compared to the seed yield obtained in 2020.

Table 9. The influence of variety and year interaction on castor been seed yield, at ARDS Teleorman in 2019 and 2020

Variety (B)	Yield kg/ha		
	Years (C)		Average variety
	2019 (c_1)	2020 (c_2)	
Teleorman (b_1)	1078	799	939
Cristian (b_2)	1730	913	1322
Dragon (b_3)	1543	1070	1307
Rivlas (b_4)	1758	1062	1410
Average years	1527	961	1244

Table 10. Limit differences for the influence of variety and year interaction on castor been seed yield, at ARDS Teleorman in 2019 and 2020

LSD	Yield kg/ha			
	Between average variety	Between average years	Interaction	
			horizontal comparisons (b_1c_1-b_1c_2)	vertical comparisons (b_1c_1-b_2c_1)
5%	251.1	27.5	55.0	181.8
1%	334.8	37.4	74.8	249.3
0.1%	468.7	50.1	100.2	339.2

CONCLUSIONS

In the specific growing conditions of South Romania, castor bean can be sown when in the soil at a depth of 10 cm, temperature reaches 7°C for three days consecutive and the general tendency of the weather is to warm. Sowing in this time gave in the performed experiences the highest seed yields regardless the climatic conditions of the year.
When the castor bean plants reach the sum of the degrees specific to a certain phenophase, they move to the next phenophase regardless of the number of days and the stage of development. Thus, sowing delay reduces the duration of the vegetative phases and the rapid entry of plants into the generative phase, directly influencing the seed yield.
Among the studied varieties, the Rivlas variety which is the latest (mid-late) of them proved to produce the highest seed yields, especially when the sowing is performed early. In the case of sowing delay, comparable seed yields with Rivlas variety gave the mid-early varieties Cristian and Dragon. Teleorman variety, which is an early variety, proved to be less productive, producing the smallest seed yields.

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RESEARCHES ON THE INFLUENCE OF HYBRID AND IRRIGATION REGIME ON MAIZE CROP

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Abstract

The research was carried out at ARDS Marculesti, Calarasi County, Romania, in 2017, 2018 and 2019 years. The experiment had two factors, namely: the maize hybrid and the irrigation regime. The investigated maize hybrids were: P9175 (FAO 330), KWS BELLAVISTA (FAO 330), KWS SMARAGD (FAO 350), KWS KASHMIR (370 FAO) and KWS DURANGO (FAO 480). Four irrigation regimes were researched: rainfed, stressed at and after flowering, stressed before flowering and full irrigated throughout the vegetation period. The yield results obtained in the three years of experimentation showed that the lowest yield was obtained for the hybrid P9175, of 10,920 kg/ha. The hybrids Durango, Kashmir and Smaragd gave very significant positive differences compared to the hybrid P9175 taken as a control; these production differences were between 890 and 1760 kg/ha. The Bellavista hybrid gave yields close to those of the P9175 hybrid. Regarding the moisture content of grains at harvest, the lowest values were obtained by the hybrid P9175.

Key words: *yield, grains moisture, irrigation regime, corn hybrid.*

INTRODUCTION

Maize occupies the third place in the world as a cultivated plant, originating from America, North of the equator. It was cultivated in Spain, after which it was also cultivated in Italy. Analyzing the evolution of production between 1939 and 2009 in the US, Assefa Y. et al., (2012) show that it increased from 1,500 kg/ha to over 8,000 kg/ha, mainly due to the application of irrigation and chemical fertilizers. In Romania, 70% of the lands cultivated with maize are in the west plain of the country and in the south of the country (Luca E. et al., 2009; Meluţ L.C. et al., 2009).

According to american researchers, when the average temperature of May falls below 12.7°C, corn production decreases by 15% (Wagger M. G. and Cassel D. K., 2008; Harold V. Eck, 1984; Jackson R.D., 1982;).

A critical period is the flowering, when the temperature must be between 18-24°C (Hall AJ et. Al., 1982; Hajibabaee M. et. Al., 2012). The high temperatures, in this phase, determine a pronounced gap between the appearance of tassel and that of silks (Jackson R.D. et. Al., 1981). At a temperature of 28-30°C, the viability of the pollen decreases. Temperature amplitudes above 30°C during the day and below 10.0ºC at night, which occur in the 6th and 7th stages of organogenesis, prevent the formation of anthers, implicitly the development of pollen grains and the normal development of fertilization processes (DeLoughery RL and Crookston RK, 1979; DeJonge KC et al., 2015; Mahdi M. Al - Kaisi and Xinhua Yin, 2003). In the grain filling phase, the lack of moisture causes them to remain undevelopped (Cakir R., 2004; Lamm F.R. et al. 2012; Mustek J.T. and Dusek D.A., 1980; Claassen M.M. and Shaw R.H., 1970).Under the conditions in our country, maize production is above average when rainfall is over 40 mm in May, 60 mm in June, 60 mm in July and less than 80 mm in August (Melut L.C. et al., 2009).

Considering that 75% of the water pumped from aquifers is used for crop irrigation, (Wallace, 2000; Howell, 2001; cited by Grassinia P. et al., 2011), as well as the fact that during the critical period for water in the months July and August, when large amounts of water need to be applied by irrigation, many researchers, especially in arid and semi-arid areas, have considered the quantitative and economic efficiency of applying irrigation to maize (DeJonge K.D. et al., 2011; Echarte L. and Andrade L.H., 2003; Grassinia P. et al., 2011; Hao B. et al., 2015). Other reserchers have taken into account the method of irrigation as well as the specific consumption per kg of grain in order to optimise the process (Howell T.A., 2001; Howell T.A. et al., 1989; Howell T.A. et al., 2002; Howell T.A. et al., 1998; Irmak S. et al., 2000; Klocke N.L. et al., 2011). The type of the hybrid, plant density, the nitrogen ammount as well as the plant morphological components were, also researched, in order to be correlate with the irrigation techniques (Lamm F.R., 2017); Marek G.W. et al., 2017; Norwood C.A., 2000; Norwood C.A. and Dumler T.J., 2002; O'Shaughnessy Susan A. et al., 2017; Samia Amiri et al., 2015; Schlegel A.J. et al., 2010; Steele D.D. et al., 1994).

MATERIALS AND METHODS

The research aimed at the yield of corn hybrids between FAO 320-450 ripening groups under irrigated conditions in different phenophases and their response during flowering to water stress.

The experiment was performed according to the method of subdivided plots with two factors and the placement scheme was in randomized blocks which assumes that all variants of a repetition are placed in a block, in this case, the repetition being the same as the block.

The A factor was considered the corn hybrid and it had 5 graduations:

a1 - P9175
a2 - KWS Bellavista
a3 - KWS Durango
a4 - KWS Kashmir
a5 - KWS Smaragd

The B factor was considered the irrigation regime and it had 4 graduations:

b1 - rainfed
b2 - stressed after flowering
b3 - stressed before flowering
b4 - full irrigated

The trial had 3 replications. The sowing and harvesting were mechanically performed, with special equipment for research for experimental plots (Mircea V. et al., 2020).

Figure 1. The BAURAL SP2100 harvester for experimental plots, on two rows

RESULTS AND DISCUSSIONS

The climatic conditions of the years of experimentation are shown in the graphs below.

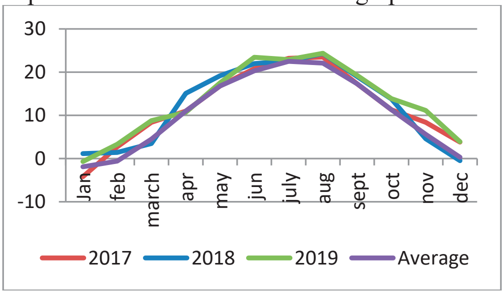

Figure 2. The average monthly air temperature in 2017, 2018 and 2019, compared to the multiannual average, at ARDS Marculesti

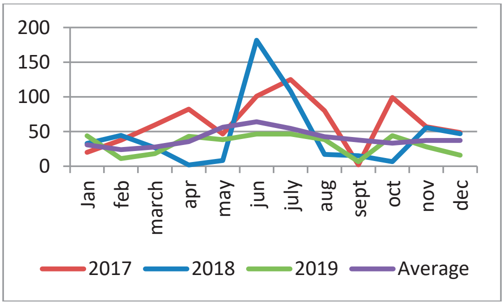

Figure 3. Monthly rainfall in 2017, 2018 and 2019, as compared to the multiannual average at ARDS Marculesti

From these graphs it can be seen that 2017 was a year with average monthly temperatures close to the multiannual average but with very abundant rainfall in the summer months, June, July and August. In September, only 2 mm of precipitation was recorded, but this did not affect the production of corn. Also, in May, 25 mm were recorded, compared to 45 mm as the multiannual average, which determined the drought phenomenon in the first phases of maize growth (Figures 2 and 3).

In 2018, the recorded monthly rainfall was extremely unevenly distributed. Thus, in the first months of spring there were very low precipitations, almost non-existent in April and May (1.80 and 8.20 mm). The average monthly temperature higher than the multiannual average, recorded in April (15.08 compared to 11.00°C) together with the low soil moisture affected the early emergence and growth of corn plants. In contrast, in June and July there were record rainfalls, three to two times higher than the multiannual average (182 compared to 65 mm in June and 108 compared to 55 mm in July). Extremely abundant rainfall during this period favored the growth and development of corn plants in optimal conditions. August and September were dry, but this did not affect corn production (Figures 2 and 3).

In 2019, in the first part of the vegetation period, the average monthly temperatures were very close or identical to the multiannual average.

Regarding precipitation, in 2019, values for the entire vegetation period were recorded below the multiannual average. Early hybrids made better use of the water reserve in the soil, this being observed in the higher yields obtained by early hybrids than late ones (Figures 2 and 3).

The grain yield per hectare registered in 2017 highlights the Durango hybrid, with the highest quantity. The lowest yield was obtained for the P9175 hybrid. The yield differences are very significant positive between the Durango hybrid and P9175 at all irrigation regimes. In rainfed conditions, all hybrids showed very significant positive differences compared to the P9175 hybrid, except for the Bellavista hybrid, which recorded a significantly positive difference (Table 1). When stressed after flowering, the Durango hybrid recorded a distinctly significant positive difference and the Kashmir hybrid recorded a significantly positive difference. At this irrigation regime, the Bellavista and Smaragd hybrids recorded insignificant differences compared to the P9175 hybrid. All the irrigation regimes gave very significant positive differences compared to the rainfed one, taken as a control. This proves the importance of irrigation on corn production. Research related to corn hybrids has also been carried out by Romanian and foreign researchers (Baumhardt R.L. et al., 2013; Domuţa C. et al., 2009; Luca E. et al., 2009).

Table 1. The influence of the hybrid and the irrigation regime on grain yield in 2017

Comparisons between hybrids at the same irrigation regime												
Hybrid	b1 - rainfed				b2 – stressed after flowering				b3 – stressed before flowering			
	Yield, kg/ha	%	Diff., kg/ha	Sign.	Yield, kg/ha	%	Diff., kg/ha	Sign.	Yield, kg/ha	%	Diff., kg/ha	Sign.
P9175	**9028**	**100**	**Mt**		**12353**	**100**	**Mt**		**12502**	**100**	**Mt**	
Bellavista	9818	108	789	*	12273	99	-80		12643	101	141	
Durango	11032	122	2004	***	13561	109	1208	**	14692	117	2190	***
Kashmir	10473	116	1444	***	13212	106	858	*	13356	106	853	*
Smaragd	10978	121	1950	***	12670	102	316		13325	106	823	*
DL 5% = 775 Kg/ha DL 1% = 1044 Kg/ ha DL 0.1% = 1386 Kg/ha												

Hybrid	b4 – full irrigated				Comparisons between irrigation regimes				
	Yield, kg/ha	%	Diff., kg/ha	Sign.	Irrigation regime	Yield, kg/ha	%	Diff., kg/ha	Sign.
P9175	**13109**	**100**	**Mt**		**Rainfed**	**10266**	**100**	**Mt**	
Bellavista	13109	100	0,0		Stressed after flowering	12814	124	2548	***
Durango	14754	112	1645	***	Stressed before flowering	13303	129	3037	***
Kashmir	14218	108	1108	**	Ffull irrigated	13755	133	3488	***
Smaragd	13583	103	473						
DL 5% = 775 Kg/ha DL 1% = 1044 Kg/ ha DL 0.1% = 1386 Kg/ha					DL 5%=346 kg/ha; DL 1%=467 kg/ha; DL 0.1%=620 kg/ha				

The moisture of the grains at harvest is a very important feature because it influences the possibility of keeping the production in silos. If the moisture content of the grains is too high, the mechanized harvesting process with the combine can also be difficult. In addition, high grain moisture requires additional costs to dry the crop in order to preserve it.

The results obtained in 2017 show that the lowest moisture of grains at harvest was recorded for the hybrid P9175 (14.28%) and the highest for the hybrid Durango (17.88%). This correlates with the yield and the FAO group. When the P9175 hybrid was taken as a control, the differences compared to all other hybrids were very significantly positive. Related to the irrigation regime, this year it is found that the rain fed treatments registered the lowest values, of 14.85%. The moisture content of the grains at harvest increased as irrigation was applied before flowering, after flowering and recorded the highest value, of 16.55% in the case of full irrigated treatment (Table 2).

Tabelul 2. The influence of the hybrid and the irrigation regime on the grain moisture content at harvest in 2017

Comparisons between hybrids at the same irrigation regime												
Hybrid	b1 - rainfed				b2 – stressed after flowering				b3 – stressed before flowering			
	U, %	%	Diff., kg/ha	Sign.	U, %	%	Diff., kg/ha	Sign.	U, %	%	Diff., kg/ha	Sign.
P9175	13.77	100	Mt		13.80	100	Mt		14.52	100	Mt	
Bellavista	14.98	108.81	1.21	***	15.63	113.26	1.83	***	16.00	110.25	1.49	***
Durango	16.99	123.41	3.23	***	17.29	125.27	3.49	***	18.04	124.29	3.53	***
Kashmir	13.98	101.51	0.21		15.13	109.60	1.33	***	15.49	106.70	0.97	**
Smaragd	14.53	105.50	0.76	*	15.39	111.51	1.59	***	15.88	109.39	1.36	***
DL 5%=0.59 %; DL 1%=0.80 %; DL 0.1%=1.07 %												

Hybrid	b4 - full irrigated				Comparisons between irrigation regimes				
	U%	%	Diff., %	Sign.	Irrigation regime	U%	%	Diff., %	Sign.
P9175	**15.04**	**100**	**Mt**		**Rainfed**	**14.85**	**100**	**Mt**	
Bellavista	16.57	110.20	1.54	***	Stressed after flowering	15.60	105.04	0.74	***
Durango	19.17	127.47	4.13	***	Stressed before flowering	16.23	109.27	1.37	***
Kashmir	16.16	107.41	1.12	***	Full irrigated	16.55	111.45	1.70	***
Smaragd	16.34	108.66	1.30	***					
DL 5%=0.59 %; DL 1%=0.80 %; DL 0.1%=1.07 %					DL 5%=0.27 %; DL 1%=0.37 %; DL 0.1%=0.49 %				

In 2018 year, too, the highest yields were obtained for late hybrids, Durango, Kashmir and Smaragd and the lowest, for early hybrids, Bellavista and P9175. The differences between the Durango, Kashmir and Smaragd hybrids and the P9175 hybrid, which recorded the lowest yield, were distinctly or significantly positive. In the case of stressed after flowering, the Bellavista and Durango hybrids did not show significant differences compared to the P9175 hybrid. Occasionally, certain climatic conditions may cause these differences. However, as an average of irrigation regimes, the Bellavista hybrid recorded a higher yield than the P9175 hybrid but this was not statistically assured (Table 3).

The irrigation regimes gave different results compared to 2017 and 2019. In this sense, if in the other two years the yield increased as irrigation was applied, from rainfed, stressed after flowering, stressed before flowering and full irrigated. This year, the stressed after flowering treatment gave a higher yield than the stressed before flowering treatment (13,038 compared to 12,096 kg/ha). This difference is very significantly positive (Table 3).

The results regarding the moisture of the grains at harvest obtained in 2018 are very similar to those obtained in 2017, in the sense that the lowest value was recorded by the hybrid P9175 (14.28%) and the highest, at the hybrid Durango (18.23%). Again, it can be seen that the hybrid that gave the lowest yield, namely P9175 recorded the lowest grain moisture at harvest and the hybrid that gave the highest grain yield also had the highest grain moisture. When the P9175 hybrid was taken as a control, all other hybrids gave very significant positive differences. Within each irrigation regime, significant, distinctly significant and very significant positive differences were also obtained compared to the P9175 hybrid (Table 4).

Regarding the influence of irrigation regimes, this year, the lowest value of grain moisture at harvest was obtained for rainfed treatments and this parameter increased steadily for irrigated varieties before flowering, after flowering and irrigated throughout the vegetation, with values between 0.70 and 0.50%. These differences are very significantly positive (Table 4).

Table 3. The influence of the hybrid and the irrigation regime on yield in 2018

Hybrid	Comparisons between hybrids at the same irrigation regime: b1 –rainfed				b2 – stressed after flowering				b3 – stressed before flowering			
	Yield, kg/ha	%	Diff., kg/ha	Sign.	Yield, kg/ha	%	Diff., kg/ha	Sign.	Yield, kg/ha	%	Diff., kg/ha	Sign.
P9175	11121	100	Mt		12323	100	Mt		10964	100	Mt	
Bellavista	11634	104	512		12034	97	-289		11779	107	814	
Durango	12098	108	976		13773	111	1449	*	12599	114	1635	**
Kashmir	12369	111	1247	*	13774	111	1450	*	12466	113	1501	*
Smaragd	12662	113	1541	**	13287	107	963		12672	115	1707	**
DL 5%=1139 kg/ha; DL 1%=1535 kg/ha; DL 0.1%=2038 kg/ha												

Hybrid	b4 – full irrigated				Comparisons between irrigation regimes: Irrigation regime	Yield, kg/ha	%	Diff., kg/ha	Sign.
	Yield, kg/ha	%	Diff., kg/ha	Sign.					
P9175	**12168**	**100**	**Mt**		**Rainfed**	**11977**	**100**	**Mt**	
Bellavista	12347	101	178		Stressed after flowering	13038	108	1061	***
Durango	13895	114	1726	**	Stressed before flowering	12096	100	119	
Kashmir	13848	113	1679	**	full irrigated	13093	109	1116	***
Smaragd	13209	108	1040						
DL 5%=1139 kg/ha; DL 1%=1535 kg/ha; DL 0.1%=2038 kg/ha					DL 5%=509 kg/ha; DL 1%=686 kg/ha; DL 0.1%=911 kg/ha				

Table 4. The influence of the hybrid and the irrigation regime on the grain moisture content at harvest in 2018

Hybrid	Comparisons between hybrids at the same irrigation regime: b1 - rainfed				b2 – stressed after flowering				b3 – stressed before flowering			
	U,%	%	Diff., kg/ha	Sign.	U,%	%	Diff., kg/ha	Sign.	U,%	%	Diff., kg/ha	Sign.
P9175	**13.77**	**100**	**Mt**		**13.80**	**100**	**Mt**		**14.52**	**100**	**Mt**	
Bellavista	14.98	108.80	1.21	***	15.63	113.26	1.83	***	16.00	110.22	1.49	***
Durango	16.99	123.41	3.23	***	18.04	130.72	4.24	***	18.73	128.99	4.21	***
Kashmir	13.98	101.50	0.21		15.13	109.60	1.33	***	15.49	106.70	0.97	**
Smaragd	14.53	105.49	0.76	*	15.39	111.51	1.59	***	15.88	109.39	1.36	***
DL 5%=0.61 %; DL 1%=0.83 %; DL 0.1%=1.10 %												

Hybrid	b4 – full irrigated				Comparisons between irrigation regimes: Irrigation regime	U,%	%	Diff., %	Sign.
	U,%	%	Diff., %	Sign.					
P9175	**15.04**	**100**	**Mt**		**Rainfed**	**14.85**	**100**	**Mt**	
Bellavista	16.57	110.20	1.54	***	Stressed after flowering	15.60	105.04	0.74	***
Durango	19.17	127.47	4.13	***	Stressed before flowering	16.12	108.55	1.27	***
Kashmir	16.16	107.41	1.12	***	Full irrigated	16.66	112.16	1.80	***
Smaragd	16.34	108.66	1.30	***					
DL 5%=0.61 %; DL 1%=0.83 %; DL 0.1%=1.10 %					DL 5%=0.27 %; DL 1%=0.37 %; DL 0.1%=0.49 %				

In 2019, the Durango hybrid, which gave the highest yields in 2017 and 2018, recorded the lowest amount of grains per hectare, namely 9,228 kg. This yield was even lower than that obtained for the P9175 hybrid, which recorded the lowest values in the first two years of experimentation. This situation can be explained by the fact that this year there was a period of drought from the second half of June until the end of the maize vegetation period, at the beginning of September. The Durango hybrid, being the latest experimented hybrid, from the FAO 450 group, suffered the most due to the drought during this period because the flowers buds and, implicitly, the grain production was affected. However, even in this situation, the application of irrigation determined the increase of the yield, from 7,727 kg/ha to rainfed treatment, to 8,418 kg/ha to stressed after, 9,845 kg/ha to stressed before flowering and 10,923 kg/ha to full irrigated treatment. All these differences from irrigated varients are very significantly positive, which highlights the importance of irrigation. Another interesting fact recorded in 2019 was the very significant positive difference recorded by the Bellavista hybrid compared to the P9175 hybrid, namely 807 kg/ha. The other two hybrids, Kashmir and Smaragd, gave very high yields compared to the Durango and P9175

hybrids, with very significant positive differences (Table 5).
Irrigation regimes have been steadily increasing, from rainfed to stressed after flowering, stressed before flowering and full irrigated throughout the vegetation period, at rates of approximately 1,000 kg/ha, from 8,122 kg/ha to rainfed, up to 11,384 kg/ha for full irrigated treatment. These differences are very significant statistically, proving the importance of applying irrigation to maize (Table 5).

Table5. The influence of the hybrid and the irrigation regime on grain yield in 2019

Comparisons between hybrids at the same irrigation regime												
Hybrid	b1 - rainfed				b2 – stressed after flowering				b3 – stressed before flowering			
	Yield, kg/ha	%	Diff., kg/ha	Sign.	Yield, kg/ha	%	Diff., kg/ha	Sign.	Yield, kg/ha	%	Diff., kg/ha	Sign.
P9175	**7700**	**100**	**Mt**		**8837**	**100**	**Mt**		**9751**	**100**	**Mt**	
Bellavista	8059	104	358		9631	108	794	*	10591	108	840	**
Durango	7727	100	26		8418	95	-419		9845	100	94	
Kashmir	8791	114	1090	**	9840	111	1002	**	10489	107	737	*
Smaragd	8332	108	631	**	9855	111	1018	**	10778	110	1027	**
DL 5%= 613 kg/ha; DL 1%= 827 kg/ha; DL 0.1%= 1098 kg/ha												

Hybrid	b4 – full irrigated				Comparisons between irrigation regimes				
	Yield, kg/ha	%	Diff., kg/ha	Sign.	Irrigation regime	Yield, kg/ha	%	Diff., kg/ha	Sign.
P9175	**10769**	**100**	**Mt**		**Rainfed**	**6578**	**100**	**Mt**	
Bellavista	12007	111	1237	***	Stressed after flowering	7634	116	1056	***
Durango	10923	101	153		Stressed before flowering	8324	126	1745	***
Kashmir	11245	104	475		full irrigated	9202	139	2623	***
Smaragd	11978	111	1208	***					
DL 5%= 613 kg/ha; DL 1%= 827 kg/ha; DL 0.1%= 1098 kg/ha					DL 5%= 274 kg/ha; DL 1%= 370 kg/ha; DL 0.1%= 491 kg/ha				

The lowest values of grain moisture at harvest were recorded, again this year, with the hybrid P9175 (10.01%), with very significant positive differences compared to all other hybrids investigated. Within each irrigation regime, there were only two situations when the differences were significant, namely, with full irrigated, between the hybrids Kashmir and P9175 and, respectively, between Smaragd and P9175.
The differences between the irrigation regimes were similar to those registered in 2018, namely, the humidity of the grains at harvest increased from the rainfed treatments (10.69%), constantly, to the full irrigated treatments (11.97%), with a rate of approx. 0.5%, considered very significant from a statistical point of view (Table 6).

Table 6.The influence of the hybrid and the irrigation regime on the grain moisture at harvest in 2019

Comparisons between hybrids at the same irrigation regime												
Hybrid	b1 - rainfed				b2 – stressed after flowering				b3 – stressed before flowering			
	U,%	%	Diff., kg/ha	Sign.	U,%	%	Diff., kg/ha	Sign.	U,%	%	Diff., kg/ha	Sign.
P9175	**9.36**	**100**	**Mt**		**9.82**	**100**	**Mt**		**10.16**	**100**	**Mt**	
Bellavista	11.77	125.76	2.41	***	12.15	123.75	2.33	***	12.54	123.46	2.38	***
Durango	10.84	115.75	1.48	***	11.74	119.57	1.92	***	12.41	122.18	2.25	***
Kashmir	10.73	114.66	1.37	***	10.91	111.10	1.09	***	11.18	110.04	1.02	***
Smaragd	10.73	114.61	1.37	***	11.13	113.32	1.31	***	11.23	110.59	1.08	***
DL 5%= 0.55 %; DL 1%= 0.75 %; DL 0.1%= 0,99 %												

Hybrid	b4 – full irrigated				Comparisons between irrigation regimes				
	U,%	%	Diff., %	Sign.	Irrigation regime	U,%	%	Diff., %	Sign.
P9175	**10.71**	**100**	**Mt**		**Rainfed**	**10.69**	**100**	**Mt**	
Bellavista	13.14	122.73	2.43	***	Stressed after flowering	11.15	104.31	0.46	***
Durango	13.04	121.80	2.34	***	Stressed before flowering	11.50	107.64	0.81	***
Kashmir	11.42	106.67	0.71	*	full irrigated	11.97	112.04	1.28	***
Smaragd	11.56	107.97	0.85	*					
DL 5%= 0.55 %; DL 1%= 0.75 %; DL 0.1%= 0,99 %					DL 5%=0.24 %; DL 1%=0.33 %; DL 0.1%=0.44 %				

CONCLUSIONS

Maize is one of the most important crops in the world. Climatic conditions play a decisive role in yield quantity.

The trial aimed to find out the influence of the hybrid and the irrigation regime on maizeyield.

The climatic conditions recorded in 2017 and 2018 determined the normal development of corn plants, leading to yields of over 13,000 kg/ha (13,510 kg/ha for the Durango hybrid).

Unfavorable climatic conditions in 2019determined the lowest yields in the three years of experimentation (9,228 for the Durango hybrid and 9,264 for the P9175 hybrid).

On average over the three years of experimentation, the lowest yield was obtained for the hybrid P9175, of 10,885 kg/ha and the highest, for the hybrid KWS Kashmir, of 11,995 kg/ha. Under the different climatic conditions of the three years of experimentation, the most stable yields were given by the hybrids KWS Smaragd (FAO 350) and KWS Kashmir (FAO 370).

Regarding the experienced irrigation regimes, the lowest production, on average over the three years, was obtained for rainfed treatment (10,121 kg/ha) and the highest, for full irrigated (12,744 kg/ha). The treatment of stressed after flowering and before flowering gave close results (11,723 and 11,897 kg/ha).

The lowest grain moisture at harvest was recorded in the earliest hybrid, in the FAO 320 group, namely P9175 (12.86%) and the highest, in the KWS Durango hybrid, the latest hybrid, in the FAO 450 group (16.04%).

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WEED CONTROL IN SUNFLOWER BY SEPARATE AND COMBINED HERBICIDE APPLICATION

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Abstract

In 2019 and 2020 two field experiments with two sunflower hybrids (Diamantis CL and P64LP130 CLP) was conducted. The trial was stated on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The efficacy and selectivity of the herbicide products Pulsar 40 (40 g/l imazamox), Pulsar Plus (25 g/l imazamox) and Viballa CE (3 g/l halauxifen-methyl), applied separately, in combinations as tank mixtures in 10 days interval and separately were evaluated. The dominating weeds on the field were Chenopodium album L., Amaranthus retroflexus L., Xanthium strumarium L., Abutilon theophrasti Medik., Datura stramonium L., Solanum nigrum L., Portulaca oleracea L., Setaria viridis L., Sinapis arvensis L., Sorghum halepense L. (Pers.), and Convolvulus arvensis L. The highest efficacy against Ch. album after the application of Viballa in both years of the study was recorded. For this product, the efficacy against D. stramonium, P. oleracea, and C. arvensis was the lowest. Both sunflower hybrids demonstrated the highest seed yield after the application of Pulsar 40 and Pulsar Plus separately followed by their tank mixture with Viballa in 10 days interval.

Key words: *sunflower, weeds, herbicides, tank mixtures, efficacy.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the four most important annual crops in the world grown primarily for edible oil (De la Vega and Hall, 2002). It is successfully grown over a wide geographical area as it is adaptable to a wide range of environmental conditions (Beards and Geng, 1982). As oilseed crop, sunflowers play a major role in Europe. Sunflower oil is regarded as high quality oil for food use, and lately there is also an increasing demand for sunflower oil for industrial uses, mainly for the bio-diesel industry.

One of the factors limiting the development of cultivated plants is the annual ubiquity and development of weeds and nutrient availability in soil (Garapova, 2020; Yanev, 2015; Yanev et al., 2014a). Also, the absolute seed mass of 1000 seeds is crucial for the yields formation (Georgiev et al., 2014). It was found that in sunflower depending on the weed density, duration of the concurrence, weed spectrum and other factors, yield losses can be up to 81% (Carranza et al. 1995). Depending on weed density, time of competition duration, weed spectrum and other factors yield loss can be up to 81% (Carranza et al. 1995). Weed management is an important component for the successful crop production and in sunflower for particular (Yanev, 2020; Jursik et al., 2015; Saudy and El-Metwally, 2009; Awan et al., 2009; Shylaja and Sundari, 2008). Sunflowers are usually planted in low densities and grow slowly during the first weeks till row closure. Weeds that emerge and establish during this time are concurrent to the crop and suppress its normal growth and development. Generally, weed control options, especially for broadleaf weeds, in sunflower are limited (Breccia et al., 2011, Pfenning et al., 2008, Bruniard, 2001).

In the sunflower fields of Bulgaria a significant dynamics of the weed species and densities that form the weed associations has occurred. In the beginning of the monitoring the dominating weed species were *Xanthium strumarium* L., *Sinapis arvensis* L., and on separate fields *Cannabis ruderalis* Janisch. in high densities was prevailing. The reasons for the mass distribution of these weed species are the violated crop rotations, seeding of sunflowers in short period of time – in 1-2 years on the same field, insufficient quality of the soil tillage, the limited choice of herbicides for their control, etc. The implementation of the alternative cropping technologies like

Clearfield® and Express® Sun, their constant improvement, as herbicide content and selection process showed positive effect for decreasing the density and range of distribution of these three weeds in the sunflower fields. From the other hand it led to clearly expressed compensatory processes and mass distribution *Chenopodium album* L. and *Portulaca oleracea* L. to a lower extent. It is known that imazamox and tribenuron-methyl have limited efficacy against *Chenopodium album* and *Portulaca oleracea* (Tonev et al, 2020).

One of the most commonly used weed control method in cultivated plants is herbicide application. The choice of proper herbicide is one of the most important and responsible moments in the crop management. The proper herbicide must meet the following requirements - to be selective for the crop; to be highly effective against the existing weeds; its application rates should not lead to the accumulation of residues in plant production and in soil; should not deteriorate the quality of production ad should be harmless to soil microorganisms and the environment (Yanev and Kalinova, 2020; Goranovska and Yanev, 2016; Hristeva et al., 2015; Yanev, 2015; Semerdjieva et al., 2015; Hristeva et al., 2014; Yanev et al., 2014b). A number of authors have studied various herbicides for weed control in *Helianthus annuus* L. (Mohapatra et al., 2020; Jursík et al., 2020; Inoue et al., 2019; Jiří et al., 2017; Kostadinova et al., 2016; Sala et al., 2012).

The usage of tank mixtures of herbicides lead to increased efficacy against some dicotyledonous weeds as *Solanum nigrum* L., *Abutilon theophrasti* L. and *Amaranthus retroflexus* L. (Mitkov et al, 2018). The efficacy and selectivity of herbicides and tank-mixture combinations at different application terms in Clearfield and ExpressSun sunflowers were evaluated. The efficacy of tribenuron (TBM) was excellent and quite rapid on *Chenopodium album.* Its efficacy on other tested dicot weeds ranged around 90%, depending on weather conditions and growth stages of weeds (Jursík et al., 2017).

The Clearfield® technology in sunflower has been developed to allow the use of imidazolinone herbicides as a post-emergence weed control option (Balabanova and Vassilev, 2015). With the current Clearfield ImiSun system, amplified herbicide performance was penalized with unacceptable crop response. Other broadleaf herbicides for a combination with Imazamox in postemergence sunflower use are currently not available. This situation restricted an improvement of the herbicidal efficacy of the imazamox based Clearfield system. The development of a higher imidazolinone tolerance with the Clearfield Plus trait allows the use of a stronger adjuvant and a better formulation for imidazolinone herbicides and consequently a more flexible and reliable weed control in sunflower by maximizing the herbicidal efficacy per ai unit without any penalty on tolerance. The increased herbicide performance on difficult-to-control weeds and possible relaxed re-cropping restrictions through potential herbicide rate reduction with the new Clearfield Plus system provide sunflower growers a better tool to manage weeds and add to the value of the new Clearfield Plus sunflower production system (Pfenning et al, 2012). When Pulsar 40 was applied separately without DASH, its efficacy against Johnson grass, corn thistle, field bindweed, hemp agrimony, rough cocklebur, white goosefoot, purslane and broomrape was significantly reduced. Referring to its efficacy against the annual broad-leaved weed species redroot pigweed, charlock mustard, wild radish, cleavers, black nightshade, etc., it was 100% and no differences were observed between the rates of 0.80, 1.00 and 1.25 l ha^{-1}. The separate use of the herbicide at a rate of 1.25 l ha^{-1} showed the same efficacy against more stubborn weeds, as that of Pulsar 40 applied at the rate of 0.80 l ha^{-1} together with 0.80 l ha^{-1} of the adjuvant Dash (Mitkov et al., 2016). The highest efficacy against *S. halepense* from rhizomes was recorded for treatment 5 (Pulsar Plus - 2.40 l h^{-1}). *S. halepense* developed from seeds, *S. viridis*, *A. retroflexus*, *Xa. strumarium*, *S. nigrum* and *S. arvensis* were successfully controlled by application of Pulsar 40 or Pulsar Plus in the low examined rates. *Ch. album* and *A. theophrasti* can be controlled by Pulsar Plus at the lower rate - 1.20 l ha^{-1} (Neshev et al., 2020).

The aim of the study is to establish the possibilities for chemical control of several weeds during the sunflower growing season

(Clearfield and Clearfield Plus technologies), using vegetation herbicides, as well as their combinations and application systems.

MATERIALS AND METHODS

During the vegetation seasons of 2019 and 2020 two field trials with the sunflower hybrids Diamantis CL (Clearfield) and P64LP130 CLP (Clearfield Plus) were conducted. The experiments were stated on the Experimental field of the department of General agriculture and Herbology at the Agricultural University of Plovdiv, Bulgaria. The studies were performed by the randomized block design in 4 replications.

The trial with the Clearfield sunflower hybrid included the following variants: 1. Pulsar 40 (40 g/l imazmox) - 1.2 l ha^{-1}; 2. Viballa CE (3 g/l halauxifen-methyl) - 1.0 l ha^{-1}; 3. Pulsar 40 + Viballa CE - 0.6 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture); 4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later; 5. Pulsar 40 - 0.6 l ha^{-1}, followed by Pulsar 40 + Viballa CE – 0.6 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later; 6. Untreated control.

The trial with the Clearfield Plus sunflower hybrid included the following variants: 1. Pulsar Plus (25 g/l imazamox) - 1.6 l ha^{-1}; Viballa CE - 1.0 l ha^{-1}; 3. Pulsar Plus + Viballa CE - 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture); 4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later; 5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later; 6. Untreated control. The herbicide application was done in BBCH 14 – 16 with size of spraying solution 250 l ha^{-1}.

Predecessor of the sunflower hybrids was the winter wheat variety "Enola" during the two years of the study. On the whole experimental field combined fertilization with 250 kg ha^{-1} with N:P:K (15:15:15), followed by deep ploughing was done. Before sowing of the crop, disking on the depth of 15 cm and two harrowings on 8 cm of depth as well as spring dressing with 250 kg ha^{-1} NH_4NO_3 was also performed.

The efficacy of the studied herbicide rates against the weeds by the 10 score scale of EWRS (European Weed Research Society) on the 14th, 28th and 56th day after application was studied. The selectivity of the herbicide by the 9 score scale of EWRS was also evaluated Biological yield was determined by harvesting the entire plot and for the four replicates of each variant.

Preliminary investigation of the experimental fields for establishing the weed species was performed. On the experimental fields 12 weed species typical for the crop were identified. For both years of the study their average densities per 1 m^2 in the untreated plots were as follows: *Chenopodium album* L. - 39 specimens; *Amaranthus retroflexus* L. - 7 specimens; *Xanthium strumarium* L. - 10 specimens; *Abutilon theophrasti* Medic. - 21 specimens; *Datura stramonium* L. - 6 specimens, *Solanum nigrum* L. - 11 specimens; *Portulaca oleracea* L. - 9 specimens; *Setaria viridis* L. - 5 specimens; *Sinapis arvensis* L. - 5 specimens; *Sorghum halepense* Pers. Developed from seeds - 10 specimens; *Sorghum halepense* Pers. Developed from rhizomes - 17 specimens and *Convolvulus arvensis* L. - 5 specimens per m^2. The total weed number on average for the untreated controls was 145 specimens per m^2.

The results of the conducted research trials were processed with software package of SPSS 19 program - module one-factorial and two-factorial analysis of variance.

RESULTS AND DISCUSSIONS

On 12 tables is presented the efficacy of the studied herbicides and their combinations against the existing weeds on the experimental field.

On table 1 is the efficacy against the weed *Ch. album* average for both experimental years. On the 14th day after the last application of the herbicide Viballa CE, as well as its combination with Pulsar 40 and Pulsar Plus the efficacy varied from 80 to 90%.

Lower and unsatisfactory efficacy was recorded for the treatment with Pulsar 40 and Pulsar Plus - 40 - 45 %. On the 56th day after the herbicide treatment from 95 to 100% efficacy after the combined herbicide application and the alone treatment with Viballa CE was reported.

Against *Ch. album* the alone application of Pulsar 40 and Pulsar Plus the observed efficacy was not sufficient enough (variant 1 for both technologies.

Table 1. Efficacy of the evaluated herbicide products against *Ch. album* average for 2019-2020 (%)

Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar 40 - 1.2 l ha^{-1}	40	50	60
2. Viballa CE - 1.0 l ha^{-1}	90	95	100
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	90	95	100
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	80	90	95
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	90	95	100
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar Plus - 1.6 l ha^{-1}	45	55	65
2. Viballa CE - 1.0 l ha^{-1}	90	95	100
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	90	95	100
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	85	90	95
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	90	95	100
6. Untreated control	-	-	-

On table 2 is presented the effect of the tested herbicides against the weed *A. retroflexus* on average for the two years of the experiment. From the product Viballa CE the reported efficiency was the lowest - 35% on the 14th day after treatments. After the combined application of the examined herbicides 70 to 80% efficacy was against the weed recorded. On this reporting date, after the alone application of Pulsar 40 and Pulsar Plus excellent efficacy was recorded. On the last reporting date the efficacy for these treatments reached from 90 to 100%. On the 56th day after treatments the efficacy of Viballa CE was 45%.

Table 2. Efficacy of the evaluated herbicide products against *A. retroflexus* average for 2019-2020 (%)

Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar 40 - 1.2 l ha^{-1}	90	95	100
2. Viballa CE - 1.0 l ha^{-1}	35	40	45
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	70	80	90
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	75	85	90
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	80	90	100
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar Plus - 1.6 l ha^{-1}	90	95	100
2. Viballa CE - 1.0 l ha^{-1}	35	40	45
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	75	85	90
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	70	85	90
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	80	95	100
6. Untreated control	-	-	-

In the field crop rotation one of the most difficult-to-control weeds is *Xanthium strumarium L.* In the present study all studied herbicide products showed excellent efficacy against this noxious weed species. The obtained results are presented on table 3. For this weed, on the 14th day after the herbicide treatments the efficacy was low 65-80%. The efficacy increased on the second reporting date, on the 56th day it reached from 80 to - 95% among the treatments.

In a trial conducted by Neshev et al. (2020) the reported efficacy of the studied herbicides reached 100% for all treatments and the authors stated that the weed can be successfully controlled by Pulsar 40 or Pulsar Plus.

Table 3. Efficacy of the evaluated herbicide products against *Xa. strumarium* average for 2019-2020 (%)

Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar 40 - 1.2 l ha^{-1}	75	90	95
2. Viballa CE - 1.0 l ha^{-1}	65	80	85
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	65	85	90
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	65	80	85
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	75	90	95
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar Plus - 1.6 l ha^{-1}	80	90	95
2. Viballa CE - 1.0 l ha^{-1}	70	80	85
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	70	80	90
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	70	80	85
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	75	90	95
6. Untreated control	-	-	-

The annual broadleaf weed species *Abutilon theophrasti* Medic. proved to be easily controllable from all tested variants of the experiment during both years of the trial (Table 4). According to Neshev et al., (2020) this weed can be successfully controlled by application of Pulsar Plus even from the rate of 1.20 ha^{-1}. From the new herbicide Viballa CE and from the established in practice herbicides - Pulsar 40 and Pulsar Plus from 85 to 100% efficacy from the first to the last reporting dates was found.

Table 4. Efficacy of the evaluated herbicide products against *A. theophrasti* average for 2019-2020 (%)

Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar 40 - 1.2 l ha^{-1}	85	95	100
2. Viballa CE - 1.0 l ha^{-1}	85	95	100
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	90	95	100
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	80	90	95
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	90	100	100
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar Plus - 1.6 l ha^{-1}	90	95	100
2. Viballa CE - 1.0 l ha^{-1}	90	95	100
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	90	95	100
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	80	90	95
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	90	100	100
6. Untreated control	-	-	-

The efficacy against the weed *Datura stramonium L.* on the 14^{th} day after the application of the herbicides, from Pulsar 40 and Pulsar Plus (var. 1 for both technologies) and in their combined application with Viballa CE (variant 5) relatively satisfactory efficacy in the range of 80 - 85% average for the two years of the experiment was found (Table 5). These efficiency results increased on the other two reporting dates, being 95% on the 56^{th} day in all four variants.

For treatments 3 and 4 (for both technologies) from 80 to 85% efficacy against this weed was observed on the last evaluation date.

From the alone use of the herbicide Vibala CE zero effect on all three reporting dates was establishd.

Table 5. Efficacy of the evaluated herbicide products against *D. stramonium* average for 2019-2020 (%)

Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar 40 - 1.2 l ha^{-1}	85	90	95
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	70	80	85
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	65	75	80
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	80	90	95
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar Plus - 1.6 l ha^{-1}	85	90	95
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	70	80	85
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	65	75	80
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	80	90	95
6. Untreated control	-	-	-

On table 6 in presented the efficacy against *Solanum nigrum L.* average for both years of the research. This weed can be successfully controlled by the application of Pulsar 40 or Pulsar Plus (Neshev et al., 2020). The obtained efficacy of the herbicide product Viballa CE was 70 % on the 14^{th} day after the treatments. On the second reporting date the efficacy increased, and on the 56^{th} day it reached 85% for the herbicide Viballa CE.

The other treatments showed 95 – 100% efficacy on the last reporting date.

Table 6. Efficacy of the evaluated herbicide products against *D. stramonium* average for 2019-2020 (%)

Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar 40 - 1.2 l ha^{-1}	85	95	100
2. Viballa CE - 1.0 l ha^{-1}	70	80	85
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	80	90	95
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	80	90	95
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	85	95	100
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar Plus - 1.6 l ha^{-1}	85	95	100
2. Viballa CE - 1.0 l ha^{-1}	70	80	85
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	80	90	95
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	80	90	95
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	85	95	100
6. Untreated control	-	-	-

From all annual weeds existing on the experimental field, the species *Portulaca oleracea L.* was the most difficult-to-control. The obtained results are presented on table 7. Satisfactory efficacy after the herbicide application for any of the treatments was not recorded. The herbicide product Viball CE showed 0% efficacy when it was applied alone. The control of the common purslane (*P. oleracea*) is mainly limited to the following measures: fertilization with manure free of seeds of the weed; qualitative and on time tillage of the stubborn; extra summer and autumn tillage after plowing if there is new infestation; on time and qualitative inter-row tillage operations, etc. (Tonev et al., 2019).

The common purslane can be controlled with the herbicides isoxaflutol, metribuzin, pendimethalin, tembotrione, etc. (Tonev et al., 2019).

Table 7. Efficacy of the evaluated herbicide products against *P. oleracea* average for 2019-2020 (%)

Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar 40 - 1.2 l ha^{-1}	15	25	30
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	10	20	25
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	10	20	25
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	20	25	30
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar Plus - 1.6 l ha^{-1}	25	35	40
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	15	20	25
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	15	25	30
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	25	35	40
6. Untreated control	-	-	-

From the data on table 8 it is clearly seen that after the alone application of Pulsar 40 and Pulsar Plus, as well as after their combined application excellent efficacy against the grass weed *S. viridis* on the 56th day was found. The herbicide product Viballa CE had no effect on this weed – it controls broadleaf weeds only. The efficacy against *S. viridis* was 0%.

In a study performed by Neshev et al. (2020) the obtained efficacy data showed that Green bristle grass (*S. viridis*) can be controlled by Pulsar 40 or Pulsar Plus.

Table 8. Efficacy of the evaluated herbicide products against *S. viridis* average for 2019-2020 (%)

Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar 40 - 1.2 l ha^{-1}	75	90	100
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	65	80	85
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	65	80	85
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	70	90	100
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar Plus - 1.6 l ha^{-1}	80	90	100
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	70	80	85
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	70	80	85
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	70	90	100
6. Untreated control	-	-	-

The efficacy data against *Sinapis arvensis* L. is shown on table 9.

Pulsar 40 and Pulsar Plus showed 100% efficacy against the weed *Sinapis arvensis* L. (Neshev et al., 2020).

For both trials, for the treatments where imazamox-containing herbicide products were applied (Pulsar 40 and Pulsar Plus), independently the combination and rate, the efficacy against this weed was 100% on the 56th day after treatments.

At variant 2 where Viballa CE was applied alone, the efficacy was 80% on the 56th day after the treatments. If there is infestation with *S. arvensis* on the field, the herbicide product have to be applied with parting herbicide for increasing the control of this widely spread weed species.

Table 9. Efficacy of the evaluated herbicide products against *S. arvensis* average for 2019-2020 (%)

Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar 40 - 1.2 l ha^{-1}	90	100	100
2. Viballa CE - 1.0 l ha^{-1}	60	70	80
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	75	90	100
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	75	90	100
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	90	100	100
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar Plus - 1.6 l ha^{-1}	95	100	100
2. Viballa CE - 1.0 l ha^{-1}	60	70	80
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	80	90	100
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	80	90	100
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	90	100	100
6. Untreated control	-	-	-

All treatments where imazamox-containing herbicide products were evaluated the efficacy against *Sorghum halepense Pers.* developed from seeds was excellent – 100% on the last reporting date.

The Viballa CE herbicide had no effect on the weed (0% efficacy) because of is broadleaf weed control spectrum (Table 10). In atrial conducted by Neshev et al. (2020) it was also found that the Johnson grass (*S. halepense*) can be successfully controlled by application of Pulsar 40 or Pulsar Plus. On the 56th day after application the efficacy against this weed was increased and reached 100% (Neshev et al., 2020).

Table 10. Efficacy of the evaluated herbicide products against *S. halepense (seeds)* average for 2019-2020 (%)

Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar 40 - 1.2 l ha^{-1}	100	100	100
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	85	95	100
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	85	95	100
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	95	100	100
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14th	28th	56th
1. Pulsar Plus - 1.6 l ha^{-1}	100	100	100
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	90	95	100
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	90	95	100
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	95	100	100
6. Untreated control	-	-	-

As opposite to *S. helepense* developed from seeds, the weed developed from rhizomes was more difficult-to-control from Pulsar 40 or Pulsar Plus (Table 11). Neshev et al. (2020) also reported low efficacy against Johnson grass (*S. halepense*) developed from rhizomes. The authors recommended if there is high infestation of Johnson grass developed from rhizomes, a partner grass herbicide product to be applied. An appropriate herbicide product is Stratos Ultra/Focus Ultra (100 g/l cycloxydim) applied at rate of 2.00 ha^{-1}.

Table 11. Efficacy of the evaluated herbicide products against *S. halepense (rhizomes)* average for 2019-2020 (%)

Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar 40 - 1.2 l ha^{-1}	60	75	80
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	30	45	50
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	25	40	45
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	60	75	80
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar Plus - 1.6 l ha^{-1}	70	75	80
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	35	50	55
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	20	45	50
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	65	75	80
6. Untreated control	-	-	-

Against the weed *Convolvulus arvensis L.* very low efficacy was observed on the first reporting date. Later in time the efficacy reached 0% for all treatments (Table 12). The weed cannot be controlled by any of the evaluated herbicide products and combinations from the study.

Table 12. Efficacy of the evaluated herbicide products against *C. arvensis* average for 2019-2020 (%)

Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar 40 - 1.2 l ha^{-1}	15	0	0
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	5	0	0
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	5	0	0
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	15	0	0
6. Untreated control	-	-	-
Treatments	Days after treatment		
	14^{th}	28^{th}	56^{th}
1. Pulsar Plus - 1.6 l ha^{-1}	20	0	0
2. Viballa CE - 1.0 l ha^{-1}	0	0	0
3. Pulsar Plus - 0.8 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	10	0	0
4. Pulsar Plus - 0.8 l ha^{-1} followed by Viballa CE - 1.0 l ha^{-1} 10 days later	10	0	0
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	20	0	0
6. Untreated control	-	-	-

There were no visible phytotoxic symptoms for any of the studied herbicide products and their combinations.

On table 13 are presented the results regarding the seed yields of the sunflower hybrid Diamantis CL on average for both experimental years. The differences in the obtained yield were determined by the herbicide selectivity and efficacy. The existing weed infestation leads to the lowest yields for the untreated control – (1.0026 t ha^{-1}).

According to the degree of mathematical proof, five separate groups are distinguished between the different variants (a, b, c, d, e). It is also seen that treatment 5 (Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later) is from group (e), farthest from the group of the untreated control (a). It had the highest seed yield - 3.9143 t ha^{-1}.

Due to the fact that the haloxyfen-methyl-containing herbicide Viballa CE applied alone is with a narrower spectrum of action and controls broadleaf weeds only, the yield is lower compared to the those of the other variants.

Table 13. Sunflower grain yield for the hybrid Diamantis CL average for 2019 and 2020

Treatments	Yields, t ha^{-1}
1. Pulsar 40 - 1.2 l ha^{-1}	3.5017* d
2. Viballa CE - 1.0 l ha^{-1}	2.4975* b
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	3.0819* c
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	3.0544* c
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	3.9143* e
6. Untreated control	1.0026 a

Legend: All values with a * sign have significant differences with the result of the untreated control. All values followed by different letters are with proved difference according to Duncan's test at $P < 0.05$

On table 14 are presented the results concerning the seed yields of the sunflower hybrid P64LP130 CLP on average for both years of the research.

The weed flora developing uncontrolled in the field is the reason for the lowest average seed yield obtained for the untreated control - 1.1113 t ha^{-1}.

For this hybrid, according to the degree of mathematical proof, also, five separate groups are distinguished between the different variants (a, b, c, d, e). Here the highest sunflower seed yield is also reported for treatment 5 (Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later) - 4.0110 t ha^{-1}.

Table 14. Sunflower grain yield for the hybrid P64LP130 CLP average for 2019 and 2020

Treatments	Yields, t ha^{-1}
1. Pulsar 40 - 1.2 l ha^{-1}	3.6223* d
2. Viballa CE - 1.0 l ha^{-1}	2.5991* b
3. Pulsar 40 - 0.6 l ha^{-1} + Viballa CE - 1.0 l ha^{-1} (in tank mixture)	3.1948* c
4. Pulsar 40 - 0.6 l ha^{-1}, followed by Viballa CE - 1.0 l ha^{-1} 10 days later	3.1759* c
5. Pulsar Plus - 0.8 l ha^{-1}, followed by Pulsar Plus + Viballa CE – 0.8 l ha^{-1} + 1.0 l ha^{-1} (in tank mixture) 10 days later	4.0110* e
6. Untreated control	1.1113 a

Legend: All values with a * sign have significant differences with the result of the untreated control. All values followed by different letters are with proved difference according to Duncan's test at $P < 0.05$

CONCLUSIONS

The herbicide product Viballa CE had excellent efficacy against the weeds *Chenopodium album* L. and *Abuthilon theophrasti* Medic.

The herbicide products Pulsar 40 and Pulsar Plus showed 100% efficacy against *Amaranthus* retroflexus L., *Abutilon theophrasti* Medic., *Solanum nigrum* L., *Setaria viridis* L., *Sinapis arvensis* L., and *Sorghum halepense* (L.) Pers from seeds.

The herbicide product Viballa CE overcomes the herbicide products Pulsar 40 and Pulsar Plus by its efficacy against *Chenopodium album* L.

The herbicide products Pulsar 40 and Pulsar Plus overcomes the herbicide product Viballa CE by their efficacy against *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Datura stramonium* L., *Solanum nigrum* L. and *Sinapis arvensis* L.

The herbicide combination of Viballa CE with Pulsar 40 and Viballa CE Pulsar Plus controlled the weeds from 80 to 100%, except the species *Portulaca oleracea* L., *Sorghum halepense* (L.) Pers. developed from rhizomes and *Convolvulus arvensis* L.

No visible signs of phytotoxicity for any of the evaluated herbicide products and their combinations were recorded.

Compared to the untreated control, mathematically proven differences for the indicator sunflower seed yield in favor of all variants treated with herbicides were reported.

For both technologies (Clearfield and Clearfield Plus) the highest yields for the herbicide combinations in treatment 5 for hybrid Diamantis CL (3.9143 t ha^{-1}) and for hybrid P64LP130CLP (4.0110 t ha^{-1}) were recorded.

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MINERAL FERTILIZATION FOR DURUM WHEAT UNDER NON-IRRIGATED CONDITIONS

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Abstract

*The aim of the study was to determine the influence of mineral fertilization in the conditions of soil type Pelic Vertisols in durum wheat, variety Progress, grown under non-irrigated conditions. The experiment was set in 1966 in two-field crop system rotation of durum wheat-cotton. The data include 2014/2019. The following fertilization rates were tested: N_{40}, N_{80}, N_{120}, N_{160}; P_{40}, P_{80}, P_{120}, P_{160}; $N_{80}P_{40}$, $N_{80}P_{80}$, $N_{80}P_{120}$, $N_{80}P_{160}$, $N_{120}P_{80}$, $N_{120}P_{120}$, $N_{160}P_{80}$, $N_{160}P_{120}$, $N_{160}P_{160}$, $N_{120}P_{120}K_{80}$. As a control variant was accepted $N_0P_0K_0$. The results of the study showed, that grain yield has the greatest effect on average for the tested period at fertilization with $N_{80}P_{40}$. The plant height, the number of grains per spike and the weight of the grains per spike showed the highest values under the influence of $N_{160}P_{120}$. The length spike is affected equally by fertilization with N_{160} and $N_{160}P_{120}$. The analysis reveals positive and proven correlations were found between all yield components. The strongest relationship was observed between length spike and number of grain per spike (0.973***).*

***Key words**: components of yield, durum wheat, grain yield, mineral fertilization.*

INTRODUCTION

Durum wheat is one of the most important crops in the world. Sardana (2000) reports that durum wheat is consumed by 35% of the world's population. Globally, most consumption is in Europe, South America and the United States (Sall et al., 2019). Filling the nutritional need and large number of the world's population depends on durum wheat (Abbass et al., 2020). Demand for wheat is projected to continue to grow over to coming decades, particularly, in the developing world to feed an increasing population (Desta and Almayehu, 2020). Durum wheat is a traditional crop in Bulgaria (Petrova, 2007). It is mainly used for the production of pasta.

Agricultural production is a dynamic system influenced by multiple factors of different nature (Stoyanov, 2020). Fertilization is one of the most practices in crop production (Jakšić et al., 2021). Nitrogen (N) is the most limiting nutrient for cereal crops (Frija and Annabi, 2020). Currently, the anthropogenic N fixation rate has increasing to 210 Tg per year (Zhang et al., 2021). However, Guerrero et al. (2021) emphasize that plant response to N is limited to a threshold; N fertilization above this level does not increase crop yield any further. Nevertheless, farmers tend to use more mineral and organic N fertilizers than biologically necessary to ensure the highest possible yield (Leslie et al., 2017). Phosphorus is the essential plant nutrient which plays major role for achieving the maximum agricultural production (Arshad et al., 2016). Indeed, the greater part of P remains in insoluble form in soil and, hence, unavailable for the plant (Cherchali et al., 2019). This leads to the use of excessive amounts of phosphorus fertilizers.

Potassium fertilizers are usually considered in third place - after nitrogen and phosphorus. However, many farmers neglect the need for durum wheat from K. Duncan et al. (2018) conclude that improved soil P and K availability has the potential to increase grain yield and improve the efficiency of N fertilizer use.

The above gives us reason to believe that fertilization is not yet developing in the right direction. The aim of our study was to investigate the effect of different fertilizer rates (alone) and different combinations of them on the productivity of durum wheat.

MATERIALS AND METHODS

The experiment was set up in 1966 at the Field Crops Institute, Chirpan, Bulgaria, as a stationary fertilizer trial. The data present

2014/2019 and Progress variety. The trial was conducted by the randomized complete block design in four replications in two-field crop system rotation of durum wheat and cotton (Stefanova-Dobreva & Muhova, 2020). Each plot was 10 m^2.

Four rates of N and P fertilizers were included in the study – alone: 0, 40, 80, 120 and 180 kg ha; and the following combinations – $N_{80}P_{40}$, $N_{80}P_{80}$, $N_{80}P_{120}$, $N_{80}P_{180}$, $N_{120}P_{80}$, $N_{120}P_{120}$, $N_{180}P_{80}$, $N_{180}P_{120}$, $N_{180}P_{180}$. In the experimental design there was a variant with K fertilizer – $N_{120}P_{120}K_{80}$. It is used as a nitrogen fertilizer ammonium nitrate, triple superphosphate was used as the phosphorus fertilizer, and as a potassium fertilizer – potassium sulfate.

The type of soil is Pellic Vertisols (Vp.). The humus content for the layer 0-20 cm is very high – 3.85% (Table 1).

Table 1. Humus content, assessment of humus stock, total nitrogen

Depth, cm	Humus, %	Humus stock in the layer 0-100 cm, t/da	Assessment of the humus stock in degree	Total N, %	$CaCO_3$, %
0-20	3.85	13.86	high	0.20	0.00
20-40	2.80	10.08	high	0.13	0.25
40-60	2.20	6.48	middle	0.10	2.25
60-80	1.95	4.50	low	0.07	4.56
80-100	1.90	3.24	low	0.04	4.56

Its depth in the profile is relatively homogeneous, decreasing in depth from 2.80% to 1.9% in the layer 80-100 cm. The salt content is negligible, which indicates that the soils are well drained and there is no danger of salinization or negative impact on crops. The amount of total carbonates does not exceed 5% in the deep soil horizons and there is no manifestation of chlorosis.

In the studied periods there is a different degree of change in the amount of precipitation and temperature sums (Table 2). During the period 2013/14, 2014/15 and 2018/19 higher temperature amounts were reported, respectively 290.8 °C, 254.4 °C and 245.9 °C than the average for a multi-year period. In 2015/16 and 2017/18 the temperature sums were, respectively, 520.7°C and 427.0 °C higher than in the period 1928/13. Different in terms of temperature is the period 2016/17, in which the temperature sum is 1,843.5 °C or 166.2 °C lower than the climatic average for the long term.

Regarding precipitation, it can be noted that the data for the period 2013/14, 2016/17 and 2018/19 show the amount of precipitation around the norm - 420.8 mm, 375.2 mm and 358.5 mm, during the periods 2014-2015 and 2017-2018 is respectively by 183.1 mm and 66.6 mm more, and in 2015-2016 it was 71.6 mm less.

Abbreviations: GY – grain yield; PH – plant height; LS – length spike; NGS – number of grain per spike; GWS – grain weight per spike.

Table 2. Temperature sum and rainfall during the durum wheat vegetation for the period 2014/2019 and over a multi-year period

Year	Mount								Σ
	XI	XII	I	II	III	IV	V	VI	
Temperature sum, Σ°C									
1928-13	215.9	61.1	-6.2	49.4	188.9	357.9	511.5	630.7	2,009.7
2013/14	275,3	3,1	95,0	160,8	278,7	369,5	510,0	608,1	2,300,5
2014/15	227.2	138.0	74.9	96.0	192.7	340.4	586.0	608.9	2,264.1
2015/16	299.3	115.1	-8.7	233.6	273.5	439.8	498.0	679.8	2,530.4
2016/17	201.7	26.2	-160.7	-46.5	289.2	355.7	513.7	664.2	1,843.5
2017/18	244.9	125.6	65.2	97.8	200.5	471.0	584.8	646.9	2,436.7
2018/19	225.3	20.7	53.8	113.1	292.6	335.2	533.4	681.5	2,255.6
Rainfall, mm									
1928-13	47.3	54.0	44.3	37.7	37.0	45.2	64.1	65.4	395.0
2013/14	49.8	8.6	47.1	1.3	105.7	83.6	99.3	25.4	420.8
2014/15	36.9	14.3	50.3	61.7	134.9	15.1	58.8	78.1	578.1
2015/16	50.2	1.3	73.9	28.3	53.1	26.6	75.0	15.0	323.4
2016/17	47.7	5.9	80.1	23.8	51.3	22.6	59.5	84.3	375.2
2017/18	48.2	38.9	23.3	109.0	83.4	8.7	62.2	87.9	461.6
2018/19	82.3	23.5	28.9	24.5	3.3	51.4	21.4	123.2	358.5

RESULTS AND DISCUSSIONS

According to Table 3, on average for the test period, durum wheat yield of 2,117.4 kg/ha without fertilization. When the mineral fertilization from the independently applied N was included, all tested rates have a confirmed statistical influence. The highest increase was fertilization with 120 kg N ha. This variation exceeds the control by 56.2%. However, the difference with the lower rate (N_{80}) was only 0.8%. The same trend was reported by Tedone et al. (2018). However, the results of our study differ from those of Panayotova & Kostadinova (2015). The authors report that the application of N_{80} leads to an increase in GY by 25-29%, N_{120} by 40-43%, and N_{180} by 45-46%.

Application of P results in a decrease of GY. Three of the tested rates showed lower values compared to the control, as follows: P_{40} by 11.0%, P_{80} by 14.9% and P_{180} by 12.5%. Only the norm of 120 kg P ha showed a slight increase, but the difference of 3.0% remained outside the statistical reliability. The results obtained are contrary to the statement of Nesme et al. (2014) that P levels always strongly influence yield.

Combined NP fertilization had the greatest effect on GY. Average test period the rate of $N_{80}P_{40}$ showed the best results, increasing the values by 64.5% above the control. It is important to note that the highest grain yield was obtained at the lowest NP combination. Panayotova, Kostadinova & Manolov (2018) reported similar results at the same fertilization rates. Analysis of the variance showed a medium degree of influence (P=1%) when fertilizing with $N_{120}P_{120}K_{80}$.

Plant height (Table 3) ranged from 74.6 to 102.6 cm. As expected, the lowest plants were reported from the non-fertilizing variant. From the independent application of fertilizers, the greatest effect was nitrogen fertilization at a rate of 120 kg/ha. This variant exceeds the control by 35.5%. Chipsa et al. (2017) observed a similar trend, reporting that as the nitrogen rate increases, plant height also increases. Desta & Almayehu (2020) also confirm our results. The authors reported that the higher plants have been measured at the highest N rate. Only the rate of 40 kg/ha remained from the independent application of P with confirmed statistical impact. Highest plants were observed at fertilization $N_{180}P_{120}$. Under this variant, the plants were 37.5% high than the control plants.

Table 3. Grain yield (kg ha) and plant height (cm) average for the period

Fertilization rate		GY, kg/ha	% of control	PH, cm	% of control
Control		2,117.4	100.0	74.6	100.0
N_{40}		2,643.5*	124.9	92.4***	123.9
N_{80}		3,289.4***	155.4	98.4***	131.9
N_{120}		3,307.7***	156.2	101.1***	135.5
N_{180}		2,997.7***	141.6	100.3***	134.5
P_{40}		1,885.4^{ns}	89.0	81.6*	109.4
P_{80}		1,801.4^{ns}	85.1	79.6^{ns}	106.7
P_{120}		2,181.2^{ns}	103.0	79.0^{ns}	105.9
P_{180}		1,853.0^{ns}	87.5	80.0^{ns}	107.2
$N_{80}P_{40}$		**3,482.8*** **	**164.5**	99.0***	132.7
$N_{80}P_{80}$		3,426.2***	161.8	96.3***	129.1
$N_{80}P_{120}$		3,375.2***	159.4	96.0***	128.7
$N_{80}P_{180}$		3,217.4***	152.0	96.7***	129.6
$N_{120}P_{80}$		3,457.8***	163.3	99.9***	133.9
$N_{120}P_{120}$		3,407.0***	160.9	98.2***	131.6
$N_{180}P_{80}$		3,322.4***	156.9	99.3***	133.1
$N_{180}P_{120}$		3,107.7***	146.8	**102.6*** **	**137.5**
$N_{180}P_{180}$		3,124.8***	147.6	99.0***	132.7
$N_{120}P_{120}K_{80}$		2,891.2**	136.6	99.2***	133.0
LSD	*5%*	*459.4*	*21.7*	*6.4*	*8.6*
	1%	*608.6*	*28.7*	*8.5*	*11.4*
	0.1%	*786.7*	*37.2*	*10.9*	*14.6*

ns - no significant; *, **, ***significant at P = 5%, P = 1% and P = 0.1%

The analysis of variance averaged over the test period showed a high degree of confidence in the length spike (Table 4). The shortest spike was reported in the control – 5.3 cm. This trait two types of fertilization have the strongest and equal influence – N_{180} and $N_{180}P_{120}$. In both fertilization rates, a 58.5% longer spike than the control was reported. This result gives grounds to report that the additional imported P fertilizer is unjustified.

The number of grains per spike varies from 25.2 to 43.2, on average for the test period (Table 4). The lowest values were reported from the control. Fertilization with $N_{180}P_{120}$ resulted in the highest NGS, with the value being 71.4% above the control. Of all the studied variants with unproven impact, the self-application of P at a rate of 40 kg/ha. The positive effect of mineral fertilization in LS and NGS has been confirmed by many researchers (Esaulko et ai., 2015; Litke et al., 2017; Chibsa et al., 2017).

The results of the dispersion analysis for grain weight per spike, on average for the test period, were similar in terms of the significant effect of mineral fertilization of LS and NGS (table 4). The lightest grains showed the variant without applied mineral fertilizer. The best results and with 98.47% heavier grains than the control was the treatment with $N_{180}P_{120}$. With this trait the influence of P fertilizer was proved statistically for all studied rates. Ibragimov & Mirzaev (2016) confirm that mineral fertilization has a strong effect on GWS.

Table 4. Length spike (cm), number of grain per spike and grain weight per spike (g) average for the period

Fertilization rate		LS, cm	% of control	NGS	% of control	GWS, g	% of control
Control		5.3	100.0	25.2	100.0	1.31	100.00
N_{40}		7.2***	135.9	35.3***	140.1	2.07***	158.02
N_{80}		7.7***	145.3	38.7***	153.6	2.35***	179.39
N_{120}		8.2***	154.7	39.8***	157.9	2.33***	177.86
N_{180}		**8.4*****	**158.5**	42.6***	169.1	2.44***	186.26
P_{40}		5.8^{ns}	109.4	26.5^{ns}	105.2	1.70*	129.77
P_{80}		6.2**	117.0	32.4**	128.6	1.83**	139.70
P_{120}		6.2**	117.0	31.4**	124.6	1.72*	131.30
P_{180}		6.1*	115.1	31.7**	125.8	1.83**	139.70
$N_{80}P_{40}$		7.0***	132.1	35.5***	140.9	2.00***	152.67
$N_{80}P_{80}$		7.4***	139.6	35.3***	140.1	1.95***	148.86
$N_{80}P_{120}$		7.6***	143.4	37.8***	150.0	2.10***	160.31
$N_{80}P_{180}$		7.7***	145.3	38.0***	150.8	2.32***	177.10
$N_{120}P_{80}$		8.0***	150.9	39.2***	155.6	2.35***	179.39
$N_{120}P_{120}$		7.8***	147.2	38.3***	152.0	2.20***	167.94
$N_{180}P_{80}$		8.1***	152.8	41.0***	162.7	2.36***	180.15
$N_{180}P_{120}$		**8.4*****	**158.5**	**43.2*****	**171.4**	**2.60*****	**198.47**
$N_{180}P_{180}$		8.3***	156.6	42.7***	169.5	2.32***	177.10
$N_{120}P_{120}K_{80}$		7.9***	149.1	42.2***	167.5	2.42***	184.73
LSD	*5%*	*0.7*	*13.2*	*4.7*	*18.7*	*0.33*	*25.19*
	1%	*0.9*	*17.0*	*6.2*	*24.6*	*0.44*	*33.59*
	0.1%	*1.1*	*20.8*	*8.1*	*32.1*	*0.57*	*43.51*

ns - no significant; *, **, ***significant at P = 5%, P = 1% and P = 0.1%

Table 5 shows the correlation coefficients of the studied traits. The analysis reveals that on average over a 6-year period, positive and proven correlations were found between all yield components.

Table 5. Correlation coefficients between the studied traits

	GY	*PH*	*LS*	*NGS*	*GWS*
GY	1				
PH	0.908***	1			
LS	0.818***	0.952***	1		
NGS	0.733***	0.905***	**0.973*****	1	
GWS	0.698***	0.907***	0.955***	0.961***	1

*** significant at 0.01% level of probability

The strongest relationship was observed between LS and NGS (0.973***). This result is confirmed by the study of Bilgin et al. (2008). On the other hand, Moosavi et al. (2020) reported an unproven correlation between the two traits in bread wheat. The lowest was the relationship between GY and GWS (0.698***). GY showed the strongest connection with PH (0.908***). The study of Turan (2018) reaches the same strong connection between the two traits.

CONCLUSIONS

Based on the results obtained, it can be concluded that the yield has been affected to the greatest extent by fertilization with $N_{80}P_{40}$. The greatest impact on plant height was reported by the variant $N_{180}P_{120}$. The fertilization with N_{180} and $N_{180}P_{120}$ had the same effect on the length of spike. Combined fertilization $N_{180}P_{120}$ had the greatest impact on the number and weight of grains per spike. Correlation analysis showed a strong and proven relationship between all studied traits, but the highest correlation coefficient was found between the length spike and the number of grains per spike.

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USE OF MEDIUM AND HIGH-RESOLUTION REMOTE SENSING DATA AND MARKOV CHAINS FOR FORECASTING PRODUCTIVITY OF NON-CONVENTIONAL FODDER CROPS

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Abstract

This paper provides the results of assessing the possibility of using remote sensing data obtained from the Sentinel-2 with a spatial resolution of 10 m and simple Markov chains to predict the degree of development of Galega orientalis within the local area. The possibility of using remote sensing data obtained with the Phantom-4ProV 2.0 to assess the productivity of Silphium perfoliatum and Zea mays biomass was also evaluated. The studies were carried out in 2017-2020 on the territory of Gorki district (Republic of Belarus). The research indicated that the use of the Markov model and the raster image of the NDVI index as a predictor makes it possible to accurately predict the areas with very poorly developed, poorly developed, moderately developed, developed and well-developed vegetation ($\chi 2_{empiric} = 0.401$; $\chi 2_{critical} = 9.488$). Vegetation indices ExG, VARI, WI, and EXGR, are suitable for creating a predictive multiple linear regression model that allows predicting the productivity of Silphium perfoliatum in the stalking phase with an error not exceeding 2%, while indices RGBVI, ExG and EXGR it is advisable to use for yield predicting of corn (MAPE=5.19%).

Key words: *forecasting, productivity, biomass, remote sensing, UAV.*

INTRODUCTION

Precision agriculture is a modern management concept that uses digital methods to monitor and optimize agricultural production processes (Maloku, 2020). In this regard, like no other industry, it needs high-precision data, of which more than 80% is geospatial data. The most reliable and demanded source of data for precision farming is remote sensing data, the share of which in the structure of precision farming technology elements has a steady tendency to increase on a global scale (Aulbur et al., 2019). In this regard the promising direction is the use of ultrahigh-resolution remote sensing data obtained from UAVs for monitoring and forecasting the productivity of grain and forage crops (Myslyva, 2020). Plant height data obtained from a digital model of the vegetation surface, which is created from the results of aerial photography, is traditionally used in assessing the biomass productivity of spring wheat (Zhaopeng et al., 2020; Hassan, 2019), winter barley (Bendig et al., 2015; 2014), corn (Furukawa et al., 2020; Zhang et al., 2020). For fodder crops, UAV data are used to assess the productivity of pasture grasses (Michez et al., 2019; Barbosa et al., 2019). However, the methodology for performing this type of work differs in the context of individual crops and their growing conditions. Moreover, it needs improvement and adaptation to the specific economic and agroecological conditions of a particular territory.

The most important indicator of the efficiency of the agricultural sector is the volume of crop production and the yield of agricultural crops. Against the background of global climatic changes, reliable forecasting of the productivity of agricultural crops is a rather difficult process, since a number of factors are involved in the formation of the yield. Improving the quality of predictive models is possible through the combined use of various types of data and forecasting techniques. In particular, to predict the yield, the capabilities of neural networks (Crane-Droesch, 2018; Shivnath Ghosh, 2014) and remote sensing data (Khaki & Wang, 2019; Ennouri & Kallel, 2019) are widely used, which provides quick analysis in the state of crops and plantations of agricultural crops over large areas. An effective and at the same time simple way to simulate random events, which includes forecasting the productivity of biomass, is modelling using Markov chains. At present, it is widely used to predict the productivity of various agricultural crops, however, such studies have not previously been performed in Belarus. In this regard, the development of predictive models that make it possible to obtain reliable estimates of crop productivity based on the joint use of remote sensing data and statistical modelling becomes relevant.

In this context, the paper presents the results of assessing the possibility of using remote sensing data with medium and ultra-high resolution and Markov chains for predicting the productivity of *Galega orientalis*, *Silphium perfoliatum* and *Zea mays* in agroecological conditions of the north-eastern part of the Republic of Belarus.

MATERIALS AND METHODS

The studies were carried out in 2017-2020 on the territory of the Gorki district, Mogilev region of the Republic of Belarus. Information about the location of the research objects is shown in Figure 1.

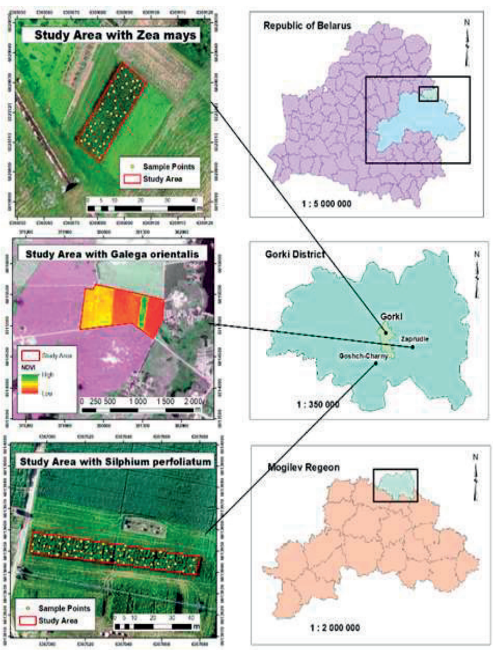


Figure1. Location of the research object

The object of the research was the development and biomass productivity of the following crops:

– *Silphium perfoliatum* L. (common name – cup plant), variety Owari giant (Hungary), development phase – full plant stem phase;

– *Zea mays* L., hybrid Rodriguez (KWS), FAO 170, development phase – R6, V13;

– *Galega orientalis* L. (common name – goat's rue), variety Nesterka (Belarus), development phase – full regrowth after the second cut.

The research was carried out in several stages, the list of which is presented in Figure 2.

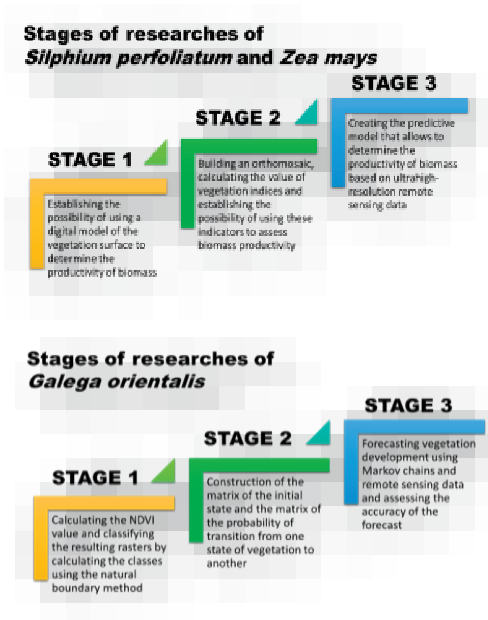


Figure 2. Stages of the researches

The UAV used in this study was quadcopter Phantom-4ProV 2.0. It is equipped with a 20 MPx digital camera with RGB-sensor. Images were captured at 50 m above ground level. Shooting spatial resolution – 2.5 cm; longitudinal and transverse overlap – 80%, the number of images obtained – 236 pcs and 216 respectively. Survey date: June 6, 2020 (*Silphium perfoliatum*) and July 27, 2020 (*Zea mays*).

The flight mission was formed using the Drone Deploy software product. Aerial photography data processing, elevation mapping and orthomosaic creation were performed using Agisoft PhotoScan Professional software.

The vegetation indices were calculated using the QGIS 3.16. The following indices were calculated: RGBVI (Red Green Blue Vegetation

Index), GLI (Green Leaf Index), VARI (Visible Atmospherically Resistant Index), NGRDI (Normalized Green-Red Difference Index) (Bendig et al., 2015); ExG (Excess Green Index), WI (Woebbecke Index) (Woebbecke et al., 1995); EXGR (Excess Green-Red Index) (Meyer & Neto, 2008); VEG (Vegetativen) (Marchant & Onyango, 2000); CIVE (Color Index of Vegetation) (Kataoka et al., 2003); COMB1 (Combined Index 1), COMB2 (Combined Index 2), GR (Ratio Green/Red Index), SAVI (Soil Adjusted Vegetation Index) (Beniaich et al., 2019).

On the day of the survey, 60 biomass samples of silphium and 42 samples of corn were randomly selected to verify the results within the study area in the field. The sampling sites were coordinated using GPS positioning. For each sample taken, its length in cm, weight in kg and density in kg/m^3 were determined.

To predict the development of plants, the methodological approaches outlined in the works of Chinese scientists (Li & Zhu, 2018) were used in relation to forecasting types of land use. A simple Markov chain, taking into account the correlation between adjacent members of the series, was used to predict the degree of development of the *Galega orientalis*. The prediction is based on the calculation of the transition matrix, the elements of which are the probabilities of transition of the predicted parameters from one state to another, from one value to another.

As a source of geospatial data with medium resolution, three scenes obtained from satellites Sentinel 2A (October 2017) and Sentinel 2B (October 2018, 2019) with a spatial resolution of 10 m (datum – WGS-84, map projection UTM 36N, processing level – 1C) were used.

The degree of vegetation cover development was assessed by the value of the vegetation index NDVI (Normalised Difference Vegetation Index) (Jinru & Baofeng, 2017).

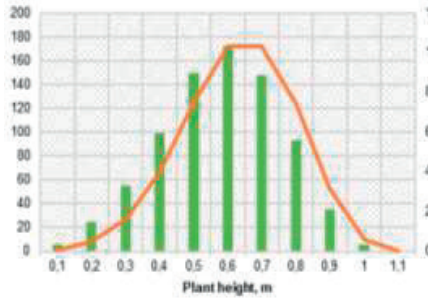
Remote sensing data were processed using the functionality of ArcGIS version 10.5. The classification of rasters with vegetation index NDVI was carried out using the method of principal components.

Statistical processing of the obtained results, construction of regression models and their cross-validation were performed in the Statistica 13.0 (TIBCO Software Inc.).

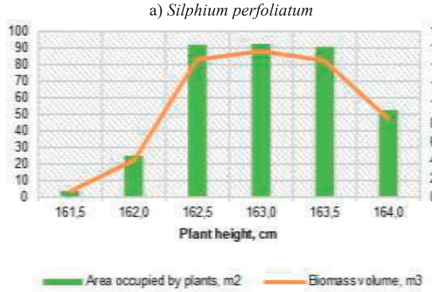
RESULTS AND DISCUSSIONS

At the first stage, crop surface model (CSM) in *tif image format with a resolution of 2.5 cm was obtained after processing the results of aerial photography. The minimum height of the constructed surface of silphium plant heights was 143.64 cm, the maximum – 144.66 cm, average – 144.17 cm, standard deviation – 0.18 cm. For maize the minimum height of the CSM was 161.59 cm, the maximum – 164.25 cm, average – 163.06 cm, standard deviation – 0.67 cm. To obtain plant heights, the difference between the vegetation heights obtained from the surface model raster and the minimum height determined within the raster was found.

The raster image of the surface model of the silphia vegetation cover was reclassified into 11 classes, since the minimum determined plant height was 0.1 m, and the maximum - 1.1 m with a step of 0.1 m. The raster image of the surface model of the maize vegetation cover was reclassified into 6 classes with a step of 0.01 m. Further, the area occupied by plants with different heights within the study area was determined, and the productivity of the biomass was calculated (Figure 3).



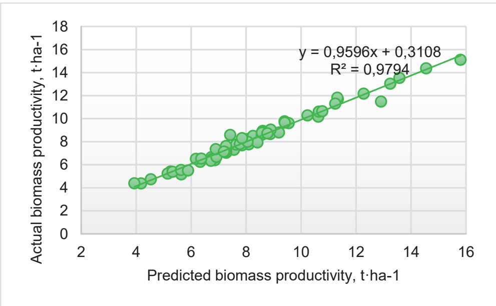
a) *Silphium perfoliatum*



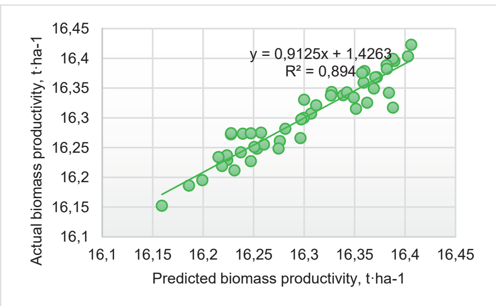
b) *Zea mays*

Figure 3. Productivity of *Silphium perfoliatum* (a) and *Zea mays* (b) biomass, determined from the data of the UAV survey

The minimum yield of raw silphia biomass was 1.49 t·ha^{-1}, the maximum – 16.4 t·ha^{-1}, and the weighted average yield was 8.63 t·ha^{-1}, while for dry biomass the minimum, maximum and weighted average yields reached 0.29 t·ha^{-1}, 3.15 t·ha^{-1} and 1.72 t·ha^{-1}, respectively.
The following results were obtained for maize: the minimum yield of raw biomass was 2.72 t·ha^{-1}, the maximum – 16.63 t·ha^{-1}, and the weighted average yield was 11.66 t·ha^{-1}, while for dry biomass the minimum, maximum and weighted average yields reached 0.82 t·ha^{-1}, 4.98 t·ha^{-1} and 3.49 t·ha^{-1}, respectively.
To determine the reliability of the assessment of biomass productivity, the actual plant height measured in the field was compared with the data obtained using the UAV, and the plant productivity was determined, calculated from the actual and predicted heights. It was found that the results obtained are in fairly good agreement with each other, and their relationship is described by a linear relationship. The correlation coefficient between the actual and predicted values of the productivity of silphia and maize was 0.98 and 0.89, and the average approximation error was 3.3 and 4.9%, respectively, which indicates the reliability of the established dependencies (Figure 4).



a) *Silphium perfoliatum*



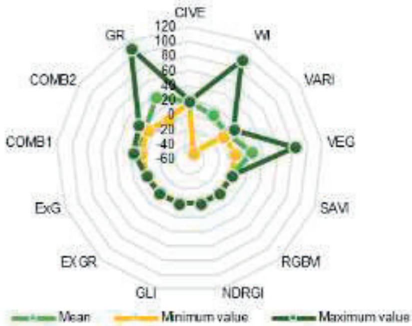
b) *Zea mays*

Figure. 4. The relationship between the actual and predicted biomass productivity

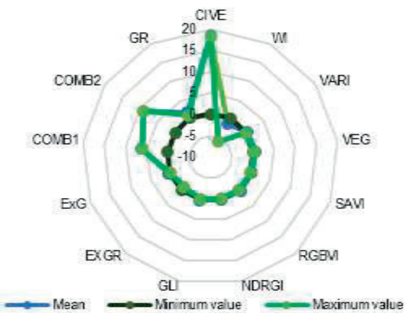
At the second stage of the researches, the possibility of using data on the value of vegetation indices for assessing biomass productivity was assessed. Vegetation indices were calculated using normalized RGB channels. The RGB bands were converted into normalized forms using the following Equation (1) (Yahui et al., 2020):

$$R=r\div(r+g+b);\ G=g\div(r+g+b);\ B=b\div(r+g+b),$$

where r, g, and b are the original digital values from the RGB images.
For silphia, the average values of the vegetation indices RGBVI, NDRGI, and GLI, as well as the vegetation indices SAVI and ExG, were quite similar to each other, while the average values of the GR and VEG indices had the highest values. At the same time, for maize, the maximum mean values were observed for the vegetation indices CIVE, COMB 1 and COMB 2, and the mean values of GLI and VARI, as well as NDRGI and SAVI, were similar to each other (Figure 5).



a) *Silphium perfoliatum*



b) *Zea mays*

Figure. 5. Statistical characteristics of the values of vegetation indices obtained from the orthomosaic created on the basis of UAV imagery in RGB

However, the results of assessing the relationship between the values of vegetation indices and biomass productivity indicate that, despite the similarity of the absolute values, these vegetation indices are characterized by varying degrees of relationship with the productivity of silphia and maize biomass (Table 1–2).

Table 1. The relationship between the values of the vegetation indices obtained from the orthomosaic and the raw biomass productivity of *Silphium perfoliatum*, n = 60

Vegetation index	R^2	RMSE, $t \cdot ha^{-1}$	MAPE, %
ExG	0.95	1.08	20.5
RGBVI	0.94	1.12	27.0
NGRDI	0.94	1.12	20.7
EXGR	0.93	1.22	23.2
CIVE	0.91	1.40	15.9
WI	0.90	1.47	19.3
GR	0.89	1.51	30.2
GLI	0.87	1.68	38.5
VEG	0.83	1.94	39.1
COMB1	0.78	2.23	44.7
COMB2	0.78	2.20	43.7
VARI	0.66	2.74	43.7
SAVI	0.44	3.53	58.7

Table 2. The relationship between the values of the vegetation indices obtained from the orthomosaic and the raw biomass productivity of *Zea mays*, n = 42

Vegetation index	R^2	RMSE, $t \cdot ha^{-1}$	MAPE, %
ExG	0.90	1.58	13.5
RGBVI	0.62	3.58	29.6
NGRDI	0.87	1.82	16.2
EXGR	0.85	1.98	21.9
CIVE	0.81	2.53	25.2
WI	0.95	1.41	12.5
GR	0.89	1.75	14.6
GLI	0.71	3.25	18.4
VEG	0.92	1.51	13.1
COMB1	0.76	2.59	15.6
COMB2	0.76	2.20	15.2
VARI	0.65	3.21	29.4
SAVI	0.56	3.87	33.3

In particular, the strongest direct linear relationship with the productivity of silphia plants was established for the ExG, RGBVI, NGRDI indices, while the productivity of maize biomass was closely related to the WI, VEG, and ExG indices. The VARI and SAVI indices are characterized by the least dependence on the biomass productivity of both silphia and maize. It should be noted that the high information content of the ExG vegetation index is also indicated by (Beniaich et al., 2019; Tumlisan, 2017), and the RGBVI index, by (Bendig et al., 2015). The SAVI vegetation index is most suitable for distinguishing between vegetated and open soil areas, however, like the combined COMB1 and COMB2 indices, it is not informative enough for assessing biomass productivity. Its low indicator capacity is also evidenced by the results of the study presented by Zhaopeng et al., 2020. The results obtained also correlate with the data of Michez et al., 2019, in which, when establishing the possibility of using vegetation indices RGBVI, GLI, NGRDI and VARI to assess the biomass productivity of meadow grasses the root-mean-square error of the estimate was 6.02 $t \cdot ha^{-1}$, 5.04 $t \cdot ha^{-1}$, 1.93 $t \cdot ha^{-1}$ and 1.42 $t \cdot ha^{-1}$, respectively.

The calculation of the average approximation error indicates that, despite the presence of a rather strong direct linear relationship between the values of individual vegetation indices and the productivity of silphia and maize biomass, the use of any one index for a reliable assessment of the productivity level of these crops is not possible, since even for the most of informative indices, the average approximation error reached 16–27% and 12–14%, respectively. In this regard, the third stage of the study was to establish the possibility of using a complex of vegetation indices to determine the productivity of biomass by performing stepwise multiple regression. As a result, a regression model of the following form was obtained (2):

$$y = -317.181 + 0.997*WI - 4.702*VARI - 389.566*EXGR + 417.682*ExG$$

The mean absolute percentage error of this model was 1.82%, the average error (SE) was 0.18 $t \cdot ha^{-1}$, and the root mean square (RMSE) was 0.13 $t \cdot ha^{-1}$, which indicates its high reliability and suitability for monitoring biomass productivity of *Silphium perfoliatum* in the full plant stem phase.

A regression model of the following type was obtained for corn (3):

$$y = 16.558 - 0.135*RGBVI + 0.119*ExG - 0.097*EXGR$$

The mean absolute percentage error of this model was 5.19%, the average error (SE) was 0.81 $t \cdot ha^{-1}$, and the root mean square (RMSE) was 2.30 $t \cdot ha^{-1}$, which indicates its high reliability and suitability for monitoring the productivity of *Zea mays* biomass in the R6 V13 phase.

The prognosis of the development of the vegetation cover represented by

Galega orientalis and the assessment of its effectiveness were also carried out in several successive stages. At the first stage, the calculation of the Normalized Difference Vegetation Index, NDVI was carried out (Myslyva et al., 2020), and raster images of the vegetation cover were obtained at different time periods (Figure 6).

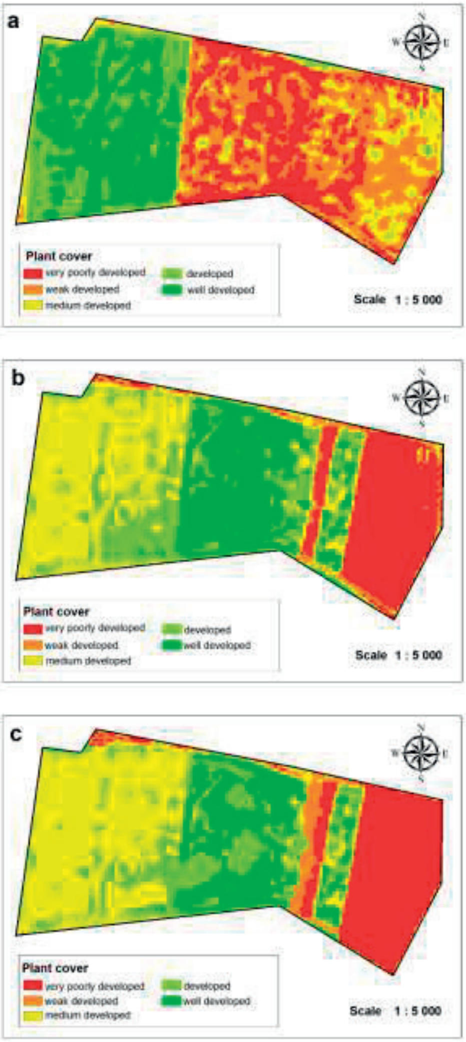


Figure. 6. Raster images of the vegetation index NDVI: a – 2017, b – 2018; c – 2019

The resulting rasters were classified according to the method of principal components. Furthermore, they were differentiated by the NDVI value by calculating the classes by the method of natural boundaries. The intervals of values of the vegetation index corresponding to one or another degree of development of the vegetation cover in the studied area are presented in Table 3.

Table 3. Intervals of vegetation index values corresponding to the degree of vegetation cover development

Plant development level	Range of NDVI values
Very poorly developed	0.15–0.30
Weak developed	0.31–0.36
Medium developed	0.37–0.50
Developed	0.51–0.60
Well developed	0.61–0.77

The transformation of the obtained raster images into vector layers made it possible to determine the areas within the raster, corresponding to one or another level of plant development (Table 4).

Table 4. Distribution of areas with different degrees of vegetation development (based on the results of determining the value of the vegetation index NDVI), %

Year	Plant development level				
	very poorly developed	weak developed	medium developed	developed	well developed
2017	20.84	27.07	12.56	16.62	22.91
2018	19.39	5.81	31.23	18.73	24.82
2019	25.48	23.46	41.08	5.083	4.88

The rasters of 2017 and 2018 were used for the prediction, and the raster of 2019 served as a basis to assess the accuracy of the prediction.
The initial state matrix S(0) was constructed based on the data presented in the Tables 3 and 4 (4):

$$S(0) = \begin{bmatrix} 17.67 \\ 22.95 \\ 10.65 \\ 14.09 \\ 19.42 \end{bmatrix} = \begin{bmatrix} NDVI - & 0.15 - & 0.30 \\ NDVI - & 0.31 - & 0.36 \\ NDVI - & 0.37 - & 0.50 \\ NDVI - & 0.51 - & 0.60 \\ NDVI - & 0.61 - & 0.77 \end{bmatrix}$$

The next stage of the research was the construction of a matrix of the probability of vegetation transition from one state to another. To achieve this goal, the rasters of 2017 and 2018 were overlaid and the areas of their mutual intersection were determined. Further, the obtained values were transformed into a matrix of transition of areas with different degrees of plant development. The matrix of the transition of areas in hectares was recalculated into the matrix of the probability of transitions of areas with different degree of development of vegetation cover in one class or another (Table 5).

Table 5. Matrix of probability of transitions of areas with different degrees of vegetation development (n = 0)

2017	2018				
	Plant development level				
	very poorly developed	weak developed	medium developed	developed	well developed
Very poorly developed	0.143875	0.528585	0.322489	0.005051	0
Weak developed	0.184886	0.370909	0.323199	0.112836	0.008169
Medium developed	0.0544338	0.034638	0.031049	0.412253	0.467626
Developed	0.226778	0.148231	0.049930	0.145621	0.429439
Well developed	0.443318	0.449301	0.087082	0.003237	0.017062

At the final stage, the forecast for the development of galega in 2019 was carried out. For its implementation, the matrix of the initial state and the matrix of the probability of transition from one state to another were used. The actual and predicted values of the areas with varying degrees of vegetation development were used to assess the accuracy of the forecast, while the χ^2 criterion was used to test the forecast model (Table 6).

Table 6. Results of estimation of accuracy of the forecast model of vegetation cover development, hectares

Plant development level	Predicted value (Y')	Actual value (Y)	Absolute error (Y' – Y)	$(Y' - Y)^2$
Very poorly developed	21.61	19.26	–2.35	5.54
Weak developed	19.89	20.35	0.46	0.21
Medium developed	34.83	32.22	–2.61	6.83
Developed	4.31	9.07	4.76	22.62
Well developed	4.14	6.78	2.64	6.98
$\chi^2_{empirical} = 0.401$; $\chi^2_{critical} = 9.488$				

The maximum value of the absolute error is typical for forecasting areas with medium developed, developed and well-developed vegetation. This phenomenon can be explained by the fact that in the process of recognizing rasters with the vegetation index NDVI by the method of principal components it is rather difficult to distinguish these classes, since their spectral brightness is in a rather close range of values. However, it is possible to improve the quality of recognition by performing preliminary raster segmentation and then applying machine learning to classify it.

CONCLUSIONS

The results obtained give reason to recommend the use of ultra-high-resolution remote sensing data obtained from UAVs to assess the biomass productivity of *Silphium perfoliatum* and *Zea mays*.

Plant height data obtained from an aerial-based crop surface model is suitable for use in estimating the productivity of silphia and maize biomass: the R^2 for the relationship between actual productivity and remotely sensed productivity is 0.98 and 0.89, respectively.

Predictive models created by the method of stepwise multiple linear regression, including a complex of several vegetation indices, make it possible to determine the silphia and maize biomass productivity according to ultra-high-resolution remote sensing data with an error of no more than 2-5%.

Medium resolution remote sensing data obtained with the Sentinel 2 and the functionality of GIS technologies allow creating adequate models using Markov chains for predicting the development of *Galega orientalis* in local areas.

The process of predicting the productivity of galega plants using Markov chains should include such stages as: obtaining a raster image; raster classification and converting it to vector layer; construction of an initial state matrix and a transition probability matrix.

Further research should focus on assessing the validity of the resulting models in the field. The results of this study can be useful both in the development of forecasting methodology and in direct forecasting of the biomass productivity of *Silphium perfoliatum*, *Zea mays* and other fodder crops, in particular *Helianthus annuus* and *Helianthus tuberosus*, as well as for assessing the productivity of pastures and creating effective pasture crop rotations.

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NEW METHOD FOR IDENTIFICATION DROUGHT-TOLERANCE WINTER WHEAT BREEDING MATERIAL

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Abstract

Drought tolerance is one of the key agricultural value traits for arid regions. Our investigation consists of two stages. During fist we worked with well-known by this drought tolerance winter wheat official realized 16 varieties, at the second one we evaluated new 88 mutant lines at M4 – M6 generations, which are potentially higher yielding as compared with the national Ukrainian standard for arid zone (Northern Steppe). It is well known that photosystem-II (PS- II) is less thermal-resistant than photosystem-I (PS-I). Since PS-II is more energy consuming and directly requires water for it active operation, this leads to a conclusion that during drought its activity predominance in the photosynthesizing system is undesirable. We evaluated potential drought tolerance by comparison photosystems activity. The simazine (Sim) 10-4(M) was used as PS-II inhibitor, represses the oxygen release processes in the photosystem. More drought-resistant winter wheat lines showed the predominance of PS-I over PS-II. It was established that the best lines in terms of drought resistance were the wheat lines 133, 157, 157-1, 185, 213, that is the lines with the lowest ratio between PS-II and PS-I were identified as being more drought-resistant.

Key words*: drought tolerance, winter wheat.*

INTRODUCTION

One of the main tasks of creating new genotypes of bread winter wheat, associated with the climate changes, escalation of global environmental problems, is the solution of the problem of adaptation and resistance, the study of environmental stress effect on plants.
With the annual production of about 783 million tons (in 2019) (USDA, 2020), bread wheat (*Triticum aestivum* L.) is one of the world’s most important cereal crops. Winter wheat is the world’s leading cereal grain and the most important food crop, occupying commanding position in Ukraine. Ukrainian agriculture takes about 48% area under cereals and contributing 38% of the total food grain production in the country (Nazarenko, 2016b).
Consequently, wheat supplies approximately one-fifth of human calories in a variety of forms. Wheat will remain a crucial component of human nutrition, and increasing its production is therefore an important requirement for food security. Wheat consumption has been steadily increasing due to population expansion and urbanization (Halford et al., 2014, Li et al., 2019).
Winter wheat is an important crop, suited to the typical weather conditions in the current climate. In a changing climate the increased frequency and severity of adverse weather events, which are often localized, are considered a major threat to wheat production (Harkness et al., 2020).
Winter wheat is not only a world’s leading cereal crop but also the most important food crop in Ukraine, which occupied leading position in the national agriculture (Nazarenko and Lykholat, 2018). As for the quality traits winter wheat is the main stable crop for our country and provides more than 20 % of calories and proteins. Focused on only yield traits we have to understand that any high yield has no sense without proper quality for food and fodder demands (Shewry et al., 2012). Wheat improvement, which bases on the principal of ecological stability of new forms and taking into account special interactions between environment and genotypes, special abilities for agroecological variability under wide ranges of conditions providing us new approaches for formation stable agroecological cereals systems without great losses at productivity (Bordes et al., 2011).

But in spite of increasing total grain productivity tolerance to the special ecological demands of new varieties have been decreased, what, consequently, influencing on the future adaptability and special interactions with environment of winter wheat (Pemental et al, 2014; Nazarenko, 2017; Nazarenko et al, 2020).

Disequilibrium in influence of different nature-agricultural factors and their interactions of region determine distinguishes summarized in different genotypes grain productivity and quality (Essam et al, 2019). Due to this fact we investigated varieties main agricultural-value traits under regional conditions. They determined balance of moisture, character of winter wheat growth and development, differences in seasons conditions, interaction between types of variety development (terms and specify of development stages) (Chope et al, 2014; Amram et al, 2015).

Key attention to pay for main agronomic-value traits such as grain productivity (and formation of this trait) and grain quality (in sense of grain protein content). These traits in interaction actually determine the overall genotypes of wheat by suitability for cultivation (Gepts & Hancock, 2006). Winter wheat yield has the most important and complex character affected directly or indirectly by genome systems present in plant (Rangare et al., 2010) as well as interaction with environment (Tester & Langridge, 2010, Serpolay et al., 2011). Thus, ecological exam (part of evaluation process in breeding program for measurements of adaptation for new lines and varieties under difference regional conditions) of new wheat lines with high yield and quality genetic potential under difference condition, it`s components (Slafer & Andrade, 1993) and interaction (Dai et al., 2015) has become a permanent task in the plant farming and breeding programs (Reif et al., 2005, Tuberosa & Salvi, 2006).

The study of drought-resistance represents one of the fundamental tasks in phytophysiology. Draughts that differ in character and duration occur annually in all regions of Ukraine. The stressing influence of the drought induces substantial reduction in yielding capacity and quality of winter grains. Potential effect of stress on the plants is constantly growing, which is caused by a severe shortage for water, rise in air temperature and pollution of environment with toxic chemicals (Desfeld et al, 2014; Daryanto et al, 2017; Jaradat, 2018).

Adaptation is only possible when an organism is capable of resistance on any level (from cell level to population level) and is able to adapt to the new environment conditions (Forsman, 2015; Pilbean, 2015; Prabhu, 2019).

The key part in field crops yield generation is played by photosynthesis. This is the only natural process resulting in growth of free energy of biological environment on account of external source – the sun, and ensures the existence both of plants and of all heterotrophs, including a human. The photosynthesis process is the biological basis of yielding capacity of agricultural crop, during which up to 95 % of dry biomass of plants is generated (Xu, 2016; Quintero et al, 2018).

It is generally thought that it is the photosynthesis that suffers most from drought: ATP synthesis decreases, while the synthesis of promoting agent grows, which causes excessive reducibility of electron-distribution chain and the decomposition of pigment-protein complex of chloroplasts (Mba et al, 2012; Richardson et al, 2017; Khalili et al, 2018).

It is well known that in the process of cells generation, the chloroplasts may be of two types according to the ratio of content of PS-I and PS-II –1:3 and 1:2 respectively, PS-II is less thermal-resistant than PS-I, which may result in changes to the electron-distribution chain (Resende, 2016; Tokalidis, 2017). The excessive content of PS-II increases the photochemical activity of chloroplasts, and under conditions of drought it causes their destruction of components of PS-II followed by the chlorophyll burn-out. Functional impairment in plants further leads to decrease of yielding capacity (Liu et al, 2016; Klcova et al, 2019; Le Gouis et al, 2020).

Non-uniform resistance of particular elements of photosynthesis system allows an assumption that the photosynthesis reaction may in general be dependent on the inhibition of the most sensitive link of the electron-distribution chain. The currently known specific inhibitors of the PS-II reaction are the monodiurons, hydroxylamine and other enzyme poisons

(Milev et al, 2014; Mickelbart et al, 2015; Nutall et al, 2017).

The enhancement of methods of winter wheat breeding material assessment in terms of drought resistance, discovering the ability of the plants to provide the acceptors with the assimilators within the system of donor and acceptor relations and the ability for cells self-maintenance in the environment of increasing water deficiency or high temperature, provide an opportunity to reasonably describe the level of drought-resistance of new lines and varieties and to forecast their behavior in corresponding environmental conditions (Tribo et al, 2003; Tsenov et al, 2015; Tencgong et al, 2020).

We suggest one of the methods of breeding material assessment in terms of drought-resistance by photosystem (PS) activity.

MATERIALS AND METHODS

Winter wheat varieties and M_4–M_5 generations of mutation lines have been sown at the end of September, at a depth of 4-5 cm. The controls were national standard by productivity 'Podolyanka' and initial variety. The working-methods in the breeding trials are satisfied to state variety exam requests. The trial was set up as a randomized block design method with three replications and with a plot size of from 5 to 10 m^2 in 2–3 replications.

Experiments were conducted on the experiment field of Dnipro State Agrarian and Economic University (village Oleksandrovka, Dnipropetrovsk district, Dnipro region, Ukraine). Normal cultural practices including fertilization were done whenever it is necessary. Weeds were manually removed where necessary, and fungicides and insecticides were applied to prevent diseases and insect damage. Evolution was conducted during 2011 – 2018 years (Nazarenko, 2016; Nazarenko and Lykholat, 2018).

Winter wheat plants of different linesand varieties were studied. Photosynthesis of plants was determined in a gasometric device developed in the laboratory of plant physiology and biochemistry MIP NAASU on the basis of the manometric method of Warburg. Simazine (Sim) 10-4 (M) was used as an inhibitor of PS-II, which sharply inhibits the processes of oxygen release in the photosystem. The intended site of action of simazine is the ETL link between the primary PS-II acceptor (Q) and the inclusion of plastoquinone. Drought resistance of the lines was determined by the advantages in the activity of individual photosystems.

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by Student's t-test, the grouping mutants cases was performed by factor analyses. In all cases standard tools of the program Statistic 8.0 were used.

RESULTS AND DISCUSSIONS

The assessment of energy state of the photosynthesis system is essential for the development of methods of diagnostics of plants' drought-resistance. The goal of our study was to assess the photosynthesis system by means of photosystems analysis using the inhibitor method for determination of winter wheat drought-resistance.

Plants of different lines were studied. For reconnaissance research, we deliberately selected varieties with low drought resistance in our region.

The addition of the inhibitor caused a peak decrease in the intensity of photosynthesis. All plants had a predominant activity of PS-II (Table 1) under the drought conditions of 2018 year.

In October-November 2017, when the photosynthetic apparatus was formed, the air temperature fluctuated sharply. It is known from the literature that in the conditions of significant daily fluctuations of air temperature in the leaves a photosynthetic apparatus with a predominant content of PS-I is formed.

Table 1. Photosynthesis winter wheat plant after treatment of PS-II inhibitor

N	Date	O_2, mkl/hour							
		Mironivska 808		Mirleben		Mironivska 33		Mironivska 65	
		Check	Treated	Check	Treated	Check	Treated	Check	Treated
1	28.10	1494	3360	2935	3002	3151	1867	3024	3472
2	25.11	4343	3394	4141	2919	4005	4005	5023	3940
3	07.05	4611	3425	-	-	3564	2828	2511	3733

Determining the intensity of photosynthesis of winter wheat plants of different lines of ecological testing showed that the crops of the 2018 harvest had almost the same number of varieties with a predominant activity of both PS-II and PS-I. The data show that in the electron transport chain, the ability of plants to withstand droughts is directly proportional to the ratio of photosystems.

According to the activity of photosynthesis in winter wheat plants, both PS-I and PS-II worked in all ecological varieties, because their ratio was about one, except for Kolos Mironivschiny, where it was equal to 1:3 (Table 2). In such ecologically plastic varieties as Podolyanka, Mironivska 808, Leganda, Pamyati Remesla and Mironivska 65 during the autumn-spring growing season the activity of PS-II slightly prevails.

Table 2. Quality of photosynthesis of winter wheat plants under ecological exam

Сорт	Intensity of photosynthesis, mkl/hour		PS-I:PSII	PS
	Check (H_2O)	Simasin		
Podolyanka	6003	4793	1,25	II
Mironivska 808	5873	4973	1,18	II
Mironivska 65	5645	6362	0,89	I
Vesta	7034	6720	1,05	II
Snizhana	5197	6720	0,77	I
Krizhinka	8064	7392	1,09	I-II
Mironivska rannostigla	1930	2726	0,71	I
Remeslivna	2334	2550	0,91	I
Demetra	8960	8064	1,11	II
Kalinova	3733	2715	1,37	II
Kolos Mironivschiny	985	2867	0,34	I
Pamyati Remesla	6630	5960	1,16	II
Legenda	8467	7302	1,16	II

Various lines of winter wheat were studied. The simazine (Sim) 10^{-4}(M) was used as PS-II inhibitor, dramatically represses the oxygen release processes in the photosystem. An envisaged center of simazine action is the link of the electron-distribution chain between the PS-II (Q) primary acceptor and the plastoquinone inclusion. For the reconnoitering study we deliberately selected the breeds of low-level drought resistance, which had been created in our region environment.

The inhibitor added caused a dramatic photosynthesis inhibition. All the plants were basically active in terms of PS-II under conditions of the droughty year 2012.

In October to November 2014, when the photosynthesizing system was being formed, the air temperature fluctuated greatly. Literature references state that under conditions of significant fluctuations of air temperature during 24 hours, a photosynthesizing system with the PS-I predominant content is formed in the leaves.

The study of photosynthesis intensity in various breeds of winter wheat showed that the winter wheat seeds of 2014 – 2016 yield had were almost equal in terms of amount of breeds with predominating activity both of PS-II and PS-I. The data proves that in the electron-distribution chain, the photosystems relation directly influences the plants' ability to resist the droughts.

As to the photosynthesis intensity in winter wheat of a previous study, in all strains both PS-I and PS-II were active, since their relation was around figure of one, except for the strains 133, 157, 185, 213 (Table 3), where this figure was much less than one. Such strains were referred to environmentally adaptive, where during the spring and autumn vegetation period the PS-II activity prevails to a small extent.

Since PS-II is more energy consuming and directly requires water for it active operation, this leads to a conclusion that during drought its activity predominance in the photosynthesizing system is undesirable.

As stated above, 133, 157, 157-1, 185, 213 showed quite good results in terms of yielding capacity, and the ratio between PS-II and PS-I indicated the predominance of the PS-I, so those strains declared themselves as drought-resistant. This is the fifth paragraph from Results and Discussions that should be replaced with your content. It only contains example text and proper formatting.

Table 3. Quality of photosynthesis of winter wheat plants under lines exam

Line	Intensity of photosynthesis, mkl/hour		PS-I:PSII	PS
	Check	Treated		
Podolyanka	5513	4276	1,29	II
130	985	2867	0,34	I
133	4553	4002	1,14	II
142-1	3017	2906	1,04	II
156	1217	1045	1,16	II
157	2715	4114	0,66	I
157-1	2334	2550	0,92	I
172	3508	3400	1,03	II
174	3300	2940	1,12	II
179	2452	1856	1,32	II
185	1234	1896	0,65	I
186	1214	1059	1,15	II
211	627	627	1,00	I
213	947	1888	0,50	I

Due to the increase of bioclimatic potential, it seems economically viable to replace the current breeds by the breeds with the photosynthesizing system that stay active for a longer period, with no destructive irregularities in various climatic conditions.

Thus, more drought-resistant strains show the predominance of PS-I over PS-II.

This is suggested as an express method of assessment of drought-resistance ability of breeds and strains of winter wheat by the ratio of photosystems activity, which enables fast and reliable determination of starting material for drought-resistance breeding.

It was established that the best strains in terms of drought resistance were the wheat strains 133, 157, 157-1, 185, 213, that is the strains with the lowest ratio between PS-II and PS-I of soft winter wheat were described as being more drought-resistant.

CONCLUSIONS

In the context of climate change associated with global warming, and possible risks of loss of winter wheat yield, the proposed method makes it possible to reliably identify drought-resistant forms already at the early stages of the breeding process on limited winter wheat plants material. It was discovered the best drought-resistant wheat lines were the 133, 157, 157-1, 185, 213 strains, that is the strains with the lowest ratio between PS-II and PS-I of soft winter wheat were described as being more drought-resistant. The method is simple enough for field diagnostics, while being based on fundamental physiological processes and fully substantiated from a theoretical basis. The use of basic patterns makes it possible to directly establish a relationship between the processes of photosynthesis and the formation of such a complex trait as drought resistance.

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EVALUATION OF THE EFFICIENCY OF FERTILIZERS BY USING THE LABELLED NITROGEN

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Abstract

It is known that extraradicular fertilizers or those containing substances that have a growth-stimulating effect indicated that only the use of biostimulants in the treatment of crops does not often lead to significant effects on production and quality. In these cases, the "explosive" vegetative development of the plant must be supported by an additional nutrients contribution necessary for nutrition. This phenomenon is obvious in the case of poor basic fertilization, on degraded soils, as well as in the case of unbalanced fertilization in macronutrients. The carried out research aimed to establish, using as a 15N isotope tracer, the contribution of foliar fertilizers containing natural organic substances, to increase the efficiency of the use of different forms of nitrogen from the soil applied fertilizer.
The efficient use of nitrogen has a positive effect on the quality of the sunflower crop. The incorporation into the NPK matrix with protein hydrolysate, substances that also have a role of biostimulator has led to increases in the production of green mass, up to 6% compared to the control fertilizer.

Key words*: fertilizer, sunflower, protein hydrolysate, isotope, labelled nitrogen.*

INTRODUCTION

Agroecosystems rely on inputs including organic and chemical fertilizers with nitrogen to support high productivity. Many fertilizers are composed of organic substances that contain, in addition to other elements, different amounts of organic nitrogen, such as, protein hydrolysate (Colla et al., 2014; Sestili et al., 2018), humic substances (Olivares et al., 2017; Tudor et al., 2017), plant or algae extracts (Craigie et al., 2011; Möller et al., 2015; Amirkhani et al., 2016). However, nitrogen is an extremely mobile element, which could make efficient management a difficult task regarding situations where significant losses can occur due to leaching such as nitrate, ammonia leakage and nitrogen gas emissions (Zhang et al., 2012; Inselsbacher et al., 2013).
Generally, the products involving biostimulant role are organic substances, which, applied in low concentrations, take part in the physiological processes of plant growth and development, with favorable effects, both quantitative and qualitative on the crops, while reducing the losses due to the transport and storage of the products, reducing the pollutant impact of chemical fertilization on the environment at the same time (Yang et al., 2013; Colla et al., 2014; Tudor et al., 2017).
There is a tendency for nitrogen overfertilization to increase production in sunflower crops but it has been observed that the yield can be about 50% (Scheiner et al., 2002).
On this line, remarkable results have been achieved during the investigation of the nutrients sources, their speed circulation in plants and interaction between them, as factors of particular importance in examining the efficiency of the imputations of nutrients in the culture system (Bengtsson & Bergwall, 2000; Takebayashi et al., 2010).
The use of nuclear techniques allows acquaintance providing great accuracy the quantities of nutrients differentially absorbed by plants from soil and from fertilizers following their evolution in plants, as well as the transformations that take place in the soil-plant-fertilizer complex system (Reddy et al., 1993; Scheiner et al., 2002; Stevens et al., 2005;

Watzka & Buchgraber, 2006; Rasmussen et al., 2008). Also, in the researches carried out using the 15N isotope on sunflower plant, the mechanisms of penetration and translocation in the different organs of the plants, of the three forms of existing nitrogen in fertilizers, amidic, nitric, ammonia have been followed.

MATERIALS AND METHODS

A scientific approach regarding rational use of fertilizers, as well as the analysis of nutritional processes can be addressed for application on soil and plant, and it can be done using stable and/or radioactive isotopes (Mihalache et al., 2019).

The paper presents the results obtained from the experiments that took place in the vegetation house on the sunflower plant. The aim was to evaluate the effect of foliar application of some organic fertilizers, containing protein hydrolysate of plant origin and various forms of mineral nitrogen marked 15N.

The two fertilizers were prepared in the laboratory:

- FERT fertilizer including an NPK matrix containing secondary elements and microelements and
- HIDROFERT including NPK type matrix containing secondary elements, microelements and hydrolyzed soy protein.

The vegetable protein hydrolysate used for introduction into the NPK-type fertilizer matrix was acquired from soybean grist with a nitrogen content ranging between 7 and 8%, following the mixed hydrolysis with a first chemical and subsequent enzymatic step with Alcalase 2.4 L, for 3 hours under 65^0C, using a pH of 8, followed by stabilization for 30 minutes under 85^0C. The degree of hydrolysis of sunflower meal ranged between 56 and 60% relative to the total nitrogen composition.

The fertilizers experimentally achieved had the following chemical characteristics, presented in the Table 1.

Table 1. Chemical characteristics of FERT and HIDROFERT fertilizers

Chemical characteristics	FERT	HIDROFERT
	Content (%)	
Nitrogen, N total, including:	18.52	21.16
ammoniacal	3.62	3.98
nitric	5.41	6.05
amidic	9.49	9.45
organic	0	1.68
Phosphorus, P_2O_5	18.34	19.16
Potassium, K_2O	18.18	18.97
Boron, B	0.01	0.01
Copper, Cu	0.005	0.006
Iron, Fe	0.047	0.052
Magnesium, MgO	0.23	0.25
Manganese, Mn	0.023	0.025
Molybdenum, Mo	0.001	0.001
Sulfur, SO_3	0.45	0.51
Zinc, Zn	0.01	0.01
Organic substances, including:	0.57	10.42
protein hydrolysate	0	9.7
free amino acids	0	0.08
pH	6.58	6.72

The experiments were organized in the vegetation house, having as a test the sunflower (*Helianthus annuus* L.) NEOMA variety. The choice of sunflower as a test plant was due to the advantage offered by the foliage, which has a great holding power of the fertilizer solution when it is applied to the leaves.

The agrochemical experiments on the sunflower culture, the NEOMA variety, were carried out on a chernozem soil with the following physico-chemical characteristics (Table 2).

Table 2. Physico-chemical characteristics of the used soil

Humus	Nitrogen	Mobile phosphorous (P_{AL})	Mobile potassium (K_{AL})	C_{org}	Mobile forms of cations in solution of ammonium acetate + EDTA at pH = 7				pH
					Zn	Cu	Fe	Mn	
(%)	(%)	(mg/kg)	(mg/kg)	(%)	(mg/kg)				pH unit
3.48	0.17	146	224	2.01	13.1	2.74	86	8.6	6.78

The experiences that have taken place have involved the following activities:

➢ agrochemistry characterization of the used soil;

➢ organizing and setting up the experience in Mitscherlich vegetation vessels type containing 10 kg cambic loam soil;

➢ basic fertilization by incorporation into the soil, before sowing ($N_{180}P_{45}K_{45}$), this means 180 kg of nitrogen, 45 kg of phosphorus and 45 kg of potassium per hectare;

➢ watering responsibilities, at about 50% of the water capacity of the soil;

➢ the sowing itself, making sure that the seed material is more uniform, calibrated (appearance, weight);

➢ fertilization using fertilizers containing ^{15}N isotopically labeled nitrogen in the amidic, ammoniacal and nitric groups after sprouting with a dose of 30 mg ^{15}N/pot and 10 mg ^{15}N/plant;

➢ the maintenance of the plants, following daily watering conditions using 70% water of the field capacity;

➢ diluted fertilizer solutions preparation and application on plants, 10 ml solution / 1% plant concentration must be established;

➢ number of foliar treatments 3 following intervals of 7 days.

For each of the combinations of experimental factors, 3 experiments of 3 plants per vessel were provided.

The experimental scheme of agrochemical testing is presented in Table 3.

Table 3. Experimental scheme of agochemically testing

No. var.	Codes	Basic fertilization	Foliar fertilization	^{15}N nitrogen species applied
V1	WBF	Without basic fertilization	Without foliar application	-
V2	WBF+FERT	Without basic fertilization	Foliar application Fert	-
V3	WBF+HFERT	Without basic fertilization	Foliar application Hidrofert	-
V4	BF + 15N-NH2	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NH_2
V5	BF + 15N-NH4	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NH_4
V6	BF + 15N-NO3	Basic fertilization ($N_{180}P_{45}K_{45}$)	Without foliar application	^{15}N-NO_3
V7	BF + 15N-NH2 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Fert	^{15}N-NH_2
V8	BF + 15N-NH4 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Fert	^{15}N-NH_4
V9	BF + 15N-NO3 + FERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Fert	^{15}N-NO_3
V10	BF + 15N-NH2 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Hidrofert	^{15}N-NH_2
V11	BF + 15N-NH4 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Hidrofert	^{15}N-NH_4
V12	BF + 15N-NO3 + HFERT	Basic fertilization ($N_{180}P_{45}K_{45}$)	Foliar application with Hidrofert	^{15}N-NO_3

The following ^{15}N labeled fertilizers applied by incorporation into soil using a dose of 30 mg / vessel were used in the experiments:

➢ 20% amide (-NH_2) labeled ^{15}N nitrogen urea;

➢ 20% nitric (-NO_3) labeled ^{15}N nitrogen ammonium nitrate;

➢ 20% ammoniacal (-NH_4) labeled ^{15}N nitrogen ammonium nitrate.

After 45 days of sprouting, these plants were harvested as green mass and dried in order to perform isotopic examination and analyze the chemical elements content. (N, P, K, Ca, Mg, Zn, Cu, Fe and Mn).

RESULTS AND DISCUSSIONS

The isotopic determinations of the dried plant material samples were performed using a Thermo Delta V mass spectrometer (IRMS) with an interface for elemental analysis NC 2500 and they were pursued:

➢ examining the isotopic ratio (R) or the percentage of atoms, AT% $^{15}N/^{14}$, in the samples of plant material depending on the ^{15}N species applied;

➢ examining the $\delta^{15}N$ (delta) parameter, which represents the accumulation of the ^{15}N isotope in the analyzed sample. This represents the corrected value of the ^{15}N isotope measured against a primary reference scale. The main reference scale for $\delta^{15}N$ used was atmospheric air. The value of $\delta^{15}N$ is given by the formula $\delta^{15}N=1000$ (Rsample-Rstandard)/Rstandard and expressed in units per million (‰);

➢ ^{15}N isotope export in sunflower plant according to the ^{15}N species applied;

➢ total nitrogen (%).

The experimentally acquired results and their interpretation are presented in the Figures 1, 2 and 3.

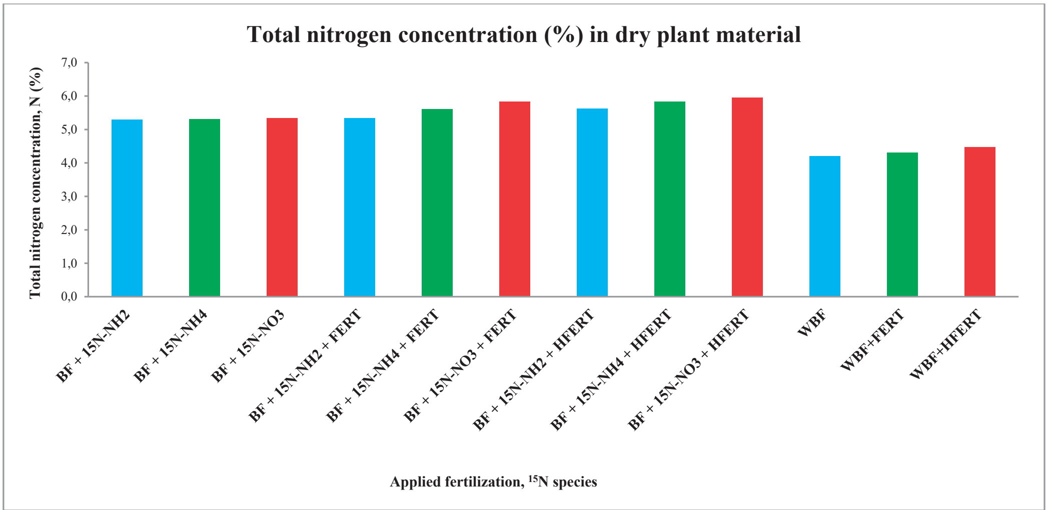

Figure 1. Evolution of nitrogen concentration in plant material depending on the basic and foliar fertilization applied

The nitrogen concentration examined in the vegetal material ranged between 4.21% for the variant without basic or foliar fertilization and 5.93% for the variant using basic fertilization and Hidrofert foliar fertilizer.

Figure 1 shows that the foliar application of the 2 fertilizers on the soil fertilized with NPK fertilizer led to an increase in the concentration of nitrogen in dry plant material for each form of nitrogen.

The highest concentrations of nitrogen accumulated in the plant biomass were recorded for Hidrofert foliar application.

A more accurate picture of the export phenomenon of nitrogen nutrient depending on the applied fertilization can be seen in Figures 2 and 3. In the case of applying foliar fertilizers on a soil without basic fertilization, nitrogen increases (relative values) ranged between 2 and 6%, and in the case of basic fertilization this increase was 32-37%. Basic fertilization led to a 20% increase in nitrogen in the unfertilized control plant, and the increase brought by foliar fertilization on a soil with basic fertilization was 5% for Fert and 9% for Hidrofert with hydrolysed protein fertilizer matrix.

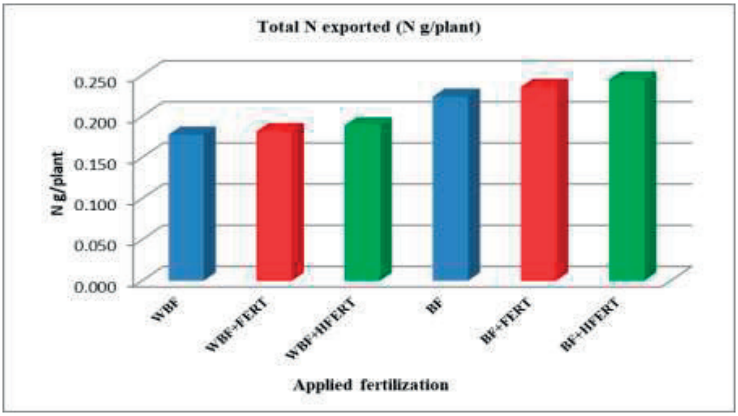

Figure 2. Total nitrogen export depending on the applied fertilization

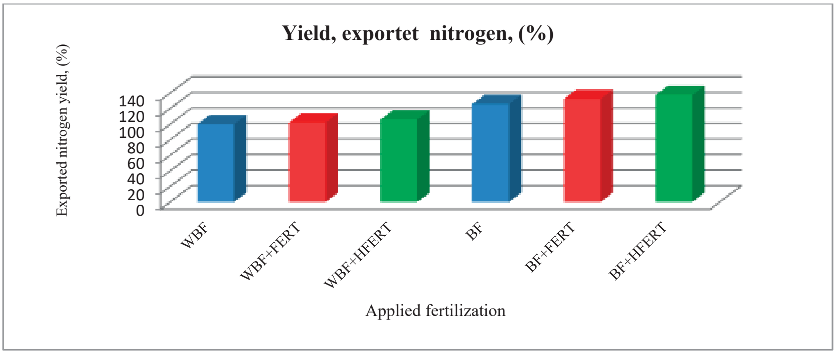

Figure 3. Exported nitrogen yield depending on the applied fertilization

The isotopic determinations of plant material regarding the content and the way of assimilation from the soil into the plant of the nitric, ammoniacal and amidic nitrogen species, have been followed:

- determination of the isotopic ratio (R) AT% $^{15}N/^{14}N$ in the plant material samples according to the ^{15}N species applied;
- determining the $\delta^{15}N$ (Δ) parameter, representing the accumulation of the ^{15}N isotope in the analyzed sample (‰);
- the export of ^{15}N isotope in the sunflower plant according to the ^{15}N species applied radically;

The experimentally acquired results and their interpretation are presented in the following figures (Figures 4 ÷ 7).

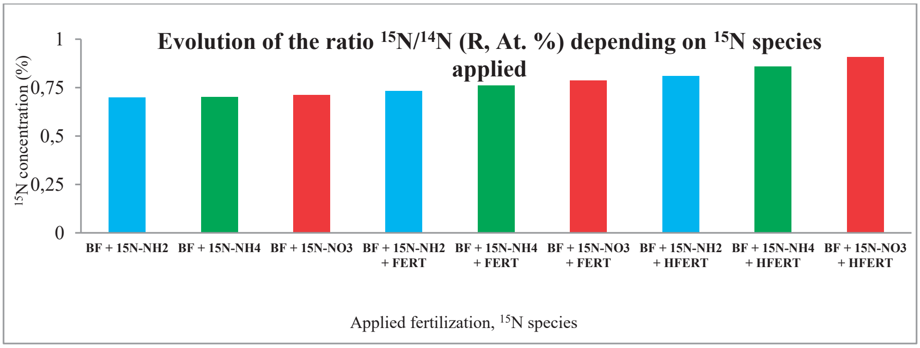

Figure 4. Evolution of the ratio (R, AT $^{15}N/^{14}N$ %) depending on the basic and foliar fertilization applied

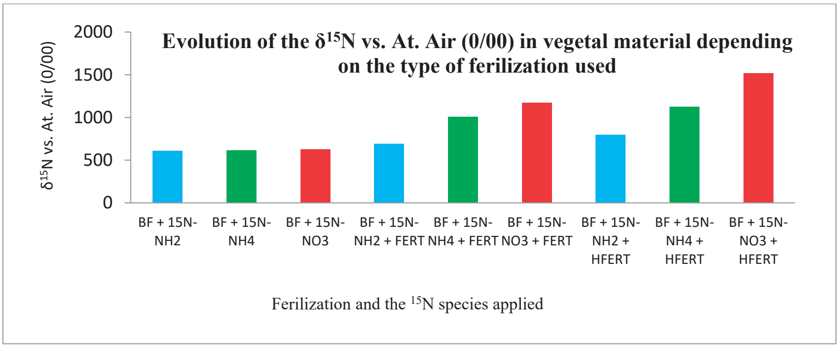

Figure 5. Evolution of the parameter $\delta^{15}N$ (Δ), representing the accumulation of the ^{15}N isotope in the analysed sample according to the species of marked nitrogen radically applied and foliar fertilization

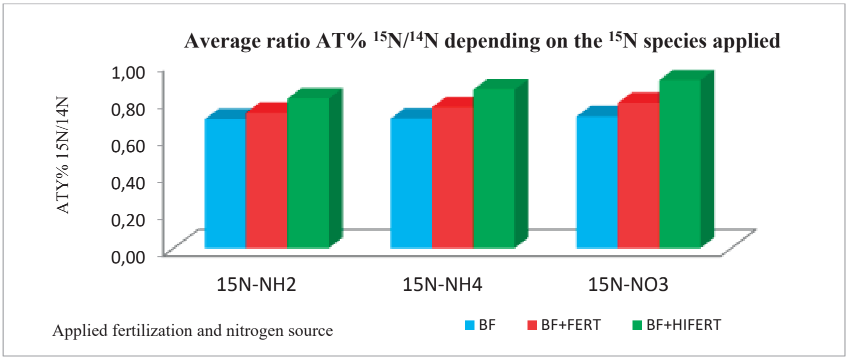

Figure 6. Evolution of the average ratio (R, AT $15N/^{14}N$ %) depending on the ^{15}N applied species

The analysis of the experimental results revealed that the isotopic ratio (R), AT% $^{15}N/^{14}$ in the plant material samples increased in the order of basic fertilization, basic fertilization including Fert fertilizer foliar application and basic fertilization including Hidrofert (HFert) foliar application (Figure 5). The average increase of the R isotopic ratio was 8% compared to only the basic fertilization variant for foliar application of Fert fertilizer and 21.9% for HidroFert (HFert) fertilizer. The foliar application of the HidroFert fertilizer (HFert) has led to an increase of 12.9% for the isotopic ratio compared to the Fert fertilizer variant.

Depending on the nitrogen species applied, an increase in the isotopic ratio (R) was noted as follows: amide nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$), with an increase of 2.1% for the variant using only basic fertilization, of 7.3% in the case of the variant in which Fert foliar fertilization was applied and of 12.2% in the case of the variant in which HidroFert (HFert) foliar fertilization was applied (Figure 6).

For the parameter $\delta^{15}N$ (Δ) (‰), representing the accumulation of the ^{15}N isotope, it increased ascending from basic fertilization, basic fertilization including Fert fertilizer foliar application and basic fertilization including HidroFert (HFert) foliar application. The increase of $\delta^{15}N$ (Δ) was 55.4% compared to only the basic fertilization variant for Fert foliar application and 86.3% for HidroFert (HFert) application. The foliar application of the HidroFert fertilizer (HFert) has led to an increase with 19.9% compared to the Fert fertilizer variant.

Depending on the applied nitrogen species, an increase of the parameter $\delta^{15}N$ (Δ) in the order of amidic nitrogen ($-NH_2$) < ammoniacal nitrogen ($-NH_4$) < nitric nitrogen ($-NO_3$) was noted, with an increase of 2.8% in the case variant using basic fertilization alone, 70.1% for the variant in which Fert foliar fertilization was applied and 90.2% for the variant in which HidroFert (HFert) foliar fertilization was applied (Figure 7).

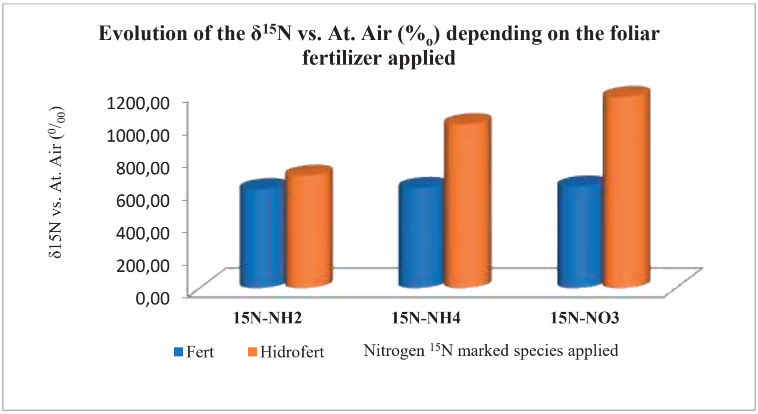

Figure 7. Evolution of the parameter $\delta^{15}N$ (Δ) (‰) depending on the foliar fertilizer used and the nitrogen ^{15}N marked species applied

CONCLUSIONS

In order to evaluate the degree of translocation of different nitrogen forms from the soil into the plant, the nuclear technique, with the ^{15}N labeled isotope applied by incorporation into the soil, was used. The degree of translocation was evaluated using the sunflower test plant (*Helianthus annuus* L.). The procedure was performed under foliar application conditions of two fertilizers containing an NPK matrix including microelements, with / without organic protein hydrolysate.

The isotopic ratio (R), AT% $^{15}N/^{14}N$, in plant material samples increased as follows: basic fertilization, basic fertilization including Fert foliar application and basic fertilization including HidroFert (HFert) foliar application, ranged from 0.698% (15N-NH_2 species) to 0.908% (15N-NO_3 species).

Depending on the nitrogen species applied, an increase in the isotopic ratio (R) as follows: amide nitrogen (-NH_2) < ammoniacal nitrogen (-NH_4) < nitric nitrogen (-NO_3), with an increase of 2.1% in the case of the variant using only basic fertilization, up to 12.2% in the case of the variant in which HidroFert (HFert) foliar fertilization was applied.

Depending on the applied nitrogen species, an evolution of the parameter δ15N (Δ) was noted as follows: amide nitrogen (-NH_2) < ammoniacal nitrogen (-NH_4) < nitric nitrogen (-NO_3), with an increase of 2.8% in the case of the variant using only basic fertilization, of 70.1% in the case of the variant in which Fert foliar fertilization was applied and of 90.2% in the case of the variant in which HidroFert (HFert) foliar fertilization was applied.

The recovery rate for nitrogen due to foliar fertilization alone ranged between 14.75% (species 15N-NH_2) and 26.53% (species 15N-NO_3). The nitrogen recovery rate due to the presence of protein hydrolysate in the fertilizer matrix was not contrasting for the ^{15}N labeled species and ranged between 24.19% (15N-NH_2 species) and 24.89% (15N-NH_4 species).

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REACTION OF RYE CULTIVARS TO LEAF RUST (*P. recondita* f. sp. *secalis*) IN THE CONTEXT OF CLIMATE CHANGE IN DRY AREA IN SOUTHERN ROMANIA

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Abstract

*Increasing temperatures and changing precipitation patterns impact plants biotic constrainers worldwide affecting host-pathogen relationship depending on geographical and temporal distribution of inoculum amount and cultivars susceptibility. Leaf rust of rye, which is caused by the obligate biotrophic basidiomycete Puccinia recondite f. sp. secalis (Roberge ex. Desmaz) has become one of the most important limiting factors for rye production in Central and Eastern Europe. During 2019-2020 growing season, a plant–pathogen interaction profile was observed on four rye genotypes in a randomized complete block design with three replications in dry area from Research and Development Station for Plant Culture on Sands Dăbuleni in south of Romania. Adult plant partial resistance was assessed through host response and epidemiological parameters as final rust severity (FRS), area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC), coefficient of infection (CI) and infection rate (IR). The response of rye genotypes to leaf rust included different variation in resistance reaction ranging from moderately resistant (Serafino, Bintto), moderately susceptible (Inspector) and very susceptible (Suceveana). A negative and highly significant correlation of AUDPC with grain yield (r =-0.9194***) was found during 2019-2020 cropping season.*

Key words*: epidemiological parameters, leaf rust, adult plant partial resistance, Puccinia recondite f. sp. secalis.*

INTRODUCTION

During the last decades global food production and food security increased significantly due to many changes in agricultural systems as a consequence of interaction among multiple factors, such as world population increase, urbanization, technical progress, income growth, genetically progress, improved cropping technologies, globalization on food production, machinery revolution, markets, consumption, faster access to the information (Matei, 2011; Matei and Roșculete, 2011; Partal et al., 2013; Partal et al., 2014; Cristea et al., 2015; Bonciu, 2018, Roșculete and Roșculete, 2018; Bonciu, 2019, Roșculete et al., 2019; Bonciu, 2020; Partal and Paraschivu, 2020).

However, this progress comes to face additional factors as climate variability and climatechanges. Worldwide the cultivation of crops, their yielding capacity and quality are directly affected by different climatic factors or extreme climatic events.

European agricultural systems are also affected by climate change through changes in temperatures (warmer than long-term means or unseasonal frosts) and precipitation (including snow, hail or extreme intensity, variable humidity) impacting directly crops production and availability and indirectly the biotic constrainers of crops, such as weeds, pests and pathogens and their relationship with plants (Coakley et al., 1999; Downing et al., 2000; Chakraborty and Pangga, 2004; Eastburn et al., 2011; Ză1ă et al., 2012; Cotuna et al., 2013; Paraschivu et al., 2015; Paraschivu et al., 2014; Paraschivu et al., 2017; Cotuna et al., 2018; Juroszek et al., 2019; Paraschivu et al., 2019a; Paraschivu et al., 2019b; Casazza et al., 2021).

Climate change together with human-induced changes is expected to cause the spread of pathogens, pests and invasive species in areas where they have not been relevant before, bringing new challenges for crop management and breeding in order to face yield losses and avoid alteration of natural landscape vegetation (EEA Report, 2017). Thus, some pathogens tend to become more aggressive even in cropping systems based on crops diversify-cation by minor cereals.

Rye (*Secale cereale*) is a minor cereal, closely related to barley and wheat, used for human consumption as rye bread and alcoholic beverages, such as beer, whiskey and vodka. Also, rye is very important feed for livestock and currently contributes to crop species diversity in temperate regions of Central and Eastern Europe, especially in marginal environ-ments where soil and climate are unfavourable for wheat production. In 2020 European Union (EU) produced 9,175,000 tons of rye grains from which 71.82% was produced in Germany and Poland (USDA, 2020).

One of the most important disease of rye in Central and Eastern Europe is Brown rust (BR), known also as Leaf rust (LR), caused by the obligate biotrophic basidiomycete *P. recondita* f. sp. *secalis* (Prs) (Roberge ex.Desmaz) (Roux et al., 2007; Roux and Wehling, 2010; Meidaner et al., 2012). Yield losses can be up to 40% in natural conditions, but they can be as high as 80% in case of early infection (Solodukhina, 2002; Wehling et al., 2003). This mainly happens to the pathogen`s ability to multiply rapidly, as well as to its air borne dispersal mechanism from one field to another (Brownand Hovmøller, 2002).

Considering important losses due to Leaf rust, genetic resistance is currently considered as the most economical and effective control measure of this disease, causing no additional cost to the farmers and reducing the use of fungicides. (Singh et al., 2005). However, cereal rusts exhibit considerable capacity for generating, recombining and selecting for resistance under the impact of climate variability and they can adapt to new environment, despite the fact that currently we are not able to predict accurately the trajectory of each pathosystem under climate change. Therefore, screening rye cultivars or using the marker-assisted selection in rye breeding program is of great importance to find new resistance genes associated with leaf rust resistance.

In this context the present paper emphasises the results of the assessment of four rye genotypes, with different origins, screened for adult plant partial resistance to *P. recondita* f. sp. *secalis* in natural infections in the sandy soils in Southern Oltenia, Romania

MATERIALS AND METHODS

The trail for screening different rye genotypes for their adult plant partial resistance to *P. recondita* f. sp. *secalis* was carried out during 2019-2020 growing season at the Development Research Station for Plant Culture on Sands Dabuleni, located in Southern Oltenia, Romania (43°48′04″N 24°05′31″E), on sandy soil, poorly supplied with nitrogen (between 0.04-0.06%), well supplied with phosphorus (between 54 ppm and 77 ppm), reduced to a medium supplied with potassium (between 64 ppm to 83 ppm), low in organic carbon (between 0.12-0.48%) and weakly acidic pH to neutral (between 5.6 and 6.93).

Technological measures applied included broadcasting the fertilizers at sowing time with $N_{80}P_{80}K_{80}$, one side nitrogen fertilization during vegetation with N_{70}, starter irrigation with 250 m^3 water/ha and supplemental irrigation with 300 m^3 water/ha at heading stage. Also, weeds control was done using Dicopur Top 464 SL (1 l/ha) applied in postemergence to control annual and perennial dicotyledons accordingly with the recommendations (cereals to the formation of the first internode and the weed species in the small phase of about 2-4 leaves and a maximum of 10-15 cm high for perennial weeds).

A plant-pathogen interaction profile was observed on four rye genotypes (Serafino, Bintto, Inspector and Suceveana), assessed for their response to natural infection with *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) in a randomized complete block design (RCBD) with three replications. Each plot had 5 m^2, a space of 1 m between blocks and 0.5 m between plots.

Disease observations were recorded since the first appearance of leaf rust infection on the susceptible rye genotypes until rust symptoms were fully developed (nearly at the early dough stage). Adult plant partial resistance for leaf rust was assessed through host response and epidemiological parameters as final rust severity (FRS), area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC), coefficient of infection (CI) and infection rate (IR).

Rye genotypes response was expressed in five infection types for cereals leaf rust according to Johnston and Browder (1966) (Table 1).

Table 1. Infection types of cereals leaf rust used in disease assessment at seedling stage adopted by Johnston and Browder (1966)

Infection type	Host response	Symptoms
0	Immune	No uredia or other macroscopic sign of infection
0	Nearly Immune	No uredia, but hypersensitive necrotic or chlorotic flecks present
1	Very resistant	Small uredia surrounded by necrosis
2	Moderately resistant	Small to medium uredia surrounded by chlorosis or necrosis
3	Moderately susceptible	Medium-sized uredia that may be associated with chlorosis
4	Very susceptible	Large uredia without chlorosis or necrosis
X	Heterogenous	Random distribution of variable-sized uredia on single leaf

Leaf rust severity (%) was recorded for each genotype from the time of rust first pustules appearance (booting stage) until the early dough stage (Zadoks scale) (Zadoks et al., 1974), assessing 10 tillers randomly selected and pre-tagged plants of the central four rows of each plot and the mean of the ten plants was considered as the value for a plot. Rust severity was determined by visual observation and expressed as percentage coverage of leaves with rust pustules (from 1% to 100%) following Cobb's scale modified by Peterson (Peterson et al., 1948) (Table 2).

Table 2. Leaf rust severity expressed as percentage coverage of leaves with rust pustules - Cobb`s scale modified by Peterson (Peterson et al., 1948)

Category	Percentage leaf rust infection relative to susceptible check	Type of resistance
1	80-100%	Susceptible
2	50-70%	Race-nonspecific, low resistance
3	30-50%	Race-nonspecific, moderate resistance
4	10-20%	Race-specific, high resistance
5	less than 10%	Race-specific, high resistance
6	less than 5%	Effective, race-specific resistance

Final rust severity values were used to calculate Area under Disease Progress Curve (AUDPC), which shows the evolution and disease quantity on each rye genotype included in the trail, following the formula (Campbell and Madden, 1990):

$$AUDPC = \sum_{i=1}^{a}\left[\left\{\frac{Yi+Y(i+1)}{2}\right\}x(t(i+1)-ti)\right]$$

where, Yi = disease severity (%) at each measurement; ti = time in days of each measurement; a = number of Leaf Rust assessments.

Relative Area Under the Disease Progress Curve (rAUDPC) was calculated using the following formula:

rAUDPC = [AUDPC check/AUDPC assessed genotype] × 100

Average coefficient of infection (CI) was calculated by multiplying the percentage of disease severity and the constant value assigned to each infection type (Saari and Wilcoxson, 1974; Pathan and Park, 2006). The constant values were considered as R=0.2, R-MR = 0.3, MR = 0.4, MS = 0.8 and S = 1.

Apparent infection rate (IR) as a function of time was also calculated from the two disease severity observations as a severity of leaf rust infection at the time of rust pustules appearance and every fifteen days thereafter. It was estimated using the following formula adopted by Van der Plank (1963).

Inf-rate (IR) = 1/t (ln x/1-x)

Where x = the percent of disease severity divided by 100; t = time measured in days. The apparent infection rate is the regression coefficient of ln x/1-x on t.

In order to characterize the evolution of climatic parameters (air temperature, rainfall, humidity, wind speed) into the experimental field it was used an automatic weather station (AWS).

Means were compared with the susceptible genotype Suceveana (control). The results were statistically analysed and interpreted using the analyse of variance and mathematical functions of MS Office Excel 2010 facilities.

RESULTS AND DISCUSSIONS

The challenges to achieve sustainable food security in dry areas meet the ones generated by the effects of climate change and climate variability on crops health, especially in vulnerable crop systems like cereals, associated by many authors with changes in pathogens life cycles, increased incidence, pathogenicity, genetically recombination and aggressiveness traits (Chakraborty and Newton, 2011; Newton et al., 2011; West et al., 2012; Chakraborty, 2013; Elad and Pertot, 2014; Fones et al., 2020; Wolfe and Ceccarelli, 2020).

The 2019-2020 cropping season was favourable to rye Leaf rust disease in the dry area in Southern Oltenia, Romania. For scouting optimization and to predict the Leaf rust disease development, rainfalls and temperatures were taken into account. Humidity was determined by the amount of rain of 383.96 mm, comparatively with multiannual average rainfall of 376.85 mm, while the monthly average temperature was 13.9ºC comparatively with multiannual average temperature of 12.7ºC (Figure 1).

Figure 1. Climatic conditions during the study period (2020 year)

During January to August 2020 the monthly average temperature increased up to +1.2ºC comparatively with multiannual average temperature for January to August between 1956-2019 for the same geographic area. This temperature increase follows the global trend in planet warming. Thus, accordingly with a report of National Oceanic and Atmospheric Administration (NOAA, September 2020) monthly average temperature for January to August 2020 increased up to +1.03ºC (+15.03ºC) at the global level comparatively with average temperature recorded on Earth in the 20th century (+14ºC).

Rainfall amount for evaluated period was slightly higher with 7,11 mm than multiannual amount for dry areas in Southern Romania. The humidity at leaf level cumulated with increased temperature favoured the development of Leaf rust disease, which exhibited the first symptoms at the end of April 2020 on the 4th and the 3th rye genotypes leaves.

Optimal environmental conditions for disease development are temperatures ranging from 15°C to 20°C, but the fungus can develop at the temperature of 2-35°C. The fungus needs approximately six hours of moisture on leaves to start developing. With much moisture and suitable temperatures, lesions are formed within 7-10 days and spore production reduplicate another uredospore generation (Kolmer, 2013).

Săvulescu (1953) showed that uredospores of leaf rust were visible on rye leaves at the end of May or the beginning of June, but the currently results show that in the context of climate change, with higher monthly average temperature and ununiform rainfalls, these fruiting bodies of the pathogen (uredinia with uredospores) appear earlier. These findings suggest a modification of life cycle of the pathogen *P. recondita* f. sp. *secalis* by many generation numbers and higher resistance of uredospores to increased temperature. Also, Harvell et al. (2002) suggested that rising temperatures will (i) increase pathogen development transmission, and generation number; (ii) increase overwinter survival and reduce growth restrictions during this period and (iii) alter host susceptibility.

P. recondita f. sp. *secalis* spores are spread by splashing water and wind leading to many successive infections. Meidaner (2012) showed that minimum wind speed for uredospores splashing is 2m/s. In the experimental field the wind speed ranged between 5-40 km/h, respectively 1.4-14 m/s (Table 3).

Table 3. Wind speed km/h recorded during April-June 2020 in the experimental field*

Time	April	May	June
the 1st decade	11-35 km/h	14-40 km/h	-
the 2nd decade	14-34 km/h	5-37 km/h	-
the 3th decade	19-34 km/h	21-37 km/h	-

*automatic weather station DRSPCS Dabuleni, Romania

Identification of the fungus *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) and its characteristics were done in the Phytopatology Laboratory of Agriculture Faculty in University of Craiova, using MOTIC microscope. The diameter of uredinia can reach even 1.5 mm, their colour is orange to brown and their shape is round to ovoid. The average size of uredospores release from uredinia is 20 mm in diameter and colour orange-brown (Figure 2). Uredospores have up to eight germ pores scattered in dense walls.

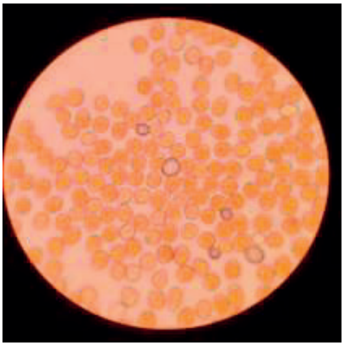

Figure 2. Uredospores of *Puccinia recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) (original photo Paraschivu Mirela, 2020)

Leaf rust pustules are small, circular to oval shape, with orange to light brown dusty spores (uredospores) on upper surface of leaves surrounded by a light-coloured halo (Figure 3).

Figure 3. Pustules with uredospores of *Puccinia recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) (original photo Paraschivu Mirela, 2020)

There can be thousands of spores in each pustule. In case of severe attack leaf rust pustules may extend also on the leaf sheaths, stalks and husks.

Following field screening, the response of rye genotypes to leaf rust included different variation in resistance reaction ranging from moderately resistant (Serafino, Bintto), moderately susceptible (Inspector) and very susceptible (Suceveana-control). These findings were also emphasised by partial resistance traits.

Adult plant data revealed that partial resistance traits (FRS, AUDPC, rAUDPC,CI and IR)showed a discrepancy in the values within parameters and genotypes (Table 4).

Table 4. Partial resistance traits to leaf rust in adult plant of four rye genotypes

Genotype	FRS	AUDPC	rAUDPC	CI	IR
Binnto	55.63**	113.75*	234.72**	22.25**	0.0373**
Serafino	43.86**	92.10**	289.90**	17.54**	0.0524**
Inspector	68.45	163.8	163.00*	54.76	0.0240
Suceveana	87.68	267.00	100	87.68	0.0083

FRS = Final Rust Severity; AUDPC = Area under disease progress curve; rAUDPC = Relative area under disease progress curve; CI = Coefficient of infection; IR = Infection rate.
**Significance level at $P \leq 0.01$
Suceveana = control

Thus, comparatively with Suceveana genotype (control), only Serafino and Binnto possessed high level of adult plant partial resistance based on the assessed traits, during 2019-2020 cropping season. Serafino recorded the lowest Final Rust Severity (FRS) (43.86%), which corresponds with low AUDPC value (92.10) and low Infection Coefficient (IC) (17.54). The differences for all resistance traits for Serafino and Binnto genotypes were highly significant comparatively with the control genotype.

Among all adult plant partial resistance traits was noticed a highly significant correlation (Table 5).

Table 5. Correlation coefficients (r)* for disease parameters of leaf rust on rye genotypes at DRSPCS Dabuleni during 2019-2020 cropping season

Disease parameter	FRS	AUDPC	rAUDPC	CI	IR
FRS	1	0.982***	-0.993***	0.979***	-0.994***
AUDPC		1	-0.957***	0.987***	-0.955***
rAUDPC			1	-0.972***	0.997***
CI				1	-0.960***
IR					1

FRS = Final Rust Severity; AUDPC = Area under disease progress curve; rAUDPC = Relative area under disease progress curve; CI = Coefficient of infection; IR = Infection rate.
* Pearsons' r_{calc}values

Negative high correlations were observed between Infection rate (IR) and FRS, AUDPC and CI in 2019-2020 cropping season. These findings indicate that although FRS, AUDPC and CI increased, the rate of infection (IR) reduced as epidemic progressed because less healthy plant tissue was available for additional infections.

The response of rye genotypes along with grain yield (t/ha) indicated the presence of inverse relation between the disease level (AUDPC) and grain yield. The highest significant loss percentages were found in susceptible genotypes Suceveana and Inspector. The value of determination coefficient ($R^2 = 0.8453$), for all rye genotypes assessed, indicated that up to 84% of variation in rye yield could be explained by AUDPC variability. It was noticed a highly significant correlation between AUDPC values and grain yield (r = -0.9194***) (Figure 4).

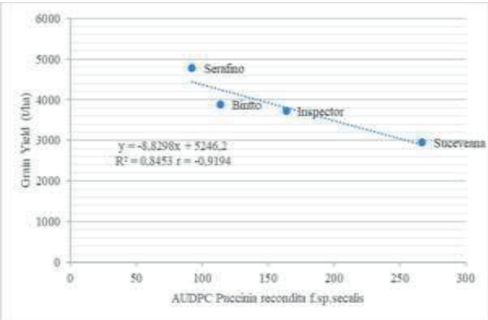

Figure 4. Relationship between Leaf rust AUDPC value and rye grain yield in 2019-2020 cropping season

Yield loses due to Leaf rust in rye in Europe were also reported previously by different authors (Solodukhina, 2002; Roux and Wehling, 2010; Meidaner et al., 2012).

However, the results of the experiment show that in the context of climate change the Leaf Rust is a serious disease of rye in dry marginal areas from Romania and climate variability can lead to further epidemics.

CONCLUSIONS

The present study was carried out to assess the adult plant response of four ryegenotypes to the attack of *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) in natural infections in dry area from Southern Romania during 2019-2020 cropping season. The study emphasized that the increase of monthly temperature with +1ºC may lead to earlier incidence of the disease starting even with the end of April.

The response of rye genotypes to the Leaf Rust (LR) included different variation in plants reaction ranging from moderately resistant (Serafino, Bintto) to moderately susceptible (Inspector) and very susceptible (Suceveana), depending on genetic background and environmental conditions.

The values of Area under Disease Progress Curve (AUDPC) ranged from 92.10 (Serafino) to 267 (Suceveana). Serafino recorded the lowest Final rust severity (FRS) (43.86%), which corresponds with low AUDPC value (92.10) and low Infection Coefficient (IC) (17.54). The Pearsons' r_{calc} values indicated a highly significant correlation among all adult plant partial resistance traits. Also, it was noticed a highly significant correlation between AUDPC values and grain yield (r = -0.9194***)

It could be concluded that the local studies on the leaf rust disease including the determination of the response of commercially rye cultivars, cultivated in marginal areas under weather factors stress, are of great benefits for both breeders and farmers, offering precious information about the impact of climatic change on the interaction between cereals and pathogens, changing host-pathogen relationship.

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THE INFLUENCE OF SOWING DATE AND PLANT DENSITY ON MAIZE YIELD AND QUALITY IN THE CONTEXT OF CLIMATE CHANGE IN SOUTHERN ROMANIA

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Abstract

Yield and quality are affected by several plant characters, environment and cultural practices. The researches were performed during the 2018-2020, in the experimental field of NARDI Fundulea and the aim of this study was to evaluate the effect of sowing date, plant density and climatic conditions on the yield and quality of maize. The experience comprised three different sowing dates (I - April 1, II – April 15 and III- May 1), using three maize hybrids (Iezer, Mostistea and F423) and three plant density (50 000, 60 000 and 70 000 plants/per hectare). The annual rainfall varied from year to year and the maximum production and quality of maize was maximized from medium plant density and earlier sowing dates, depending on the hybrid. The early sowing date led to increase of quality values, which was depending on climatic conditions. The results showed that yield and quality from maize was very significantly affected by year conditions, sowing date, plant density and hybrids, as well as by most interactions between these factors.

***Key words**: maize, sowing dates, plant density, yield and quality, climatic conditions.*

INTRODUCTION

Maize (*Zea mays*) is one of the most important crops and ranks second in the world, cultivated on 160 million ha and producing about 800 million tons of grain (www.fao.org). In Romania, maize is cultivated on 2.7 million ha (www.madr.ro).
The results of various researches show the role of sowing time and plant density on increasing maize production in different climatic conditions. The sowing season is a very important technological link to maximize the yield of maize (Van Roekel and Coulter, 2012) and therefore research is concentrated about the response to maize yield at sowing (Paraschivu et al., 2017; Abendroth et al., 2017).
The optimal time for sowing crops can vary from one agricultural area to another due to differences in soil and climatic elements (Paraschivu et al., 2015; Burns and Abbas, 2006, Tsimba et al., 2013). Maize seed needs positive soil temperatures to germinate and grow (Abendroth et al., 2017), but early sowing is now widely applied by farmers to take advantage of solar radiation (Fabian & Gomoiu, 1981). Research has shown that early planting leads to increased yields during photosynthesis and avoids stagnation of seed germination and degradation of their quality (Lauer et al, 1999). However, in conditions of too low temperatures in the soil, the seeds have the ability to absorb water, but will delay the growth of roots, which leads to the installation of seed rot and a problematic emergence (Abendroth et al., 2017; Hall et al., 2016). The availability of water in the soil is one of the limiting factors that affects the optimal density of plants and implicitly the yield of maize. The amount and distribution of rainfall, soil water and plant density interact and directly influence the period of active crop growth (Lobell and Burke, 2008; Huzsvai & Nagy, 2005). Yield differences between hybrids are determined by the genetic characteristics of the hybrid, environmental conditions, nutritional space, soil fertility and applied technology (Partal & Paraschivu, 2020; Paraschivu et al, 2019; Bene et al., 2014). The impact of climate change on agricultural yields can have various consequences from one area to another (Donatelli et al., 2012). Thus, in this paper we present the results recorded in the last three

years on the influence of sowing time, plant density and climatic conditions on maize yield and quality.

MATERIALS AND METHODS

The field tests were established in the period 2018 - 2019 - 2020, on a specific soil for southern Romania (cambic chernozem). Regarding the physical characteristics of the soil, the humus content is higher in the first 15 cm due to the former bedding and gradually decreases to depth.
The soil consists of several horizons:
- Ap+Aph - 0-30 cm, clay-clay-dust with 36.5% clay and permeability 492, pH 5.9.
- Am - 30-45 cm, clay-clay with 37.3% clay, compacted, DA 1.41g / cm^3, pH 5.9.
- A/B (45-62 cm), Bv1 (62-80 cm), Bv2 (82-112 cm), Cnk1 (149-170 cm), Cnk2 (170-200 cm).

Depending on the agricultural year, the water supply of the soil is favorable for field crops, groundwater at 10-12 meters.
The experimental material included three maize hybrids (Iezer, Mostistea and F423), developed at the National Institute for Agricultural Research and Development in Fundulea. Hybrids were sown at three different dates (SD I - April 1, SD II - April 15 and SD III - May 1) and at three plant density (50 000, 60 000 and 70 000 plants / hectare). The size of the plot was 56.0 m^2 (with 20 m long, 4 rows, 70 cm distance between rows). The cultivation of maize followed the wheat, in the rotation of 4 years.
In terms of quality, samples were taken from each repetition and variant and determined:
- The mass of 1000 grains was weighed with the Kern precision electronic scale.
- The hectolitre weight was determined with the special cylinder, followed by weighing on the Kern scale.

Using the electronic device INFRATEC 1241 Grain Analyzer, the elements of seed analysis were determined: - Starch content (%) - Protein content (%) - Fat content (%).
The dynamics of water loss of cereals in the studied hybrids was performed directly in the experimental field during the last month of the vegetation period.
The data were statistically evaluated using analysis of variance procedure - ANOVA, using MSTATC software. The significant differences among means of variant was tested by Duncan's multiple range test.

RESULTS AND DISCUSSIONS

Climatic aspects

The 2018 was a dry year. Temperatures recorded average values 1.0ºC higher than the multiannual average. The variation of temperatures differs from one year to another, so that in 2018, the high temperatures of the period of intense vegetation associated with the deficit of precipitation led to a significant decrease in yields.
In 2019, the months with the lowest amounts of precipitation were September with 6.2 mm, compared to 50.9 mm multiannual average and August with 12.6 mm compared to 51.5 mm multiannual average. The highest amounts of precipitation were recorded in July with 87.4 mm, about 14.7 mm above the multiannual average. Regarding the thermal regime, in the period June - September, the registered values show that the average monthly temperatures were higher than the multiannual average, in June by 3ºC above the multiannual average.
The year 2020 was a dry one, with accentuated water deficit and high temperatures, compared to the multiannual average. The months with the lowest rainfall were 14 mm compared to 44.6 mm on average, August with 5.4 mm compared to 49.7 mm on average and July with 34.2 mm compared to 71.4 mm on average. In May and June there were precipitation amounts close to normal, 57.8 mm and 68.4 mm, respectively. The precipitation deficit affected the installation and development of crop plants in the first phases after emergence, which had a negative impact on the final production. Higher than average annual temperatures have exacerbated the drought. In July and August, due to the severe drought, the development of maize crop was affected. The average temperatures recorded in the agricultural year 2020 were 20.6ºC, compared to the multiannual average of 18.5ºC and an increase was 2.1ºC.
In order to establish the influence of the climatic elements, on the evolution of the maize culture, the values obtained in different phenological phases of the plants with the final production were analyzed and corroborated, in terms of quantity and quality.

Table 1. The meteorological parameters in the experimental period (Fundulea, 2018–2020)

	Years/Months	April	May	June	July	August	September	Total/Average
Precipitations (mm)	2018	2.4	34.0	120.6	84.9	2.8	28.6	273.3
	2019	51.4	124.2	74.6	87.4	12.6	6.2	356.4
	2020	14.0	57.8	68.4	34.2	5.4	68.6	248.4
	50 years average	**44.0**	**60.0**	**73.0**	**72.7**	**51.5**	**50.9**	**352.1**
Temperatures (°C)	2018	15.8	19.3	22.6	22.8	24.2	19.0	20.4
	2019	11.2	17.2	23.6	22.9	24.7	19.3	19.8
	2020	12.4	16.8	21.8	25.1	25.5	20.8	20.6
	50 years average	**11.1**	**16.9**	**20.7**	**22.7**	**22.1**	**17.3**	**18.5**

Production and Quality

The yields registered significantly different values from one year to another, depending on the evolution of precipitations and temperatures, the sowing dates, the density of the plants and the characteristics of the hybrid.

Thus, in 2018, considered a dry year, with a rainfall deficit of 78.8 mm compared to the multiannual average and a negative distribution in May, July and August and temperatures above the multiannual average, the yield of maize recorded values between 3.1 – 6.5 to/ha, the best technological variant was registered at the association between the sowing date I, the density of 60 0000 plants/ha and the F423 hybrid or the Mostistea hybrid. The lowest value of the yield was at the associated variant between the sowing date III, the density of 70 0000 plants/ha and the hybrid Mostistea, with 3.1 to/ha (Figure 1).

In 2019, a year considered normal, with an average rainfall of 356.4 mm, the yield of maize recorded values between 4.6 – 9.4 to/ha, depending on the technological variant. The variations were between 6.3 – 9.4 to/ha at the sowing date I, of 5.3 – 7.0 to/ha at the sowing date II and 4.6 – 6.5 to/ha at the sowing date III. The best technological variant was registered at the association between the sowing date I, the density of 60 0000 plants/ha and the F423 hybrid or the Mostistea hybrid.

In 2020, considered a dry year, with a precipitation deficit of 103.7 mm compared to the multiannual average and with an average temperature of 2.1°C above the multiannual average, the maize yield recorded the highest value, of 3.9 to / ha, for the F423 hybrid in the technological variant with the sowing date II and with a density of 60 0000 plants/ha, followed equally by the Mostistea and Iezer hybrids with 3.8 to/ha in the same technological variant.

The sowing date I had yield values of 3.6 to/ha for the F423 hybrid and 3.5 to/ha for the Mostistea hybrid, both at a density of 60 000 plants/ha. The lowest value of the yield was for the technological variant with the sowing date III, the density of 70 000 plants/ha and the hybrid Mostistea, followed by the technological variant with the sowing date III, the plant density of 50000 plants/ha for the Iezer and Mostistea hybrids (Figure 1).

The analysis of variance shows a very significant influence of the climatic conditions on sowing dates (SD), plant density, hybrids and interactions of these factors (Table 2).

The average number of days from sowing to emergence of plants was a maximum of 19 at sowing date I and decreased to 14 and 11 days for sowing date II and III. The sowing date was distinctly significant positive for the final production, the harvest index and the moisture at seed harvest. The highest average value of the productions was registered at the second sowing date, with 7.40 to/ha, followed by the value from the first sowing date I with 4.70 to/ha.

The associated influence of year, hybrid and sowing date was significantly positive for final production and grain humidity at harvest. As the research data show, the height of maize plants increases with late sowing dates. Thus, the height of maize plants increased significantly (7%) from an average of 2.20 m for sowing date I to 2.35 m for sowing date III.

The variation recorded was due to the influence of climatic conditions, warm weather can stimulate plants to develop large vegetative mass. Hybrids showed differences in plant height as an average over three years, so that F423 recorded the highest value of 2.40 m followed by Iezer hybrid with 2.32, in the favorable years.

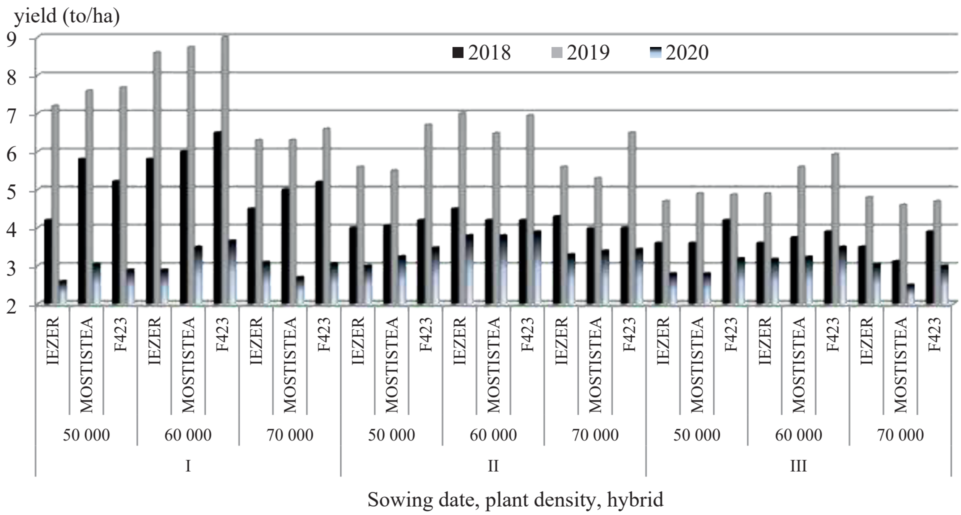

Figure 1. Yields obtained to maize hybrids under experimental conditions during 2018-2020

Table 2. ANOVA for sowing date (SD), grain yield, humidity, the emergence days and plant height – average the years 2018-2020

Sowing Date (SD)	Grain Yield	Harvest Index	Grain Humidity	Emergence	Plant Height
	(to/ha)		(%)	(days)	(m)
2018	6.10	0.59	17.2	21	2.22
2019	4.30	0.57	16.8	19	2.26
2020	3.80	0.60	14.1	20	2.12
Average SD I	**4.70**	**0.58**	**16.0**	**19**	**2.20**
2018	8.90	0.53	17.6	13	2.30
2019	6.80	0.52	17.4	13	2.33
2020	6.50	0.50	14.6	16	2.14
Average SD II	**7.40**	**0.52**	**16.5**	**14**	**2.26**
2018	3.40	0.50	19.5	10	2.35
2019	3.80	0.49	20.4	12	2.40
2020	5.50	0.47	19.0	12	2.31
Average SD III	**4.20**	**0.49**	**19.6**	**11**	**2.35**
ANOVA / SEMNIFICATIONS					
Year (Y)	**	*	**	**	**
Error					
Sowing date (SD)	**	**	**	*	*
SD*Y	*	*	*	**	*
Error					
Hybrid (H)	*	ns	**	ns	*
H*SD	*	ns	**	ns	ns
Y*H	ns	ns	ns	ns	*
Y*H*SD	*	ns	*	ns	ns

*- Significat at P-value <0.05; ** Significat at P-value <0.01; ns – not significat;

The hectolitric weight registered an average of 76.7 kg / hl at the sowing date I with values between 74.0 – 80.0 kg / hl, an average of 76.8 kg/hl at the sowing date II with values between 74.0 – 79.4 kg/hl and the last average of 75.7 kg/hl at the sowing date III with values between 72.6 – 79.4 kg/hl.

The highest value of the hectoliter weight was registered in 2019, with 80.0 kg/hl, at the sowing date I, and the lowest in 2020, with 72.6 kg/hl, at the sowing date III (Table 3).

The weight of one thousand grains (WTG) recorded an average of 287.2 g at the sowing date I with values between 260.1 – 316.0 g, an average of 296.1 g at the sowing date II with values between 257.9 – 332.1 g and an average of 281.8 g at the sowing date III with values between 252.4 – 306.9 g.

The protein content registered an annual average of 7.9% at the sowing date I with values between 7.1 - 8.8%, an average of 8.6% at the sowing date II with values between 7.6 - 9.7% and an average of 7.8% at the sowing date III with values between 7.2 - 8.9%. The highest value of protein content was registered in 2020, with 9.7%, at the sowing date II, and the lowest in 2019, with .71%, at the sowing date I.

The starch content registered an average of 68.7% at the sowing date I with values between 64.3 - 72.3%, an average of 69.2% at the sowing date II with values between 65.4 - 72.5% and an average of 68.2% at the sowing date III with values between 63.7– 72.1%. The highest value of the starch content was registered in 2020, with 9.7%, at the sowing date II, and the lowest in 2019, with 7.1%, at the sowing date I.

The fat content in the grain registered an average of 4.2% at the sowing date I with values between 4.0 - 4.5%, an average of 4.2% at the sowing date II with values between 3, 9 - 4.5% and average of 3.9% at the sowing date III with values between 3.6 - 4.3%. The highest value of fat content in grain was registered in 2020, by 4.5%, at the sowing date I, and the lowest in 2018, by 3.6%, at the sowing date III (Table 3).

The correlation between the values of the starch content is negatively correlated with the values of the protein and fat content, due to the climatic characteristics of the agricultural year.

The values of the variants were very significantly positive when associating the year with the sowing date for the starch content and the fat content in the grain.

The influence of the year was very significantly positive for the hectolitric weight, the protein content, the starch content and the fat content in the grain.

The influence of the hybrid was insignificant for the weight of a thousand grains and the starch content.

The dynamics of grain water loss in the studied hybrids: F423, Iezer and Mostistea was decisively influenced by the climatic evolution, the technological elements and the characteristics of the hybrid (Figures 2 and 3).

Table 3. ANOVA for hectoliter weight (HW), weight thousands grain (WTS) and the protein, starch and fat content – average 2018-2020

Years	HW	WTG	Proteine	Amidon	Fat content
	(kg/hl)	(g)	(%)	(%)	(%)
2018	76.1	285.5	7.8	69.5	4.0
2019	80.0	316.0	7.1	72.3	4.0
2020	74.0	260.1	8.8	64.3	4.5
Average SD I	**76.7**	**287.2**	**7.9**	**68.7**	**4.2**
2018	77.1	298.5	8.5	69.6	3.9
2019	79.4	332.1	7.6	72.5	4.1
2020	74.0	257.9	9.7	65.4	4.5
Average SD II	**76.8**	**296.1**	**8.6**	**69.2**	**4.2**
2018	75.0	286.0	7.5	69.0	3.6
2019	79.4	306.9	7.2	72.1	3.8
2020	72.6	252.4	8.9	63.7	4.3
Average SD III	**75.7**	**281,8**	**7.8**	**68.2**	**3.9**
ANOVA / SEMNIFICATIONS					
Year (Y)	**	*	**	**	**
Error					
Sowing date (SD)	*	*	*	*	*
SD*Y	*	*	*	**	**
Error					
Hybrid (H)	*	ns	*	ns	*
H*SD		ns	*	ns	ns
Y*H	*	*	*	*	*
Y*H*SD	*	*	*	*	*

*- Significat at P-value <0.05; ** Significat at P-value <0.01; ns – not significat;

At the sowing date I, the F423 hybrid had a humidity percentage of 23%, and the Mostiştea hybrid had 2.2% more, a difference that was maintained on September 13th.
At the sowing date II, the F423 hybrid had a humidity percentage of 24%, and the Mostiştea hybrid had 1.7% more, a difference that was maintained on September 15th. At the sowing date III, the F423 hybrid had a humidity percentage of 22%, and the Iezer hybrid had 2.2% more, a difference that was maintained on September 21st. For the all sowing dates, the Iezer hybrid recorded intermediate values, compared to other hybrids.
At the time of harvest, the moisture of the grains became uniform, the difference between hybrids being insignificant, between 14.3% and 15.2%, regardless of the sowing season.

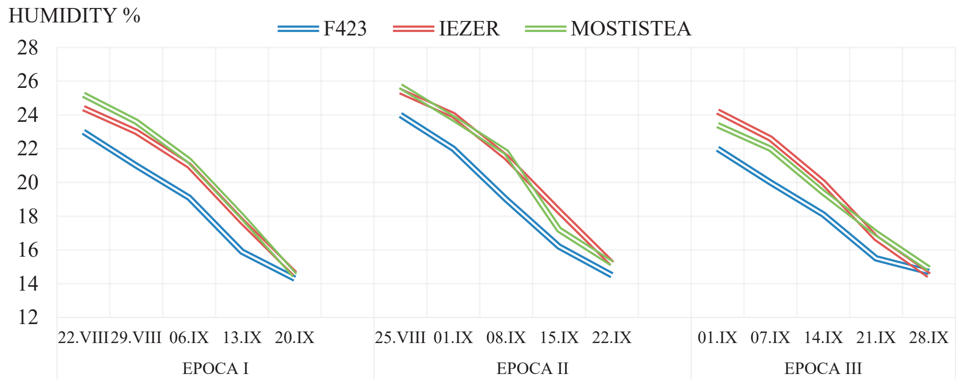

Figure 2. Dynamics of grain water loss in the hybrid F423, Iezer and Mostistea, sowing in the optimal year – average 2019

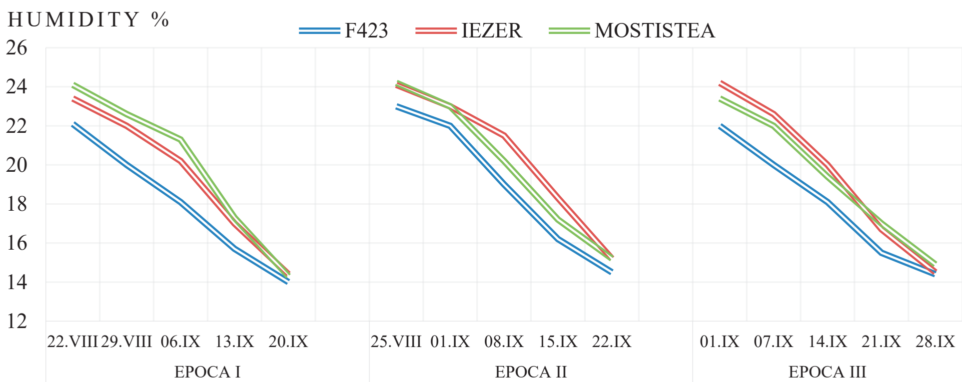

Figure 3. Dynamics of grain water loss in the hybrid F423, Iezer and Mostistea, sowing in the dry year – average 2020

CONCLUSIONS

The results study and analyses shown in this paper showed that sowing date and plant density interact with water supply and affect quantity and quality of the maize yield.
Climatic conditions affect maize plants in any phase of vegetation, with a negative impact on the final production and its quality. The drought phenomenon influences by intensity and duration, sometimes up to the compromise the crop and in this case the drought resistance of the hybrid has a decisive role. In the year 2020, the drought afected the final productions no matter the technological links applied.

Early sowing data (SD I) increased the number of days from sowing to plant emergence, which reduced the density of crop plants and is closely dependent on soil temperature that conditions of the germination process. Late sowing data (SD III) reduced the number of days from sowing to the appearance of plants, increasing the moisture content of cereals at harvest.

The highest yields were obtained in the normal climatic year and in the associated technological variant of the sowing date I, the plant density of 60 000 plants/ha and the hybrid F423 or Mostistea.

The yield quality registered high values in a normal climatic year and in the associated technological variant of the sowing date II and the plant density of 60 000 plants/ha.

The dynamics of water loss of grains in the studied hybrids highlighted the hybrid F423 which recorded lower values compared to the hybrids Mostistea and Iezer, by 1.7 - 2.2%, depending on the sowing date. The humidity in the grain was influenced by the climatic evolution, the technological elements and the characteristics of the hybrid.

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THE EFFECT OF HYDROXYAPATITE AND IRONE OXIDE NANOPARTICLES ON MAIZE AND WINTER WHEAT PLANTS

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Abstract

In the last few decades, the intensive use of agricultural lands affected crop productivity and thus raised serious concerns due to competing demands for food to feed the ever-growing world population (projected to be 9.7 billion by 2050). In this context, the development of nanotechnology-based fertilizers for crop nutrition has been suggested as an alternative tool to overcome the drawbacks arising from the current agricultural practices. Unfortunathelly, there is little studies about the effects of nanomaterials on plants.
In this study we presented the effect of hydroxyapatite (nHA) and iron oxides (nIO) nanoparticles obtained in Romania on growth and photosintesis of corn and winter wheat plants.
The results show that hydroxyapatite nHA) and iron oxides (nIO) treatments applied by watering the soil had a positive effect on the photosynthesis of maize and winter wheat plants.
In the case of treatments with solutions of iron oxides a negative effect on the length of main root was observation, but a compensating effect was found by increasing root density. This and also the higher chlorophyll content, led to a positive effect on height of maize and winter wheat plants.

Key words: *hydroxyapatite and iron oxides nanoparticles, maize, winter wheat, chlorophyll content, growth.*

INTRODUCTION

In modern agricultural production, the application of nitrogen and phosphorus fertilizers is a necessary measure to ensure high and stable yields for cereals and beyond. Unfortunately, the plants do not take all the amount of nutrients from the applied fertilizers but less than 50%, depending on the crop species and soil conditions.
Conventional fertilizers have low nutrient uptake efficiencies and are often associated with high losses in the environment. Therefore, is necessary the avoid these nutrient losses and the increase its uptake by crops. In this sense in last time nanofertilizers were been studied.
However, the extremely appealing prospects of use nanofertilizers in large part have still to be experimentally demonstrated in field conditions.
Hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ nanoparticles (nHA) have an interesting potential to be used as nanofertilizers (Marchiol et al., 2019). With regard to plant macronutrients, studies on hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ nanoparticles (nHA) explored their potential use as carrier of nitrogen (N) or as phosphorus (P) fertilizer. The main advantage of using nHA with respect to other nanomaterials is that they are widely renowned for their intrinsic biocompatibility and biodegradability, being the main component of human bones and teeth (Tampieri et al., 2016).

Other nanoparticles used are those based on iron oxides which have been used for environmental remediation's (Chen et al., 2008).
Most of the current published studies regarding nanoparticles and plants are centred around the effects of this on seed germination and vegetative plant growth.
Several studies were showed positive effect of hydroxyapatite nanoparticles (applied alone or in combination with urea) on the germination rate, biomass yield for several plants or the effects of iron oxide nanoparticles on vegetative growth in maize plants under optimal and hydric stress conditions (Gunaratne et al., 2016; Kottegoda et al., 2017; Petcu et al., 2019).
Also, it has been reported that daily additions of Fe3O4-NPs in the presence of static magnetic fields, increased the growth of *Zea mays* and the levels of chlorophyll (Răcuciu and Creangă, 2007). No trace of toxicity but translocation into soybean stems was reported (Mahmoudi, 2013). Furthermore, it has been found that aqueous suspensions of Fe3O4-NPs can be translocated throughout pumpkin plant tissues and accumulated into the roots and leaves (Zhu et al., 2008). In Romania few studies concerning the effects of nHA or other nanoparticles on plants have been carried out. In this study, the effects of different treatment with hydroxyapatite and iron oxide nanoparticles were evaluated on growth, chlorophyll content and biomass accumulation of maize and winter wheat plants.

MATERIALS AND METHODS

In this study we used the solutions of hydroxyapatite and iron oxide nanoparticles provided by National Institute of Materials Physics.
Seeds of maize and winter wheat were shown in pots filled with a soil-sand mixture (3:1).
The experimental variants were: pots which were watering with tap water (control), pots watering with solution of hydroxyapatite (T1), pots watering with iron oxides solution (T2).
The treatments were applied at soil once one week.
The height of plant (mm), leaf area (cm^2), length of main root (mm), root volume (in cm^3, measured by water displacement), chlorophyll content and dry matter (g/plant) were determined. The chlorophyll content was carried out by using chlorophyll matter Minolta and results are expressed in SPAD units.
The statistical significance of the treatments was evaluated by Analysis of Variance (ANOVA). Means were compared by Least Significant Difference (LSD), according to Fisher's statistical test.

RESULTS AND DISCUSSIONS

The results of the analysis of variance showed that the treatments applied have not a significant influence on the growth of corn plants and rather, the source of the variant was the analysed organ and the interaction between these factors, which were statistically significant (Table 1).

Table 1. Analyses of variance for stem and root length

Source of variance	DF	Sum of squares	Mean square	*F value and significance*
Factor A (treatments)	2	9209	4604	*1.27*
Error A	8	28932	3616	
Factor B (organ)	1	18233	18233	*9.59**
Interraction AxB	2	46917	23458	*12.34***
Error B	12	22800	1900	

There was an insignificant positive effect of the treatments with hydroxyapatite and iron oxide solutions on the growth of the aerial part of the plants. As compared with the positive effect of hydroxyapatite it was obviously that the treatment with iron oxide nanoparticles decrease the length of the main root compared to the control (Figure 1, Photo 1).

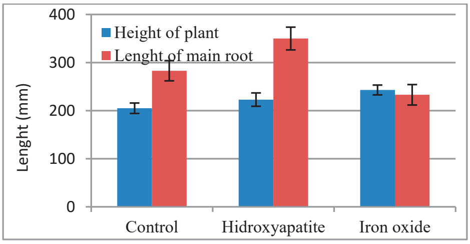

Figure 1. The effect of treatments with hydroxyapatite and iron oxide solutions on growth of maize seedling

The result obtained by Marchiol et al., (2019) showed that the germination percentage was not influenced by the increase in concentration of nHA, but the root elongation of tomato seedlings was clearly affected.
Treatment with iron oxides, although it negatively influenced the growth in root length

(Photo 1), had a beneficial effect on the volume of the root (Table 2).

Photo 1. The effect of treatments with hydroxyapatite and iron oxide solutions on growth of maize seedling (left = control; middle, 1 = hidroxyapatite; right, 2 = iron oxide)

Table 2. The effect of treatments with hydroxyapatite and iron oxides on the volume of the root system

Variants	Volume of root (cm^3)	
	cm^3	%
Control	1.6	100
T1. Hidroxyapatite	1.4	89
T2. Iron oxides	2.0	125
LDS 5%	0.22	
LDS 1%	0.37	

The biomass accumulation increase compared to the control amounted to +12%, for hydroxy-apatite and iron oxide nanoparticles (Figure 2).

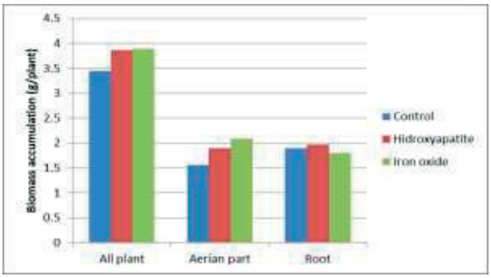

Figure 2. The effect of treatments with hydroxyapatite and iron oxide solutions on biomass accumulation

The treatment with irone oxide nanoparticle decrease the dry matter content of root by 10%, compared to the control. Although on the whole plant there was a positive effect on the dry matter content of plants grown on iron oxide solution, which had a content of 1.7 g dm/plant, while plants grown in water (control) had a content of 1, 4 g dm/plant. This also explains the positive correlation between the dry matter content and the volume of the root system, the correlation coefficient being very significant (r = 0.98***, Figure 3).

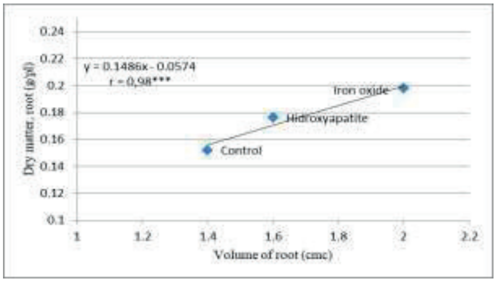

Figure 3. The correlation between the dry matter content of the roots and the volume of the root system

This means that corn plants compensate for the reduction in root length due to iron oxide treatments, with an increase in its weight. The higher amount of dry matter was due to the thickening of the main root and an increased number of absorbent hairs. This is favorable for the plant, because the absorbent hairs are the ones that achieve the absorption of water and nutrients from the soil.

The results regarding the effect of the studied substances on the photosynthesis process showed that they favorably influence this process, the leaf area and the chlorophyll content being superior to the control (Table 3).

Table 3. Effect of hydroxyapatite and iron oxide nanoparticles treatments on leaf area and chlorophyll content

Variants	Leaf area (mm^2)	Chlorophyll content (SPAD units)
Control	1676.25	29.6
T1. Hydroxyapatite (nHA)	1933.5	30.1
T2. Iron oxide (nIO)	2631	33.2

Some research has been showed that iron oxide nanoparticles caused to enhancement of growth and photosynthesis of peanut. Iron oxide nanoparticles in compared with treatments such as organic fertilizer and iron citrate facilitated the transferring of materials and increased iron transporting to leaves (Liu et al., 2005). Oil and protein production depend on the quality characteristics of seeds (oil and protein) and grain yield (Morshedi's, 2000) showed that the usage of iron caused to significantly increase in oil and protein in Rape seeds (25% in oil and 20% in protein) in compared with control.

In contrast the results reported in watermelon (Li et al., 2013) and Chinese mung bean (Hong-Xuan et al., 2011) showed that treatments with magnetite NPs of 9 and 18 nm, was not significantly effect on chlorophyll content compared to control.

Analysis of variance showed that plant height, length of main root, root volume and chlorophyll content in wheat plants were significantly influenced (at the probability level of 5% and 1%, respectively) by the applied treatments (Table 4).

Table 4. Analysis of variance for the physiological characteristics studied in wheat

Source of variance	FD	Height of plants		Length of main root		Root volume		Chlorophyll content	
		Mean square	F factor and significance	Mean square	F factor and significance	Mean square	F factor and significance	Mean square	F factor and significance
Treatments	2	1849	12.38**	1022.11	187.73***	0.0661	14.60**	46.77	60.14***
Error	4	149.33		5.44		0.0045		0.77	

Wheat plants treated with iron oxide solution had a smaller height of plants, by 13 mm compared to the untreated control, (Table 5).
The applied treatments had a positive effect on the root volume and the chlorophyll content, the values for the root volume being 0.54 cm^3 for the treatment with nanoparticles with iron oxides and 0.41 cm^3 for the treatment with hydroxyapatite compared to only 0.25 cm^3 for the control. The highest chlorophyll content was recorded in plants treated with hydroxyapatite nanoparticles (Table 5).
Similar results were obtained in lettuce plants treated with Fe/Fe3O4 NPs treatment, the chlorophyll content increased with respect to the control by 12% (Trujillo-Reyes et al., 2014).

Table 5. The effect of treatment with nHA and nIO on studied physiological traits

Variants	Height of plants		Length of main root		Root volume		Chlorophyll content	
	mm	Differences	mm	Differences	cm^3	Differences	SPAD units	Differences
Control	273	0	175	0	0.25	0	30	0
T1. Hydroxyapatite (nHA)	289	+16	180	+5	0.41	+16	37	+7
T2. Iron oxide (nIO)	260	-13	167	-8	0.54	+29	34	+4

The applied treatments had a very significant positive effect on dry matter content (Table 6).

Table 6. Analysis of variance for dry matter

Source of variance	FD	Mean square	F factor and significance
Treatments	2	0.292	19.58***
Error	4	0.014	

The analysis of the correlations showed that between the dry matter and the root volume there is a positive correlation (r = -0.98***, figure 4), and also with chlorophyll content (r = 0.75*, figure 5).
From our observations it was seen that there was a compensation of the reduction of the length by increasing the density of the root system, respectively the winter wheat plants treated with iron oxide had more adventitious roots.
This and the higher chlorophyll content may explain the positive effect of the treatments applied on the height of plants.

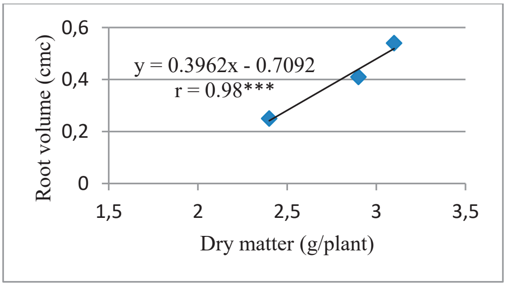

Figure 4. Relationship between dry matter and root volume

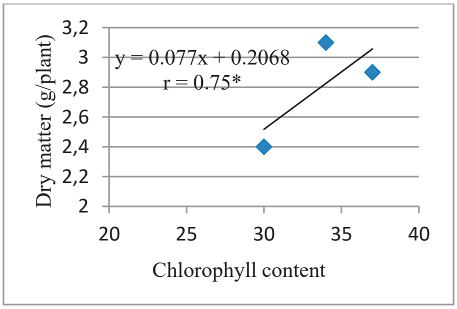

Figure 5. Relationship between dry matter and chlorophyll content

CONCLUSIONS

The results show that hydroxyapatite and irone oxide nanoparticle treatments had a positive effect on chlorophyll content of maize and winter wheat plants.

In the case of treatments with solutions of iron oxides nanoparticles, although negative effects were reported on the plant height and root length, a compensating effect was found by increasing root density. but also by higher chlorophyll content. This and also the higher chlorophyll content, led to a positive effect on height of maize and winter wheat plants.

The results suggested that use of treatments with hydroxyapatite and iron oxide nanoparticle in agriculture could be possible.

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GRAIN SIZE STABILITY OF A WINTER BARLEY GENOTYPES ASSORTMENT UNDER DIFFERENT SEED RATES

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Abstract

The grain uniformity of the malting barley has a decisive role in determining the malting quality and the process efficiency. One of the main technological factors which influence malting barley quality, including the grain size and the grain uniformity is the seed rate. Starting from these ideas, the objective of this study was to evaluate the response of different winter barley genotypes to different seed rates and to identify winter barley genotypes with high stability regarding seed size fractions as related with grain yield across two seed rates. In this respect, eighteen winter barley genotypes were tested for grain yield and size seed fractions for three consecutively years (2017, 2018, 2019) at National Agricultural Research and Development Institute Fundulea (NARDI Fundulea). In order to be used in brewing industry, the value of barley seeds is estimated based on their size, namely assortment (size of seed >2.8 mm and >2.5 mm/assortment I+II). The contribution from the average yield of each genotype, representing the percentage of seeds larger than 2.5 mm and 2.8 mm, was evaluated and the response of studied genotypes to the different seed rates were determined on the ANOVA and coefficient of variation basis. ANOVA showed that year (Y), genotype (G), year x genotype (Y x G), seed rate x genotype (S x G) and triple interaction year x seed rate x genotype (Y x S x G) significantly influenced the seed size >2.8 mm and 2.5 mm with a coefficient of variation of 6.47% and 31.7%, respectively. As source of variation, the seed rates (S) and interaction between year x seed rate (Y x S) was insignificant for both category seed size. The most winter barley genotypes had a positively response to the lower seed rate, both for yield and seed assortment, especially for seed size >2.8 mm.

Key words: *winter barley genotype, assortment, seed rate, grain size, yield.*

INTRODUCTION

Barley (*Hordeum vulgare* L.) has three main uses: feed, food, and malt production (Newton et al., 2011).

With more than 51 million ha harvested area in 2019 and with a production of about 159 million tons (FAOSTAT, 2019), barley is the fourth cereal crop worldwide.

A significant challenge for delivering grain with consistent yield and quality in the future is climate change due to the complex effects of atmospheric CO_2 and changing temperature and rainfall patterns on barley development (Nuttall et al., 2017). Multiple traits such as yield and quality of grains are routinely evaluated in barley breeding programs (Bhatta et al., 2020), but grain yield and its components are highly influenced by environmental factors and agronomic management practices (Khumalo, 2020).

In Romania, there is a reference document for malting barley (SR ISO 13477) and in the Grading Manual of Grains for Consumption (2017), three grades are mentioned based on minimum percent seeds larger than 2.5 mm, respectively: Grade I, Grade II, and Grade III, with minimum 85%, 75%, and respectively 70% seeds larger than 2.5 mm. The malt and beer industry use as raw material the seeds >2.5 mm (Petcu et al., 2019). For brewery industry, there will be used only barley grains which reach the standardized rules such as disease-free seeds, low protein content, plumpness of grains and none of the less seed uniformity (Brewing and Malting Barley Research Institute, 2010).

Grain size is an important quality parameter of malting barley, this being dependend on genotypes, environmental factors and their interactions (Stupar et al., 2017). However, this grain quality parameter has to be associated

with the grain content of starch and protein, the starch content having to be as high as possible, while the protein content having to be less than 11.5%, but no less than 9%. Also, it has to be considered that during the brewing process, starch is converting in malt extract and this is facilitated by uniform and plump grains and this conducts to an increase extract levels (Mather et al., 1997).
The size and uniformity of the grains have a decisive role in determining the level of malting process efficiency. Thick grains have a high content of extractable substances and a higher germination energy. A reduced uniformity of the grains translates into appreciable losses of raw material and low yields. The I+II grain assortment (percent of grains larger than 2.8 mm and 2.5 mm, respectively) constitutes an important index both for uniformity and size of the grains (Drăghici et al., 1975).
One of the main technological factors which influence malting barley quality, including the grain size is the seed rate, respectively the seed density. Wade & Froment (2003) had been found that seed rate influenced malting barley quality, respectively the grain size uniformity.
A study on relationship between malt quality and seed rates conducted by McKenzie et al. (2005) in southern Alberta revealed that increased seed rates from 150 to 350 viable seeds m^{-2} generally provided small yield gains, slight reductions in grain protein concentration and reduced grain size. As the sowing rate increases, it is expected that the grain size decreases.
O'Donovan et al. (2011) performed a field experiment during 4 years and stated that for CDC Copeland and AC Metcalfe barley varieties grain weight and plumpness were lower at the higher seeding rate (400 seeds/m^2) but seeding rate had no effect on grain yield. Another multi-location research was made to study the effect of seed date and seed rate on malting barley production in western Canada which showed that a 300 seed/m^2 maintained the yield and increased the grain size which improve the potential for higher economic returns for barley growers (O'Donovan et al., 2012; O'Donovan et al., 2009). Spaner et al. (2001) obtained similar results when barley density increased, the seed size (plumpness) being lower and grain size being significantly affected by seed rate. Paynter et al. (2013) indicated a specific seed rate which maximized the yield per ha and how influenced the phenotypic traits and grain quality (grain and hectoliter weight, protein content and seed size).
Grain yield represents the interaction between yield components and, at their turn, these interact with each other. The result can be an increase of one of them leading to a decrease of other, but it is possible to breed a character independently by other (Zhou et al., 2016). The yield capacity is an important trait that barley varieties must possess and express to a higher level to finally appreciate the agronomic value of the genotypes. A valuable barley genotype must have the capacity to grow in normal conditions of cultivation, having in the same time a higher adaptability when the climatic conditions are unfavorable with large variations of temperature and rainfall of a given region (Drăghici et al., 1975). One of the most important factors in maximizing crop yield is the high quality seed (Zareian et al., 2013). The optimal number of seed used for sowing has a significant impact in the final yield. Using a lower seed rate can cause an irregular and unbalanced uniformity and ripening of the grains due to extension of tillering and forming the first spikes in different periods of time. A higher plant density can determine lodging of the plants specially to genotypes who do not have lodging resistance (Drăghici et al., 1975).
In this respect, the objective of this study was to evaluate the response of different winter barley genotypes to different seed rates and to identify winter barley genotypes with high stability regarding seed size fractions as related with grain yield across two seed rates. It was intended to find out which is the seed rate that can maintain barley grain size to acceptable required levels for registered varieties, but also for promising breeding lines, and to compare the stability of their yield and the grain size.

MATERIALS AND METHODS

Eighteen winter barley genotypes (six-rows varieties and lines) developed at National Agricultural Research and Development Institute Fundulea (NARDI Fundulea) were sown in field conditions for 3 consecutively

years, respectively 2017, 2018, and 2019 under two different seed rates: lower seed rate using 350 seeds/m^2 (TS1 = technological sequence 1) and classical seed rate for barley in the specific growing conditions of Romania, with 500 seeds/m^2 (TS2 = technological sequence 2).
The researches were performed within Barley Breeding Experimental Field (BBEF) belonging to NARDI Fundulea, which is situated in South Romania, Fundulea town (44°33' Northern latitude and 24°10' Eastern longitude). The analysed genotypes were created at NARDI Fundulea and included eight winter barley varieties (Dana, Cardinal FD, Univers, Ametist, Smarald, Simbol, Onix, Lucian) and ten advanced lines (F8-2-12, F8-3-01, F8-3-12, F8-4-12, F8-5-12, F8-6-12, F8-10-12, F8-11-12, F8-5-13, F8-6-17).
Each seed rate was established every year for each variety and line based on their grain weight and the plot area. The harvested plots had 4.5 m^2 harvested area in three replications. After harvest, the grain yield (GY) was determined by weighing each replication and it was expressed in kg/ha at 14% moisture content.
To assess seeds size, one hundred grams of grains in three replicates were analysed with Sortimat machine which is designed for sieving based on plumpness of grains. After sieving within 3 different dimensions (seeds size >2.8 mm, seeds size >2.5 mm, seeds size >2.2 mm), the seeds were weighted using the electronic balance with 2 decimals and each seed category was reported as percent.
The obtained data were analysed with MSTAT C program. In order to evaluate and compare winter barley yield and seed size stability of the varieties and lines grown under different seed rates and climatic conditions, for each studied parameter (grain yield and the two seed size dimensions >2.8 and >2.5 mm) the standard deviation was calculated. A low standard deviation indicates that the values tend to be close to the mean of the studied barley genotypes sets, while a high standard deviation indicates that the values are spread out over a wider range.
The meteorological data were provided by a weather station located nearby experimental fields of NARDI Fundulea. The evolution of climatic data during barley growing period in the spring (period March - June) indicates different distribution of rainfall each month comparing with long term average (60 years). The lowest level of rainfall has registered in April and May, 2018 year, with a difference of 42.2 mm (March) and 27.5 mm (May) comparing with multiannual average (Figure 1). The exceeding of rainfall occurs in May, 2019 year, being registered almost double quantity of rainfall compared to the average of multiannual rainfall.
Temperatures tended to gradually grow each year in March from 3.3 to 9.3°C, comparing to multiannual average being higher with 4.6°C in 2019 and with 2.4°C in 2017 (Figure 2). In June, all average temperatures tended to grow, from 22.2 to 23.6ºC, in all the years the temperatures being higher than the multiannual average. In April and May, in 2018 there were registered the highest temperatures, while the temperatures in these months in the years 2017 and 2019 being closed to the multiannual average.

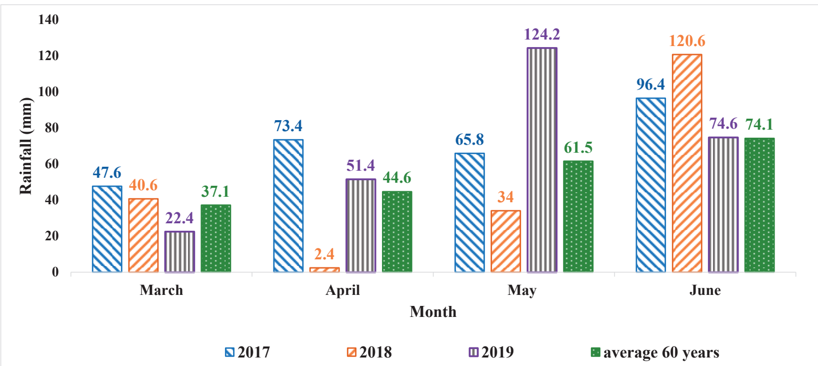

Figure 1. Average rainfall during barley growing period in the spring and multiannual average (1957-2017)

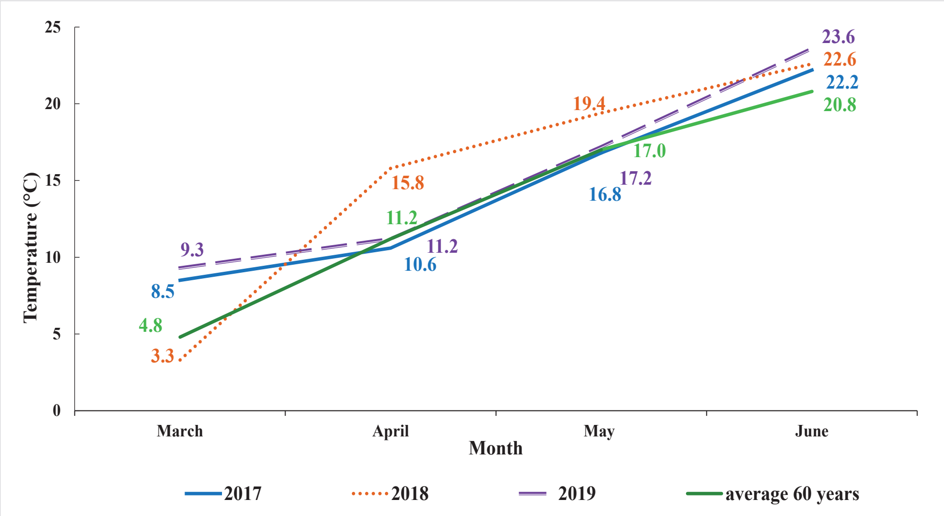

Figure 2. Average temperature during barley growing period in the spring and multiannual average (1957-2017)

RESULTS AND DISCUSSIONS

Analyze of variance showed that grain yield was influenced by seed rate, genotypes and their interaction with the climatic conditions of the year (Y x S and Y x G) (Table 1). The triple interaction Y x S x G was unsignificant for grain yield. As source of variation, year, genotypes, and the interactions Y x G, S x G and Y x S x G have significantly influenced on all three seed size, while seed rate and interaction between Y x S had an unsignificant influence of them.

The coefficient of variation registered the smallest values for the seed size >2.8 mm and for the grain yield, while the seed size categories >2.5 but especially >2.2 mm registered the highest values (Table 1).

Table 1. Analyse of variance for yield and seed size

No.	Source	Degree of freedom	F factor			
			Grain yield	Seed size >2.8 mm	Seed size >2.5 mm	Seed size >2.2 mm
1.	Year (Y)	2	1.8571 n.s	99.8767 **	85.9850 **	273.0696 **
2.	Seed rate (S)	1	15.9087 **	0.4268 n.s	0.8077 n.s	0.3806 n.s
3.	Y x S	2	9.0871 **	0.0829 n.s	1.6240 n.s	0.3752 n.s
4.	Error	10	-	-	-	-
5.	Genotypes (G)	17	5.2125 **	7.8963 **	16.8496 **	16.5117 **
6.	Y x G	34	5.0453 **	3.8809 **	7.4529 **	10.3322 **
7.	S x G	17	1.1044 n.s	2.2680 *	4.6245 **	2.1184 *
8.	Y x S x G	34	0.8270 n.s	2.4043 **	4.5593 **	1.6107 *
9.	Error	204	-	-	-	-
		CV%	10.47	6.47	31.77	45.52

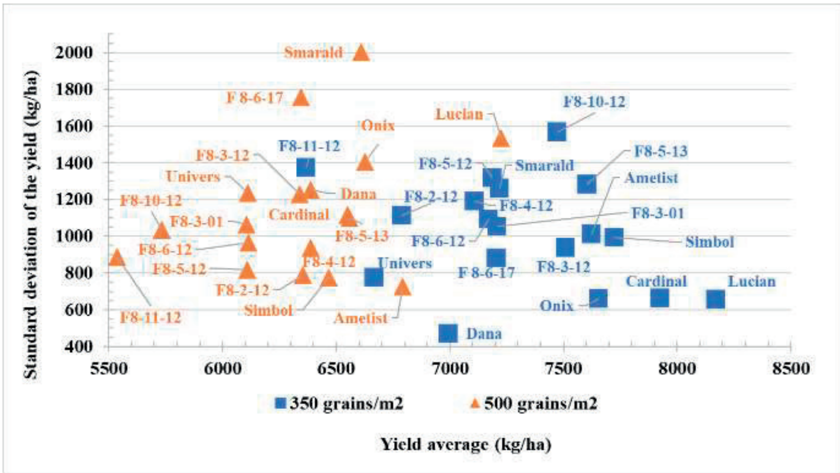

Figure 3. Yield results for TS1 and TS2 as average for 3 years

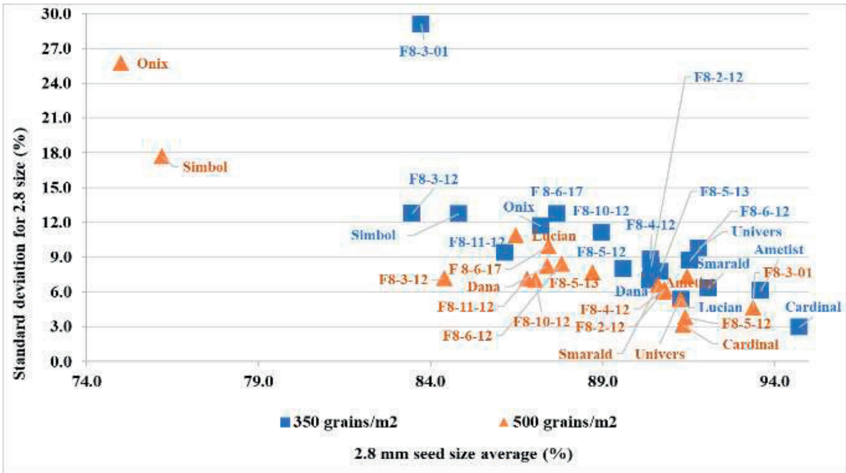

Figure 4. Grain size average (2.8 mm) and standard deviation (%)

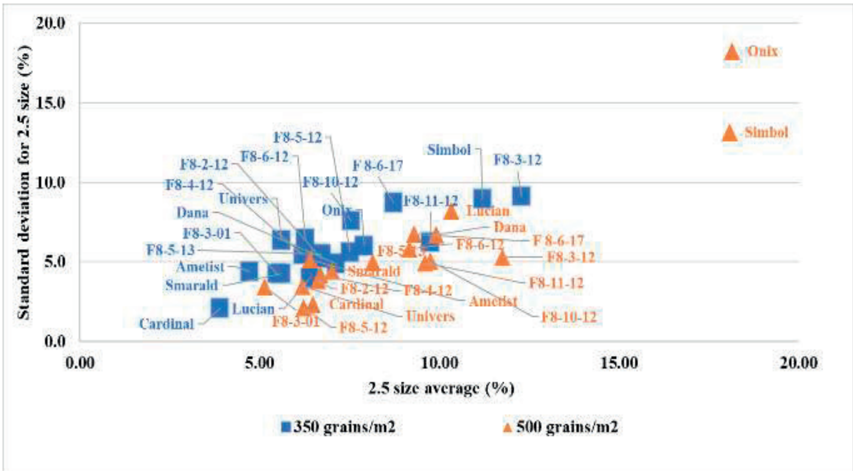

Figure 5. Grain size average (2.5 mm) and standard deviation (%)

In Figure 3, the tested winter barley genotypes are graphic represented by two different colours: blue colour for TS1 - 350 grains/m^2 and brown colour for TS2 - 500 grains/m^2. Average yield level (average of 3 years) was plotted against standard deviation of each barley genotype. Almost all genotypes had a good response regarding their yield potential under TS1 (350 grains/m^2) compared with the TS2 (500 grains/m^2) except the line F8-11-12 which registered the lowest yield and also was very instable, having a higher standard deviation value (almost 1400 kg/ha). The winter barley varieties Onix, Lucian and Cardinal with high yields had the best stability during the tested period under TS1 comparing with TS2, where only Lucian variety has registered a high yield but a smaller stability, and the varieties Simbol and Ametist has registered better stability (with lower yield standard deviation) but lower yields. The check variety, Dana, had the most stable yield, with lowest standard deviation (470 kg/ha) under TS1. The most instable genotypes under TS1 condition were the line F8-10-12 and Smarald variety for TS2 condition.

The analyze for obtained values for 2.8 mm seed size showed that genotypes responded differently to the seed rate (Figure 4). For seed size of 2.8 mm, values ranged between 75% (Onix variety - TS2) and 94.7% (Cardinal variety - TS1). The most stable genotype regarding this parameter was Cardinal with a standard deviation percent below 5% both for TS1 and TS2, the percent of plumpness grains being over 90% for both conditions. Regarding stability of seed size percent of seeds >2.8 mm can be remarked also other genotypes as Smarald, Univers, F8-5-12 under TS2 and Smarald, Ametist and Lucian under TS1 with values over 90%. The highest values of standard deviation have registered under TS2 in the case of Simbol and Onix varieties (17.7 and 25.7% respectively).

The analyze for obtained values for 2.5 mm seed size showed a different tendency of genotypes to the applied seed rate (Figure 5). In general, the grain size of 2.5 mm registered a standard deviation below 10% (TS1) while the same seed size has registered a standard deviation of 18.2% (TS2) and the values ranged between 3.9% (Cardinal - TS1) and 18.14% (Onix - TS2). The most stable genotypes regarding this parameter were also Lucian, Smarald and Ametist with a percent below 5% for TS1 and TS2 (except Lucian variety with 10.32% under TS2). Regarding stability of seed size percent of seeds >2.5 mm, it can be remarked Simbol and Onix varieties with a percent of seed size values over 18.07, respectively 18.14%. The highest values of standard deviation have registered under TS2 in the case of those varieties being 13.2 and 18.2%.

CONCLUSIONS

The response of winter barley genotypes was different for each analised parameter depending on used seed rate (classical density and lower density) and therefore it could be a key to successful and increased yield. The most winter barley genotypes had a positively response to the lower seed rate, both for grain yield and seed assortment, especially for seed size >2.8 mm. Choosing the right genotype adapted to the proper seed rate is one of technological sequence which can provide a high grain yield that meets required raw industry standards.

During the tested period, the most stable winter barley genotypes regarding grain yield level under TS1 (lower density) were Cardinal, Ametist, Simbol, Onix and Lucian varieties.

Developing regionally best management practices regarding seed rate will increase farmer's knowledge base and can be seen as a management tool for maximizing net return for farmers.

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SOYBEAN SEED SCANNING FOR SIZE, GENOTYPE COLOR AND *Cercospora blight* DETECTION

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Abstract

Soybean cultivation in Europe has increased his importance due to the protein demand. Therefore, the scaling of different changes in cultivation technology and cultivar choosing is concerning to extend the areas of soybean crop zone by overcoming the biotic and abiotic stress. A number of 30 soybean genotypes had been studied by scanning a batch of seeds with GrainScan software in order to assess the seed size, color variation and mottling degree of grains. Further laboratory tests for the assessment of resistance of low temperature has been performed to check the relations between color code and degree of cold resistance of soybean genotypes. The work procedure is a fast option and low cost analysis for a primary seed health assessment.

Key words: *soybean, GrainScan, seed scan, Cercospora blight, mottling, germination rate, low temperature, correlation.*

INTRODUCTION

In Europe the importance of soybean increased with the deficit of protein crops, estimated at 60-80% (Jerzak et al., 2020) and the increasing demand for the epoxidized soybean oil as a biobased and biodegradable polymer material. (Zych et al., 2021). 19 EU States signed the Europe Soya Declaration, a common agreement to reduce soybean imports (estimated at over 40 mil. tones) by cultivating non-GMO crops. (Dima, 2016; Bittner, 2019). Research works are following to extend the cultivated soybean area in colder climates like North of France and Poland (Jerzak et al., 2020; Boulch et al., 2021). Mature seeds of soybean cultivars vary in large colour groups of yellow type, green-type and black seed coat that reveals significant differences in composition of carotenoids components (Monma et al., 1994) and on common beans the microelements like Fe, Zn, Ca (Silva et al., 2012). Soybean seed mottling could have various causes from fungus and viruses pathogens. Often, purple colour of soybean grains is an indicator of purple seed stain (PSS) of soybean which is a major seed disease that cause grains value depreciation due to the purple discoloration and reduces vigor and quality. It is known that purple colour of soybean seeds is a light activated pigment named cercosporin and is produced by *Cercospora kikuchii* fungal pathogen.

Seed size is an important yield component, linked to the seedling vigour. On ideal moisture conditions larger size soybean seeds develops more vigorous seedlings with higher germination speeds (Pereira et al., 2013) although in the field conditions, planting methods and different conditions could advantage small seeds to obtain higher yields (Madanzi et al., 2010). In the research activity there is needed fast analysis methods for assessment and evaluation of different soybean cultivars. Seed dimensions also are correlated with vegetation period and therefore maturity group of the soybean cultivars (Greilhuber et al., 1997). Seed size measurement and description of colour and morphology is difficult to be performed by conventional methods due to the time and effort resources even for a small amount of samples. (Baek et al., 2020). To overcome this inconvenience and obtaining objective datasets about size, colour and seed shapes phenotypical analyses of grains using images was made for 30 soybean genotypes in order to find out the characters that could be used for soybean description and breeding process and finally emphasis and correlate with agronomical advantages of different genotypes.

MATERIALS AND METHODS

A total of 30 soybean genotypes were evaluated in this study, of which 10 are registered varieties and 20 are lines obtained in the soybean breeding program from NARDI Fundulea, Romania. (Table 1)

Field experiment was conducted at National Agricultural Research and Development Institute Fundulea, on a chambic chernozem soil in 2020 (44°26'39.07"N; 26°30'54.25"E). For all experiments, seeds were planted at a rate of 55 seeds/m^2. The field experiment was laid out in a Randomised Complete Block Design (RCBD). The plot size was 9 m^2. Planting date was at beginning of April (03 April). Applied crop technology was low input, without irrigation and additional nutrients. Two mechanical and two manual weeding works were performed after crop emerged.

Table 1. Breeding lines and registered varieties studied

No.	Soybean genotype	Maturity group
1	Safta F	0
2	F10-1443	0
3	F13-908	00
4	F13-993	00
5	Ilaria F	0
6	F13-1114	0
7	F13-1117	00
8	F13-1124	0
9	F13-1163	0
10	F13-1174	00
11	F14-878	00
12	F14-883	00
13	F14-892	0
14	F14-918	0
15	F14-924	0
16	F15-428	0
17	F15-749	0
18	F15-792	0
19	F15-828	0
20	F15-1026	0
21	Anduța F	0
22	Camelia F	0
23	Carla TD	000
24	Fabiana F	I
25	Flavia	00
26	Florina F	0
27	Larisa TD	0
28	Ovidiu F	0
29	Ricky	I
30	Teo TD	00

GrainScan software was used as low cost, and fast method for measurement of grain size and colour (Whan et al., 2014; Kinnikar et al., 2015). For capture the images of soybean genotypes studied a regular Canon MX390 PIXMA scanner was used. In order to avoid the seed shadows the equipment was covered by a black polyethylene sheet. A random number of soybean seeds from each genotype was scanned images files no bigger than 500 kilobytes. (Figure 1). After scanning and adjusting each file with the genotype name, the data has been generated with batch processing of files in separate .csv files. The class of values for size measures obtained are: area (mm^2), perimeter (mm), maximum length of the grain ellipse – noted with majellipse (mm) and the width – the minor axe of the best fit ellipse, noted as minellipse (mm). For the colour three values for red, green and blue codes was obtained.

Further, data editing was performed with Query instruments of MS Excel software (Figure 2).

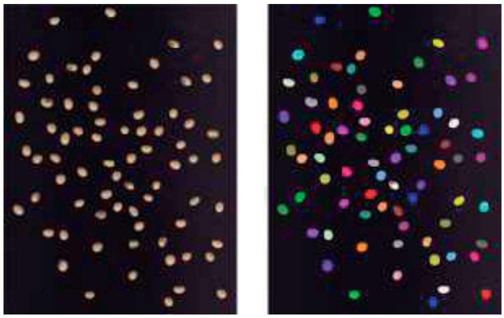

Figure 1. Scanned image of soybean seeds, variety Camelia F

Figure 2. Batch editing of .csv data at once from all images with Query instruments of MS Excel

A macro script was used to generate in a single cell each seed colour (Williams, 2015) (Figure 3).

```
Function myRGB(r, g, b)

    Dim clr As Long, src As Range, sht As String, f, v

    If IsEmpty(r) Or IsEmpty(g) Or IsEmpty(b) Then
        clr = vbWhite
    Else
        clr = RGB(r, g, b)
    End If

    Set src = Application.ThisCell
    sht = src.Parent.Name

    f = "Changeit(""" & sht & """,""" & _
                  src.Address(False, False) & """," & clr & ")"
    src.Parent.Evaluate f
    myRGB = ""
End Function

Sub ChangeIt(sht, c, clr As Long)
    ThisWorkbook.Sheets(sht).Range(c).Interior.Color = clr
End Sub
```

Figure 3. Macro script for combining three Red, Green, Blue values into a single cell colour, after Williams.

Number of spotted grains in red-brown and purple-black colours was counted from the scanned images and grains were analysed for purple seed stain (*Cercospora kukuchii*) and other fungal diseases by a classical method: seeds has been sterilized in a solution of 0.5% sodium hypochlorite (NaOCl) for 4 minutes and washed after with distilled water. Further was assured a potato dextrose agar environment and incubated at 25°C in the dark for 10 days. (Jordan et al., 1986). SPSS software was used for boxplots graphical representation of the seed dimensions results.

RESULTS AND DISCUSSIONS

The average daily maximum air temperature and total precipitation for each month during growing season from 2019 as compared with multiannual average are shown in Figure 4.
When plants were at the first growth stages in May, the amount of precipitation (124.4 mm) in 2019 was approximately 1.9 times that of multiannual average (62.5 mm). The average daily maximum air temperatures in May were 28.9˚C and 30.7˚C for MA, respectively.
When plants reached R5 or R6 growth stage in August, the total precipitation (12.6 mm) in 2019 was under normal of the zone (49.7 mm) and relative humidity was 60% in 2019 as compared cu 68% for MA. The average air temperatures in August were 35.2˚C and 35.3˚C for normal of the zone.
In conclusion, the year 2019 was characterized by abundant rainfall, high relative humidity and slightly lower temperatures in the first part of the soybean growing season, then from July was the drought until harvest.

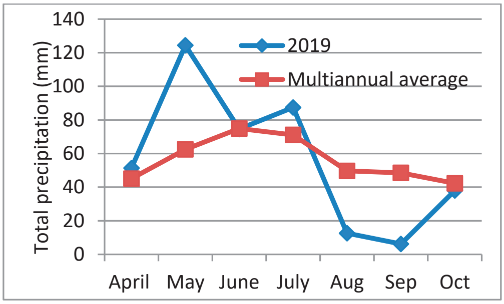

Figure 4.a. Total precipitation during soybean growing seasons

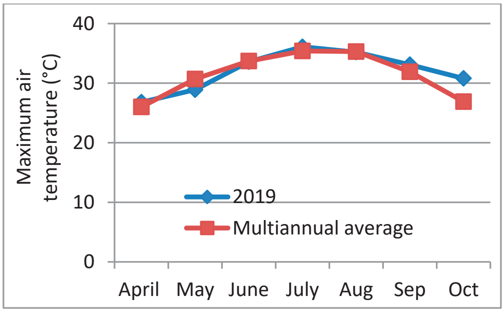

Figure 4.b. Maximum air temperature during soybean growing seasons

These conditions are favorable for *Cercospora* sp infection, which has been reported in the experimental field (Figure 5).

Figure 5. Aspect with soybean plants infested with *Cercospora sp*.

Soybean seed dimensions results and mottled grains
The seed dimensions resulted values from the image analysis: area (mm^2), perimeter (mm),

length of seeds as majellipse (mm), and wide of seed as minellipse (mm) are presented in this paper in boxplots graphics. The outlier values, that differs from the lower bound and higher bound of each soybean genotype are explained partially as errors due to the different spots found on grains. Purple-black and brown-red spots (Figure 6) influenced the dimension results, due to the grain recognition area of Grainscan software. Spotted genotypes seems to have a wider range that characterize their dimensions and more values that are very far from the median and close range (outliers). For further soybean and other grain software development it will be necessary to integrate dimension analysis with characteristic of particular symptoms that could change the seed colour.

An initial manual counting of mottled seeds have been performed (Figure 6) also in order to

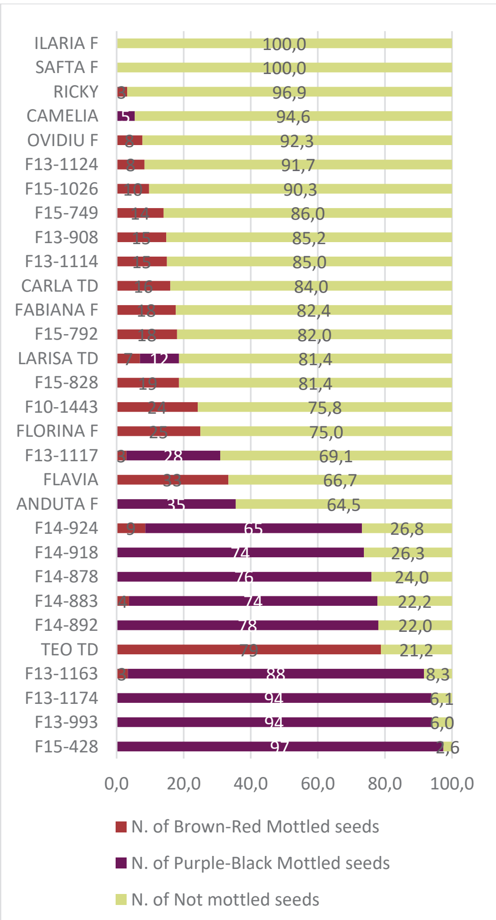

Figure 6. Percentage (%) of mottled soybean seeds of analysed soybean genotypes

Grains area

For grain area of soybean genotypes, analysed values range is spread from the lowest band of F15-792 (17.91 mm^2) to the highest band of upper limit of genotype Ilaria F (41.56 mm^2). Longer boxes of genotypes F13-1163, F13-1174, F13-993 although belongs to the susceptible group of very mottled seeds could also show that the breeding lines are heterogenous, as the seeds dimensions within the same genotypes differs. Scanned images from genotypes Ilaria F and Safta F didn't had mottled seeds. Therefore, their lower and higher bound limits (Ilaria F – between 37.11 and 41.56 and Safta F between 33.08 and 39.89) could be confidentially considered as reference values. Shorter boxes (Figure 7) of genotypes F15-749, F15-792 and their bottom position indicates that the grain area is smaller and seeds are uniform. Particularly, F15-426 bottom position in the graphic and larger large of values with many outliers is linked to the fact that the mottled seeds of this soybean breeding line was at 97.4%. (Figure 6).

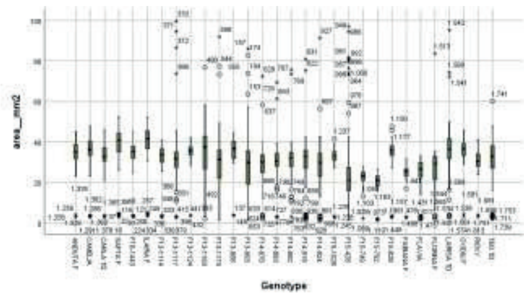

Figure 7. Area values of soybean grains (mm^2) of analysed genotypes

Grains perimeter

For grain perimeter of soybean genotypes, analysed values range is spread from the lowest bound of F14-883 (12.04 mm) to the highest bound of upper limit of genotype Anduta F (30.17 mm) (Figure 8). Large range of values for breeding line F13-993 outliers and spotted grains over 93.9% (Figure 6) place this genotype as the most heterogenous compared to others. Perimeter values for Anduta F, F13-114, F13-908, F15-792 and Fabiana F are closer to the median, that shows the uniformity of grains to this genotypes and a higher degree of heterogeneity for other studied lines.

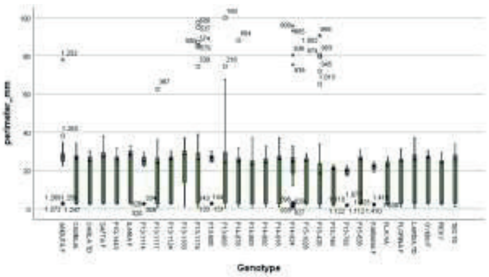

Figure 8. Perimeter values of soybean grains (mm) of analysed genotypes

Inner seed dimensions: Length and wideness of ellipse

Length and wideness values derived from the data of scanned images follow the same range pattern (Figure 9, Figure10), but as it was expected with lower values for the minimum of ellipse dimension (wideness). For both values genotypes F13-1174, F13-908, F13-993, F15-428, and Ovidiu F presented a high degree of heterogeneity, while other soybean grains values were less spanned.

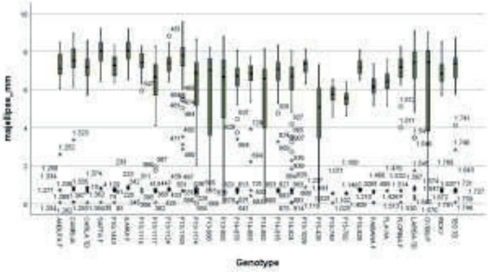

Figure 9. Length of seeds as majellipse (mm) values of soybean grains of analysed genotypes

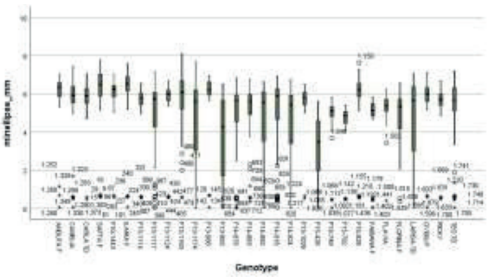

Figure 10. Wideness of seeds as minellipse (mm) values of soybean grains of analysed genotypes

In Table 2 are shown the mean RGB values and resulted color. As it was expected for genotypes Safta F and Ilaria F the color is close to natural because the scanned images did not show spots.

The color analysis also revealed purple color in case of the minimal mottled seeds of Ricky genotype but in genotypes Anduta F, Camelia, F13-114, F13-1124, F13-908, Florina F and Ovidiu F there are no perceptible color differentiations due to the frequency lower than 15% of mottled seeds.

Table 2. Mottled grains genotypes color generated by the mean of RGB codes

Genotype	grain ch1 code RED	grain ch2 code GREEN	grain ch3 code BLUE	Genotype color
ANDUTA F	185	156	146	
CAMELIA	183	156	133	
CARLA TD	148	136	774	
F10-1443	182	150	199	
F13-1114	185	153	141	
F13-1117	167	150	490	
F13-1124	189	159	128	
F13-1163	175	147	173	
F13-1174	174	154	187	
F13-908	191	161	128	
F13-993	164	161	366	
F14-878	172	142	354	
F14-883	172	158	394	
F14-892	172	160	412	
F14-918	174	152	274	
F14-924	181	197	294	
F15-1026	192	162	129	
F15-428	159	347	637	
F15-749	149	131	696	
F15-792	151	130	781	
F15-828	183	150	125	
FABIANA F	152	149	802	
FLAVIA	175	235	743	
FLORINA F	191	160	133	
ILARIA F	187	160	126	
LARISA TD	181	151	176	
OVIDIU F	192	158	126	
RICKY	159	129	604	
SAFTA F	185	158	124	
TEO TD	168	196	313	

Correlations between seeds color and germination, seedling weight and degree of soil cover

Seed germination is one of the important components of soybean seed quality.

In our study, there was a significant correlation between percent seed brown red colored and germination at low temperature, the correlation coefficient (r) was by 0.43 (significantly for P <0.1).

Some genotypes had high levels of coloration, but also had high seed germination (TEO TD, Flavia), while some genotypes had low levels of seed coloration, but had high percentages of seed germination (FD 15-792), (Figure 11).

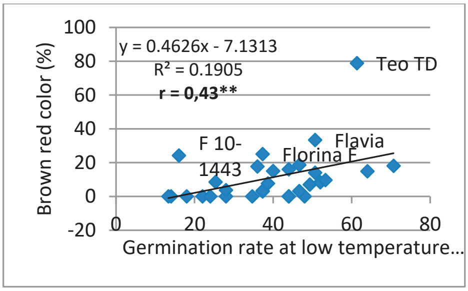

Figure 11. Correlation between germination rate and brown red color of seeds

There was a significant negative correlation between percent seed colored purple black (probably infected by Cercospora spp.) and germination (r = -0,46***), (Figure 12).

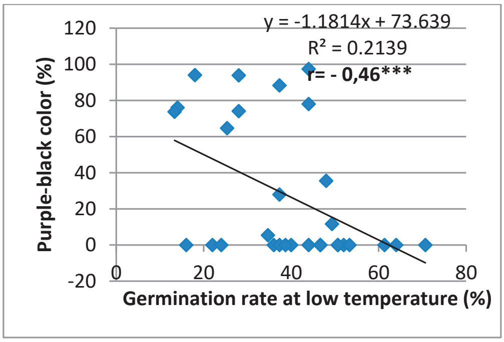

Figure 12. Correlation between germination rate and seeds purple black colored

A negative correlation was between seedling weight at low temperature and purple black color of seeds (Figure 13).

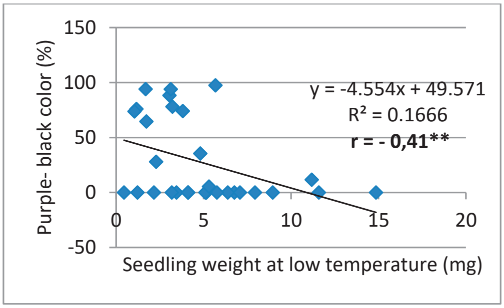

Figure 13. Correlation between seedling weight and purple black color of seeds

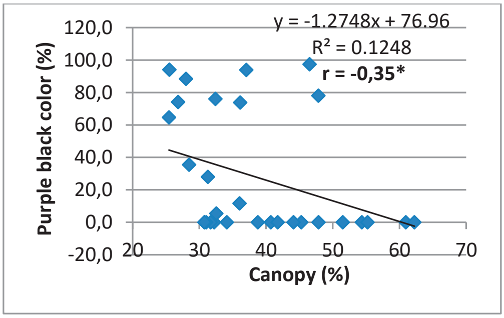

Figure 14. Correlation between degree of soil cover and purple black color of seeds

A negative correlation was between seedling weight at low temperature and purple black color of seeds (Figure 14).

CONCLUSIONS

There were differences in seed size and color among studied genotypes, that has been revealed by a simple scanner method and computer facilities.

Batch seed scanning for seed dimensions could be used as a tool for differentiate the uniformity and heterogeneity of different soybean breeding lines and as a fast method to detect the frequency above 15% of mottled seeds.

The correlations obtained shown that generally soybean genotypes more resistant to low temperature had low levels of coloration purple black which suggested probably low level of infection with *Cercosporea sp*.

Further research and algorithms are needed to fine tune the specific soybean grains color, obtain more precise dimensions of mottled seeds and diseases symptomatology.

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STUDY OF THE INFLUENCE OF YARA MINERAL FERTILIZER RODUCTS ON BIOMASS, PRODUCTIVITY AND QUALITY INDICATORS OF GRAIN FROM COMMON WINTER WHEAT

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Abstract

Three variants of fertilization were carried out at common winter wheat of Avenue variety on a total area of 9 ha. Pre-sowing application was performed with Yara Mila Triple (16% N, 16% P_2O_5, 16% K_2O) and double nitrogen feeding with Yara Vera Amidas (40% N, 14% SO3) and Yara Bela Sulfan (24% N, 15% SO_3, 11% CaO). The pre-sowing study of soil indicators showed that phosphorus can be blocked and more difficult to access for plants due to medium acid reaction of the soil. High levels of potassium have been reported, but additional mineral fertilization is still needed. The values of magnesium are very high, which requires saturating the soil solution with potassium ions until the normal ratio K: Mg of 3: 1 is reached. Very low sulphur content has a direct effect on nitrogen efficiency. The presented biomass maps show its increase after fertilization. Higher productivity, hectolitre weight and protein content in the grains were found for the harvest from field 1 (highest nitrogen fertilizer rate), which confirms the leading role of the macronutrient as a yield factor.

***Key words**: macronutrients, wheat, biomass, productivity.*

INTRODUCTION

Wheat is a main constituent part of the agrarian system of production, which provides the feeding of the world population and the functioning of the chains for production. The high yield potential, the diversity of possibilities for application and the great plasticity of wheat to the climatic and soil conditions carry as a consequence the fact that different technologies for production are practiced on a world scale. In Bulgaria is sown winter wheat, which is a constant constituent part of the grain-production, while in south Europe prevails the durum wheat, which is most frequently cultivated as a spring crop. The winter common wheat is most frequently based on higher investments of production amounts, but in any cases it realizes significantly higher incomes of yield.

As a matter of principle, for obtaining higher yields is required optimum feeding, this can be carried out only by the right reporting of the necessities. For this purpose the most important elements are those, which are expected to be extracted with the yield, as well as the nutrient reserves in the soil and their circle (Nikolova and etc., 2014). In comparison with the rest grain crops wheat is characterized by high requirements to the N quantity. Following namely the context of the production purpose, the nitrogen fertilization with the wheat, as well as its concrete “distribution in portions” is a significant factor for the reaching of the set yield and quality. A big part of the necessary N after blossoming is taken by the plants before blossoming and after this it is reutilized (DuPont&Altenbach, 2003; Xu et al., 2005). It deserves to be drawn attention that during the drought, which becomes more frequent, in the early summer months the fertilization in phase grain filling is with decreased impact. In similar abiotic stressful conditions is reduced namely the productiveness (Porter&Gawith, 1999), which gives us grounds to admit decrease of the usage efficiency of the applied N. The redistributed nitrogen in the plant tissues is an important source of N namely in phase grain filling in connection with the restricted intake of soil N because of the stress of drought (Dalling et al., 1975; Heitholt et al., 1990). The nitrogen feeding is an important factor for the good tillering and the creation of optimum sowing as per thickness (Ur&Vasileva, 2014). The researches show also reduction of the potential yield with high N levels with early application (Gooding&Davies, 1997; Maidl et al., 1998). The division of the fertilization with nitrogen in portions has for purpose to provide the wheat quantitatively with this macroelement according to its necessities in each phase of development, to synchronize the availability of soluble nitrogen in the soil and the necessities of the plant (Sticksel et al., 2000; Golba et al., 2013; Lopez-Bellido et al., 2005), and also to increase the efficiency of nitrogen usage. The feeding with nitrogen significantly influences the absolute content of protein, but the quality of the protein (the formation of gluten) and the sedimentation number are genetically and varietal conditioned (Uhlen et al., 2004). The thickness of the sowing, the number of the grains in the ear and the weight of the grain determine the yield (Desheva&Kachakova, 2013). The grain production is also character-rized by a narrow connection between the phosphorous feeding and the yield. The potassium feeding show restricted efficiency on the yield, but has impact on the qualitative level. Besides, there is determined a positive effect on the yield with the application of sufficient magnesium fertilizer norms. For high yield and especially on poor in sulphur, light soils, the feeding with sulphur is a promising factor for guaranteeing the raw protein content (Järvan et al., 2012). The experiments carried out show positive impact of the sulphur in the generating of proteins in the gluten, which on its behalf, has positive impact on the bread-making qualities of the wheat (Wieser et al., 2004).

The optimum storing with nutrient substances is a constant part of the successful wheat cultivation, based on the right zoning and directed to reaching the yield potential of the variety. Efficiency of fertilization mainly means that the used fertilizer for a given crop improves the yield and quality of the basic production, and also less quantity of nutrient substances are lost from the system soil-plants (Kostadinova, 2011). Yara Mila Triple is a balanced NPK 16/16/16 formula. Thanks to the good results as high yield and quality, the prime cost per decare becomes significantly lower in comparison with the traditional fertilizers. Yara Vera Amidas contains 40% total nitrogen, of which 35% is in amidic form, which has to transform in assimilable form for the plants – from ammonium in nitrate, and 14% sulphur. The granulated form of Yara Vera Amidas allows even distribution on the soil surface and thus the uneven assimilation by the crops is avoided. Yara Bela Sulfan is a complex nitrogen fertilizer for simultaneous application of three important nutrient elements – nitrogen (12% nitrate, 12% ammonium), sulphur (15%) and potassium (11%), which are completely soluble and provide optimum dynamics of the absorption.

The purpose of the study is research on the impact of Yara mineral fertilizer products on the biomass, productivity and qualitative indicators of grain of common winter wheat.

MATERIALS AND METHODS

Three variants of fertilization with common winter wheat variety Avenue are set on total area of 90 da. A pro-sowing bringing in of Yara Mila Triple (16%N, 16%P_2O_5, 16%K_2O) is carried out and double nitrogen feeding with Yara Vera Amidas (40%N, 14%SO_3) and Yara Bela Sulfan (24%N, 15%SO_3, 11%CaO). Variety common winter wheat Avenue is used (selection of company Limagrain). The sowing is done on 11 October 2018 with sowing norm 600 germinating seeds/m^2.

Within the frameworks of the experimental area is carried out a pro-sowing bringing in of Yara Mila Triple (16% N, 16% P_2O_5, 16% K_2O) – 25 kg/decare (4 kg/decare N active substance, a.s.). The distribution of the experimental variants, each of which with area of 30 decare, is as follows:

<u>Variant 1 (Field 1):</u>

First fertilization 09 March 2019: Yara Vera Amidas (40% N) – 20 kg/decare (8 kg/decare N a.s.);

Second fertilization 18 April 2019: Yara Bela Sulfan (24% N) – 25 kg/decare (6 kg/decare N a.s.).

Total active substance nitrogen for the whole period of vegetation: 18 kg/decare N

Variant 2 (Field 2):
First fertilization 09 March 2019: Yara Vera Amidas – 15 kg/decare (6 kg/decare N a.s.);
Second fertilization 18 April 2019: Yara Bela Sulfan – 25 kg/decare (6 kg/decare N a.s.).
Total active substance nitrogen for the whole period of vegetation: 16 kg/decare N
Variant 3 (Field 3):
First fertilization 09 March 2019: Yara Vera Amidas – 14 kg/decare (5.6 kg/decare N a.s.);
Second fertilization 18 April 2019: Yara Bela Sulfan – 20 kg/decare (4.8 kg/decare N a.s.).
Total active substance nitrogen for the whole period of vegetation: 14.4 kg/decare N
The methods for the wide-spectrum analysis of soil samples, taken before sowing, include analysis of P, K, Mg, S, Ca, B, Mo, Mn, Zn, Cu, Fe, Na, pH and exchange cationic capacity of the soil and are presented in Table 1.

Table 1. Standard analytical methods for examination of micro- and macronutrient elements in the soil, pH and exchange cationic capacity of the soil

Element	**Digestion Extractor**	**Analytical Technique**
Calcium	1 M ammonium nitrate	Atomic absorption or ICP
Magnesium	1 M ammonium nitrate	Atomic absorption or ICP
Manganese	1 M ammonium acetate with 2 gr/l quinoline	Atomic absorption or ICP
Boron	Hot water (80 °C)	Spectrophotometry of the solution after mixing with azomethine or ICP
Copper	0.05 M EDTA disodium salt	Atomic absorption or ICP
Molybdenum	Ammonium acetate (24,9 gr/l) + oxalic acid (12.6 gr/l)	Atomic absorption with dinitrogen oxide or ICP
Iron	0.05 M EDTA disodium salt	Atomic absorption or ICP
Zinc	0.05 M EDTA disodium salt	Atomic absorption or ICP
Cobalt	0.05 M EDTA disodium salt	Atomic absorption or ICP
Iodine	Hot water (80 ℃)	Ion-selective electrode
Phosphorus	OLSEN (sodium hydrogen-carbonate)	Spectrophotometry of the solution after mixing with ammonium molybdate
Potassium	1 M ammonium nitrate	Atomic emission spectrometry with flame
Sulphur	Calcium tetrahydrogen diortophosphate	Spectrophotometry of deposited barium sulphate solution
pH	Water	pH electrode
Organic matter	WALKEY BLACK METHOD – oxidation of organic matter calcium dichromate+ sulphuric acid; method of DUMAS	Spectrophotometry
Nitrogen	Decomposition to orthophosphorus acid; method of DUMAS	Method of Kjeldahl; CNS analyzer
Cationic exchange	It is washed by 1M ammonium acetate	Atomic absorption or ICP
$CaCO_3$ total	Hydrochloric acid concentrate	Capacity of the released CO_2
$CaCO_3$ active	Ammonium oxalate	Titration with potassium permanganate after adding of 5% sulphuric acid
EC el. conductivity	Water or calcium sulphate	Meter for conductivity
Nitrates	Water or calcium sulphate	Colorimetric analysis

The determining of the grain qualitative indicators includes analysis of: hectoliter weight (kg/hl), content of moisture (%), raw protein (%) and yield wet gluten (%) for each of the set variants. The qualitative indicators of the grain are carried out in Agroecological laboratory of Technical University - Varna. A NIR-analyzer, model: DA7200 NIR is used for the purpose.
A complete monitoring of the field is carried out during the vegetation of the wheat. The platform AtFarm is used for the purpose of a spectral analysis of satellite images in moments, when nitrate feeding is not carried out.

RESULTS AND DISCUSSIONS

The wide-spectrum analysis of the soil samples, taken before sowing, which include analysis of P, K, Mg, S, Ca, B, Mo, Mn, Zn, Cu, Fe, Na, pH, exchange cationic capacity of the soil and content of organic matter is presented in table 2.

Table 2. Values of a wide-spectrum soil analysis

Indicators	Result	Reference value	Inter-pretation	Comments
Calcium (ppm)	4459	1600	Normal	Normal level.
Magnesium (ppm)	371	50	High	(Index 6.0) Above the normal levels.
Manganese (ppm)	110	15	Normal	Normal level. During stress or periods of vigorous growth the spraying with YaraVita Gramitrel may be advisable.
Boron (ppm)	1.50	1.60	Slightly low	Low necessity with this crop.
Copper (ppm)	12.1	4.1	Normal	Normal level. During stress or in periods of vigorous growth the spraying with YaraVita Gramitrel may be advisable.
Molybdenum (ppm)	0.18	0.60	Very low	Low necessity with this crop. The next crop may experience deficiency.
Iron (ppm)	315	50	Normal	Normal level.
Zinc (ppm)	2.0	4.1	Very low	URGENT APPLICATION of 50 - 100 ml/decare YaraVita Zintrac. When the deficiency is very strong, repeat in 10-14 days.
Sulphur (ppm)	3	15	Very low	Review your soil fertilization with a representative of KBC Agro Bulgaria.
Phosphorus (ppm)	21	16	Normal	(Index 2.5) Normal level.
Potassium (ppm)	274	121	High	(Index 3.2) The magnesium assimilability may decrease.
Sodium (ppm)	60	90	Low	This is not a problem for this crop.
pH	5.9	6.5	Low	The acidic soil may decrease the assimilability of N, P, K, Mg, Ca and S. Liming is recommended.
Organic Matter (%)	3.6	2 – 4	Average stock	Normal level.
Exchange cationic capacity (meq/100g)	32.1	15.0	Normal	The cationic exchange capacity of the soil shows good ability for keeping nutrient elements.

The soil reaction is interpreted as medium acid (Table 2). For the development of the wheat this is not a problem, but this presumes that there is a danger the phosphorus to be blocked and to be more difficult for access for the plants. The phosphorus as an example, in triple superphosphate is calcium phosphate, this is a not accessible compound for the plant and barely 15 % of it is in a form, accessible for the crop for the current year. I.e. the literal recalculation of the active substances is not very correct (6.9 to 7.5% is the active form of phosphorus, with normal environment pH above 6.5). When there are unfavourable soil conditions we rely on formulas, in which the phosphorus is in long chains and is easily mobile, in order to be available and free for the plants before it is fixed. The passing of active elements, as they are in mineral fertilizer Yara Mila Triple (NPK 16/16/16) provide a good start for the plants and setting of maximum yield.

Potassium is the other macroelement, which has an important role for the normal crop development. In the sample are reported high levels, but despite this an additional mineral fertilization is necessary. The high levels of magnesium make impression. This element is secondary and the wheat necessities are less, and the levels in the soil are quite high. This requires the soil solution to be saturated with potassium ions. The normal ratio of K:Mg is 3:1, as an example if 240 mg/l soil is the norm for potassium, then 80 mg/l is the norm for the magnesium. This shows that the plants do not assimilate normally the available potassium. If each element is examined individually, the concrete levels shall be reported. Therefore it is more important the nutrient elements to be examined as one complete system. All elements create connections of antagonism or synergism among them. In this connection three out of the chemical elements, which are positively charged, are in high levels – potassium, calcium and magnesium. Thus are created conditions of competition and the plant cannot feed normally. The lack of sulphur and potassium as elements has direct impact on the efficiency of the nitrogen.

The levels of the organic matter (humus) are in normal boundaries.

Sodium shows us the salting degree of the areas, but it is not examined like a nutrient element in this situation.

The zinc levels are very low and the crop would show sensitiveness. The symptoms may be expressed in shortened internodes, small leaves, inter-nerve chlorosis.

We have determined in a parallel study for the same experimental fields, that the content of nitrogen, phosphorus and potassium assimilable forms in the soil after usage of Yara mineral fertilizers is increased in comparison with the control sample before setting of the experiment (Naskova et al., 2021). The trend of increase continues up to phase ear formation, as in phase after harvesting is observed decrease of the reserve, which is an adequate process on basis the wheat botanical characteristics and the vegetation phases. The best results are obtained with the variant fertilization of field 1 regarding ammonium nitrogen, field 3 – nitrate nitrogen, assimilable phosphorus and potassium – field 2.

The impact of the fertilization on the wheat productivity, however, is variable in a long period of time, because of strong yield dependency on the meteorological situation, in particular the precipitations (Toncheva et al., 2010; Koteva, 2012) and the soil fertility (Koteva, 1993). According to Filipov and Vasileva (2005), the wheat yields are influenced most by the nitrogen feeding, followed by the meteorological conditions and the variety. The nitrogen fertilization has a significant impact on the wheat productivity, leads to increase of the yields in comparison with variants without fertilization (Koteva, 2001; Yanchev et al., 2014; Panayotova, 2004). The following of the temperatures, precipitations, winds and snow cover for the present analysis has shown that the precipitations are the limiting factor for the wheat development and they are extremely insufficient as quantity.

The AtFarm platform is used for a complete monitoring of the field during the vegetation in moments when nitric feeding is not carried out. The observations for homogeneity of the field (united passing from phase into phase) are of extreme importance for the yield. All phases from sowing to harvest are followed by the AtFarm platform of Yara and are observed the differences in the development without them being visible at the very field. This allows to be taken into consideration the future feeding or another important activity.

Figures 1-5 are maps of the biomass, shot during the respective period in the active vegetation of the wheat. The rectangle is the whole block of 507 da – at the upper side one next to another as a united, merging line are the three fields x 30 da (or totally this contour is a strip of 90 da and is limited to the right by the forest belt). By the rest part of the rectangle is presented the 417 decare control.

The first nitrogen feeding of the experimental field in village of Gurkovo is carried out on 9 March 2019 with Yara Vera Amidas (40N+14SO_3), and the map of the biomass as of 24 March 2019 visually clearly shows the effect of the carried out activity on the experimental area – the strip of 90 da is coloured in light green to green colour, corresponding to average level of the biomass index, published for comparison below. Unlike it, the control is coloured in yellow-orange, brown in some places, which falls in the low segment of the biomass index (Figure 1).

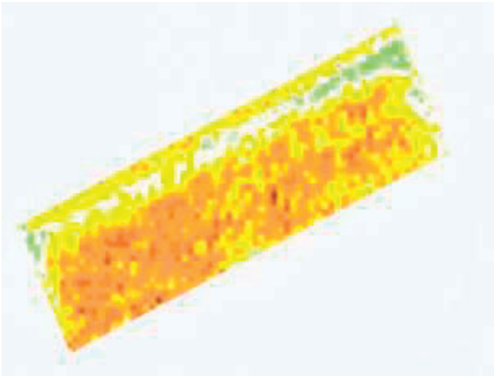

Figure 1. Map of the biomass as of 24 March 2019

The next map is documented on 28 April 2020 and clearly visualizes the nitric feeding with Yara Bela Sulfan (24N+15SO_3+11CaO) from 18 April 2020 (Figure 2). The experimental strip of 90 decare is coloured in green to shades of blue and begins to distinguish clearly in colour, following ascending the scale of biomass index, and in the control are noticed even spots in yellow, which clearly prove the nutrient deficiency of nitrogen, despite that until this moment of its development this part of the field is fertilized with 19.52 kg/decare active substance N.

Figure 2. Map of the biomass as of 28 April 2019

As of date 28 April 2020 more and more clearly is distinguished a difference in the advancing development of the plants from the fertilized plots with Yara fertilizers, which shading is already green to dark green. The control slowly begins to colour in light green.
On 3 May 2020 the plot of 90 decare is in shades of the green colour – a characteristic for a vigorous vegetation growth and it is clearly distinguished from the rest part of the field, in which light spots are still noticed (Figure 3).

Figure 3. Map of the biomass as of 3 May 2019

The last maps from 28 May and 2 June 2020 clearly show the differences in the two techniques in the assimilation of the nitrogen by the plants and the better, equalized vegetation growth, expressed by the index of the wheat biomass (Figures 4 and 5).

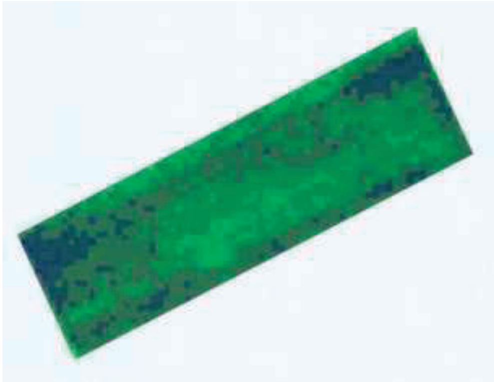

Figure 4. Map of the biomass as of 28 May 2019

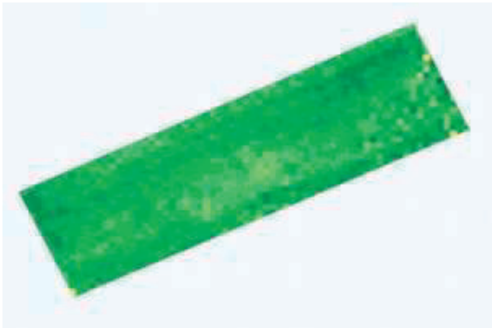

Figure 5. Map of the biomass as of 2 June 2019

In table 3 are presented the data for grain productivity and quality of wheat variety Avenue with the three variants of fertilization.

Table 3. Productivity and qualitative grain indicators

Indicators	Field 1	Field 2	Field 3
Yield, kg/decare	711	612	622
Moisture, %	10.5	10.8	11
Hectoliter weight, kg/hl	76.5	76.1	76
Protein, %	12.7	11.8	12.5
Wet gluten yield, %	25.8	24.4	25.5

The results of the productivity show that the yield from filed 1 is the highest in the carried out experiment (711 kg/decare), which is a result of the used higher nitric fertilizer norm (18 kg/decare N a.s.). The other two variants are with results similar between them – 612 kg/decare (field 2) and 622 kg/decare (field 3), as the differences in comparison with field 1 (respectively lower by 99 kg/decare and 89 kg/decare) are proven. Therefore there is present a statistically reliably higher productivity of the wheat plants in the present experiment with usage of the higher nitric fertilizer norm, which confirms the leading role of the macronutrient element as a factor of the yield. The differences in the yield between field 2 and 3 is only 10 kg/decare, as the same are not proven statistically. For comparison the obtained yield from the same field with usage of the same variety Avenue outside the carried out experiment with the used conventional scheme of fertilization is 596 kg/decare. The data about the grain moisture with the three variants indicate for similar values (10.5 – 11.0%). The hectoliter weight as an indicator of the milling properties of wheat provides the effective minimum of total yield flour with the wheat processing, which for the three groups as per quality (I, II and II B group) according to Bulgarian State Standard 602-87 is 76 kg/hl – a limiting value under which the efficiency of the technological process drops. In the present experiment the hectoliter weight of the grain is the highest with field 1 (76,5 kg/hl), but with the rest two fields the values of the indicator cover the required standard of 76 kg/hl. The results for content of protein indicate the same regularity – the grain collected from field 1 distinguishes with higher protein (12,7%). The value of the indicator with field 3 is close (12.5%), as both variants completely cover the base standard for bread grain. Only the content of protein is lower (11.8%) with field 2. The analysis of chemical technology of the grain shows presence of positive correlative connection between the content of protein and the wet gluten yield (WGY), which is absolutely reasonable, since both indicators are relevant to the proteins. The indicator wet gluten yield provides, on the one hand, the protein content and the nutrient value, and on the other hand guarantees respective quantity of gluten in the flours. The grain obtained from field 1 is with the highest wet gluten yield (25.8%), as it is a little bit lower with field 3 (25.5%), but the differences are insignificant. The results for the harvested grain from field 2 are lower (24.4%), but despite this they show satisfying values. At another our experiment (Naskova et al., 2019) the highest productivity in an experiment with wheat was determined with the variant with usage of liquid nitric fertilizer UAN, in comparison with unfertilized variants and variants, which are fertilized with granulated nitric fertilizers. According to a study of Horvat et al. (2006) the proteins content increases significantly by the application of 80 kg N ha^{-1}, 120 kg N $ha^{-}1$ and 160 kg N ha^{-1}. The index of gluten is increased only by application of 80 kg N ha^{-1}, while the highest levels of N (200 kg N ha^{-1} and 240 kg N ha^{-1}) show drastic weakening of gluten. Taking into account the solidity of the grain, only the treating by 80 kg N ha^{-1} has significant ($P <0,001$) impact. Simultaneously with this P and K do not influence the yield and the quality of the grain according to the authors' study.

CONCLUSIONS

The pro-sowing study of the soil indicators shows that phosphorus may be blocked and to be more difficult for access for the plants, because of the medium acid reaction of the soil. A high potassium level are reported, but despite this additional mineral fertilization is necessary. The magnesium values are very high, which imposes the soil solution to be saturated with potassium ions up to reaching the normal ratio of K:Mg at 3:1. The very low sulphur content renders direct impact on the nitrogen efficiency.

On the grounds of the obtained results may be affirmed that the fertilizer variants, suggested for testing, have exceptionally favourable impact on the soil reaction and the content of macroelements in the soil. The presented maps of the biomass show its growth after fertilization. Higher productivity, hectolitre weight and protein content in the grains are determined for the yield from field 1 (the highest nitric fertilizer norm), which confirms the leading role of the macronutrient element as a factor of the yield.

The data of the productivity in the carried out experiment show biggest values of the yield from field 1 (711 kg/decare), where the highest nitric fertilizer norm is used. The other two variants are with close results between them (611-622 kg/decare), but with statistically proven lower yield from field 1. The productivity is the lowest from the harvested wheat from the same field outside the carried out experiment (596 kg/decare), where a conventional scheme of fertilization is followed.

The analysis of the chemical technology of grain shows that the wheat production is with the best results, regarding the indicators hectoliter weight (76.5 kg/hl), protein (12.7%) and wet gluten yield (25.8%) as a result of fertilization with the higher nitric fertilizer norm in the experiment.

Therefore in the present experiment the variant with usage of the biggest nitrogen quantity active substance/decare distinguishes with the highest values for the yield quantities and quality of the obtained production.

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STARCH, PROTEIN AND LIPID CONTENT OF CERTAIN MAIZE HYBRIDS CULTIVATED IN DIFFERENT PEDOCLIMATE AREAS OF ROMANIA IN THE PERIOD 2018-2019

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Abstract

This paper aims to bring to the fore new data on the content of starch, protein and lipids in corn grains from 24 hybrids from different maturity groups, grown in period 2018-2019 in ten areas of our country, in different pedoclimatic conditions. The highest average obtained regarding the starch content in the two years of study (2018-2019) was 68,865% for the P9241 hybrid, and the lowest 66.315%% for the LG30315 hybrid, resulting in a variability of 2.55%. Although the maize hybrid EVO3617, obtained the highest average protein content in the two years of study, in terms of lipids, obtained the lowest content (3,920%). During the study period, it was found that tardy hybrids have grains with a higher protein content *compared early hybrids. To the 24 corn hybrids studied in the two years, the lipid content showed a variability of 0.475%, being the lowest compared to the variability of the starch and protein content.*

Key words*: corn, grain, starch, protein, lipids.*

INTRODUCTION

Romania has a very important role on the corn market considering the significant quantities it produces, being in full growth in terms of production and exports, reaching in 2017 the largest corn producer in the EU, with an estimated production of 13 million tons (Popescu et al., 2018).
Research on agricultural production in Romania reflects a continuing concern for ensuring the internal and external market of Romanian agricultural products (Toth and Cristea, 2018; Alexandru et al., 2018; Țeican et al., 2019; Chiriac et al., 2018; Arghiroiu and colab, 2018).
Over the the time, corn has proven to be a valuable plant both in terms of productivity and in terms of its economic importance, due to its multiple uses in human nutrition, in animal feeding and in industry.
This plant ranks third in importance among plants grown on Earth. This position is acquired through a series of peculiarities: it has a high production capacity, it has a great ecological plasticity, which allows a wide spreading area, it is a good precursor plant for most crops, it supports monoculture, it can be cultivated 100% mechanized, harvesting is done without shaking, capitalizes well on fertilizers and water (Chilba et al., 2019).
Over 50% of human caloric needs are provided by cereal products, their chemical composition establishing the specificity and value of each.
Corn grains are used in industry to obtain alcohol, starch, dextrin, glucose and other products (plastics, glue, acetone, dyes, etc.), and a good quality dietary oil is extracted from embryos to prevent the accumulation of cholesterol in the blood (Muntean et al., 2008).
In the case of corn, the chemical composition of the grains is very varied and can be influenced by: the pedoclimatic conditions, variety, variety and agrotechnics used, but it is similar to that of other cereals from a structural point of view.
The endosperm occupies the main part of the bean (80-84%), composed of cells with starch and protein storage tissue. The general appearance of the endosperm is presented in two variants: a corneous part and a floury one, predominating one or the other, depending on the variety.
Carbohydrates in corn represent about 80% of the grain, of which starch has the largest share. In addition to starch, there are 3% sugars and dextrins, 6% pentosans, 3% cellulose.
Proteins present between 9 and 13.5% (with variation limit between 8-14%), being

represented in a proportion of approx. 45% prolamine (zein), approx. 35% glutenin and approx. 20% globulin.
Zein is the main protein in corn kernels, which has a high content of glutamic acid and leucine, but a very low content of tryptophan and almost devoid of lysine. Over 73% of proteins are located in the endosperm (Ion, 2010).

MATERIALS AND METHODS

Our research aimed to at analyzing the chemical composition of corn on starch, protein and lipids in the 24 corn hybrids studied in 2018-2019, hybrids grown in different pedoclimatic zones of Romania (Figure 1).
The experiences were placed in ten locations, according to the randomized blocks method in 3 repetitions at a density of 70,000 plants / ha.
Harvesting was done manually, taking samples to determine: production, grain moisture at harvest, table of 1000 grains, hectoliter table and chemical composition (starch, protein, lipids, ash).

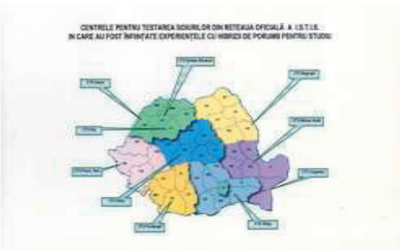

Figure 1. The locations where the research was conducted. Source: www.istis.ro

The determination of the protein, lipids and starch content was performed with the help of the INFRA NEO JUNIOR automatic analyzer having as operating principle the infrared scanning of the seeds of which the sample is constituted.
This paper is based on own data obtained through laboratory analysis and interpretation of data related to the study of corn hybrids in period 2018-2019.

RESULTS AND DISCUSSIONS

In the period 2018-2019, a number of 24 hybrids from different precocity groups were studied, cultivated in 10 areas of our country, in different pedoclimatic conditions.
During the cultivation of the studied hybrids, it was used as a precursor plant (peas), the plants were fertilized with ammonium nitrate at a dose of 200 kg / ha and and a unitary agrotechnics was used.
Similar research was carried out in the period 2003-2005 for a number of 21 maize hybrids from different maturity groups (FAO 300-400, FAO 200-300 and FAO 100-200), obtaining in the studied period an average of 69 % starch content, 4% protein and 12.4% lipids (Haș and colab., 2010).
Also, research on the variability of the chemical composition of grains in certain maize hybrids was performed and in the period 2009-2013, obtaining the following values: starch 69%, fat 5.2% and protein 11.3% (Haș and colab., 2019).
For 2018, the starch content varied between 65.9 and 69.4% for the 24 hybrids studied as shown in Table 1
The highest content was registered for the INVENTIVE hybrid with a starch percentage of 69.4% in the location in Cogealac, Constanta county, closely followed by the hybrid P9241 with a 69.3% starch percentage for the same location, Cogealac in Constanța county, but also by SY ORPHEUS hybrid with a starch percentage of 69.1% (Inand locality).
The EVO 3517 hybrid stood out with the lowest starch content, with 65.9% in the location from Dalga, Calarasi county.
Analyzing the averages obtained on the 10 locations where the experiences were located, the highest starch content was obtained by the hybrid P9241 (69.03%), and the lowest was recorded at the hybrid EVO 3517 (66.71%).
In 2019, the corn hybrid P9241 was noticed, registering the highest starch content, of 69.1% in the location from Cogealac, Constanta county, and at the opposite pole, the lowest starch content was registered by the hybrid EVO 3517, of 64.8% , in the location from Portaresti.
Analyzing the averages obtained on the 10 locations where the experimences were located, the lowest starch content was recorded by the hybrid EVO 3517, of 65.92%, and the highest percentage of starch, of 68.70%, the hybrid P9241.

Table 1. Starch content of analyzed hybrids, 2018-2019 (%)

Hybrid	Year	Starch (%)										Average
		Cogealac	Dâlga	Inand	Portărești	Peciu Nou	Mircea Vodă	Dej	Sibiu	Negrești	Simleul Silvaniei	
EVO 3517	2018	66.9	65.9↓	67.0	67.2	66.7	66.9	67.0	66.3	66.5	66.7	**66.71**
	2019	66.5	65.7	66.2	64.8↓	65.3	66.1	66.4	65.8	66.1	66.3	**65.92**
LG30315	2018	68.4	67.9	68.1	68.8	67.9	68.5	68.1	68.3	68.6	68.1	**68.27**
	2019	68.1	67.3	67.2	68.0	67.9	68.4	67.6	68.3	68.4	67.9	**67.91**
P9241	2018	69.3	68.7	69.2	69.1	69.0	69.2	69.0	69.2	68.7	68.9	**69.03**
	2019	69.1↑	68.3	68.0	68.4	69.0	68.7	69.0	69.2	68.7	68.6	**68.70**
INVENTIV E	2018	69.4↑	68.9	68.2	68.5	68.9	69.1	68.3	68.5	68.2	67.7	**68.57**
	2019	68.4	68.0	67.9	68.3	68.5	68.3	67.7	67.7	68.0	67.5	**68.03**
SY ORPHEUS	2018	68.9	68.5	69.1	68.5	68.2	67.2	68.3	67.8	68.1	68.3	**68.29**
	2019	68.5	67.8	68.3	67.9	67.4	68.2	68.4	67.6	67.8	68.0	**67.99**
TURDA 201	2018	67.9	68.0	68.2	67.9	67.6	68.0	67.2	67.9	67.6	68.1	**67.84**
	2019	67.8	68.0	68.2	67.8	68.1	68.3	67.9	67.7	67.6	67.8	**67.92**
FARADAI	2018	68.7	68.3	68.1	68.0	67.8	67.9	67.7	68.1	68.6	68.3	**68.15**
	2019	67.3	68.0	67.6	68.3	68.1	67.5	67.7	67.9	68.0	67.7	**67.81**
LG30369	2018	68.9	68.4	68.0	67.9	68.0	67.6	68.0	67.6	68.1	67.7	**68.02**
	2019	68.9	68.5	68.2	68.3	68.7	68.5	68.3	67.9	68.2	68.1	**68.36**
P9903	2018	67.9	68.1	68.2	67.6	67.5	67.9	67.5	67.6	67.9	68.0	**67.82**
	2019	68.5	68.2	68.4	68.3	68.6	67.6	67.7	68.2	68.4	68.2	**68.21**
EVO 3617	2018	68.7	68.5	67.8	67.7	67.8	67.2	67.9	67.5	68.2	67.6	**67.89**
	2019	68.6	68.9	68.5	68.3	68.5	68.6	68.2	67.9	68.2	67.9	**68.36**
OLT	2018	68.2	67.8	67.3	67.5	67.6	68.1	67.7	67.5	68.5	67.6	**67.78**
	2019	67.8	68.0	67.9	67.8	68.2	68.1	67.8	67.7	68.2	67.8	**67.93**
SENSOR	2018	67.8	68.0	68.0	67.8	67.6	67.7	67.9	67.5	68.5	67.4	**67.82**
	2019	68.2	67.9	68.3	67.7	67.9	68.0	67.9	67.7	68.1	67.3	**67.90**
LG 30389	2018	67.9	68.3	67.9	67.7	68.2	68.5	68.0	67.8	68.8	67.9	**68.10**
	2019	68.1	68.0	67.9	67.9	68.0	67.6	67.9	68.2	68.0	67.7	**67.93**
P9911	2018	68.3	67.8	67.7	67.8	68.0	68.6	67.5	67.3	68.3	68.0	**67.93**
	2019	67.9	68.1	68.0	67.9	68.1	68.2	67.9	68.1	68.0	67.9	**68.01**
ZEPHYR	2018	67.9	68.5	67.6	67.5	67.8	68.7	67.3	67.4	68.7	68.5	**67.99**
	2019	67.5	68.1	67.8	68.0	68.1	67.6	67.8	68.0	68.1	67.9	**67.89**
FUNDULE A 376	2018	67.9	67.8	67.5	67.9	67.6	68.1	67.9	67.5	68.0	67.9	**67.81**
	2019	67.9	68.0	67.7	67.8	68.0	68.1	67.8	67.8	68.1	67.8	**67.90**
LAGOON	2018	68.0	68.2	67.8	67.9	68.0	68.7	67.9	67.7	68.3	68.2	**68.07**
	2019	67.6	68.0	67.8	67.7	67.9	68.1	68.0	67.8	68.1	67.7	**67.87**
P 0412	2018	68.1	68.2	67.9	68.1	67.5	68.7	67.9	67.9	68.3	68.0	**68.06**
	2019	68.1	67.8	67.9	68.1	68.1	67.8	68.0	67.9	68.1	67.9	**67.97**
LG 31377	2018	68.2	68.0	67.7	67.9	67.5	68.0	67.7	67.8	68.5	67.9	**67.92**
	2019	67.9	68.0	67.7	67.9	68.0	68.3	67.8	68.1	68.1	67.7	**67.95**
DKC 5830	2018	68.3	68.0	67.7	67.5	68.0	68.0	67.6	67.9	68.1	68.0	**67.91**
	2019	67.9	68.3	68.3	67.7	67.9	68.2	67.8	67.9	68.3	68.0	**68.03**
P 0725	2018	68.0	68.4	67.8	67.6	68.0	68.2	67.9	67.8	68.4	68.0	**68.01**
	2019	68.1	67.9	68.0	67.8	68.2	68.3	67.7	67.8	68.0	67.9	**67.97**
LG 30500	2018	68.3	68.2	67.9	67.6	67.5	68.2	67.9	67.5	68.3	67.9	**67.93**
	2019	67.9	68.2	67.9	68.0	68.1	67.7	67.8	68.1	68.0	67.7	**67.94**
ZLATAN	2018	68.2	68.2	67.9	67.9	68.0	68.4	67.9	67.6	68.1	67.9	**68.01**
	2019	67.9	67.9	68.1	68.2	68.2	68.0	68.1	67.9	68.0	68.2	**68.05**
TOMASOV	2018	68.4	68.2	67.8	67.9	67.8	67.9	67.9	67.8	68.4	67.9	**68.00**
	2019	67.9	67.8	68.0	68.1	67.8	68.0	67.8	67.9	68.1	67.9	**67.93**

TURDA 201 and EVO 3617 hybrids obtained the highest protein content, respectively 11.23% in the locations in Cogealac and Mircea Voda, followed by the hybrid LG30369 with 11.21% in the location in Cogealac, while the lowest percentage, respectively 9.91%, at obtained the hybrid LG30315, in the location from Dej, Cluj county.

From the point of view of the average on the ten locations where the experiences were located, the highest protein content was obtained by the hybrid EVO 3617 (11,165%), and the lowest percentage was registered by the hybrid LG30315 (10,110%), as shown in Table 2.

Table 2. Protein content of analyzed hybrids, 2018-2019 (%)

Hybrid	Year	Protein (%)										Average
		Cogealac	Dâlga	Inand	Portărești	Peciu Nou	Mircea Vodă	Dej	Sibiu	Negrești	Simleul Silvaniei	
EVO 3517	2018	10.35	10.30	10.30	10.23	10.28	10.31	10.30	10.29	10.32	10.24	**10.292**
	2019	10.28	10.31	10.30	10.12	10.28	10.26	10.29	10.24	10.31	10.19	**10.258**
LG30315	2018	10.21	10.14	10.20	10.00	10.10	10.00	9.91↓	10.15	10.21	10.18	**10.110**
	2019	10.10	10.07	10.12	9.85↓	10.00	9.96	9.87	10.05	10.10	10.12	**10.024**
P9241	2018	10.81	10.70	10.83	10.75	10.82	10.83	10.81	10.88	10.93	10.82	**10.818**
	2019	10.76	10.69	10.81	10.72	10.79	10.83	10.80	10.86	10.89	10.82	**10.797**
INVENTIVE	2018	11.07	11.10	10.96	11.08	11.02	10.96	10.99	11.12	11.10	10.94	**11.034**
	2019	11.07	10.95	10.98	11.01	11.02	10.95	10.99	11.06	11.10	10.96	**11.009**
SY ORPHEUS	2018	11.10	11.00	10.77	10.95	10.99	11.09	10.94	11.09	11.01	10.99	**10.993**
	2019	11.02	11.00	10.87	10.90	10.85	11.00	10.91	11.03	10.94	10.98	**10.950**
TURDA 201	2018	11.23↑	11.15	11.10	11.12	11.10	11.00	11.02	11.12	11.15	11.10	**11.109**
	2019	11.21	11.12	11.08	10.99	11.18	10.98	10.99	11.12	11.10	11.10	**11.087**
FARADAI	2018	10.91	10.99	10.96	10.92	10.90	10.99	10.97	11.00	10.87	10.89	**10.940**
	2019	10.82	10.95	10.92	11.00	10.87	10.99	10.99	11.01	10.86	10.89	**10.930**
LG30369	2018	11.21	11.12	11.10	11.01	11.10	11.00	11.19	11.12	11.04	10.99	**11.088**
	2019	11.24↑	11.03	11.01	11.01	11.12	10.95	11.21	11.08	11.15	10.98	**11.078**
P9903	2018	11.19	11.17	11.00	11.08	11.11	11.18	11.07	11.12	11.14	11.11	**11.117**
	2019	11.12	11.08	11.00	11.12	11.15	11.09	11.12	11.06	11.16	11.14	**11.104**
EVO 3617	2018	11.20	11.00	11.14	11.21	11.16	11.23↑	11.08	11.22	11.21	11.20	**11.165**
	2019	11.24↑	10.99	11.12	11.20	11.08	11.21	11.10	11.27	11.20	11.21	**11.162**
OLT	2018	11.10	11.06	11.12	10.98	11.09	10.96	11.02	11.13	11.00	10.99	**11.045**
	2019	11.02	10.90	10.98	11.06	11.09	10.98	11.02	11.06	11.00	10.97	**11.008**
SENSOR	2018	10.89	10.93	10.89	11.00	11.09	10.92	10.92	10.90	10.89	10.95	**10.938**
	2019	10.87	10.91	10.89	10.93	10.95	10.90	10.92	10.99	10.88	10.89	**10.913**
LG 30389	2018	11.02	10.98	11.10	11.04	10.99	10.95	10.98	11.00	10.85	10.99	**10.990**
	2019	11.00	10.91	10.86	11.02	10.90	10.95	10.88	11.01	10.89	10.97	**10.939**
P9911	2018	11.08	10.94	10.98	10.92	11.09	10.87	11.10	11.06	10.97	10.99	**11.000**
	2019	10.95	11.00	10.99	11.00	11.01	11.10	11.04	10.89	11.04	10.95	**10.997**
ZEPHYR	2018	11.00	10.98	11.10	11.06	11.00	10.99	11.10	11.12	11.01	10.98	**11.034**
	2019	11.12	10.87	11.22	11.10	10.95	10.96	11.09	10.87	11.05	11.00	**11.030**
FUNDULEA 376	2018	11.07	10.99	11.12	11.00	11.10	11.02	11.12	11.06	10.98	10.97	**11.043**
	2019	10.92	11.00	10.91	11.10	11.00	10.97	11.10	11.08	11.00	11.01	**11.090**
LAGOON	2018	11.05	11.00	10.99	11.00	11.09	10.90	11.09	11.11	11.05	10.99	**11.027**
	2019	11.03	10.97	11.02	11.12	11.15	10.99	11.10	11.10	11.00	11.03	**11.051**
P 0412	2018	11.08	10.99	10.90	11.06	11.11	10.90	11.09	11.12	11.09	10.89	**11.023**
	2019	11.02	10.95	11.02	11.09	11.10	10.97	10.89	11.00	10.99	11.00	**11.003**
LG 31377	2018	11.08	10.90	10.99	11.12	11.15	10.98	11.06	11.10	11.00	10.95	**11.033**
	2019	11.12	11.02	11.05	11.06	11.10	11.11	11.09	11.10	11.03	11.05	**11.073**
DKC 5830	2018	11.10	10.90	11.08	11.06	11.09	10.90	10.92	11.06	11.09	10.91	**11.011**
	2019	11.10	10.78	10.81	11.09	11.00	10.92	11.01	11.06	10.98	10.87	**10.962**
P 0725	2018	11.10	10.98	10.98	11.12	11.09	10.87	11.11	11.06	11.01	10.97	**11.029**
	2019	11.08	11.07	11.00	11.12	11.18	10.98	11.02	11.06	11.00	10.97	**11.048**
LG 30500	2018	11.09	10.91	10.98	11.12	11.16	10.98	11.05	11.10	11.00	10.98	**11.037**
	2019	11.10	11.03	11.05	10.95	11.00	10.96	11.15	11.10	11.07	10.99	**11.040**
ZLATAN	2018	11.10	10.93	10.98	11.12	10.91	10.98	11.02	11.10	10.93	10.99	**11.006**
	2019	11.08	11.03	11.10	11.12	11.18	11.02	11.00	11.13	11.00	10.99	**11.065**
TOMASOV	2018	11.09	10.90	11.12	11.06	11.10	10.98	11.00	11.13	11.09	10.99	**11.046**
	2019	11.12	11.10	11.15	11.09	11.16	11.08	11.12	11.14	11.11	11.18	**11.125**

In 2019, the hybrids LG30369 and EVO 3617, respectively 11.24%, both located in Cogealac, obtained the highest protein content, and the lowest percentage of protein, respectively 9.85%, was obtained by the hybrid LG30315, located in Portaresti .

From the point of view of the average on the ten locations where the experiences were located, the highest protein content was obtained by the hybrid EVO 3617 (11,162%), and the lowest percentage was registered by the whole hybrid LG30315 (10,024%).

From the point of view of the average on the ten locations where the experiences were located, the highest protein content was obtained by the hybrid EVO 3617 (11,162%), and the lowest percentage was registered by hybrid LG30315 (10,024%).

The highest lipid content was registered in 2018, respectively 4.6% for the SY ORPHEUS hybrid in the location in Cogealac and the SENSOR hybrid in two locations out of the ten, respectively Dalga and Mircea Voda. The lowest lipid content was registered for EVO 3517 and ZEPHYR hybrids, respectively 3.7% in the locations from Negresti and Simleul Silvaniei, as shown in Table 3.

From the point of view of the average on the ten locations where the experiences were located, the highest lipid content was registered in 2018 for the hybrid P9241 (4.27%), and the lowest was registered for the hybrid EVO 3617 (3.94%).

In 2019, with the highest lipid content was the SENSOR hybrid, with a percentage of 4.6% in the location in Mircea Voda, and the lowest lipid content was registered in the SY ORPHEUS hybrid, respectively 3.6% in the location in Negresti.

From the point of view of the average on the ten locations where the experiences were located, the highest lipid content registered at the SENSOR hybrid (4.37%), and the lowest was registered for the EVO 3617 hybrid (3.90%)

Table 3. Lipid content of analyzed hybrids, 2018-2019 (%)

Hybrid	Year	Lipid (%)										Average
		Cogealac	Dâlga	Inand	Portărești	Peciu Nou	Mircea Vodă	Dej	Sibiu	Negrești	Simleul Silvaniei	
EVO 3517	2018	4.3	4.1	3.9	4.1	4.3	4.3	4.1	4.2	4.5	4.4	**4.22**
	2019	4.2	4.0	3.7	4.1	4.3	4.0	3.9	4.2	4.3	4.0	**4.07**
LG30315	2018	3.9	4.1	3.9	4.0	4.2	4.1	3.9	4.3	4.2	4.2	**4.08**
	2019	3.7	3.9	3.7	4.0	4.1	3.8	3.9	4.3	4.1	4.3	**3.98**
P9241	2018	4.4	4.2	4.1	4.3	4.2	4.2	4.3	4.4	4.2	4.4	**4.27**
	2019	4.3	4.1	4.1	4.4	4.2	4.1	4.3	4.1	4.2	4.2	**4.20**
INVENTIVE	2018	4.3	4.1	4.4	4.2	4.3	4.0	4.1	4.0	4.2	4.2	**4.18**
	2019	4.0	4.2	4.2	4.4	4.3	4.0	4.3	4.0	3.9	4.1	**4.14**
SY ORPHEUS	2018	4.6↑	4.2	3.9	4.0	4.1	4.5	4.0	4.2	4.5	4.2	**4.22**
	2019	4.4	4.2	3.8	4.1	3.7	4.2	4.3	4.0	3.6↓	3.9	**4.02**
TURDA 201	2018	4.4	4.2	4.0	4.0	4.2	4.1	4.3	4.0	4.0	4.1	**4.13**
	2019	4.3	4.2	4.1	4.0	4.3	4.2	4.3	4.1	4.2	4.1	**4.18**
FARADAI	2018	3.9	4.1	4.2	3.9	4.2	4.1	4.0	3.8	3.9	4.1	**4.02**
	2019	3.9	4.2	4.0	3.8	4.3	4.0	4.2	4.1	3.9	4.3	**4.07**
LG30369	2018	3.8	4.0	3.9	4.1	4.2	4.0	3.8	4.0	3.8	3.9	**3.95**
	2019	3.8	4.0	4.3	4.1	4.3	4.0	3.9	4.0	3.8	4.0	**4.02**
P9903	2018	4.0	4.1	3.8	3.8	4.1	4.0	4.2	3.9	4.1	4.0	**4.00**
	2019	3.9	4.2	4.0	3.8	4.2	4.0	4.4	3.9	3.9	4.0	**4.03**
EVO 3617	2018	3.8	4.0	3.9	3.9	4.0	4.1	4.0	3.9	3.7↓	4.1	**3.94**
	2019	3.7	4.0	3.9	3.8	4.1	3.9	4.0	3.7	3.9	4.0	**3.90**
OLT	2018	4.0	4.2	4.2	4.3	4.1	3.9	4.1	4.1	3.9	3.8	**4.06**
	2019	4.2	4.3	4.2	4.3	4.3	4.1	4.3	4.1	3.9	4.0	**4.17**
SENSOR	2018	4.4	4.6↑	4.4	4.3	4.3	4.6↑	4.5	4.3	4.3	4.5	**4.42**
	2019	4.5	4.3	4.2	4.5	4.3	4.6↑	4.4	4.2	4.3	4.4	**4.37**
LG 30389	2018	4.0	4.4	4.1	4.0	3.9	4.0	3.8	4.1	3.9	4.1	**4.03**
	2019	4.1	4.3	4.0	3.8	3.9	4.0	4.2	4.1	3.8	3.9	**4.01**
P9911	2018	4.0	4.3	4.1	4.3	4.2	3.9	4.2	4.1	4.0	4.1	**4.12**
	2019	3.9	4.1	4.0	3.9	4.2	4.0	4.1	3.8	4.1	4.0	**4.01**
ZEPHYR	2018	4.2	4.1	3.9	4.3	4.2	3.8	4.3	4.2	3.9	3.7↓	**4.06**
	2019	3.9	4.2	4.0	4.1	3.8	4.0	4.1	4.1	3.9	4.1	**4.02**
FUNDULEA 376	2018	4.0	4.3	4.1	3.9	4.1	4.1	3.8	4.0	4.2	4.3	**4.08**
	2019	4.4	4.2	4.1	4.3	4.2	4.3	4.2	4.0	4.1	4.0	**4.18**

LAGOON	2018	3.9	4.1	4.2	4.0	3.8	4.0	3.9	4.1	4.2	4.0	**4.02**
	2019	3.9	4.3	4.0	3.8	3.8	4.2	3.8	4.1	3.9	4.1	**3.99**
P 0412	2018	4.0	4.3	4.2	3.9	4.0	4.1	3.9	3.8	3.9	4.2	**4.03**
	2019	4.1	4.2	4.1	4.1	4.3	4.0	4.3	3.9	4.0	4.1	**4.11**
LG 31377	2018	3.8	4.4	4.3	3.9	4.0	4.1	4.0	3.9	3.9	4.2	**4.05**
	2019	4.0	4.1	4.2	4.1	4.0	3.9	4.0	3.8	3.9	3.8	**3.98**
DKC 5830	2018	3.9	4.3	4.0	4.1	4.0	4.4	4.3	4.0	4.2	4.1	**4.13**
	2019	4.0	3.8	4.1	4.0	3.9	3.9	4.0	4.0	4.2	4.1	**4.00**
P 0725	2018	3.9	4.3	4.2	4.0	4.0	4.3	4.0	3.8	3.9	4.1	**4.05**
	2019	3.7	4.0	4.1	4.0	3.8	4.2	4.0	4.1	4.2	4.3	**4.04**
LG 30500	2018	3.8	4.3	4.2	3.9	4.0	4.1	3.9	4.0	3.9	4.1	**4.02**
	2019	4.0	3.9	4.1	4.3	4.2	4.3	4.0	3.9	4.1	4.3	**4.11**
ZLATAN	2018	3.9	4.3	4.2	3.8	4.3	4.0	3.9	4.1	4.0	4.2	**4.07**
	2019	3.8	4.0	3.8	3.9	3.9	4.1	4.0	3.9	4.1	4.2	**3.97**
TOMASOV	2018	3.9	4.3	4.0	4.1	4.0	4.4	4.1	4.1	3.8	4.2	**4.09**
	2019	4.3	4.2	4.0	4.1	4.2	4.1	4.2	4.0	4.1	4.0	**4.12**

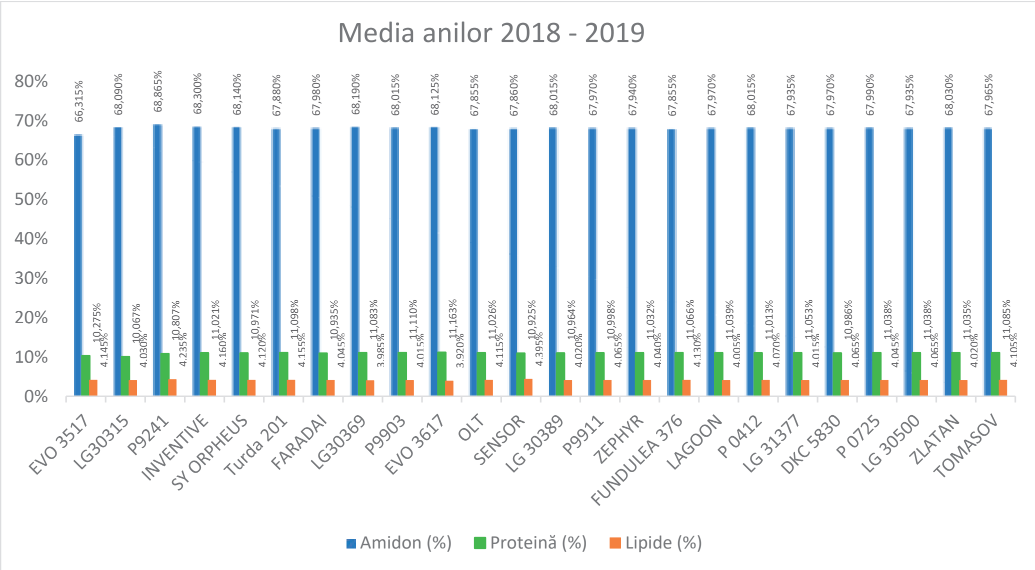

Figure 2. Average of the two years of study regarding the content of starch, protein and lipids in the studied maize hybrids

The highest average regarding the starch content in the studied hybrids, in the period 2018-2019, was registered at the hybrid P9241, respectively 68,865%, followed by the hybrid INVENTIVE with 68.3% and the hybrid LG30369 with 68.19%, and the lowest average of was registered to the hybrid EVO3517, respectively 66.315%.

In terms of protein content, the EVO 3617 hybrid stood out, recording the highest average of the two years of study, respectively 11,163%, followed by a difference of 0.053% by the hybrid P9903, and the lowest average for the two years of study. was obtained by the hybrid LG30315, of 10,067%.

The highest average lipid content in the two years of study was 4,395%, obtained by the SENSOR hybrid, followed by the INVENTIVE hybrid (4,160%) and the TURDA 201 hybrid (4,155%), and the lowest average lipid content in the two years of study it was 3,920% related to the hybrid EVO 3617 (Figure 2).

CONCLUSIONS

The highest average obtained starch content in the two years of study (2018-2019) was 68,865% for the P9241 hybrid, and the lowest 66.315%% for the LG30315 hybrid, resulting in a variability of 2.55%.

Analyzing the average on the ten locations where the experiences were located, it was found that the hybrid EVO 3617 obtained in the two years of study, the highest protein content, respectively 11,163% (11.23% in 2018 and 11.24% in 2019), and the lowest average was obtained by the hybrid LG30315, respectively 10,067, the variability being 1,096%.

Although the maize hybrid EVO3617 obtained the highest average protein content in the two years of knowledge, it obtained the lowest average lipid content (3,920%).

To the 24 corn hybrids studied in the two years, the lipid content showed a variability of 0.475%, being the lowest compared to the variability of starch and protein content.

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STUDY OF THE INFLUENCE OF PRE-SOWING ELECTROMAGNETIC TREATMENTS ON THE PROPAGATING QUALITIES OF BEAN SEEDS AFTER NATURAL AGING

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Abstract

Research has been carried out on seeds of the Bulgarian variety "Obraztsov Chiflik 12". The seeds are divided into two groups. They are stored in conditions of natural aging. The first group of seeds remained for 5 years, and the second group for 6 years until the date of the study. It was found that the pre-sowing electromagnetic treatment stimulated the laboratory germination, the lengths of the sprouts and the accumulated dry mass of the plants, which sprouted from the seeds that remained for 6 years. After a stay of the seeds for 5 years and performed pre-sowing electromagnetic treatment, an increase in the lengths of the roots and sprouts was achieved, respectively to 121.20% and 135.43% compared to the remaining untreated seeds. The regime parameters of the pre-sowing electromagnetic treatments, which stimulate the sowing qualities of old seeds, have been established. The results of the research show that the pre-sowing electromagnetic treatments have a stimulating effect on the sowing qualities of bean seeds, which have been stored for a long period.

***Key words**: bean seeds, pre-sowing electromagnetic treatments, electromagnetic field effect.*

INTRODUCTION

Among the legumes grown in Bulgaria, beans are of the highest economic significance. This is essentially due to the high nutritional value of the seeds used as protein food. The amount of protein contained in beans varies from 20% to 30%, depending on the variety and the conditions of growing. Beans are a major plant source of protein in human nutritional balance. In addition to proteins, beans contain carbohydrates - 62%, small amounts of fatty acids - about 1.8%, and mineral salts - 9.9% in average. Nowadays, different cultivation approaches are applied to increase the yields of agricultural crops. Such methods are fertilization with mineral and foliar fertilizers (Dobreva et al., 2018), intensive irrigation (Saldzhiev et al., 2014), rotation of crops, etc.

In terms of crop rotation, the use of legumes is increasingly brought into focus. Beans are a good forerunner of almost any agricultural crop. They are a particularly appropriate forerunner of cereal grains. This is due to the fact that beans are a source of biological nitrogen readily available to plants. Under the conditions of increasing rates of nitrogen fertilization of the soil, legumes have an important and mandatory role for increasing yields in the system of organic farming (Saldzhiev & Muhova, 2013).

For a number of reasons, including the ongoing climate change, it is not always possible to provide quality seeds for the next production year. In such cases it becomes necessary to use old seeds, i.e. ones that are stored under the conditions of natural aging. Such seeds have reduced biological properties, and need activation.

The rate of natural aging of seeds is determined by genetics and is phenotypically plastic, i.e. it reflects the specificity of the species, variety and production conditions (Реймерс, 1979; Фурса, 1974).

It has been established (Powell & Mattewa, 1984) that the mechanism and the biochemical nature of the aging processes of seeds, stored under normal or extreme conditions, are unidirectional.

A number of studies have shown that seeds can be stimulated in different ways before sowing. This helps to improve the qualitative composition of the seeds, which is a prerequisite for higher yields.

In Spain, for example, (Flórez et al., 2014) and (Martinez et al., 2017) conducted studies on the pre-sowing magnetic treatment of triticale seeds. In Bulgaria, on the other hand, the effect of electromagnetic pre-sowing treatments of triticale seeds (Muhova et al., 2016) on certain laboratory parameters and on the germination energy and laboratory germination capacity (Sirakov, 2006; Sirakov et al., 2016) was studied. Studies in both countries have shown the positive effects of magnetic and electromagnetic treatment of the seeds on plant development. Similar results for the lengths and number of roots and lengths of sprouts have been found for the electromagnetic treatment of triticale seeds of the variety Kolorit (Sirakov et al., 2019).

It is a known fact that certain changes take place in the seeds during and after the pre-sowing electromagnetic treatments, which can intensify the processes of growth and fruit-bearing of the plants. Generally speaking, pre-sowing electromagnetic treatments with appropriate values of the control factors stimulate plant development.

The aim of this study is to determine the effect of pre-sowing electromagnetic treatments on the sowing qualities of bean seeds stored for several years after their production.

MATERIALS AND METHODS

For the purposes of this study, seeds of field beans of the Bulgarian variety "Obraztsov chiflik 12" (Добрев & Патенова, 2003), were used. During the study, the seeds were divided into three groups (**A**, **M** and **L**) and left to age naturally in storage. The seeds from group **A** were produced in the year preceding the study and therefore remained stored for 1 year. The seeds from group **M** were stored for 5 years, and those from group **L** – for 6 years.

The seeds from group **A** were used as controls. They did not undergo electromagnetic treatment. The seeds from groups **M** and **L** were subjected to three-stage electromagnetic treatment, as per invention patent No. 42681 (Палов et al., 1998). The voltage U, kV between the electrodes, and the duration of the electromagnetic treatment τ, s., were taken to be the controlled factors.

The electromagnetic treatment of the seeds from groups **M** and **L** was carried out with such values of the controlled factors (Table 1) that had produced positive results in prior studies (Палов et al., 2012; Патенова et al., 2009; Palov & Sirakov, 2004).

The results of the pre-sowing electromagnetic treatment of the bean seeds in the groups **M** and **L** were compared with the results obtained from the seeds in the control group – **A**.

After the pre-sowing electromagnetic treatment, the seeds remained stored for further 14 days (Палов et al., 2009; Палов et al., 2012; Патенова et al., 2009) and then put to germinate by the standard methodology (using a thermostat).

Tests were performed on the laboratory parameters lengths of roots ℓ_{root} and lengths of sprouts ℓ_{spr}, and the indicator germination capacity.

Table 1. Values of controlled factors in the pre-sowing electromagnetic treatment of bean seeds of the variety "Obraztsov chiflik 12", groups **M** and **L**

For seeds from group L						
Variant	U_1, kV	τ_1, s	U_2, kV	τ_2, s	U_3, kV	τ_3, s
1	4	5	2,5	15	2	25
2	5,5	5	4	15	3,6	25
3	5,5	5	4	15	5,5	25
4	Control, untreated seeds from group **L** – C_L					
For seeds from group M						
5	5,5	5	4	15	3,6	25
6	Control, untreated seeds from group **M** - C_M					
7	Control, untreated seeds from group **A** - C_A					

Measurements were taken, following which the germinated seeds were left to dry naturally under laboratory conditions until there was no further change in their dry mass. At that point, the dry mass $\mathbf{m}_{dry}$ of the plants was measured for the specified test variants.

All data from the measurements and tests were given as a percentage of the control – untreated seeds from the respective group, for example the control seeds from group **L** are coded as C_L, and those from group **M** as C_M.

To determine how the duration of storage affected the seeds, tests were also made on the untreated control seeds from group **A** (1-year old), coded as C_A.

RESULTS OF THE TESTS

Table 2 shows the results of the laboratory tests of the average lengths of the roots ℓ_{root} and sprouts ℓ_{spr} and the dry mass $\mathbf{m}_{dry}$ of the control seeds from the groups **A** and **L**.

Table 2. Laboratory test results of control (untreated) bean seeds “Obraztsov Chiflik 12” from the groups **A** and **L**.

Observed parameter	Seeds from group:	
	L	A
Germination capacity, %	70,0	92,5
$\overline{\ell}_{root}$, mm	76,5	113,5
$\overline{\ell}_{spr}$, mm	51,4	71,5
$\mathbf{m}_{dry}$, g	38,1	46,7

It can be concluded that the extended 6-year period of storage of the seeds from group **L** has had a suppressive effect on all observed indicators of the control seeds (Table 2).

The extended period of storage – 6 years – resulted in lower germination capacity of the seeds in group **L**. At the time of testing, their germination capacity under laboratory conditions was 70%, and for the 1-year old seeds from group **A** it was 92,5%. The determined average lengths of the roots and sprouts in the control seeds from group **L** were $\overline{\ell}_{root\,L}$ =76,5mm and $\overline{\ell}_{spr\,L}$=51,4mm, respectively. They were considerably shorter from the corresponding lengths measured in the seeds of the same variety from group **A**, in particular: $\overline{\ell}_{root\,A}$=113,5mm and $\overline{\ell}_{spr\,A}$=71,5mm. Also lower was the dry mass $\mathbf{m}_{dry}$ of the sprouted seeds from group **L**, its value was $\mathbf{m}_{dryL}$=38,1g. It measured 81,6% of the dry mass of the seeds from group **A**, which was $\mathbf{m}_{dryA}$=46,7g.

The results of the observed average values of the lengths of roots $\overline{\ell}_{root}$ and sprouts $\overline{\ell}_{spr}$, the dry mass $\mathbf{m}_{dry}$ of the plants, and the laboratory germination capacity of the control seeds from group **L** expressed as a percentage of the controls from group **A**, i.e. $C_{L/A}$ are shown in (Figure 1).

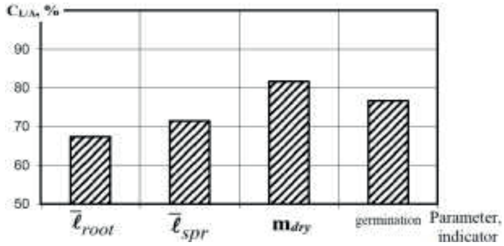

Figure 1. Test results of the control bean seeds from group **L**, expressed as a percentage of the corresponding ones from group **A**

The extended 6-year period of storage affected adversely the observed parameters $\overline{\ell}_{root}$ and $\overline{\ell}_{spr}$ of the control seeds C_L from group **L**.

To compare the control seeds C_L and the corresponding control seeds C_A from group **A,** the following average values for the lengths of roots and sprouts were measured: $\overline{\ell}_{root\,L}$= 67,4%/$C_A$ and $\overline{\ell}_{spr\,L}$= 71,5%/C_A.

The foregoing results lead to the conclusion that seeds “age”, which affects their vital indicators. Thus, the germination capacity of the seeds from group **L** (6-year old) also dropped to just 76,7%/C_A by comparison to the germination capacity of the beans from group **A**.

The impaired vitality of the seeds from group **L** resulted in the accumulation of less dry mass during their sprouting under laboratory conditions, $\mathbf{m}_{dryL}$ = 81,7%/C_A.

Table 3 shows the test results for the germination capacity, lengths of roots and sprouts, and accumulated dry mass of the germinated seeds after their treatment by variants 1…3 (Table 1). All collected data are given as a percentage of the corresponding control seeds from group **L** (C_L) and the control seeds from group **A** (C_A).

It can be established from Table 3 that the pre-sowing electromagnetic treatment of the seeds by variants 1 and 2 (Table 1) helped to increase their laboratory germination capacity to 103,6%/C_C and 110,7%/C_L. respectively. Therefore, the germination capacity of the control seeds from group **L** was 70,0%, while for the seeds treated by variants 1 and 2 it was higher: 72,5% and 77,5%, respectively.

Table 3. Laboratory test results obtained after the pre-sowing electromagnetic treatment of bean seeds "Obraztsov chiflik 12" from group **L** (stored for 6 years)

Observed parameter and indicator	Treatment variants		
	1	2	3
Germination capacity, %	72,5	77,5	50,0
$\%/C_L$	103,6	110,7	71,4
$\%/C_A$	78,3	83,8	54,1
$\overline{\ell}_{root}$, mm	60,3	51,5	52,6
$\%/C_L$	78,9	67,4	68,8
$\%/C_A$	53,1	45,4	60,6
$\overline{\ell}_{spr}$, mm	52,8	52,6	55,2
$\%/C_L$	102,8	102,3	107,3
$\%/C_A$	73,9	73,5	77,2
$\mathbf{m}_{dry}$, g	36,0	39,7	25,7
$\%/C_L$	94,6	104,3	67,3
$\%/C_A$	77,3	85,2	55,0

However, the pre-sowing electromagnetic treatment did not mitigate the biological aging of the seeds over the 6-year long period of storage. In comparison with the control seeds from group **A**, which remained in storage for 1 year only, the laboratory germination capacity for the older seeds from group **L** was 78,3%/C_A for variant 1, 83,8%/C_A for variant 2 and 54,1% C_A for variant 3.

The discussed pre-sowing treatment had a suppressive effect on the development of the roots and their lengths were smaller compared to those of the controls C_L.

As with germination capacity, the pre-sowing treatment stimulated the growth of the sprouted seeds from group **L**; for variants 1 and 2, the lengths of the sprouts were above 102%/C_L.

Compared with the lengths of the sprouts of the controls from group **A**, the sprouts of the plants in group **L** were shorter in length, whereby the length for variant 1 was 73,9%/C_A, for variant 2 - 73,5%/C_A, and for variant 3 - 77,2%/C_A.

The analysis of the data on the accumulated dry mass $\mathbf{m}_{dry}$ of the sprouted plants shows that it increased to 104.3% / C_L due the treatment by variant 2. In this variant, the highest value of dry mass, as compared to that of the seeds produced and stored for 1 year, was achieved - 85.2% / C_A.

The received results and the performed analysis lead to the conclusion that the pre-sowing electromagnetic treatment of the seeds from group **L** by variant 2 (Table 1) helped to improve the germination capacity and increase the lengths of sprouts and the accumulated dry mass.

According to Table 1, variants 5 and 6 were applied to test bean seeds of the variety "Obraztsov chiflik 12", stored for 5 years – group **M**. The seeds from group **M** were subjected to electromagnetic treatment by variant 5, using the parameters of variant 2 (group **L)** for which the best results had been obtained. The seeds from variant 6 were left untreated and were used as controls for the respective group – C_M.

The results of the observed average lengths of the roots $\overline{\ell}_{root}$ and sprouts $\overline{\ell}_{spr}$, the dry mass $\mathbf{m}_{dry}$ of the plants and the laboratory germination capacity of the control seeds from group **M,** expressed as a percentage of the controls from group **A,** are shown in (Figure 2).

It can be concluded from Figure 2 that the shorter period of storage of the seeds, reduced from 6 to 5 years, had a less suppressive effect on the lengths of the sprouts. The sprouts of the control seeds of group **L** (stored for 6 years) had lengths of $\overline{\ell}_{spr\,L}$=71,5%/C_A, which were smaller than the ones of the controls from group **M** (stored for 5 years) $\overline{\ell}_{spr\,M}$ =92,5%/C_A.

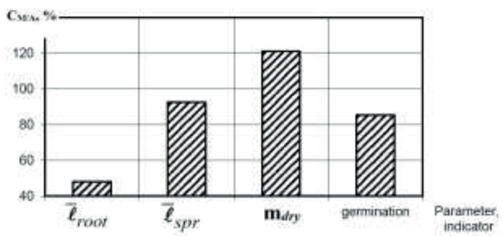

Figure 2. Test results of control bean seeds from group **M**, expressed as a percentage of the corresponding ones from group **A**

The fact that the roots of the sprouts in group M $\overline{\ell}_{root\,M}$ =48,1%/C_A were of smaller lengths could be deemed compensated by the larger quantity of accumulated mass $\mathbf{m}_{dryL}$= 120,9%/C_A. Its value is greater when compared with the dry mass of the control seeds from group **L** ($\mathbf{m}_{dryL}$= 81,7%/C_A – (Figure 1.).

In comparison with the seeds having remained in storage for 6 years, those stored for 5 years

had a higher germination capacity - for the seeds from group **L** it was 76.7%/ C_A, and for the seeds from group **M** - 86.4% / C_A.
The results of the treatments of the seeds from group M are shown in (Table 4).

Table 4. Laboratory test results after pre-sowing electromagnetic treatment of bean seeds "Obraztsov chiflik12" from group **M** (stored for 5 years)

Treatment variant 5 (Table 1)					
$\overline{\ell}_{root\,M}$, mm			$\overline{\ell}_{spr\,M}$, mm		
mm	%/C_M	%/C_A	mm	%/C_M	%/C_A
66,1	121,2	58,3	89,6	153,4	125,3

Based on the performed laboratory studies is was found that the electromagnetic treatment had a beneficial effect on the lengths of the grown roots and sprouts. With the control variant 6 for group **M**, the length of the roots was 54,6mm, and the length of the sprouts 66,1mm. The roots and sprouts that grew from the seeds subjected to electromagnetic treatment, had higher values of the average length $\overline{\ell}_{root\,M}$ =66,1mm, and $\overline{\ell}_{spr\,M}$ =89,6mm. These data, expressed as a percentage of the controls from group **M,** are $\overline{\ell}_{root\,M}$ =121,2%/C_M and $\overline{\ell}_{spr\,M}$ = 135,4%/C_M, respectively,
It can be concluded from Table 4 that the pre-sowing electromagnetic treatment of the seeds from group **M** had a positive effect also on the lengths of roots. By comparison with the lengths of roots of the controls from group **A**, there was an increase to $\overline{\ell}_{root\,M}$ = 58,3%/C_A. It was found that the pre-sowing treatment had a stronger stimulating effect on the growth of the sprouts. Compared to the controls from group **A**, their length was $\overline{\ell}_{spr\,M}$ =125,3%/C_A. The parameters of the electromagnetic treatment were the ones given for variant 5 in (Table 1).
The analysis of the data contained in Table 3 and Table 4 confirm the existence of a pattern - the pre-sowing electromagnetic treatments have a stronger stimulating effect on the growth of the sprouts of bean seeds rather than of their roots.

CONCLUSIONS

1. It was found that the extended storage period of 6 years had an adverse effect on the observed parameters and indicators of the control (untreated) seeds from group **L**. Compared to the control seeds from group **A** (C_A), the average lengths of the roots and sprouts were, respectively, $\overline{\ell}_{root\,L}$= 67,4%/$C_A$ and $\overline{\ell}_{spr\,L}$= 71.5%/C_A, the germination capacity was 76.7% / C_A, and the accumulated dry mass $\mathbf{m}_{dryL}$ = 81,7%/C_A.
2. It was found that the performed pre-sowing electromagnetic treatment of seeds from group L (having remained 6 years in storage) by variants 1 and 2 (table 1):
a) helped to increase the laboratory germination capacity to 103,6%/C_L and 110,7 %/C_L, respectively.
b) the pre-sowing electromagnetic treatment stimulated the growth of the sprouts of the seeds from group **L,** with length of the sprouts exceeding 102%/C_L for variants 1 and 2.
c) it can be seen by the accumulated dry mass $\mathbf{m}_{dry}$ of the sprouted plants that the treatment by variant 2 contributed for its increase to 104,3%/C_L. In this variant, the highest value of dry mass (compared with that of the control group **A**) was obtained - 85,2%/C_A.
3. The pre-sowing electromagnetic treatments did not compensate for the biological ageing of the seeds over their extended 6-year period (**L**) of storage:
a) in comparison to the control seeds from group **A,** the old seeds (group **L**) showed lower laboratory germination capacity: for variant 1 it was 78,3%/C_A, for variant 2 – 83,8%/C_A and for variant 3 – 54,1%/ C_A.
b) compared with the lengths of sprouts of the seeds from group A, the sprouts grown from the seeds in group **L** were of smaller length: for variant 1 it was 74,0%/C_A, for variant 2 - 73,5%/C_A, and for variant 3 - 77,2%/C_A.
4. The shorter period of 5 instead of 6 years of storage of the seeds in group **M**, in combination with the applied pre-sowing electromagnetic treatment, helped to:
a) increase the lengths: of the growing roots to $\overline{\ell}_{root\,M}$= 121,2%/$C_M$ and of the sprouts to $\overline{\ell}_{spr\,M}$= 135,4%/C_M as compared to the controls - **M**.
б) better stimulate the growth of the sprouts. Compared with the controls from group **A**, their length is equal to $\overline{\ell}_{spr\,M}$ = 125,3%/C_A.

5. The test results show that the pre-sowing electromagnetic treatments have a stimulating effect on the sowing qualities of bean seeds that have been stored for an extended period after their production.

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EFFECT OF DROUGHT ON YIELD AND YIELD COMPONENTS OF TRITICALE IN THE CONDITIONS OF SOUTH DOBRUDZHA

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Abstract

In order to determine the effects of drought on triticale under the conditions of South Dobrudzha, eleven cultivars of this crop were studied during six contrasting periods of growing. The following traits were determined: number of tillers, yield, 1000 kernel weight, number of grains in spike. Based on the used indices (DAASDI and DAADPI), moderate to high effect of drought on the growth and development of the crop was determined in comparison to the period favorable for growing of triticale (2014/2015). In some of the studied genotypes, the reduction in yield exceeded 30%. The intensive drought influenced all yield components, the effect being highest on 1000 kernel weight. Comparatively low was the effect on the trait number of productive tillers regardless of the unfavourable conditions during its formation. Cultivars Bumerang and Doni 52 were characterized by the highest drought resistance based on all periods, in which drought was observed. The reduction of yield in these genotypes was moderate, revealing their high tolerance and suitability for growing under the conditions of South Dobrudzha.

Key words*: drought, triticale, yield.*

INTRODUCTION

Drought is among the most serious problems of agriculture worldwide. This is due to the fact that the most important element for the physiological processes in the plant organism is the availability of water (Kadrev, 1985; Bassett, 2013). Therefore, less than the optimal amount of water in the plant organism causes a large number of metabolic disturbances and inability to carry out the normal physiological processes (Kadrev, 1967). The various forms of such type of stress are the reason for different reactions in the cultural plants. Yield, regardless of the crop, as a complex value is affected by drought to the highest degree. In this respect, it is important to distinguish between biological and agronomic drought tolerance. The first type is associated with the ability of the plant to complete its life cycle overcoming the effect of drought, and the second type – with the ability of the plant to realize sufficient production, overcoming the drought. The agronomic drought tolerance is particularly important from a breeding point of view since it allows for selecting those genotypes, which would realize the highest productivity under stress. There are different methods for determining this type of drought tolerance. A set of indices and coefficients, which give varied information about the behavior of a certain group of genotypes, are most often being mentioned in literature (Khavarinejad and Babajanov, 2010; Farshadfar et al., 2012; Parchin et al., 2013; Mursalova et al., 2015). A major point in all indices, however, is that they are formed on the basis of productivity or the components of productivity, as an obligatory ratio between check (irrigated) variant and drought variant (Anwar et al., 2011).

Triticale, in contrast to other cereal plants, is characterized by a very high tolerance to abiotic stress. Since on a global scale droughts are becoming increasingly common, such crops as triticale would endure water deficiency, which significantly affects their productivity (Arseniuk, 2015). Fayaz and Arzani (2011) and Lonbani and Arzani (2011) reported a significant variation in the response among the triticale genotypes they studied. Nevertheless, these authors also pointed out that triticale exceeded bread wheat by drought resistance. Royo et al. (2000) and Zhang et al. (2009) emphasized the serious variability of triticale under conditions of drought, especially with regard to the traits pertaining to growth – plant height, roots, leaf mass. Drought resistance of triticale, according to Giunta et al. (1992), was due to the higher earliness of the studied genotypes, the earlier date to heading and also to the ability of the roots of triticale to extract water from soil. In spite of these data, Arseniuk (2015) stated that the effect of soil moisture on triticale was not sufficiently studied, which imposes the necessity to study in detail the processes of soil drought, which influence the productivity of this crop.

The indices for evaluation of drought resistance ensure a quantitative approach based on the reduction of a given trait under the effect of drought according to normal or controlled conditions of watering (Mitra, 2001). There are few researches in triticale based on specialized indices. Kutlu and Kinaci (2010) determined through indices the wide response of yield and its components in three studied genotypes with regard to drought. Grzesiak et al. (2012) observed that the reaction of the triticale genotypes to drought can be differentiated through the values of the indices, allowing their efficient grouping.

In Bulgaria, indices for evaluation of the drought effects on triticale have been used by Stoyanov (2018) in a controlled field-laboratory experiment. The research determined that the different triticale genotypes responded with considerable reduction of the studied traits in comparison to the irrigated variant. Under natural conditions, the duration and intensity of drought often are not a controlled factor and unlike the field-laboratory experiments, the results from the separate periods cannot be adequately compared and averaged. At the same time, drought can have a negative effect only on a certain element of yield, but the value of yield itself as a whole may not be affected. Therefore, the results from different field experiments should be analyzed on the basis of the degree of drought during a studied period. Besides yield, other traits should also be evaluated in order to obtain adequate information about the behavior of the studied set of genotypes.

The aim of this study was to determine the effects of the natural processes of drought under the conditions of South Dobrudzha in Bulgarian triticale cultivars.

MATERIALS AND METHODS

Plant material and biometric analysis

To implement the above goal, eleven Bulgarian triticale cultivars were used (Kolorit, Atila, Akord, Respekt, Bumerang, Irnik, Dobrudzhanets, Lovchanets, Doni 52, Blagovest, Borislav). The studied cultivars were grown as a whole-surface crop in experimental plots of 10 m^2, in four replicates in a standard block design, within a competitive varietal trial. Sowing was mechanized within the standard dates for triticale, at density 550 seeds per m^2. Besides the above cultivars, the competitive variety trial also involved the triticale check cultivars AD-7291, Vihren and Rakita, as well as the world checks Lasko and Presto. The number of productive tillers per m^2 (NPT) were determined for each experimental plot using a 0.25 m^2 sampling frame. 1000 kernel weight (g) (M1000) and number of grains in spike (NGS) were also determined. The plots were harvested at full maturity, reading the yield (Y) from each of them separately.

Growing conditions

The trial was carried out for six successive harvest years - 2014/2015, 2015/2016, 2016/2017, 2017/2018, 2018/2019, 2019/2020. The data presented on the mean monthly air temperature and the sum of precipitation (Table 1) shows the contrasting nature of the studied periods. The highest differences according to the long-term tendency with regard to temperature were observed during December – March, and with regard to precipitation – in December and May. The differences between these periods are sufficient to consider that the vegetative growth of plants during the separate years occurred in different ways. Certain events and processes in meteorological respects are clearly outlined; they were of single occurrence and were not repeated over periods; they were also able to affect the physiological processes in the plant organism.

Economic years 2018/2019 and 2019/2020 are worth mentioning due to the extreme intensive spring drought. Highly unfavorable for growing of triticale was economic year 2019/2020 due to the rather long-lasting drought during March – April. At the same time, favorable for growing of triticale were the conditions in 2014/2015 and 2016/2017, when a lower number of negative events during the vegetative growth of plants was observed.

Table 1. Mean monthly air temperature and sum of precipitation for the studied period

Parameter	Year	Aug	Sep	Oct	Noe	Dec	Jan
Mean monthly air temperature, °C	2014/2015	22,70	17,50	11,20	5,60	3,10	1,40
	2015/2016	22,80	19,50	10,90	9,30	3,40	-0,80
	2016/2017	22,20	18,10	10,60	6,50	-0,60	-4,10
	2017/2018	22,50	19,00	11,80	7,50	4,70	1,70
	2018/2019	23,60	17,70	13,30	5,40	1,20	1,00
	2019/2020	22,80	17,90	13,40	11,70	5,20	1,80
	1960/2020	21,13	16,88	11,68	6,77	1,97	-0,21
Sum of precipitation, mm	2014/2015	19,30	31,40	57,90	33,20	87,00	33,20
	2015/2016	42,00	20,80	78,30	55,10	0,40	86,30
	2016/2017	5,00	35,80	72,20	43,30	12,50	48,40
	2017/2018	12,40	69,90	50,50	57,20	55,80	75,40
	2018/2019	1,10	54,70	11,70	66,20	43,80	19,20
	2019/2020	7,80	36,70	27,60	35,40	21,80	2,80
	1960/2020	36,95	46,26	42,08	43,41	41,66	36,37
Parameter	Year	Feb	Mar	Apr	May	Jun	Jul
Mean monthly air temperature, °C	2014/2015	2,00	5,00	10,10	16,40	19,40	22,40
	2015/2016	7,30	6,80	13,20	14,70	20,90	22,80
	2016/2017	2,00	7,30	8,70	15,00	20,20	21,80
	2017/2018	1,10	4,60	13,40	17,70	20,40	22,20
	2018/2019	3,50	8,20	9,00	16,00	22,30	22,00
	2019/2020	5,10	8,00	10,00	15,40	19,60	22,30
	1960/2020	1,19	4,71	9,87	15,24	21,99	21,40
Sum of precipitation, mm	2014/2015	79,50	67,70	8,50	12,90	31,30	27,20
	2015/2016	40,70	52,70	20,80	117,10	55,70	2,80
	2016/2017	27,40	48,90	38,40	29,00	87,70	66,30
	2017/2018	48,80	4,90	30,90	90,80	59,60	59,60
	2018/2019	16,30	16,10	49,40	31,70	37,50	54,00
	2019/2020	28,10	28,30	5,80	48,00	51,30	2,70
	1960/2020	34,18	35,46	39,89	52,00	60,93	51,34

Developing a model

According to Stoyanov (2018) number of indices were developed, which provide varied information on how the conditions of drought influence a certain studied trait. In our previous researches (Stoyanov, 2018; Stoyanov et al., 2019), the sensitivity drought index (SDI) developed by Farshadfar and Javadinia (2011) was preferred as an index for evaluation (Formula 1). This index allows evaluating the degree to which the value of a given studied trait decreases under drought.

$$SDI = \frac{x_n - x_s}{x_n} \qquad (1)$$

where

x_n – value of the index in the watered check variant

x_s – value of the index in the variant with drought

The indices as SDI, give an idea about the direct effects of drought without being able to evaluate the levels of a given studied trait. Therefore, the genotypes evaluated as highly drought-tolerant turn out to be with low values of yield or of other studied trait. This gave Stoyanov (2018) reasons to develop the specific index DPI (drought parameter index), which combined in its values the effects of both drought and the potentials of a given trait (Formula 2).

$$DPI = \frac{x_n + x_s}{2} . \frac{x_s}{x_n} \qquad (2)$$

Thus developed and applied under controlled conditions, the index give valuable information about the behavior of the genotypes according to a certain level of drought. On the other hand the indices could also be applied to two different periods of study, one of which is selected as a basic one (check), while the other is with a clearly expressed drought. Under natural conditions, however, drought is not a controlled process, and its duration and intensity can be rather different during the separate periods of vegetative growth. Therefore, the question arises – is it possible the values of the indices for the separate dry periods, in comparison to the basic favorable periods, to be compared and averaged? Such differing effects require the indices referring to different periods to be corrected based on the intensity and duration of the respective period of drought, and to select a period meeting certain criteria as a basic (check or favorable) one.

One of the most common methods for evaluation the drought duration and intensity is by using the aridity index of De Martonne (I_{DM}) (Formula 3) (Croitoru et al., 2012). In its original form, this index gives an idea about drought during the entire calendar or economic year (12 month-period). It can be calculated for another specific period, too. When evaluating the vegetative growth period from October to July (10 month-period), this index would satisfy Formula 4.

$$I_{DMY} = \frac{P_Y}{T_Y + 10} \quad (3) \qquad I_{DMGP} = \frac{1,2 P_{GP}}{T_{GP} + 10} \quad (4)$$

where

I_{DMY} – De Martonne index for an economic year

P_Y – Sum of precipitation of an economic year

T_Y – Mean air temperature of an economic year

I_{DMGP} – De Martonne index of a vegetative growth period

P_{GP} – Sum of precipitation of a vegetative growth period

T_{GP} – Mean air temperature of a vegetative growth period

Since each period is characterized by different levels of intensity and duration of drought, it is highly important which of these periods will be taken as a basic (check, favorable) one, to which the rest will be compared. The use of the mean long-term data also allows calculating the I_{DM}. As a basic should be preferred the period, which, by its I_{DM} values is closest to the the long-term period I_{DM} values. In this case, the correction of SDI and DPI should be based on the difference between the I_{DM} calculated for the basic period (I_{DMb}) and the index calculated for the drought period (I_{DMi}). The correction of SDI and DPI for each of the compared periods allows calculating the mean value adjusted with the mean long-term effect of drought on a given genotype according to a certain basic period. The difference between I_{DMb} and I_{DMi} should be of relative character. This makes the ratio an immeasurable value allowing for more adequate interpretation of the obtained data. The mathematically corrected long-term mean value of the two indices SDI and DPI can be presented as Formulae 5 and 6.

$$DAASDI = \frac{\sum_{i=1}^{S}\left(SDI_i \cdot \frac{I_{DMb}}{I_{DMi}}\right)}{S} \quad (5)$$

$$DAADPI = \frac{\sum_{i=1}^{S}\left(DPI_i \cdot \frac{I_{DMi}}{I_{DMb}}\right)}{S} \quad (6)$$

where

DAASDI – De Martonne Adjusted Average SDI
DAADPI – De Martonne Adjusted Average DPI
SDI_i – SDI calculated for a given period i
DPI_i – DPI calculated for a given i
I_{DMi} – De Martonne index for period i
I_{DMb} – De Martonne index for the basic check period b
S – number of compared periods according to the favorable period

Thus calculated, the value of DAASDI shows the maximum reduction of the studied trait based on the intensity and duration of drought in each of the compared periods according to the favorable check period. Simultaneously, DAADPI shows the model values of the studied trait, provided that its actual means are adjusted with the maximum intensity of the drought during a given period (according to the definition of Stoyanov (2018) on DPI) but corrected according to the differences of the separate periods in comparison to the basic period. It can be determined from Formulae 5 and 6 that the corrective factor for the two indices is different, the factor for SDI being reciprocal to the factor for DPI. This is necessary because the two indices have opposite meanings with regard to drought – SDI expresses a reduction, which is increasing with the increase of the effects from drought, while DPI exhibit the model value, which decreases with the increase of the drought effects. Simultaneously, it is necessary to determine for what period I_{DM} should be calculated. Although the vegetative growth period, throughout which the plants develop from planting to harvesting is the preferred variant, it should be emphasized, that the meteorological effects are not limited only to this period. The drought effects may have a durable impact on the condition and moisture reserves of soil, which may influence the pre-sowing tilths and thence – the setting of optimal condition for the emergence and initial development of plants. Therefore, I_{DM} should be calculated for the overall vegetative growth period (which is 10 months for triticale, October-July), but also for the economic year, which in cereals begins with the harvesting of the previous crop or with the initial soil tillage after the previous crop (in this experimental setting the period started in August and its duration was 12 months). The use of the two periods would allow for more precise determining of the basic check period and for more efficient evaluation of the studied genotypes under conditions of drought in the separate compared periods.

Application of the model and statistical analyses

The results obtained on yield and the other studied traits of the studied triticale cultivars were summarized and averaged over genotypes and periods. The aridity index of De Martonne was calculated for each studied period separately both based on a 10-month vegetative growth period (October – July), and for a 12-month economic year (August – July). Analysis was carried out on drought over the years and the basic periods were determined. The indices DAASDI and DAADPI were calculated with a correction factor based on a 10-month vegetative growth period ($DAASDI_{GP}$ and $DAADPI_{GP}$) and a respective 12-month economic year ($DAASDI_{Y}$ and $DAADPI_{Y}$) for each studied trait. A close analysis was carried out on the effect of drought on the yield and its elements. A $DDADPI_{GP}$-$DAADPI_{Y}$ biplot was constructed, determining the genotypes, which were most tolerant under the conditions of long-term forms of drought. MS Office Excel, 2003 was used for all analyses.

RESULTS AND DISCUSSIONS

Peculiarities of drought during the period of investigation

The results obtained on I_{DM} (Table 2), which was calculated on the basis of a 10-month vegetative growth period and a 12-month economic year, revealed extreme differences in drought during the studied periods. The I_{DMY} values of economic years 2018/2019 and 2019/2020 showed that the meteorological conditions in these two years were indicative of an arid type of climate (according to Pellicone et al., 2019), which is not typical for the region of South Dobrudzha as a whole. The data for the long-term period (1960/2020) (Table 2) characterize the region where the experiment was carried out as moderately dry (Mediterranean type according to Pellicone et al., 2019). In this respect, by values of I_{DMY}, economic years 2015/2016 and 2016/2017 were closest to the long-term period, the difference with economic year 2016/2017 being lowest. Therefore we chose this economic year as a basic period, ***most typical meteorologically***, according to which to analyze the effects of drought during the rest of the economic years.

Table 2. Index of De Martonne for the studied periods

Year	I_{DMY}	I_{DMGP}
2014/2015	22,86	26,76
2015/2016	25,38	29,35
2016/2017	24,94	30,36
2017/2018	27,72	31,21
2018/2019	18,31	20,56
2019/2020	13,09	14,22
1960/2020	24,73	26,97

A similar tendency with regard to the separate studied periods was observed also for the values of I_{DMGP}. The data on vegetative growth periods 2018/2019 and 2019/2020 emphasized the extreme dry conditions of growing the studied genotypes. The results on the long-term period, however, showed that the period of vegetative growth of the crop was considerably more humid than the whole of the economic year. Such a peculiarity relates to the fact that the months of August and September, when the crop was not sown yet, were extremely dry. Nevertheless, it is worth mentioning the fact that by values of I_{DMPG}, vegetative growth period 2014/2015 was closest to the long-term period (1960/2020). Respectively, periods 2015/2016, 2016/2017 and 2017/2018 were considerably more humid. When comparing the results on yield (Table 3), it becomes clear that the highest yield from the studied triticale cultivars was realized in 2014/2015. This indicated that this vegetative growth period was ***the most favorable*** for growing of the crop and should also be chosen as a basic period for determining the effects of drought.

The above peculiarities allow calculating the model indices DAASDI and DAADPI on the basis of the two basic periods: 2016/2017, which was ***the most typical*** in a meteorological respect, and 2014/2015, which was ***the most favorable*** for growing of the crop. This, on its part, allows to define in detail the effects of drought and to estimate to what degree drought leads to unfavorable consequences for yield and the other studied traits.

Specificity of the studied traits and effect of drought on them

Yield

Yield was highly influenced by the different conditions of drought (Table 3). During the two periods with the most intensive and long-lasting drought (2018/2019 and 2019/2020), the average yields from the studied cultivars were rather low. It is worth mentioning the facts, however, that the lowest yield was registered in economic year 2015/2016. Comparatively low was also the yield in 2017/2018. These two periods were marked by unfavorable rainfalls in June and July with a highly negative effect on grain filling and on 1000 kernel weight and test weight, respectively. This indicated that the periods with intensive drought, as well as the periods with excessive rainfalls, were rather unfavorable for the formation of yield from the studied cultivars. This is also emphasized by the fact that in the most favorable period, as well as in the period with the most typical meteorological conditions, the productivity of the triticale cultivars was highest.

Table 3. Means of studied traits by years

Cultivar	NPT	Y, kg/da	NGS	M1000, g
2014/2015	689	728	24	43,6
2015/2016	828	510	19	32,7
2016/2017	609	682	23	49,2
2017/2018	726	649	21	42,5
2018/2019	699	539	19	39,7
2019/2020	545	542	27	37,8
Average	683	608	22	40,9
LSD 0,05	*78,3*	*71,8*	*2,4*	*4,48*
LSD 0,01	*102,9*	*94,3*	*3,1*	*5,88*
LSD 0,001	*131,4*	*120,5*	*3,9*	*7,51*

NPT – Number of productive tillers; Y – Yield; NGS – Number of grains in spike; M1000 – Thousand kernels weight.

Table 4. Means of studied traits by cultivars

Cultivar	NPT	Y, kg/da	NGS	M1000, g
AD-7291	622	569	24	38,9
Vihren	633	586	21	43,4
Rakita	641	647	25	40,5
Lasko	712	596	21	40,2
Presto	762	621	20	41,4
Kolorit	610	596	24	40,4
Atila	681	633	21	43,9
Akord	632	618	24	41,8
Respekt	655	525	21	37,7
Bumerang	690	660	23	42,6
Irnik	651	600	25	38,3
Dobrudzhanets	724	599	21	40,7
Lovchanets	787	530	19	37,8
Doni 52	719	673	23	40,7
Blagovest	713	623	22	39,6
Borislav	690	655	21	46,5
Average	683	608	22	40,9
LSD 0,05	*25,1*	*20,9*	*0,9*	*1,16*
LSD 0,01	*33,0*	*27,5*	*1,2*	*1,52*
LSD 0,001	*42,2*	*35,1*	*1,5*	*1,94*

NPT – Number of productive tillers; Y – Yield; NGS – Number of grains in spike; M1000 – Thousand kernels weight.

Averaged for the period of study (Table 4), the highest values of yield were registered in the check cultivar Rakita and in cultivars Atila, Bumerang, Doni 52 and Borislav, and the lowest – in Respekt and Lovchanets. The other cultivars were by their productivity close to some of the involved checks or to the mean value of all genotypes. A tendency towards maintaining high productivity under intensive drought in comparison to the favorable period ($DAADPI_{GP}$) was observed in Atila, Bumerang and Doni 52, as well as in the check cultivars AD-7291, Vihren and Rakita (Table 5). In comparison to the most typical growing period ($DAADPI_{Y}$), such a tendency was observed in the check Rakita, the world check Lasko, and in cultivars Bumerang, Irnik, Doni 52 and Borislav.

In both indices, registering a reduction according to drought, averaged for the period of investigation ($DAASDI_{GP}$ and $DAASDI_{Y}$), comparatively high values were observed, indicating that the effects of drought had a considerable influence on yield. The lowest reduction according to the two basic periods was determined in AD-7291 and Kolorit, and the highest – in Respekt.

The results from different experiments (Kutlu and Kinaci, 2010; Villegas et al., 2010; Akbarian et al., 2011; Lonbani and Arzani, 2011; Fayaz and Arzani, 2011; Shchypak et al., 2016; Munjonji, 2017; Shanazai et al., 2018; Ramazani and Izanloo, 2019) under conditions of drought show that the yield of triticale is affected by drought. Blum (2014) found out, investigating 24 hexaploid triticale lines under drought, that triticale gave higher yields than common wheat. When determining the correlations of the yields under stress and without stress, the data are characterized by high variation, which emphasizes that triticale as a crop is capable of responding adequately to stress. Martyniak (2002), on the other hand, related the reaction to drought to the specific stage when stressed occurred.

The differing tendencies with regard to the two model indices ($DAADPI_{GP}$ and $DAADPI_{Y}$) calculated for the two different basic periods showed that there was a considerable difference in the productivity of the cultivars and their ranking by productivity during the favorable period and the period with most typical meteorological conditions. The period favorable for growing was characterized by I_{DM}, lower than the period with most typical conditions. This demonstrated that the lack of rainfalls (drought) during a certain period of the vegetative growth of triticale was a key moment for higher productivity. In this respect, the most typical conditions observed in economic year 2016/2017, although deviating comparatively little from the long-term tendency, were characterized by a higher level of abiotic stress than in 2014/2015, when, during heading and grain filling a short period of drought was observed. Therefore, drought tolerance should be considered not an absolute breeding criteria but a relative corrective. This

means that according to the most favorable growing period, drought tolerance should be considered ***agronomic tolerance to drought***, i.e. which are the cultivars maintaining high productivity according to a certain basic period. On the other hand, the drought tolerance according to the period with most typical conditions should be considered ***biological tolerance to drought***, i.e. which are the cultivars reacting to the effects of drought that are different from those in the meteorologically most typical period. Such a concept means that the values of $DAADPI_{GP}$ would show to what degree a given cultivar would maintain its optimal productivity under the effect of drought, and the values of $DAADPI_{Y}$ – to what extent the typical productivity of a given genotype would react under the effect of drought. The differences in these two concepts are presented in Figure 1 through biplot, on which the values of $DAADPI_{GP}$ and $DAADPI_{Y}$ are respectively given.
The cultivars were distributed along the entire graph showing varied combinations of agronomic and biological drought tolerance. A tendency to maintain higher productivity in comparison to the two basic periods was observed in the checks Vihren and Rakita and in cultivars Bumerang, Doni 52 and Borislav. The exact opposite tendency was registered in Respekt and Lovchanets.

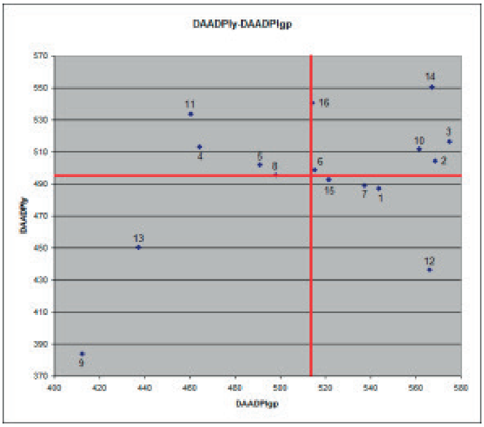

Figure 1. Biplot combining values of $DDADPI_{GP}$ and $DAADPI_{Y}$ on yield
1. AD-7291; 2. Vihren; 3. Rakita; 4. Lasko; 5. Presto; 6. Kolorit; 7. Atila; 8. Akord; 9. Respekt; 10. Bumerang; 11. Irnik; 12. Dobrudzhanets; 13. Lovchanets; 14. Doni 52; 15. Blagovest; 16. Borislav

With low agronomic tolerance to drought but with high biological one were Lasko, Presto, Akord and Irnik, and with high agronomic but low biological tolerance to drought were AD-7291, Atila, Dobrudzhanets and Blagovest. Specific was the behavior of cultivar Kolorit, which was almost at the interception point of the mean values of indices $DAADPI_{GP}$ and $DAADPI_{Y}$. Such a response makes it suitable for using as a check of drought resistance of this crop.
A similar concept can be observed also with regard to the indices showing reduction of yield according to the two basic periods ($DAASDI_{GP}$ and $DAASDI_{Y}$). In this respect, $DAASDI_{GP}$ would show to what degree a given genotype would reduce its optimal productivity under the effect of drought. On the other hand, the values of $DAASDI_{Y}$ would reflect the degree, to which the productivity typical for a genotype under the most typical conditions of the environment would be reduced when influenced by drought. Both groups of indices allow determining the biological and agronomic tolerance to drought of the studied genotypes. However, yield is a rather complex value. Therefore, for a better understanding of the effects of drought, it is necessary to apply the concept of long-term mean effect of drought to the main components of yield, too.

Number of productive tillers

During the period of the most intensive drought (2019/2020), the number of productive tillers, averaged for the studied cultivars, was lowest according to all other periods (Table 3). Respectively, during the two most humid economic years (2015/2016 and 2017/2018), the highest values of the trait were registered. During the other three of the studied periods, the values were considerably close. There were no significant differences between the number of productive tillers averaged for the cultivars during the most favorable and the most typical growing period, their number being lower during the second period. In practice, drought, especially in the early stages of growing, and the insufficient soil moisture in the pre-sowing period were the main reason for the decrease of the number of productive tillers in the studied genotypes.

The highest values of NPT were observed in the world checks Lasko and Presto and in cultivars Dobrudzhanets, Lovchanets, Doni 52 and Blagovest, averaged for the entire period of study (Table 4). Respective low NPT were registered in checks AD-7291 and Vihren and in cultivars Kolorit and Akord.

Concerning the index $DAADPI_{GP}$, a tendency toward maintaining a high NPT according to the favorable period was observed in Presto, Dobrudzhanets and Lovchanets, and a tendency toward lower NPT under the effect of drought was observed in Kolorit and Akord (Table 5). According to the most typical growing period, similar tendencies were registered according to index $DAADPI_{Y}$, but in Bumerang and Borislav it was in a negative direction, while in Akord, the tendency was positive. The lowest reduction caused by drought according to the favorable period ($DAASDI_{GP}$) was observed in the check Rakita and cultivars Irnik and Borislav, and the highest – in Lasko and Akord, Doni 52 and Blagovest. According to the most typical growing period, the lowest reduction ($DAASDI_{Y}$) was observed in Irnik and Blagovest, and the highest – in Bumerang and Borislav. In the greater part of the cultivars, the values of this index were negative indicating that during the typical growing period the number of productive tillers was lower in comparison to the other periods. The results from the calculated indices showed that the different genotypes had a comparatively low response to drought. The agronomic drought tolerance according to this index was comparatively high (only 4.40 % average reduction). In a biological sense, the index responded positively, averaged for the studied periods, because the effect of drought on it was observed only in the period with the longest and most intensive drought (2029/2020). It should be emphasized that the warmer and more humid weather conditioned the higher NPT. On the other hand, in the dryer periods, although being reduced to a certain degree, the number of productive tillers reached moderate values related to the better seed set.

The data obtained by Kirchev et al. (2012); Baychev, (2013); Aggrawal and Sinha (1987); Fayaz and Arzani, (2011) confirm our results. Baychev (2013) observe a very high effect of spring drought on Bulgarian triticale cultivars. The high number of productive tillers resulting from the warmer winter was considerably reduced, especially in cultivar Bumerang. This author also pointed out that the effect of drought was not always identical but was strictly dependent on the conditions of the environment. According to data from our previous research (Stoyanov, 2018), the results on the behavior of the cultivars showed that under controlled conditions this trait was least influenced in cultivars Atila, Bumerang, Dobrudzhanets and Doni 52.

Table 5. Values of the indices applied to the studied triticale cultivars for yield and number of productive tillers

Cultivar	Yield			
	$DAADPI_{GP}$	$DAADPI_{Y}$	$DAASDI_{GP}$	$DAASDI_{Y}$
AD-7291	543,47	487,39	8,39	11,45
Vihren	568,53	504,38	9,86	14,74
Rakita	574,61	516,59	17,12	19,51
Lasko	464,07	513,35	31,52	5,71
Presto	490,84	501,98	27,87	13,04
Kolorit	515,26	498,75	21,60	13,61
Atila	537,04	489,03	26,35	26,43
Akord	497,96	495,63	28,14	16,75
Respekt	412,34	384,04	37,14	34,92
Bumerang	561,40	511,64	22,00	22,58
Irnik	460,18	533,76	34,96	2,74
Dobrudzhanets	565,90	436,29	13,72	35,45
Lovchanets	437,06	450,68	26,74	10,53
Doni 52	567,19	550,34	25,05	16,86
Blagovest	521,28	492,81	24,89	20,31
Borislav	514,20	540,65	31,98	13,42
Average	514,46	494,21	24,21	17,38
Cultivar	Number of productive tillers			
	$DAADPI_{GP}$	$DAADPI_{Y}$	$DAASDI_{GP}$	$DAASDI_{Y}$
AD-7291	637,11	645,66	-0,94	-19,71
Vihren	635,72	570,27	-6,24	-2,50
Rakita	637,14	624,17	5,65	-5,82
Lasko	639,75	844,86	18,81	-49,87
Presto	754,51	820,64	6,20	-25,53
Kolorit	536,32	586,95	12,85	-17,62
Atila	693,75	631,31	-2,25	-1,39
Akord	582,66	712,39	10,15	-45,32
Respekt	622,75	666,16	7,46	-19,96
Bumerang	652,70	563,49	9,85	17,95
Irnik	665,95	616,95	2,24	0,03
Dobrudzhanets	782,19	775,41	-14,80	-30,80
Lovchanets	845,11	887,22	-8,81	-36,59
Doni 52	654,10	735,96	16,93	-18,11
Blagovest	688,68	666,43	10,26	1,09
Borislav	672,65	593,35	3,07	8,77
Average	668,82	683,83	4,40	-15,34

Number of grains in spike

In the dry year 2018/2019, the number of grains in spike was considerably lower (Table 3). In economic year 2019/2020, when drought was much longer and intense, the number of grains in spike was highest in comparison to all years of study. This was related to the fact that in this period lower number of productive tillers were formed in comparison to 2019/2020. This causes the formation of larger spikes and higher number of grains in spike. At the same time, similar to yield during the two most humid economic years, the number of grains in spike was also lower. Such a phenomenon was due to the specific anthesis of triticale, which is strongly influenced by the high air humidity, and to the higher number of productive tillers. The two basic periods were with the optimal value of the trait.

The highest values of this trait (Table 4) were that of the check cultivars AD-7291 and Rakita, and of cultivars Kolorit, Akord and Irnik, and the lowest – of the world check Presto and cultivar Lovchanets. In comparison to the favorable period, under the effect of drought, this tendency was not interrupted according to the values of $DAADPI_{GP}$ (Table 5). Nevertheless, a positive tendency was observed in Bumerang and Doni 52, while a negative one was present in Atila, Respekt and Borislav. According to the most typical period, there was a positive tendency in Kolorit, Bumerang and Irnik, and a negative one in Respekt and Dobrudzhanets.

The lowest reduction of the values of the trait under drought, in comparison to the most favorable period, was observed in Akord, Doni 52 and Blagovest, and the highest – in Atila, Irnik and Borislav. According to the most typical period, the highest reduction was in Akord and Dobrudzhanets, and the lowest in Kolorit and Doni 52. A great part of the cultivars realized negative values of $DAASDI_{Y}$. This was an indication that in the period with the most typical meteorological conditions, a lower number of grains were formed as compared to the periods with expressed drought. This means that for a better seed set, drought (lack of rainfalls) during heading – grain formation is rather a necessity than a stress factor.

The results on the values of the indices emphasized the fact that the number of grains in spike could be influenced largely by the effects of drought. From and agronomic point of view, a reduction of more than 8% was determined, averaged for the studied cultivars, which reached 24% in cultivar Irnik. At the same time, from a biological point of view, the different genotypes responded to drought in accordance with the most typical conditions, but often the lack of rainfalls during anthesis and pollination had a better effect on the values of the trait. This contradicts to some extent the data we obtained under controlled drought (Stoyanov, 2018).

The formation of grain in triticale is related to the considerably more open pollination due to specificity in the biology of the florets. Therefore, drought during anthesis, pollination and fertilization causes severe damages on the pollen and the stigmas of the florets (Barnabas et al., 2008). On the other hand, however, the intensive rainfalls during anthesis and long periods of humid and cool weather impede normal pollination (Stoyanov, 2018). Baychev (2013) emphasized that in periods with stronger drought, the seed set was considerably influenced in cultivars Kolorit and Akord. A large number of researches (Saleem, 2003; Kutlu and Kinaci, 2010; Fayaz and Arzani, 2011) confirmed that the seed set in triticale was highly influenced by the effect of drought but was directly dependent on the specific genotype.

1000 kernel weight

During the two basic periods (favorable and most typical), the highest values of 1000 kernel weight were registered (Table 3). This was due to the fact that during these two vegetative growth periods the most suitable conditions for grain formation and filling were observed. Respectively, under the effect of drought during economic years 2018/2019 and 2019/2020, the values of the trait were lower; significantly lower they were in 2019/2020. On the other hand, the lowest values of this trait were determined in economic year 2015/2016, which was characterized by high precipitation during grain formation and filling. Lower than the basic periods were also the values in economic year 2017/2018 due to the long-lasting rainfalls in July of 2018, which impeded harvesting. These peculiarities show that both drought and excessive rainfalls cause extreme unfavorable effects on grain filling. Therefore, it may be argued that drought is only a part of the complex of stress factors, which have impact on the values of 1000 kernel weight. Averaged for the period, the check cultivar Vihren and cutivars Atila, Bumerang and Borislav were with the highest values of this trait, while Respekt, Irnik and Lovchanets were with the lowest (Table 4). A tendency of maintaining high values of 1000 kernel weight according to the favorable period (Table 6) was observed in the world check Presto and in cultivars Atila and Borislav, and a tendency toward low values – in Respekt, Irnik and Lovchanets. According to the most typical period, the tendency, both in positive and negative directions, remained almost the same, cultivar Bumerang also giving a positive response to drought according to this index. The reduction in the trait based both on the favorable and the most typical periods was however different. This was related to the differing conditions during grain filling in the two basic periods.

Table 6. Values of the indices applied to the studied triticale cultivars for number of grains in spike and 1000 kernel weight

Cultivar	Numer of grains per spike			
	$DAADPI_{GP}$	$DAADPI_{Y}$	$DAASDI_{GP}$	$DAASDI_{Y}$
AD-7291	23,03	18,37	-13,08	10,14
Vihren	19,58	18,61	12,44	6,98
Rakita	22,57	19,33	1,08	11,62
Lasko	18,81	16,55	1,78	8,34
Presto	15,79	16,23	17,25	0,54
Kolorit	22,72	21,53	7,25	1,88
Atila	17,25	16,90	19,27	9,63
Akord	22,31	17,78	3,42	23,01
Respekt	17,86	15,63	11,87	17,94
Bumerang	20,74	21,47	-3,07	-24,75
Irnik	18,13	23,30	24,28	-30,35
Dobrudzhanets	18,02	15,76	17,62	23,46
Lovchanets	14,45	17,25	21,68	-19,38
Doni 52	20,90	19,14	0,21	0,56
Blagovest	18,99	19,52	4,60	-14,54
Borislav	17,38	19,58	11,24	-24,64
Average	19,28	18,56	8,62	0,03
Cultivar	Thousand kernels weight			
	$DAADPI_{GP}$	$DAADPI_{Y}$	$DAASDI_{GP}$	$DAASDI_{Y}$
AD-7291	35,62	31,86	13,28	16,36
Vihren	39,72	35,25	5,82	10,48
Rakita	36,87	32,25	7,60	14,26
Lasko	37,27	28,20	9,01	33,64
Presto	39,61	30,03	4,00	30,01
Kolorit	37,25	31,33	5,89	18,39
Atila	40,22	33,88	10,75	22,26
Akord	37,52	31,18	11,84	24,93
Respekt	33,19	27,28	21,75	34,63
Bumerang	37,34	32,12	16,58	24,78
Irnik	35,55	28,29	7,47	26,74
Dobrudzhanets	38,93	28,86	5,96	33,95
Lovchanets	35,46	27,05	9,62	33,40
Doni 52	38,84	30,08	4,16	27,54
Blagovest	37,08	29,40	6,97	26,76
Borislav	41,30	34,73	16,27	27,24
Average	37,61	30,74	9,81	25,34

During the favorable period, comparatively drier conditions were observed, while during the most typical period the amount of rainfalls was higher and with comparatively even distribution. This was the reason why the values of the reduction were higher in comparison to the most typical period. In contrast to the previous two traits, from an agronomic point of view the reduction of the 1000 kernels weight was significantly lower than the reduction in biological sense. This was due to the fact that the moderate drought, although favorable for optimal values of the number of tillers and a better seed set, restricted the proper grain filling regardless of its duration and intensity.

Thousand kernel weight was characterized by very high variation under the effect of the environmental conditions (Baychev, 2013; Giunta et al., 1993; Villegas et al., 2010). This was related to the long period of formation of the values of the trait and to the sensitivity of the physiological processes of grain filling to a large number of factors of the environment (Moayedi et al., 2009; Dogan et al., 2012). A large number of the researches on triticale (Kutlu and Kinaci, 2010; Fayaz and Arzani, 2011) and other cereals (Dencic et al., 2000; El-Kareem and El-Saydi, 2011) confirm our results that 1000 is influenced considerably by the effects of drought.

The data obtained on yield and its components showed that as a result from the long-term mean effects of drought, under natural conditions the triticale genotypes reacted in a number of different ways depending on their specificity. According to the favorable period, a great part of the cultivars managed to maintain their optimal productivity and moderate levels of productive tillers and number of grains in spike, the reduction of the studied traits not being too high.

From a biological point of view, the studied triticale cultivars reacted significantly stronger; with regard to the NPT and the number of grains in spike a negative correlation was observed. This correlation allowed the separate cultivars to respond adequately to drought, the negative effect in one of the traits being compensated for by the other. Only in periods with severe and long-lasting drought, the effects on both traits could not compensate for the negative ones. In spite of this correlation, in 1000 kernel weight much higher biological effects were observed than agronomic ones. This indicated that grain filling was a highly susceptible period with regard to grain formation, when insufficient moisture and thermal stress were the reason for lower productivity. This thesis was supported by the results of Martyniak (2002), who established that triticale was most influenced by the stages of heading and milk maturity, when the greatest decrease in grain yield was observed. Drought at the initial stages of development affected yield only insignificantly. Stankova and Stankov (2002) pointed out that drought caused significant deviations in traits such as number of grains in spike and weight of grains in spike. Lower is the effect of drought on the same traits during grain filling, without observing effect on the structure of the plants. Dhindsa et al. (1998) found out that under conditions of natural drought on the territory of India, the components of yield, which were least affected, were number of productive tillers per m^2, weight of grains in spike and the harvest index. The presence of compensatory mechanisms between the number of productive tillers and the number of grains in spike and the high sensitivity of 1000 kernel weight indicated high complexity of the effects of drought on the yield from the studied cultivars. In this respect, worth mentioning as genotypes, which reacted adequately both with regard to yield and separate elements of it are Bumerang, Irnik, Dobrudzhnets, Doni 52 and Borislav. The long-term effects of drought, both from agronomic and biological point of view, were less prominent in them, which makes these genotypes suitable for growing under the conditions of South Dobrudzha.

CONCLUSIONS

A model for averaging and comparison of the effects of drought occurring during separate periods under natural conditions was developed. The model was applied on two basic periods – the most favorable for growing of the crop, and the most typical meteorologically. The effects of drought were registered as two separate concepts – agronomic effect and biological response. It was found out that with regard to yield, drought had a significant effect, the agronomic effects being considerably higher. The number of productive tillers was less influenced in comparison to the number of grains in spike. Thousand kernel weight was highly affected by drought, which was essential for the productivity of the crop. Cultivars Bumerang and Doni 52 were with the highest drought tolerance calculated for all periods when drought was observed; this was an indication for their high tolerance to drought and respective suitability for growing under the conditions of South Dobrudzha.

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ASSESSMENT OF YIELD AND WATER USE EFFICIENCY OF DRIP-IRRIGATED COTTON (*Gossypium hirsutum* L.)

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Abstract

Climate change raises the question of demand for more economical ways to use irrigation water. Against the background of different levels of fertilization, cotton varieties have been tested. The experiment was performed under irrigated and non-irrigated conditions. The experiment was performed in the experimental field of the Trakia University, Stara Zagora during the period 2018-2020. Cotton productivity has been established under non-irrigated and irrigated conditions. The analysis of the results shows that the naturally colored variety Isabel, when realizing irrigations, on average for the period forms a yield 39.9% higher than the non-irrigated one. Under irrigated conditions, the Darmi variety increased productivity by 34.3%. An increase of 27.9% was registered for the Helios variety. The Isabel variety has the highest values of the irrigation water efficiency coefficient (0.88-1.11). Against the background of different levels of fertilization, the Darmi variety forms an efficiency coefficient from 0.9 to 1.03. Helios variety is responsive to irrigation, but on average for the three-year period stands out with the lowest values of efficient use of water resources (0.53 - 0.95).

Key words: *cotton, fertilizing, irrigation, productivity, efficiency.*

INTRODUCTION

Negative consequences of global climate change affect crop development in the following two aspects: reduced yields and deterioration of product quality due to lack of soil moisture during the growing season and reduced rainfall, a sharp decline in water resources, accumulation of water, small volumes of water in the dams in annual and perennial aspect, limiting the possibilities for using the water for irrigation. Climate change raises the question of demand for more economical ways to use irrigation water. One of the areas where new or updated technologies and policies can have a significant impact on more efficient use of water resources is crop irrigation (Ziad et al., 2010; Moteva et al., 2016; Kireva et al., 2018; Gospodinova et al., 2020).

Irrigation is an effective means of limiting or preventing the stressful effects of drought on crops. Determining the parameters of growth and development of generative organs under irrigated conditions is crucial, according to Hameed et al. (2017). Results indicated that inter culturing + chiseling produced the highest significant seed cotton production (17.8%) more bolls plant-1 (14.3%) and water intake (17.7%) than no chiseling with inter culturing. However, irrigation interval after eight days produced the maximum yield of seed cotton (14.2%), more 14.3%bolls plant-1and water retention (35.6%) than 12 days irrigation interval. Water stress reduces leaf area, dry matter accumulation, number of knots, number of boxes, and reduce fiber length (Pettigrew, 2004; Mert, 2005). Pettigrew (2004) also reported that varieties produce additional boxes at higher nodes in response to additional irrigation, leading to higher overall yields.

The effect of drip irrigation on fiber yield and quality has been studied by a number of authors. Tracing the water resource in the soil profile in the 0-30 cm layer Ibraginmov et al. (2007) recommend an optimal regime for drip irrigation (70–70–60% of FC) for each of the three main plant growth periods (germination - budding; budding - flowering; onset of ripening - ripening). Seed-lint cotton yield was increased 10-19% relative to that for furrow irrigated cotton. The irrigation scheduling rule developed here should be considered an improved practice for drip irrigated cotton that is applicable to irrigated Calcic Xerosols of Uzbekistan.

Papastylianou et al. (2014) registered a decrease in the leaf area index. The length of the fiber is also shortened in response to water stress, while the index of strength, fineness and uniformity of the fibers are not affected by irrigation levels.

Basal et all. (2009) find that irrigation of cotton with a drip irrigation method at 75% level had significant benefits in terms of saved irrigation water without reducing yield, and high WUE indicated a definitive advantage of employing deficit irrigation under limited water supply conditions.

Increasing irrigation costs and reducing water resources are forcing producers to adapt irrigation strategies to maximize crop yields and increase water efficiency.

The advantage of drip irrigation was pointed out by a team of scientists from India after three years of experiments (Ramamurthy et al. 2009). Irrigation scheduling in hybrid cotton throughdrip based on 0.6 evapotranspiration enhanced seed cotton yield by 37% over broad bed furrow and 72% over thefarmer's practice. The water-use efficiency with drip-irrigated cotton was 28-58% higher than broad bed furrow and 45-68% higher than the flood method of irrigation.

Soil fertility is a prerequisite for achieving high and quality yields. Jayakumar et al. (2015) established the effectiveness of cotton fertilizers in drip irrigation with different irrigation rates. It is recommended that drip fertigation of inorganic fertilizers in combination with biofertigation be used as a viable technique to realize the yield potential of cotton and sustenance of soil fertility.

The reduction in water available for cotton production is forcing researchers to focus on increasing water efficiency by introducing new varieties of drought-resistant cotton or water management. New irrigation technologies are being developed, suitable for limited water supply, as well as for specific topographic and soil conditions. O'Shaughnessy et al. (2010) created algorithms for automatic cotton irrigation.

In a market economy, the efficient use of water is associated with the optimization of mineral nutrition, labor and energy. Optimization of irrigation water, analysis of the impact of sufficient available moisture on productivity and product quality, determining the efficiency of water resources is embedded in the developments of many researchers (Matev et al., 2014, Matev et al., 2018; Kuneva et al., 2015; Earl et al., 2018).

The main goal in the present study is: to study the productivity of three varieties of cotton under non-irrigated and irrigated conditions; establishing the effect of irrigation.

MATERIALS AND METHODS

The experiment was performed in the experimental field of the Trakia University, Stara Zagora, which is located in the region of Eastern Central Bulgaria, covering the Thracian lowland, located at 42°41'51.75" north latitude, 23°19'18.722" east longitude and 169m above sea level. According to the climatic zoning, the region falls within the European-continental area and the Transitional-Continental sub-area from it.

The three-year field experiment was performed with three varieties of cotton. Against the background of different levels of fertilization were tested varieties Bulgarian selection (2007-2010) - Helius, Darmi and Isabell. Isabell is characterized by a naturally colored light brown to creamy fiber that is short, medium fine, with good uniformity and extensibility. The vegetation period of this early variety is 109 - 111 days. In Helios and Darmi the vegetation period is on average 131 days, respectively, and in Darmi it varies in the range of 116 to 122 days.

The experiment was performed under irrigated and non-irrigated conditions. The experiment was set by the method of fractional plots in 4 replications, with a plot size of 15m^2 (1.80 x 8.34m) in the period 2018/2020. The soil type is typically meadow-cinnamon soil with a clearly formed humus horizon of 0 to 45 (50) cm. The maximum field moisture content in the layer 0 - 40 cm is 31.6%. The soil is on average stocked with humus - 3.42% - 4.04%. The supply of irrigation water is realized with a drip

irrigation system at 75% FC in the layer 0-50cm.
The main fertilization was done with triple superphosphate in normal P8 in the fall. Nitrogen fertilization was performed once before sowing with nitrogen fertilizer (NH_4NO_3). Four levels of fertilizer rate were tested ($N_{0,8,16,24}$). Herbicide treatments and double hoeing of the crop were carried out. The beginning of the harvest begins in September, after cracking of 50-60% of the boxes.

RESULTS AND DISCUSSIONS

Climatic characteristics
In terms of climate, Bulgaria belongs to the zone of unstable humidification, in case of drought in July-August due to which obtaining high and stable yields of cotton are impossible without irrigation. On the other hand, the limited temperature resources set specific requirements and features of cotton irrigation in our country in order to achieve high profitability with a relatively good early maturity.
The air temperature in the soil of the experimental field is not characterized by significant differences in the values measured for the period 2006-2020 (Figure 1). The fluctuations are larger in the first and third experimental years.

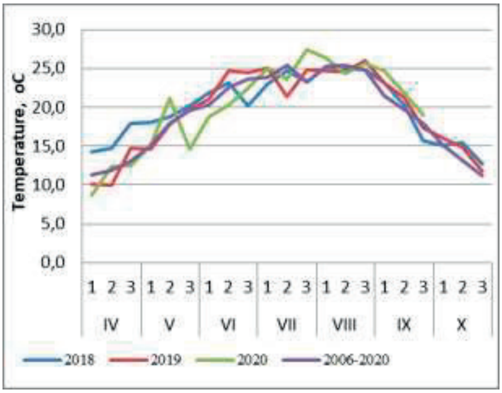

Figure 1. Temperature conditions in the area of the town of St. Zagora during the cotton vegetation for the research period and the period, 2018-2020

In 2018, sowing and the beginning of the vegetation took place at higher than normal temperatures. The average monthly temperatures for April and May exceed by 3.68 and 1.94ºC, respectively, the annual norm set for a period of 90 years. During this year, the vegetation of cotton took place during prolonged summer drought and higher temperatures. In June, July and August the total amount of precipitation was 187.2 mm, with only 27 mm of precipitation recorded in August.
The vegetation period of cotton in 2019 passed under relatively favorable humidity and temperature conditions. The measured average monthly temperatures show an excess for all months compared to the average values, the most significant differences being in May (+1.94°C), June (+ 2.27°C), August (+1.83°C) and September. (+1.53°C). In 2019, the peak of the fallen rainfall was established in June by 108.7 mm. In August, the amount of precipitation was only 11.2 mm, which at high temperatures led to dripping of some of the knots and reduced yields.

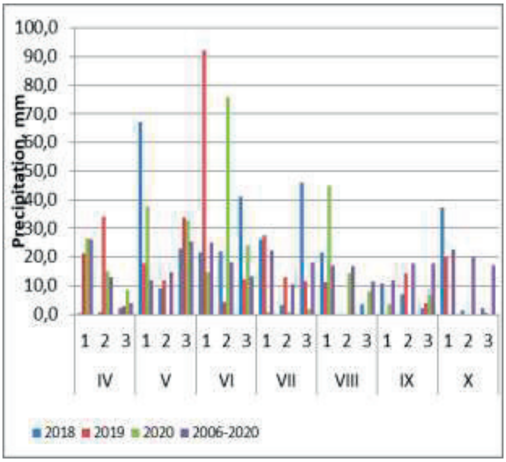

Figure 2. Dynamics of precipitation during the experiment period 2018-2020 in the region of Stara Zagora

In the third year the temperature sum from May to September is 3375ºC, which is only 3ºC less than in 2019 and 3ºC more than in 2018. The high temperatures had an adverse effect in July (average monthly temperature 25.5ºC at 23.7 and 23.8ºC for 2018 and 2019) and the lack of precipitation, which caused heavy dripping of buds and flowers. The year is characterized as the driest for the study period. A peak of 114.5mm was registered in June, which is the highest amount of precipitation per month during the considered three-year period. Figure 2 shows the uneven distribution of precipitation during the growing season.

The influence of climatic factors on the growth and development of crops is expressed in coefficients.

The hydrothermal coefficient (according to Selyaninov) is established by the formula:

HTK = P*10/∑To ,

The humidity coefficient of Ivanov (1941) is determined by the following dependence:

E = 0,0018*(t + 25)2*(100-a),

When calculating the humidity coefficient and the moisture balance coefficient, the active temperature sums (ºC), relative humidity (%), the sum of precipitation (mm) were used.

Table 1. Coefficients characterizing the humidity during the period 2018-2020

Years	Months																	
	IV			V			VI			VII			VIII			IX		
	I	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Humidity coefficient of Ivanov																		
2018	1,14	0,91	1,36	1,00	1,07	1,40	1,23	1,59	1,14	1,37	1,87	1,01	1,86	2,21	2,01	1,79	1,33	1,25
2019	0,69	0,64	1,19	1,02	0,99	1,26	1,10	1,73	1,72	1,94	1,43	1,74	1,87	2,30	2,57	2,00	2,08	1,10
2020	0,86	0,98	1,06	1,01	1,42	0,90	1,11	0,89	1,42	1,98	2,17	3,55	2,57	1,97	2,12	2,31	1,98	1,36
Hydrothermal coefficient (HTC)																		
2018	0,01	0,04	0,12	3,71	0,48	1,14	0,99	0,95	2,03	1,13	0,13	1,97	0,86	0,00	0,14	0,46	0,35	0,13
2019	2,09	3,41	0,18	1,22	0,67	1,71	4,39	0,17	0,50	1,10	0,60	0,46	0,45	0,00	0,00	0,00	0,67	0,23
2020	3,06	1,22	0,66	2,49	0,00	2,24	0,78	3,73	1,07	0,03	0,03	0,06	1,70	0,59	0,32	0,14	0,00	0,36

According to Selyaninov's HTC, it is accepted that at values of the coefficient lower than 0.5, drought is reported, and at HTC> 2.0, overwetting is observed. HTC according to Selyaninov for the three economic years is calculated only for the period with a biological minimum of 10 °C, ie. for the months of April-September.

Ivanov's coefficient is more accurate, because the average daily temperature, the amount of precipitation and the relative humidity of the air are involved in determining the values of the coefficients. The empirical formula for calculating the evaporation of Ivanov shows the degree of moisture in ten days for the period during which the plants need a sufficient amount of moisture in the soil. In the last ten days of July, the humidity in the first year is 70% lower than in the second. The water deficit during these periods requires irrigation to increase the water supply in the soil.

The climatic conditions during the cotton growing season are specific and differ during the three years, both in terms of temperature and in terms of the amount and distribution of precipitation. Particularly vulnerable are plants grown in conditions of natural moisture. In the formation of cotton yields, the temperature sums during the vegetation and the moisture during the flowering-beginning of ripening period, which takes place during the months of July-August, are of great importance.

To determine the security (P) in the present study, data on the amount of precipitation and the sum of the average daily temperatures for a 91-year period were used. Collateral calculation was performed according to the following formula:

P = ((n-0,3)/(m+0,4))*100, where

m is the consecutive number of the year in the ascending series, and n is the total number of years in the series.

Depending on its security, the years can be in terms of precipitation: wet years - at P <15%, medium wet - at P <25%, average years at P =

50% and in the range between 25 and 75%, medium dry at - P> 75%, dry years at P> 90%.
The statistical evaluation of the experimental years in terms of the temperature sum was made for the period for the period May-October (Table 1). The analysis shows that for the 90-year series of data (from 1930 to 2020) the three years are characterized as warm, with security of 10.6%, 9.5%, respectively, and the last year is the warmest with security 5.1%. Temperature is crucial for the development and maturation of cotton. During the vegetation period and during the three years no extremely high temperatures were measured, which would damage the development of the plants.
The analysis shows that the cotton is grown in conditions of optimal temperature resources and unstable moisture. Although cotton is a relatively drought-resistant crop, the results show its responsiveness in optimizing the water factor. In order to assess the water supply of plants, it is necessary to know not only the total value of precipitation during the cotton growing season, but also their distribution throughout the period.
The provision with precipitation for the period May-October in the first year is 29.2%, which defines the period as average, but with values close to moderately humid. With 42.3% and 52.2% security, the second and third experimental years are characterized as average in terms of the growing season. For the period characterized by higher average daily temperatures in June-August, the provision with precipitation in the first year characterizes the period with values close to moderately humid (27.0%). Regardless of the registered precipitation in the second year of the field survey, the period is characterized by an average coverage of precipitation (31.4%). The lower amount of precipitation in the third experimental year defines it as average in terms of rainfall (50.0%). The uneven distribution of precipitation determines the need for irrigation for soil moisture supply.

Cotton productivity

The productivity of the three varieties of cotton, under natural moisture supply and irrigation, is presented in Figures 3 and 4. The different stresses of the meteorological conditions predetermine the higher productivity in the first year of the Polish experiment.

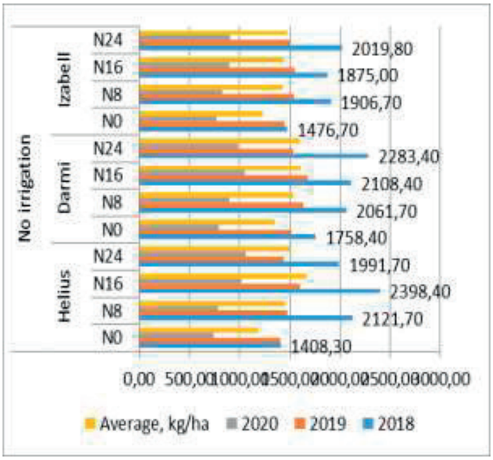

Figure 3. Productivity of cotton under non-irrigated conditions, kg/ha

With natural moisture content, the three varieties of cotton form a high yield. In Helios, the average yield for 2018 of the four levels of fertilization is 20.3% higher than that obtained under irrigated conditions.

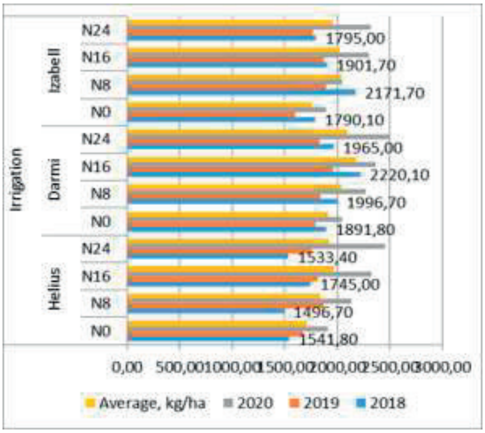

Figure 4. Productivity of cotton under irrigated conditions, kg/ha

Darmi achieved a higher yield by 1.7% compared to irrigated cotton, on average for fertilization levels. Only naturally colored Isabelle increases irrigation productivity by 5.7%. Therefore, it is necessary to analyze the effect of the supplied irrigation water.

Table 3. Irrigation parameters, productivity and the effect of irrigation on three cotton varieties, for the period 2018-2020, for the Stara Zagora

Variants		Yield without irrigation (Yn)	Yield by irrigation (Yir)	Irrigation norm (M)	Additional yield (Yad)	Efficiency factor (EF)
Variety	Fertilization rate	kg/ha	kg/ha	mm	kg/ha	
Helios	N_0	1185,30	1707,30	550,00	522,00	0,95
	N_8	1459,30	1833,20	550,00	373,90	0,68
	N_{16}	1672,70	1963,00	550,00	290,30	0,53
	N_{24}	1497,80	1915,10	550,00	417,30	0,76
	Average	*1453,80*	*1859,40*	*550,00*	*405,60*	*0,74*
Darmi	N_0	1355,60	1908,60	550,00	553,00	1,01
	N_8	1533,70	2035,50	550,00	501,80	0,91
	N_{16}	1613,50	2179,10	550,00	565,60	1,03
	N_{24}	1601,10	2094,70	550,00	493,60	0,90
	Average	*1526,00*	*2054,50*	*550,00*	*528,50*	*0,96*
Izabell	N_0	1231,3	1760,2	550,00	528,90	0,96
	N_8	1427,6	2037,4	550,00	609,80	1,11
	N_{16}	1440,8	2023,5	550,00	582,70	1,06
	N_{24}	1474,4	1959,6	550,00	485,20	0,88
	Average	*1391*	*1945,2*	*550,00*	*554,20*	*1,01*
		1456,9	1953	550,00	496,10	0,90

Unproductive water consumption is particularly worrying against the background of reduced water resources. Over the next two years, yields are higher under irrigated conditions. In the last year, the optimization of soil moisture has contributed to increased productivity at Helius by 144.3%, Darmi by 145.7%, Isabelle by 156.4%, on average for the four levels of fertilization.

On average for the three-year period, characterized by different security of the climatic elements, an increase in productivity was registered at Helius by 27.9%, Darmi - by 34.3% and Isabel by 39.9%. Against the background of natural moisture and different levels of nutrients, Helius and Darmi have the highest average yield for three years in the N_{16} variant.

Isabelle is responsive to fertilization and the average yield of this variety is the highest at N_{24}. In conditions of optimizing the moisture in the soil horizon, a similar trend is reported for all three varieties. Not the highest fertilization rate guarantees high yields, and N16 has the highest productivity. The available moisture in the soil creates conditions for better absorption of the available nitrogen.

During the three years of the field experience, a different number of irrigations were realized, depending on the amount and distribution of the registered precipitation. In the first year, two irrigations were applied, in the second four, and in the fifth five irrigations were carried out to maintain optimal conditions in the soil horizon. Table 3 presents the results of irrigated and non-irrigated variants for the studied varieties.

The ratio between the additional yield and the irrigation rate gives us the efficiency coefficient. The parameters of the additional yield depend on the stress of the meteorological factors and on the levels of fertilization.

The table presents the average yields and the additional yield formed under the influence of irrigation. Helios forms 405.6 kg/ha of additional yield under the influence of the realized irrigations during the vegetation. Darmy and Isabella are more responsive to irrigation, with 528.5 kg/ha and 554.2 kg/ha additional yield, on average for the varieties. Regarding the levels of nitrogen fertilization, against the background of optimization of the water resource at Helios, it was found that the lowest additional yield was registered when N16 was introduced. Nitrogen (N_{16}) imported from Darmi was most effectively utilized. Isabelle, when optimizing the water factor, formed an additional yield of 582.2 kg/ha and 609.8 kg/ha when fertilizing with N16 and N8, respectively.

The values of the efficiency coefficient vary from 0.53 to 1.11. On average for the period with the lowest EF, Helios stands out (0.74). Isabel is the variety that responds best to irrigation, with an EF of 1.01 on average for all levels of fertilization. The reduction of the unproductive costs of irrigation water depends on the analyzes of the productivity of irrigation water for each variety.

CONCLUSIONS

The following conclusions can be drawn from the field study:

Naturally colored variety Isabel, when watering, on average for the period forms a yield of 39.9% higher than non-irrigated. Under irrigated conditions, the Darmi variety increased productivity by 34.3%. An increase of 27.9% was registered for the Helios variety.

The Isabel variety has the highest values of the irrigation water efficiency coefficient (0.88-1.11). Against the background of different levels of fertilization, the Darmi variety forms an efficiency coefficient from 0.9 to 1.03. Helios variety is responsive to irrigation, but on average for the three-year period stands out with the lowest values of efficient use of water resources (0.53 - 0.95).

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LOSS OF GRAIN AT HARVESTING WHEAT WITH A COMBINE HARVESTER

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Abstract

The article investigates grain losses in wheat harvesting with combine Claas Lexion 660. A thousand grain mean mass has been established, which is 49 g, whereas the mean mass of wheat in 1 m^2 is as follows: at operation speed of 4.5 km/h it is 2.98 kg, at operation speed of 5.2 km/h it is 3.67 kg and at operation speed of 6.7 km/h it is 4.56 kg. In wheat harvesting these are between 0.49% and 0.75%. In addition, by increasing operation speed from 4.5 to 6.7 km/h, losses had increased by 34%. A linear relationship has been established between the percentage of grain losses and the working speed of the combine, as the coefficient of determination is R^2 = 0.9945, i.e. 99% of the change in grain losses is due to the operating speed of the combine.

***Key words**: grain harvester, grain losses, method for loss determination, harvesting wheat, productivity.*

INTRODUCTION

Harvesting is the last stage of all cultivation activities (Delchev et al., 2016). Of great importance is also the timely harvesting. This event is of great importance since the end results of the year-round work are harvested (Vasilevich, 2011). The harvest itself is characterized by campaign nature, high energy intensity, production losses and multivariance (Ishpekov, 2013).

It is one of the main activities in agriculture and should be carried out within short term - a maximum of 10-12 days, in order to avoid high losses from grain spillage and to reduce the risk of crop destroying by natural conditions (Nikolov et al., 1974). Improper harvesting method, delayed harvesting campaign or poor organization may result in significant material losses of the product (Vakarelski et al., 1976; Delchev & Trendafilov, 2013; Li et al., 2013; Petrovna, 2014; Paixão et al., 2016; Trendafilov & Dragoev, 2017; Dragoev, 2018).

In the process of harvesting cereals, different types of losses occur: biological losses, which represent losses due to adverse climatic and natural conditions, and losses of nutrients and physical losses caused by the mechanical impact of machines in the process of crop harvesting. It is known that if cereals remain, for example, 5 days after full maturity, loss from shattering is up to 3.7%, after 10 days they increase up to 21%, and a delay of 20 days leads to losses over 30% (Kolev, 1999).

Grain losses during harvest represent a direct loss of income for the farmer. In some countries it is perceived that the reasonable small grain loss should reach a maximum of 3% of the total crop yield (Delchev & Trendafilov, 2013). By extending harvesting periods, grain losses sharply increase (Kehayov & Mehmedova, 2010).

Harvester losses are not great, they are usually indicated at 2-3% when working with normal load under relatively favourable conditions (Vasilev, 1987). The same author reports that in studies several times higher losses have been found. For example, when harvesting wheat, losses were between 3-6% and losses due to meteorological and organizational reasons were 4-5%.

The testing of the combine harvesters is carried out according to the standard in force for the country. For modern conditions in agriculture it is appropriate to apply the methods specified in the international standard ISO 8210:1989. The main normative provisions of this standard are also laid down in the Russian standard for combine harvesters GOST 28301-2007. The quality of the harvest is one of the mandatory

evaluations to be given in the test machine to harvest (Beloev et al., 2018).
The objective of this study is to investigate the grain losses during wheat harvesting with combine Claas Lexion 660 depending on the harvester operation speed.

MATERIALS AND METHODS

The study is carried out during the 2020 harvesting campaign in harvesting wheat (*Renaissance* variety) grown in the region of Byalo pole village, Opan municipality, Stara Zagora District. The total area of the field is 92 ha. The crop was harvested with a Claas Lexion 660 harvester (Figure 1), which had a working width of the header 6.1 m. The average yield was 6.5 t/ha and the average humidity recorded by the on-board hydrometer of the harvester was 12.1%.

Figure 1. Claas Lexion 660 grain harvester

Grain losses were examined. The actual measurements are made at three different harvester speeds of 4.5, 5.5 and 6.7 km/h. The measuring location was always located at a minimum distance of about 50 meters from the end of the section in order to stabilize the operation speed and performance of the harvester. Figure 2 shows the "Mihovi bryasti" plot, where grain losses were measured. The figure clearly shows that grain loss measurements are made at the three measurement points (1, 2 and 3), after which the results obtained were averaged.

Figure 2. Map of "Mihovi bryasti" plot with an area of 92 ha

For the purpose of the study, special trays were made to collect the mass falling behind the harvester with an area of 1 m^2. They were 4 pieces with a diameter of 56 cm. These trays stood under the harvester and after entering 50 meters into the crop, they remained behind it (Figure 3). The fallen mass in the trays was collected in bags. After that grains were separated and counted (Koryčanský, 2010).

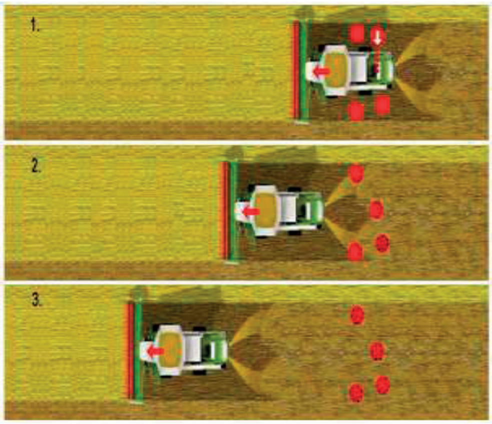

Figure 3. Check of grain losses after the grain cleaning device

After the harvester had been adjusted for wheat harvest, it intuitively set off at a speed that was appropriate for that harvest and for this crop, at this yield and on this terrain. When the harvester enters the crop 50 meters into it, it stops to allow the operator to assess grain losses visually. After he decided that the losses were acceptable for the respective harvest conditions, the harvester was setting off at the speed it had worked before.

The percentage of grain losses was calculated by the formula (Delcev & Trendafilov, 2013):

$$z = \frac{100.A.n}{B.\beta.N.D}, \quad \% \qquad (1)$$

where z are grain losses, %;

A – the width of the sieves of the combine when leaving the straw in the slope or the width of the header of the combine when spreading the straw, m;

n – number of grains in chaff and straw behind the grain harvester, pcs./m^2;

B – working width of the grain harvester header, m;

β – coefficient of utilization of the working width;

N – number of grains in 1 kg of grain, pcs./kg

D – the yield per hectare, t/ha.

To determine the number of grains in 1 kg, 1000 grain taken were from the harvested crop and weighed. On the basis of this the number of grains in 1 кg of grain were determined. A total of 5 samples have been taken and their number was calculated using the formula:

$$N = \frac{100000}{m}, \quad pcs./kg \qquad (2)$$

where m is the mass of 1000 grains, g.

Since losses are collected and reported from an area with width equal to the harvester "tunnel" width and have been obtained from an area the width of which is equal to the width harvested by the harvester, it is necessary to determine the coefficient of utilization of the header working width β. It was determined by the following formula:

$$\beta = \frac{L}{10.B} \qquad (3)$$

where L is the distance measured for 10 harvester moves, m;

B – structural working width of the harvester header, m.

RESULTS AND DISCUSSIONS

Table 1 presents the experimental data of the coefficient of utilization of the harvester working width. For greater accuracy these were made on the basis of 10 harvester moves.

Table 1. Coefficient of utilization of the header working width in wheat harvesting with Claas Lexion 660 harvester

Harvester	Mean value of the length of 10 moves L, m	Coefficient of header utilization β
Claas Lexion 660	56.95	0.93

The table shows that the average value of the utilization coefficient of the header working width is 0.93. It is evident that this coefficient is very low. In this case, the structural working width of the harvester header is 6.1 m, while the actually used one $B_{actual}=B.\beta= 6.10.0.93 = 5.67$ m, i.e. 7 % of the header working width of the grain harvester was not used. This shows that operators did not put enough effort and experience into driving the harvester in the field since high productivity was not a priority. Quite often, when conducting the experiments, we found out that during harvest, there was also a second person next to the harvester operator who was distracting him. It is difficult to find out the real reason for the low utilization of the header working width, but it is a fact. In our opinion, it is mainly due to the fact that high productivity is not a priority. Incomplete use of the header working width leads to a decrease in the harvester productivity.

Table 2 gives the number of grains in 1 kg of wheat. It also shows the mass of 1 grain and 1000 grains. Table 2 shows that the mean mass of 1000 grains is 49 g, which corresponds to 20408 pieces of grain in 1 kg.

Table 2. Number of grains in 1 кg of wheat *Renaissance* variety

Crop	Weight of 1 grain, g	Weight of 1000 grains, g	Number of grains in 1 kg N
Wheat	0.049	49	20408

Table 3 presents the averaged results of the baseline data and parameters used in the study of wheat harvest losses. In order to simplify calculations, the table shows the number of grains per 1 m^2 at the three operating speeds of the harvester. From the samples taken, it can be seen that the mean number of grains ranged from 61 to 93 grains. The average mass of wheat in 1 m^2 at operating speed of 4.5 km/h is 2.98 kg, at a speed of 5.2 km/h it is 3.67 kg and at a speed of 6.7 km/h it is 4.56 kg.

Table 3. Total number of grains and mass of grain in 1 m^2 in various operating speeds of the grain harvester

Operating speed of the grain harvester, v_p , km/h	Total number of grains N, pcs.	Weight, kg / 1 m^2
4.5	61	2.98
5.2	75	3.67
6.7	93	4.56

Table 3 also shows that grain mass differs significantly at the three harvester operating speeds or between 2.98 and 4.56 kg/1 m^2. This difference is due to the fact that the crop was weeded and particles of weeds were sticking on the working surface of the straw sieves, which made the work of the straw sieves (separation system) difficult. This results in a decrease in the separation rate and an increase in grain losses.

Figure 4 shows the percentages of total wheat harvesting losses depending on the grain harvester operating speed. The results obtained shown in Figure 4 reveal that as the working speed of the grain harvester increases, losses increase. Figure 4 also shows in wheat harvest these are between 0.49% and 0.75%. This is perfectly logical and completely confirms the results of other researchers that operating speed is a key factor. In this case, the increase of the operating speed of the grain harvester from 4.5 km/h to 6.7 km/h, i.e. by 2.2 km/h, losses increased by 34%.

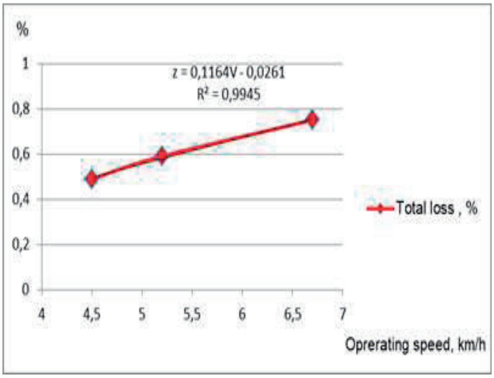

Figure 4. Percentage of total losses in harvesting wheat depending on the working speed of the grain harvester

Separation losses increase with increasing speed of the threshing drum (Zaman et al., 1992). This finding is also supported by Wrublcski & Smith (1980). Data from other authors revealed higher grain losses in Pak 81 - 4.18% than those of Punjab 85 - 3.04% (Zaman et al., 1992).

According to Koryčanský (2010), the lowest grain losses of 0.6% were achieved at an operating speed of the combine of 3 km/h, and the highest of 0.8% at an operating speed of 6 km/h. These results were due to the dense sowing and the fact that the threshing machine was overloaded. Similar results were obtained by Basavaraja (2007) who identified the factors influencing post-harvest losses of rice and wheat. The losses were maximum at the farm level (3.82 kg/q in rice and 3.28 kg/q in wheat) accounting for 73.57 per cent and 75.93 per cent of the total post-harvest losses, respectively.

Davoodi & Houshyar (2010) estimate grain losses on New Holland TC56 combine when harvesting wheat. Their results show that the grain losses were the lowest in the operating speed of the combine of 3 km/h and at a frequency of rotation of the reel of 25 min^{-1} and at 850 min^{-1} turnovers of the rotor. They also suggested that farmers choose them at harvest.

The results presented in figure 4 also show the relationship between the percentage of grain losses and the working speed of the combine. It is evident that the relation between the two parameters is linear one,

$$z = 0,1164.v_p - 0,0261 \quad (4)$$

i.e. with increasing the working speed of the combine, the percentage of grain losses increases. The regression model is adequate at significance level p=0.05, as the coefficient of determination is R^2 = 0.9945, i.e. 99% of the change in grain losses is due to the operating speed of the combine.

Figure 5 presents graphically the mass of wheat losses from 1 ha depending on the grain harvester operating speed. It can be seen that the mass at an operating speed of 4.5 km/h is 29.8 kg/ha and at an operating speed of 6.7 km/h it is 45.6 kg/ha. The graph also shows that as the harvester operating speed increases, the grain mass increases by 34%.

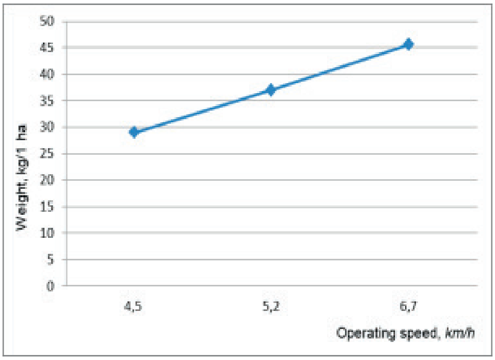

Figure 5. Mass of wheat depending on the grain harvester operating speed

Studies confirm that grain losses at harvest are directly related to the working speed of the combine. In order to achieve a high productivity harvest, a precise calibration of the combine loss reporting system is required.

CONCLUSIONS

It was established that: (i) 7% of the header working width of the grain harvester was not utilized. Incomplete use of the header working width results in a decrease in the harvester productivity; (ii) the mean mass per 1000 grains is 49 g; (iii) mean mass of wheat in 1 m^2 is as follows: at an operating speed of 4.5 km/h it is 2.98 kg, at an operating speed of 5.2 km/h it is 3.67 kg and at an operating speed of 6.7 km/h it is 4.56 kg; (iv) the reported grain losses during wheat harvest are between 0.49% and 0.75%; (v) by increasing the speed from 4.5 km/h to 6.7 km/h losses had increased by 34%; (vi) a linear relationship has been established between the percentage of grain losses and the working speed of the combine, as the coefficient of determination is $R^2 = 0.9945$, i.e. 99% of the change in grain losses is due to the operating speed of the combine; (vii) by increasing the speed from 4.5 km/h to 6.7 km/h, i.e. losses had increased by 34% and grain weight increased by 34%.

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GROWING SEVERAL RAPESEED HYBRIDS FOR GREEN FODDER IN THE CONDITIONS OF CENTRAL SOUTHERN BULGARIA

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Abstract

This experiment aims to establish the productivity and quality of several rapeseed hybrids grown for green fodder in the region of Central Southern Bulgaria. The experiment was conducted for three years (2017-2020) in the experimental field of the Department of Plant Growing at AU Plovdiv, by the block method, in 4 replications, with a harvest plot size of 20 m^2. The subject of the experiment is the hybrids PT225, PT234, PR44W29, and PT271. Harvesting of rapeseed for green mass is done in the flowering phase. It was found that the best ratio of individual parts of plants combined with the highest content of crude protein and the lowest crude fiber was reported in the hybrid PT225. During the three years of the experiment and on average for the study period, the highest yield of green mass was obtained from the hybrid PT234 (69 250 kg/ha).

***Key words**: rapeseed, hybrids, green fodder, quality.*

INTRODUCTION

Along with repko, winter rapeseed provides farm animals with the first green fodder, before alfalfa and pre-crops rye, winter peas, and barley. In terms of biochemical properties, rapeseed is superior to many forage crops and is one of the high-protein feeds.

One kilogram of green mass contains 0.16 food units and 30 g of protein, which exceeds that in the green mass of corn, sunflower, barley, and peas. Its coefficient of digestibility of protein in the green mass is one of the highest (80-86%). In its fresh mass, winter rapeseed contains from 9 to 12.5% sugars (glucose, fructose, sucrose) (Nikiforov, 2004; Ivanova et al. 2009; Shpaara, 2012; Ivanova, 2008, 2012; Delchev, 1988).

The yield and quality of the green mass depends on the climatic conditions, the varietal characteristics, the phase of development, the quantity of input fertilizers, and other agrotechnical factors (Ivanova et al., 2009; Todorov, 2012; Jeromela et al., 2010; Mikić et al., 2010).

MATERIALS AND METHODS

The study was conducted in the period 2017 – 2020 in the Educational, Experimental and Implementation Base of the Department of Plant Growing at the Agricultural University Plovdiv.

The experiment is based on a block method, with 4 repetitions with the size of the experimental plot 20 m^2.

Subject of the experiment are hybrids PT234, PT271, PR44W29 and PT 225.

The experiment was carried out after a predecessor of wheat cultivated on the generally accepted cultivation technology.

Sowing is carried out in the optimal timing for rapeseed, at a row spacing of 12-15 cm and sowing rate 12 kg/ha.

Harvesting of rapeseed for green mass is done in the flowering phase.

The analysis of the feed was performed by the so-called Weende method. It determines crude protein, crude fat, crude fiber.

During the years of the experiment, the combination of climatic factors is favourable for the cultivation of rapeseed for green fodder, which allows obtaining high yields.

The data characterizing these factors during the years of the experiment in the areas of the Educational, Experimental and Implementation Base are indicated in (Figures 1 and 2).

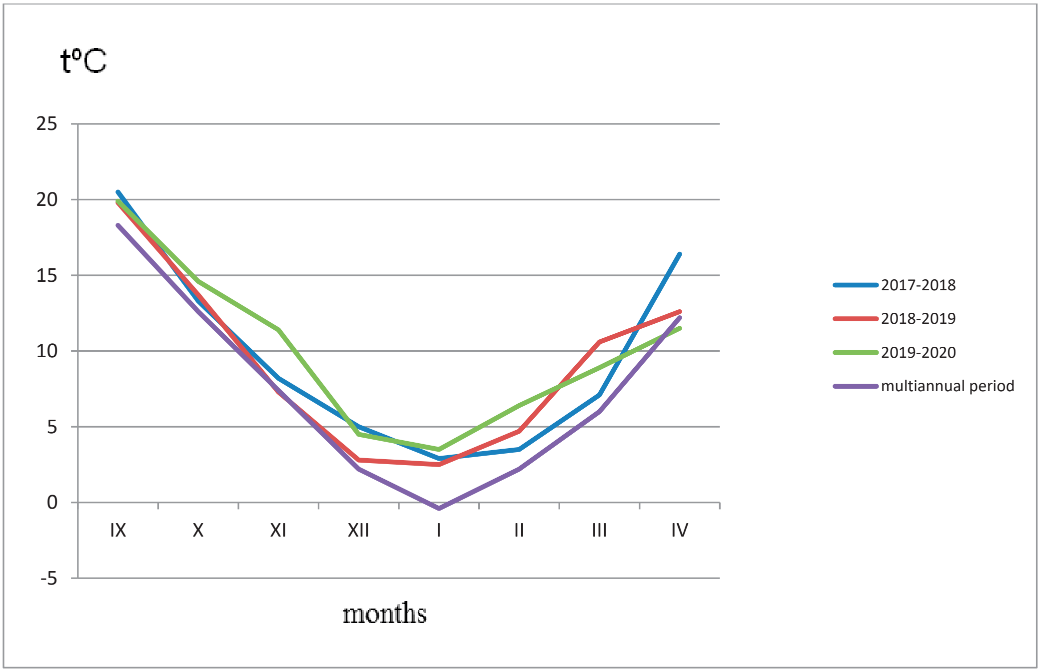

Figure 1. Average monthly temperatures in the region of the Training, experimental and implementation base

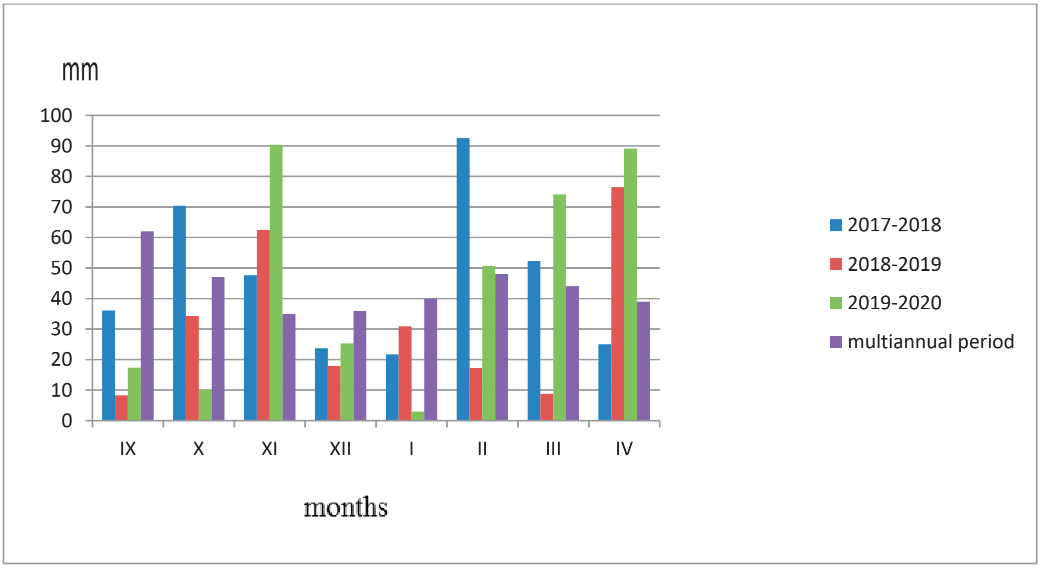

Figure 2. Quantity of rainfall during the years of survey in the region of the Training, experimental and implementation base

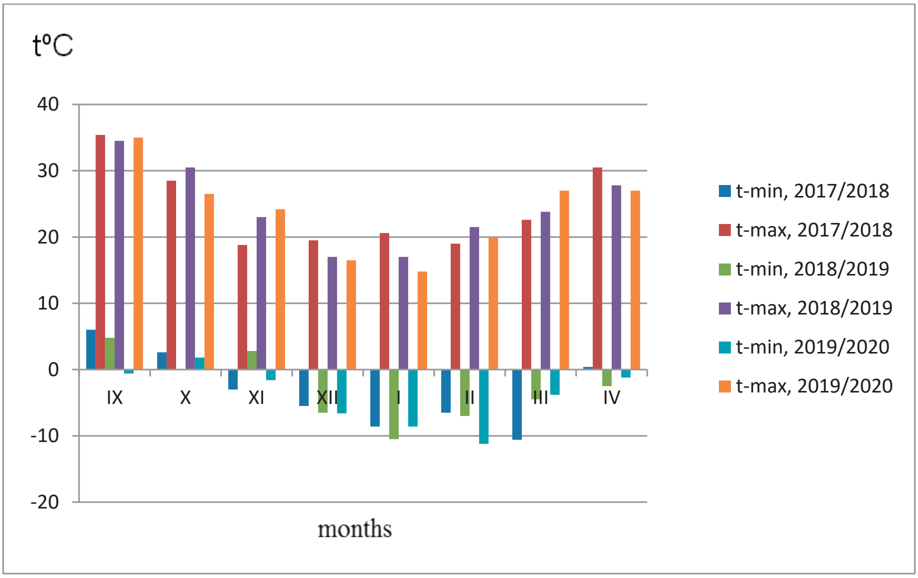

Figure 3. Absolute minimum and maximum temperatures by months 2017/2020

In the growing area, the amount of precipitation before sowing and during the vegetation of rapeseed grown for green fodder was 360.2 mm in 2019-2020, 256.4 mm in 2018-2019, and 369.3 in 2017-2018.

During the second and third year it exceeds by 18.3 mm and 9.2 mm, while in the second it is by 94.6 mm less than the multiannual period (351 mm) (Figure 2).

An absolute minimum temperature during the three years of the study was reported in March 2018 (-10.6ºC), in January 2019 (-10.5ºC) and in February for 2020 (-11.2ºC) (Figure 3).

RESULTS AND DISCUSSIONS

The phases of plant development in the different years occurred at different times (Table 1).

All tested hybrids in the experimental 2018 germinate on 20.09, in 2019 on 27.09, and in 2017 on 28.09.

In all three experimental years, all specimens reached the 6th-8th leaf stage about one month after germination.

During the three years of the experiment the tested hybrids enter phase 6-8th leaf at a different time PT234 (29.10; 26.10; 29.10); PT271 (29.10; 27.10; 29.10); PR44W29 (29.10; 27.10; 30.10) and PT 225 (30.10; 28.10; 30.10).

In the first ten days of December, with a permanent drop in temperature, rapeseed ceases its vegetation. In the first year of the study this period occurs at 12.12, in the second on 9.12, and in the third on 8.12.

A prerequisite for the resumption of vegetation in 2019 on (7.03) was the increase in temperatures in early March 2019 up to 10.9°C

In 2018 and 2020, the resumption of vegetation occurred later on (10.03 and 9.03), respectively.

Stem formation phase lasts for three days, from 24.03 to 27.03.

Table 1. Development stages

Development stages	Years											
	2017/2018				2018/2019				2019/2020			
Hybrids	PT225	PT234	PR44W29	PT271	PT225	PT234	PR44W29	PT271	PT225	PT234	PR44W29	PT271
Sowing	5.09	5.09	5.09	5.09	3.09	3.09	3.09	3.09	5.09	5.09	5.09	5.09
Germination	28.09	28.09	28.09	28.09	20.09	20.09	20.09	20.09	27.09	27.09	27.09	27.09
Sixth to eighth leaf	30.10	29.10	29.10	29.10	28.10	26.10	27.10	27.10	30.10	29.10	30.10	29.10
Termination of vegetation	12.12	12.12	12.12	12.12	9.12	9.12	9.12	9.12	8.12	8.12	8.12	8.12
Restoration of vegetation	10.03	10.03	10.03	10.03	7.03	7.03	7.03	7.03	9.03	9.03	9.03	9.03
Stem formation	27.03	26.03	25.03	25.03	27.03	25.03	26.03	25.03	25.03	24.03	25.03	24.03
Budding	11.04	10.04	10.04	10.04	11.04	9.04	10.04	10.04	10.04	8.04	9.04	9.04
Beginning of flowering - 10%	19.04	18.04	17.04	17.04	20.04	17.04	18.04	18.04	17.04	15.04	16.04	16.04
Mass flowering – 75%	27.04	25.04	26.04	26.04	28.04	25.04	26.04	27.04	26.04	23.04	25.04	25.04
Vegetation period from germination to mass flowering	212	209	210	211	221	218	219	220	213	210	212	212

Table 2. Structural elements of yield of the studied hybrids

Hybrids	Plant height /cm/				Number of branches per plant /pcs/				Number of leaves per plant /pcs/				Stem thickness /mm/			
	Years															
	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average	2017 2018	2018 2019	2019 2020	Average
PT225	140	137	142	140	6.9	6.8	7.0	6.9	18.4	18.0	18.7	18.4	16.9	16.8	17.1	16.9
PT234	143	140	144	142	7.3	7.1	7.4	7.3	18.9	18.8	19.1	18.9	17.4	17.3	17.5	17.4
PR44W29	141	138	143	141	7.1	7.0	7.2	7.1	18.5	18.3	18.8	18.5	17.1	17.0	17.2	17.1
PT271	138	135	143	139	6.8	6.6	7.1	6.8	18.2	17.9	18.7	18.3	16.8	16.6	17.2	16.9

In the beginning of April (08.04 -11.04) the hybrids enter the budding phase. Depending on the year of study, PT234 hybrid reaches the earliest this phase (10.04; 09.04; 08.04), followed by PR44W29 and PT271 hybrids (10.04; 10.04; 9.04), and PT225 hybrid at the latest (11.04; 11.04; 10.04).

Of the three years of the experiment, the earliest phase of mass flowering occurred in the third year - 2020, and depending on the studied hybrids - PT234 (23.04 - 25.04), followed by hybrids PR44W29 (25.04 – 26.04); PT271 (25.04 – 27.04), and PT225 (26.04 – 28.04).

Of the tested hybrids, the longest vegetation period was reported for PT225 from 212 to 221 days, while the shortest - for PT234 from 209 to 218 days.

The structural analysis of the plants in the flowering phase during the years of the experiment is presented in Table 2.

The height of the plants of the tested hybrids on average for the study period varied from 139 to 142 cm.

In the three years of study, the highest plants were registered for PT234 hybrid (from 140 to 144 cm) followed by the hybrids PR44W29 (from 138 to 143 cm), PT225 (from 137 to 142 cm), and PT271 (from 135 to 143 cm).

The number of branches per plant in 2020 is higher than in 2018 and 2019.

In 2020, the number of branches varies from 7.0 for the PT225 hybrid to 7.4 for the PT234.

The average for the study period the largest number of branches (7.3 pieces) is registered for PT234 hybrid, and the smallest – for PT271 (6.8 pieces).

With regards to the indicator number of leaves per plant, higher values were reported in 2020 (from 18.7 to 19.1 pieces) compared to 2018 (from 18.2 to 18.9 pieces), and in 2019 (from 17.9 to 18.8 pieces).

On average for the study period, the number of leaves was highest for PT234 hybrid (18.9 pieces), and the lowest - for PT271 hybrid (18.3 pieces).

The thickness of the stems during the three years of study in different hybrids varies within small limits.

Both on average for the period and in the three separate years, the highest values of the structural elements are reported for PT234 hybrid, which also affects the size of the yield.

The morphological analysis of the plants of the cultivated hybrids shows that the highest relative share in the individual parts of the plant belongs to the stems (from 56.6 to 57.5%) followed by the leaves (from 35.2 to 36.0%), and blossoms (7.2 to 7.4%) (Table 3).

The highest percentage of the total mass of the plant for PT225 hybrid consists of the leaves (36.0%) followed by the hybrids PR44W29 (35.9%), and PT234 (35.7%), while the smallest percentage is reported for PT271 hybrid (35. 2%).

The relative share of the blossoms in the four hybrids is almost the same (7.2 – 7.4%).

The ratio between the individual organs of plants in the cultivation of rapeseed for green fodder is most appropriate when the percentage of stems is lowest, and the leaves and flowers - the highest.

Of the tested hybrids, PT225 hybrid best meets these requirements, the morphological analysis of which is closest to the above requirements, and appears as the most suitable for growing for green mass.

With slightly lower values, the other hybrids are also very suitable for growing green fodder.

The results of the experiment show that, depending on the meteorological conditions, the yield of green mass obtained from the cultivated hybrids changes in the years of study.

The more favourable combination of climatic factors and the better distribution of precipitation in 2020 created conditions for obtaining higher green mass yields (from 68 310 kg/ha to 69 810 kg/ha) compared to 2018 (67 600 kg/ha to 69 230 kg/ha), and in 2019 (67 240 kg/ha to 68 720 kg/ha) (Table 4).

On average for the study period, the highest green mass yield was reported for PT234 hybrid (69 250 kg/ha), and the lowest for PT271 hybrid (67 740 kg/ha).

From the mathematical processing performed in terms of green mass yield, the differences between PT234 and all hybrids at CD5%, are mathematically proven.

Table 3. Morphological analysis of plants on average for the period of growing in the flowering phase

Hybrids	Weight of one plant (g)	%	Weight of the stems of a plant (g)	%	Leaf weight of a plant (g)	%	Weight of flowers of a plant (g)	%
PT225	159.4	100	90.2	56.6	57.4	36.0	11.8	7.4
PT234	162.8	100	93.0	57.1	58.1	35.7	11.7	7.2
PR44W29	160.0	100	90.9	56.8	57.5	35.9	11.6	7.3
PT271	158.3	100	91.0	57.5	55.8	35.2	11.5	7.3

Table 4. Green mass yield kg/ha

Hybrids	Years			
	2018	2019	2020	Average
PT225	67850	67500	68310	67890
PT234	69230	68720	69810	69250
PR44W29	68470	67950	68940	68450
PT271	67600	67240	68390	67740
GD 5%	710.51	690.10	800.33	

The data on the chemical composition show that there are small differences between the tested hybrids in terms of the content of crude protein in the dry matter of rapeseed (Table 5).

Table 5. Chemical analysis of the gre§en mass, average for two years

Hybrids	Absolute dry matter %	Crude protein %	Crude fat %	Crude fibre %
PT225	87.4	17.2	3.45	30.93
PT234	86.8	17.0	3.58	31.27
PR44W29	87.0	17.0	3.53	31.45
PT271	86.9	16.8	3.50	31.50

The values of crude protein on average for the study period in the tested hybrids vary from 16. 8 to 17.2%, which is due to differences in climatic conditions, and the percentage of individual plant parts (stems, leaves, blossoms).
The higher relative share of leaves in PT225 hybrid is also the reason for the higher crude protein content (17.2%).
The other three hybrids have lower crude protein values compared to the PT225 hybrid, but the differences between them are insignificant.
Regarding the content of crude fat in the dry matter, there are no significant differences between the individual hybrids. The values range from 3.45-3.58%.
The content of crude fibre in the dry matter between the individual hybrids varies from 30.93 to 31.50%.
The higher percentage of stems in the aboveground biomass of PT271 hybrid creates a prerequisite for the highest fibre content (31.50%).
This confirms the trend that plants with a higher percentage of leaves have a higher protein content, and these with higher percentage of stems - a higher crude fibre content.
Therefore, in the case of PT225 hybrid, with the lowest percentage of stems, the lowest fibre content is reported (30.93%).
Summarizing the data on the chemical composition of the green mass, we come to the conclusion that hybrid PT225 is characterized by the highest quality of green fodder, due to having the highest content of crude protein and the lowest content of crude fibre.
The green mass obtained from the other hybrids also meets all the indicators for very high quality fodder.

CONCLUSIONS

The duration of the vegetation period in 2017-2018 is from 209 to 212 days, in 2018-2019 - from 218 to 221 days, and in 2019-2020 - from 210 to 213 days.
The highest values of the structural elements both in the three separate years and on average for the period are reported for PT234 hybrid.
From the morphological analysis of the plants of the cultivated hybrids it is observed that the highest relative share in the individual parts of the plant belongs to the stems (from 56.6 to 57.5%), followed by the leaves (from 35.3 to 36.1%), and the blossoms (from 7.1 to 7.4%).
The best ratio of the individual parts of the plants is reported for PT225 hybrid.
During the two years of the experiment and on average for the study period, the highest green mass yield was obtained by PT234 hybrid (69270 kg/ha).
Highest crude protein content and lowest crude fibre content are reported for PT225 hybrid.
All four hybrids tested are high yielding and of high quality, and can be grown for green fodder.

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WEED ASSOCIATION DYNAMICS IN THE OILSEED RAPE FIELDS

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Abstract

The monitoring shows that in the oilseed rape fields of Bulgaria the dominating weed species are Sinapis arvensis L., Raphanus raphanistrum L., and on separate fields Papaver rhoeas L. The most common cereal weeds are Lolium ssp., Avena fatua L.. and wheat self-seeding. Perennial weeds are relatively limited. Species of the genus Phelipanche are most common in southern Bulgaria. The reasons for the mass distribution of these weed species are the violated crop rotations, seeding of oilseed rapes in short period of time - in 1-2 years on the same field, insufficient quality of the soil tillage, limited choice of herbicides for control of parasitic weeds, etc. The implementation of alternative cropping technologies like Clearfield®, their constant improvement, as herbicide content and selection process showed positive effect for decreasing the density and range of distribution of these weeds in the oilseed rape fields.

Key words: *oilseed rape, weed associations, dynamics, Clearfield®.*

INTRODUCTION

Over the last four decades, oilseed rape (*Brassica napus* L.) is established as one of the most important oilseed crops in the world, ranked as the second most important source of vegetable oil around the globe (Brennan and Bolland, 2007). The growing role of oilseed crops is also due to their use as a raw material for biodiesel production (Abbadi and Leckband, 2011; Vinnichek et al., 2019). Oilseed rape is also a potential source of specific proteins and industrial raw materials, biopolymers, surfactants and adhesives (Wu и Muir 2008).

In Bulgaria for 2019 the oilseed rape is grown on an area of 151,174 ha with a total production of 428,256 t (MZH, 2020). One of the factors limiting the normal growth and development of the crop is weed infestation. Weeds are the main competitors of *B. napus* in terms of vegetation factors like water, nutrients, space and light, etc. (Tonev et al., 2019). In addition, they cause indirect damage, as many weed species are hosts of diseases and pests of the crop (Kalinova et al., 2012).

Oerke (2005) points out that the largest crop losses in agriculture, around 34%, are due to the high weed infestation in crops, while the reduction in yields due to pests and diseases is lower and is approximately 16-18%. Moradi, et al. (2020) found that weed competition not only reduced grain yield but also the seed oil content by 5%. If weeds, are not controlled, can lead to a 50% loss of seed yield and reduce the quality of the production (Llewellyn et al., 2016; Konstantinović et al., 2007; Blackshaw et al., 2002; Radosevich et al., 1996). In order to obtain an optimal yield from B. napus, on time and precise weed control is necessary to be accomplished (Tonev et al., 2019; Pavlovic´ et al., 2015; Dimitrova et al., 2014a; Hamzei et al., 2010; Maataoui et al., 2003). An important element for successful weed control in oilseed rape is the establishment of the weed species and associations (Tonev, 2000). There is a dynamics in the weed species composition depending on the latitude in which the crop is grown. Studies on showed that *Lolium rigidum*, *Vulpia myuros*, *Avena fatua* are the most abundant weed species in oilseed rape in Australia (Lemerle et al., 2001).

The most distributed weeds in Hubei, China are *Alopecurus aequalis*, *Veronica persica*, *Polypogon fugax*, *Malachium aquaticum*, *Beckmannia syzigachne*, *Galium aparine*, *Poa annua* (WenDa, et al., 2008).

In Tehran province (Iran) dominant weeds in *B. napus* fields are presented by *Descurainia sophia, Cardaria draba, Rapistrum rugosum, Goldbachia laevigata, Erysimum rependum, Capsella bursa-pastoris, Lamium amplexicaule, Malva neglecta, Veronica persica, Sonchus spp., Galium tricornutum,* *Vicia villosa, Silene conoidea, Fumaria officinalis, Convolvulus arvensis, Avena ludoviciana*, *Bromus tectorum, Euphorbia helioscopia,* etc. (Salimi and Sajedi, 2005).

Also in Iran, but in Golestan Province the most distributed weed species are *Phalaris minor* Retz., *Melilotus officinalis* (L.) Pall., *Rapistrum rugosum* (L.) All., *Avena sterilis* subsp. *ludoviciana*, *Veronica persica* Poir. and *Sinapis arvensis* L. (Ataie et al., 2018). In District Swat-Pakistan major weeds found in oilseed rape are *Sinapsis arvense*, *Chenopodium* spp., *Convolvulus arvensis*, *Coronopus didymus*, *Medicago denticulata*, *Fumaria indica*, *Vicia sativa*, *Poa annua*, etc (Saeed et al., 2011).

In areas of north-east Scotland in winter oilseed rape the most frequently recorded and abundant species are *Poa annua*, *Stellaria media*, *Viola arvensis*, *Hordeum vulgare* [volunteer barley], *Matricaria* spp., *Capsella bursa-pastoris* and *Fumaria officinalis*. Several mainly spring-germinating species are also recorded, e.g. *Spergula arvensis*, *Polygonum aviculare*, *Galeopsis tetrahit*, *Veronica arvensis* and *Capsella bursa-pastoris* (Gillian et al., 1992).

In the United Kingdom, the most common broadleaf weeds in oilseed rape fields are *Stellaria media*, *Matricaria* spp., and *Veronica persica*, while the volunteer cereals and *Poa annua* are the most frequently recorded grass weeds (Whitehead and Wright, 1990).

The weeds infesting oilseed rape in Germany are mainly represented by *Matricaria* spp., *Viola arvensis*, *Capsella bursa-pastoris*, *Stellaria media*, etc. The predominant winter weeds are *Poa annua* and *Apera spica-venti* (Hanzlik, et al., 2010). Hanzlik and Gerowitt (2012) report that in areas with intensive oilseed rape cultivation prevalence of *Geranium* spp., *Sisymbrium* spp. and *Anchusa arvensis* is recorded.

Goerke et al., (2008) found that most of the weed species found in winter oilseed rape in Germany are not evenly distributed. *Sisymbrium officinale*, *Descurainia sophia*, *Centaurea cyanus*, *Anchusa* spp., *Sonchus* spp., *Rumex* spp. or *Euphorbia* spp. are some of the weed species of predominantly regional importance. In contrast, statistically proven differences in weed density in the Germany were recorded for *Capsella bursa-pastoris, Chenopodium album*, *Galium aparine*, *Matricaria* spp., etc.

In the Lublin (Poland) the weeds infesting oilseed rape are *Matricaria maritima*, *Athemis arvensis*, *Centaurea cyanus*, *Agropyron repens* (Kapeluszny, 2005).

In France between the 1970s and the 2000s the most distributed weed species was *Geranium dissectum* (Fried et al., 2015).

In addition to weeds with autotrophic nutrition in oilseed rape, there are also weeds with heterotophic nutrition. Broomrapes are without chlorophyll, root, obligate parasitic plants. In Swat-Pakistan District *Orobanche* spp. parasitizes on rapeseed (Saeed et al., 2011). Kohlschmid et al., 2011 found that the oilseed rape grown in Germany is parasitized by *Phelipanche ramosa*. Gibot-Leclerc et al. (2012) report that broomrape parasite can cause approximately 80% loss of oilseed rape yield.

In order to protect the environment and human health, the efficient use of material and energy resources in the cultivation of oilseed rape for the control of weeds, non-chemical methods and biological products are applied (Marcinkevičiene et al., 2017; Velička et al., 2016; Marcinkevičiene et al., 2015.

Not all the time organic oilseed rape cultivation can easily and effectively applied. That is the main reason for controlling the weeds mainly by the chemical method. The application of herbicides in oilseed rape is one of the most important and responsible points during the vegetation (Frisen, et al, 2003; Heard, et al., 2003; Harker, et al. 2003; Senior and Dale, 2002; Tonev, 2000). The successful weed control in oilseed rape depends on the herbicide application time, i.e. from the optimal stages of the weeds and the crop. The soil or early-vegetation herbicide application is more effective in comparison to the spring treatment (Freeman and Lutman, 2004; Franek, 1994).

The choice of herbicide depends on that if that grown oilseed rape hybrid is selected to be grown by the conventional or Clearfield® technology. In dependence of the existing weed flora at the time of herbicide application a great number of herbicides for weed management in the conventional oilseed rape production technology are evaluated. Such herbicides are propisochlor, trifluralin, haloxyfop-p-methyl; metazachlor; bifenox; clomazone, napropamide, dimethachlor; alachlor; isoxaben; halauxifen-methyl, picloram; propyzamide, aminopyralid; clopyralid; ethametsulfuron-methyl; clethodim and propaquizafop (Bardsley et al., 2018; Lourdet and Rougerie, 2016; Koleva-Valkova et al., 2016; Zotz et al., 2016; Koprivlenski et al., 2015; Dimitrova et al., 2014b; Dimitrova et al., 2014c; Werner, 2014; Duroueix et al., 2013; Lourdet, 2013; Drobny and Schlang, 2012; Stormonth et al., 2012; Bijanzadeh et al., 2010; Majchrzak and Jarosz, 2010; Majchrzak et al., 2008; Konstantinovic´, 2007; Franek and Rola, 2002a; Franek and Rola, 2002b; Franek and Rola, 2001).

At the conventional oilseed rape hybrids some of the registered selective herbicides cannot ensure effective control of the cruciferous weeds like *Sinapis arvensis*, *Raphanus raphanistrum* and *Descurainia sophia*. An alternative for solving this problem is the Clearfield® technology at oilseed rape (Pfenning et al., 2012). In this cropping system, the oilseed rape hybrids are IMI-tolerant (Imidazolinone-tolerant).

During the growing season, for the CL oilseed rape hybrids, Cleranda SC (375 g/l metazachlor + 17.5 g/l imazamox) in rate of 1.50 – 2.00 l/ha + Dash (adjuvant) is successfully applied (Schönhammer et al., 2010; Ádámszki et al., 2010).

The application of imazamox-containing herbicides also provides very good control of the blue wrist in rapeseed. Yanev et al. (2020) found that Pulsar® Plus - 2.00 l / ha (BBCH 51) showed good efficacy against *Ph. ramosa* when applied at Clearfield oilseed rape.

The aim of current research is to study the weed association dynamics in oilseed rape fields under the agro-ecological conditions of the Republic of Bulgaria.

MATERIALS AND METHODS

The study was performed during the period of 2010-2019 in 12 municipalities and lands of 17 settlements in Republic of Bulgaria.

Table 1. Description of the examined area with winter oilseed rape in the period of 2010 – 2019

Year/ Municipalities	Settlements	Oilseed rape hybrid	Examined area, da	Total area, da
2010/Ivanovo	Village of Shtraklevo	LG Architect	320	1640
2010/Ivanovo	Village of Shtraklevo	Ambassador	320	1360
2011/Sliven	Village of Kovachite	Imminent CL	200	650
2012/Yambol	Village of Malomir	Imminent CL	100	1050
2012/Nova Zagora	Village of Omarche	Visbi, Kodiak	500	2500
2013/Nova Zagora	Village of Omarche	PT 200 CL, Sunset CL	500	2500
2013/Bolyarovo	Village of Stephan Karadzhovo	Vectra	100	460
2014/Bolyqrovo	Village of Gorska polyana	Imminent CL	100	1050
2014/Plovdiv	Trial field of Agr. University	PT 279 CL	60	200
2014/Rodopi	Village of Krumovo	Imminent CL	200	600
2015/Silistra	Village of Ivanski	PT 228CL	90	310
2015/Silistra	Village of Neophit Rilski	PX 100 CL	130	1000
2015/Silistra	Village of Vaglen	PT 200CL	130	700
2015/Silistra	Village of Smolnitsa	PX 100 CL	160	520
2016/ Plovdiv	Village of Krumovo	PT 200CL	200	2000
2016/Stara Zagora	Village of Pastren	DK Impression CL	350	1000
2017/Plovdiv	Trial field of Agr. University	PX 111CL	50	50
2017/Svishtov	Village of Bulgarsko slivovo	PX 100 CL	100	150
2017/Ihtiman	Village of Chernyovo	Imminent CL	200	650
2018/Pazardzhik	Village of Simitovo	Imminent CL	150	900
2018/Ivanovo	Village of Krasen	Ambassador	140	1500
2019/Plovdiv	Village of Krumovo	PT 200 CL	200	2000
2019/Plovdiv	Trial field of Agr. University	PT 225	10	30
12 total	17 total		4 310 da 18.88%	22 820 da/ 100%

The criteria for choosing the pigeonholed areas were to be typical for the oilseed rape production and with optimal soil-climatic conditions. The weed infestation monitoring of the fields on which conventional oilseed rape hybrids are grown is in period of 10 years and the weed infestation monitoring of the areas with Clearfield® oilseed rapes is in period of 10 years also.

Weed mapping was performed according to “Methodology for reporting and recording the weeds in major field crops” (Dimitrova et al., 2004).

The efficacy of herbicide products with active substances imazamox + metazachlor and clopyralid + aminopyralid + picloram was recorded by the visual score scale of EWRS.

The data on table 1 shows that the weed infestation research in fields with winter oilseed rape is conducted on an area of 22 820 da. The mapping and identification of the wed species covers an area of 4 310 da, which is 1.88% of the total area.

RESULTS AND DISCUSSIONS

The weed species composition in the areas of the studied regions is very diverse (Table 2). Tatal of 22 weed species from 6 biological groups have been identified. The ephemerals are presented by three species: ivyleaf speedwell - *Veronica hederifolia* L., common henbit – *Lamium amplexicaule* L. and common fumitory – *Fumaria officinalis* L.

From the early spring weeds, widely distributed species in South Bulgaria were: wild oat – *Avena fatua* L., meadow fescue - *Festuca pratensis L. and* cleavers - *Galium aparine* L.

Eight species presenting the weeds from the winter-spring group were reported (7 broadleaf and 1 grass): ryegrass - *Lolium rigidum* Gaud., wild radish - *Raphanus raphanistrum L. and* wild mustard - *Sinapis arvensis* L. These three weeds were found to in low density. This was probably due to the fact that the applied herbicide products for weed control had high efficacy when applied in nonconventional winter oilseed rape hybrids.

The most distributed weeds from the late-spring group were fat-hen - *Chenopodium album L.)* and common purslane - *Portulaca oleracea L.*

In separate regions from South Bulgaria wild hemp - *Cannabis ruderalis Janisch. and* cornflower - *Centaurea cyanus* L.

In the winter oilseed rape fields in Bulgaria the only presenter of the perennial weeds was creeping thistle - *Cirsium arvense (L.) Scop.*

Table 2. Weed species of the infestation of winter oilseed rape fields in areas not treated with herbicides for the period 2010 - 2019

	GRASS WEEDS	BROADLEAF WEEDS
ANNUAL		
Ephemerals		Ivyleaf speedwell - *Veronica hederifolia* L.
		Common henbit – *Lamium amplexicaule* L.
		Common fumitory – *Fumaria officinalis* L.
Winter-spring	Annual ryegrass - *Lolium rigidum* Gaud.	Corn chamomile - *Anthemis arvensis* L.
		Corn-cockle - *Agrostema githago* L.
		Wild radish - *Raphanus raphanistrum* L.
		Shepherd's purse - *Capsella bursa-pastoris* (L.) Med.
		Cornflower – *Centaurea cyanus* L.
		Common poppy - *Papaver rhoeas* L.
		Oriental knight's-spur – *Consolida orientalis* L.
Early-spring	Wild oat – *Avena fatua* L.	Muskweed - *Myagrum perfoliatum* L.
	Fescue - *Festuca pratensis L.*	Hairy vetch - *Vicia hirsuta* (L.) S.F. Gray
		Wild mustard - *Sinapis arvensis L.*
		Cleavers - *Galium aparine* L.
Late-spring		Fat-hen – *Chenopodium album* L.
		Common purslane – *Portulaca oleracea* L.
PERENNIAL		
Root-sprouted		Creeping thistle – *Cirsium arvense* (L.) Scop.
PARASITIC		Branched broomrape – *Phelipanche ramosa* (L.) Pomel
		Mutel's Broomrape – *Phelipanche mutelli* (Schultz) Pomel

Data from the ten-year mapping of the root parasites *Phelipanche ramosa* (L.) Pomel. and *Phelipanche mutelli* (Schultz) Pomel do not lead to the establishment of an exact algorithm for their distribution. Exceptions are seen from the observations in the region of Stara Zagora and Plovdiv, where due to impaired rotation of oilseed rape and its cultivation over a period of 2-3 years, as well as after precrop vegetable crops such as tomatoes, cabbage and others, infestation pressure of species from genus *Phelipanche* is observed and increased.

The analysis of the obtained data for the weed flora before treatment in the areas with oilseed rape grown by the Clearfield technology showed the following. On average for the period of the study period, the following species of weeds are reported in high-density: the winter-spring weeds are the corn chamomile, common poppy, oriental knight's-spur and ryegrass.

From the group of ephemerals only the ivy-leaved speedwell. From early-spring weeds in higher density wild oat and cleavers are found, and from late-spring weeds in some parts of the country the density of fat-hen was high. The root parasite broomrape is also found to be in high density in some areas of South Bulgaria, and especially in regions of Stara Zagora, Yambol, Plovdiv and Pazardzhik. Excellent efficacy of the herbicide products applied in the oilseed rape grown by the Clearfield technology is observed. The controlled weeds in this technology are corn chamomile, common poppy, oriental knight's-spur, cleavers and fat-hen (Figure 1).

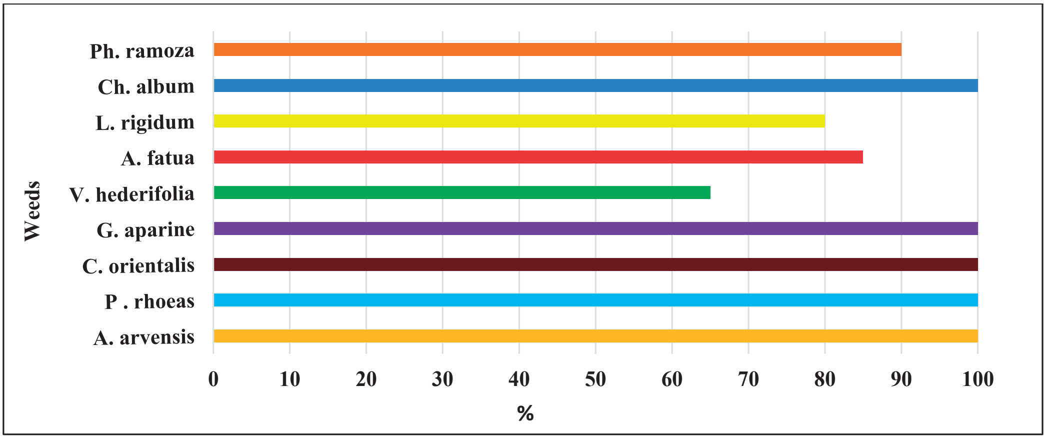

Figure 1. Efficacy of imazamox + metazachlor average for the research period

In a trial conducted by Yanev (2020) the highest herbicide efficacy against *Anthemis arvensis* L., *Papaver rhoeas* L., *Galium aparine* L., *Capsella bursa-pastoris* (L.) Medik, *Lolium temulentum* L., *Avena fatua* L. and the *Triticum aestivum* L. volunteer after the application of Cleranda SC + Dash in rates of 2.00 + 1.00 l/ha, Cleranda SC + Dash in rates of 1.40+1.00 l/ha, as well as Cleravis + Dash in rates of 2.00+1.00 l/ha was found.

It is worth noting that for obtaining high herbicide efficacy against corn chamomile, the herbicide application should be performed before 2nd – 4th leaf stage of the weed. After the growing stage rosette, the efficacy of the herbicides is severely decreased. Ivyleaf speedwell is relatively more resistant to the application of herbicides with active substance imazamox + metazachlor when compared to the above mentioned weeds, but it forms low aboveground biomass and cannot concur the oilseed rape at a higher extend. The grass weeds wild oat and ryegrass are relatively difficult to control with imazamox + metazachlor treatments. For obtaining satisfactory herbicidal effect it is necessary to apply the herbicides before tillering stage of the grass weeds. At the time when these grass weeds are in high density on the field several graminicide herbicides can be applied - cycloxidim, fluazifop-P-butyl, quizalofop-P-ethyl, etc. Very often in the practice these herbicides are used for control of winter wheat volunteers, grown as a predecessor before oilseed rape. Against the parasite broomrape, at the Clearfield technology is relied entirely on the mode of action of the herbicide containing imazamox + metazachlor. According to Mitkov et al. (2017) the effect is high enough and the herbicide applied in the spring controls an average of 90% of the parasite (Yanev et al., 2020). Many scientific studies have shown that the broomrape that remains in the field after treatment with imazamox - containing products forms sterile seeds, which also has economic significance.

Herbicide efficacy of the most often used herbicide product for broadleaf weed control containing is presented on Figure 2. The presented herbicide has no efficacy against the grass weeds. As well as for the Clearfield technology, here the herbicides that control grass weeds like cycloxidim, clerhodim, fluazifop-P-butyl, quizalofop-P-ethyl, etc. The herbicide product containing clopyralid + aminopyralid + picloram shows excellent mixing abilities with the grass herbicides cycloxidim and fluazifop-P-butyl.

At the conventional technology for control of the parasite broomrape it is relied entirely on the genetic resistance of some rapeseed hybrids. The control of the weeds at the conventional oilseed rape fields during the period of the study is conducted by soil herbicides with active substances napropamide, metazachlor, clomazone, dimethachlor, etc.

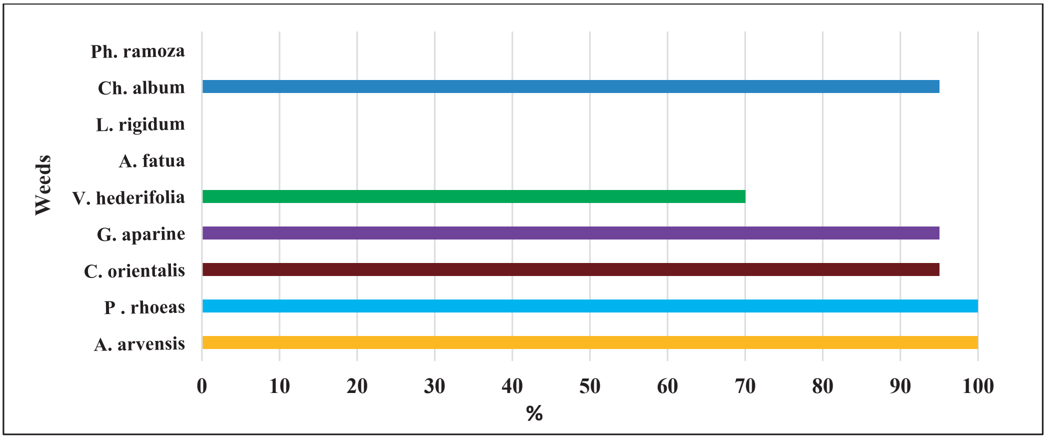

Figure 2. Efficacy of clopyralid + aminopyralid + picloram average for the research period

CONCLUSIONS

The data analyses from the research showed that in the oilseed rape fields of Bulgaria a significant dynamics of the weed species and densities that form the weed associations has occurred. The dominating weed species are wild mustard (*Sinapis arvensis* L.), wild radish (*Raphanus raphanistrum* L.) and on separate fields the wild hemp (*Cannabis ruderalis* Janisch.), common poppy (*Papaver rhoeas* L.) in high density was prevailing. Perennial weeds are relatively limited. Species of genus *Phelipanche* are most common in southern Bulgaria.

The implementation of alternative cropping technologies like Clearfield®, their constant improvement, as herbicide content and selection process showed positive effect for decreasing the density and range of distribution of these weeds in the oilseed rape fields. In Clearfield technology, the herbicides that control cereal weeds wary well are cycloxydim, cletodim, fluazifop-P-butyl, quizalofop-P-ethyl.

At the conventional technology for control of the parasite broomrape it is relied entirely on the genetic resistance of the rapeseed hybrids.

The control of the weeds at the conventional oilseed rape fields during the period of the study is conducted by soil herbicides with active substances napropamide, metazachlor, clomazone, dimethachlor.

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THE QUALITY OF MEADOW FESCUE, *Festuca pratensis*, UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

The main objective of this research was to evaluate the quality of green mass, prepared hay, silage and haylage from meadow fescue, Festuca pratensis 'Tâmpa', cultivated in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova. The results revealed that the harvested green mass contained 25.0-29.8 % dry matter. The dry matter of the whole plant contained 112-120 g/kg CP, 361-394 g/kg CF, 78-79 g/kg ash, 374-381 g/kg ADF, 622-630 g/kg NDF, 34-38 g/kg ADL, 340-343 g/kg Cel, 241-256 g/kg HC, with nutritive and energy value 59.0-61.5% DMD, 54.2-58.1% DOM, RFV=88-89, 9.63-9.71 MJ/kg ME and 5.65-5.73 MJ/kg NEl. The biochemical composition, nutritive and energy value of prepared hay: 113-125 g/kg CP, 378-386 g/kg CF, 88-91 g/kg ash, 407-418 g/kg ADF, 651-673 g/kg NDF, 41-43 g/kg ADL, 366-375 g/kg Cel and 244-255 g/kg HC, 9.0-61.5% DMD, 54.2-58.1% DOM, RFV=88-89, 9.63-9.71 MJ/kg ME and 5.65-5.73 MJ/kg NEl. The fermented fodder, silage and haylage, are characterized by pH = 4.06-4.23, 8.0-8.1 g/kg acetic acid, 29.5-31.2 g/kg lactic acid, 106-119 g/kg CP, 380-399 g/kg CF, 89-96 g/kg ash, 402-414 g/kg ADF, 677-679 g/kg NDF, 28-31 g/kg ADL, 68-76 g/kg TSS, 374-383 g/kg Cel, 402-414 g/kg HC, with nutritive and energy value 56.9-62.2% DMD, 49.2-53.5% DOM, RFV=78-79, 11.27-11.45 MJ/kg DE, 9.63-9.71 MJ/kg ME, 5.65-5.73 MJ/kg NEl. The meadow fescue substrates used for anaerobic digestion have optimal C/N ratio, amount of lignin and hemicellulose. The biochemical methane potential of green mass substrates achieved 346-352 l/kg ODM, silage substrate – 362 l/kg ODM and haylage substrate – 355 l/kg ODM. We consider that the biomass of meadow fescue Festuca pratensis 'Tâmpa' may be used as multi-purpose feed for livestock, and also as feedstock for biogas reactors and renewable energy production.

Key words*: biochemical composition, biomethane potential, Festuca pratensis, green mass, hay, haylage, nutritive value, silage.*

INTRODUCTION

The human population on Earth is steadily growing, which leads to an increase in food and energy demands and aggravates the environmental challenges. Grasslands have a wide range of ecological functions and are home to highly diverse, specialized ecosystems. *Poaceae* is clearly one of the largest and most important families, accounting for about 24% of the Earth's vegetation, containing 777 plant genera and 11461 accepted species. *The Plant List* has mentioned 1741 species of the genus *Festuca*. Among them, 646 are accepted species names. In the spontaneous flora of the Republic of Moldova, there are 8 species.

Meadow fescue, *Festuca pratensis* Huds. (syn. *Lolium pratense* (Huds.) Darbysh.; *Tragus pratensis* (Hudson) Panzer ex B.D.Jackson; *Bromus pratensis* (Huds.) Spreng; *Bucetum pratense* (Huds.) Parn.; *Festuca elatior* subsp. *pratensis* (Huds.) Hack. *Schedonorus pratensis* (Huds.) P. Beauv.) is a long-lived, robust, glabrous, cespitose, perennial grass, native to Europe, C_3 photosynthetic pathway. Stems erect or slightly curved, 40 – 115 cm tall with 3-5 thickened nodes. Leaves narrowly linear, scaberulous along margin, 3-6 mm broad 22-30 cm long with at least 16 distinct veins on the upper part. The leaf blade is dark green, sheaths of inferior leaves – purple red. Ligule short, sometimes missing. Auricles well developed. Panicle-like inflorescence, up to 8-20 cm long, erect, sometimes 1-sided, contracted, drooping at the tip, develops by 2 branches on the lower nodes, the biggest one is half the length of the panicle and has 4-5 spikelets, and the smallest one has 1-3 spikelets. The spikelets are lanceolate or linearly oblong, sometimes laterally compressed, short pedicellate, 9-13 mm long and 2-2.5 mm wide, with 7-8 pale greenish flowers with purple tints, the glumes are 2.5-5.0 mm long, with notched margins, the palea is narrowly lanceolate, sharp, 6-8 mm long. Blossoms in May-June, and bears fruit in July-August. Cross-pollinated by wind. The seeds are cayopses, 4.8-6.3 mm long and 0.9-1.7 mm wide. The weight of 1000 seeds is 1.3-2.2 g. The roots grow 80 cm deep into the soil. The root system is more developed in the upper layer of the soil, the growth rate – medium. This grass is mesophyte, tolerant of waterlogged soils, very well resistant to cold. It has been cultivated since the beginning of the 20th century and is one of the most important forage grasses in the temperate regions of the world. It is used in pasture mixtures on wetlands and for erosion control. *Festuca pratensis* goes well with clover, *Lotus corniculatus*, alfalfa and grasses, such as *Phleum pratense* and *Dactylis glomerata*, producing a large proportion of leafy bottom growth (Stone, 2010; Marușca et al., 2010, 2011; Ţîţei&Roşca, 2021)

The goal of the current study was to evaluate the quality of meadow fescue, *Festuca pratensis*, and the possibility of using it as feed for livestock and feedstock for the production of biomethane by anaerobic digestion.

MATERIALS AND METHODS

The cultivar *'Tâmpa'* of meadow fescue *Festuca pratensis* created in the Research-Development Institute for Grassland Brasov, Romania, and grown in monoculture on the experimental land of National Botanical Garden (Institute) Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″ longitude, served as subject of the research. The samples were collected in pre-anthesis stage (1-st cut) in the second and third growing seasons. The prepared hay was dried directly in the field. The haylage was prepared from wilted mass. For ensiling, the green mass and wilted mass were chopped into 1.5-2.0 cm pieces by using forage chopping unit, shredded and compressed in well-sealed glass containers. The dry matter content was detected by drying samples up to constant weight at 105°C. After 45 days, the containers were opened, and the sensorial and fermented indices of conserved forage were determined in accordance with Moldavian standard SM 108. Some assessments of the main biochemical parameters: protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) have been evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 at the Research-Development Institute for Grassland Brasov, Romania. The concentration of hemicellulose (HC) and cellulose (Cel), relative feed value (RFV), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl)) were calculated according to standard procedures. The carbon content of the substrates was determined using an empirical equation reported by Badger et al. (1979). The biochemical biogas potential (Yb) and methane potential (Ym) were calculated according to the equations of Dandikas et al. 2015, based on the chemical compounds – protein, acid detergent lignin (ADL) and hemicellulose (HC) values:

biogas $Yb=670+0.44PB+0.16HC-3.02ADL$

methane $Ym=370+0.21PB+0.05HC-1.61ADL$

RESULTS AND DISCUSSIONS

In the second growing season, the cultivar 'Tâmpa' of *Festuca pratensis* resumed growth and development in spring, in the middle of March, when the average temperature was about 5-6°C, but in the third growing season – at the end of March. In the spring of the third year of growth, the weather conditions, characterized by optimal amount of rainfall and air temperatures as compared with the previous year, helped the plants produce more shoots and were favorable for their growth, development and biomass production. We would like to mention that the cultivar 'Tâmpa' of *Festuca pratensis*, in second year reached 73.5 cm in height, but in the third growing season, the plants were taller – 118.9 cm. In the harvested biomass, the leaf content was 33.0-35.6%, the amount of dry matter – 25.0-29.8%.

The green mass yield at the first cut, in the second growing season, reached 3.10 kg/m^2, but in the third year – 4.07 kg/m^2.

Several literature sources have described the productivity of *Festuca pratensis.* According to Maruşca et al. (2011) the potential yields of cv. 'Tâmpa' in Romania were 45-50 t/ha green mass or 10 t/ha dry matter and 800 kg/ha seeds. Drapeau & Belanger (2009) reported that meadow fescue in monoculture had produced 4.3 t/ha DM dry matter. Berzins et al. (2015) found that studied cultivars yielded were 5.83-6.49 t/ha. Coblentz et al. (2020) revealed that the dry matter yield of meadow fescue had been 5.58 t/ha and of tall fescue – 5.76 t/ha. De Boer et al. (2020) mentioned that herbage mass of meadow fescue was 5.0 tons/acre DM.

The biochemical composition, nutritive and energy value of the green mass and hay from *Festuca pratensis* cv. 'Tâmpa' are presented in Table 1. Analysing the results of the biochemical composition of green mass, we found that the dry matter contained 112-120 g/kg CP, 361-394 g/kg CF, 78-79 g/kg ash, 374-381 g/kg ADF, 622-630 g/kg NDF, 34-38 g/kg ADL, 340-343 g/kg Cel, 241-256 g/kg HC. Digestibility is the most important factor influencing nutritive and energy value, performance of animals. In our case, the harvested mass was characterized by 59.0-61.5% DMD, 54.2-58.1% DOM, RFV=88-89, 9.63-9.71 MJ/kg ME and 5.65-5.73 MJ/kg NEl. The concentration of crude protein was high in the green mass harvested in the third growing season. It contained a low amount of acid detergent fibre and lignin, but a high amount of hemicellulose in green mass, in the second growing season, which have a positive effect on dry matter digestibility, relative feed value and energy content.

Table 1. The biochemical composition and nutritive value of green mass and hay from *Festuca pratensis* cv. 'Tâmpa'

Indices	Second growing season		Third growing season,	
	green mass	hay	green mass	hay
Crude protein, g/kg DM	112	125	120	113
Crude fibre, g/kg DM	394	378	361	386
Minerals, g/kg DM	78	88	79	91
Acid detergent fibre, g/kg DM	374	407	381	418
Neutral detergent fibre, g/kg DM	630	651	622	673
Acid detergent lignin, g/kg DM	34	41	38	43
Total soluble sugars, g/kg DM	-	-	133	66
Cellulose, g/kg DM	340	366	343	375
Hemicellulose, g/kg DM	256	244	241	255
Digestible dry matter, g/kg DM	615	545	590	495
Digestible organic matter, g/kg DM	581	503	542	471
Relative feed value	88	82	89	79
Digestible energy, MJ/kg	11.83	11.37	11.73	11.21
Metabolizable energy, MJ/kg	9.71	9.34	9.63	9.21
Net energy for lactation, MJ/kg	5.73	5.35	5.65	5.22

Literature sources indicate considerable variation in the chemical composition and nutritional value of harvested *Festuca pratensis* plants. According to Burlacu et al. (2002), the nutritive composition of *Festuca pratensis* was: 16.8-22.1% DM, 7.9-10.5% ash, 11.4-19.5% CP, 3.3-4.8 % fats, 21.4-30.0% CF, 43.8-47.5% NFE, 59.6-64.1% NDF, 39.6% ADF, 3.8-4.7% ADL, 31.5-37.2% Cel, 16.0% HC, 18.3-18.4 MJ/kg GE, 11.74-14.11 MJ/kg DE, 9.61-11.41 MJ/kg ME. Podkówka et al. (2011), reported that the dry matter content and the nutritive value of meadow fescue were: 300.98-310.48 g/kg DM, 82.16-86.92 g/kg CP, 21.72-23.03 g/kg fat, 291.42-311.29 g/kg CF, 534.87-542.86 g/kg NFE, 554.34-563.31 g/kg NDF, 283.58-295.93 g/kg ADF, 19.93-20.80 g/kg ADL. Sosnowski (2012) found that *Festuca pratensis* contained 32.7-35.9% ADF, 49.7-54.2% NDF, with 61.0-63.4% DMD, RFV=104-119. Guo et al. (2013) mentioned that mixed-crop 60% tall fescue and 40% meadow fescue at the first cut contained 179 g/kg DM, 20.6% CP, 54.3% NDF, 13.9% NFC and at the second cut – 291 g/kg DM, 13.9% CP, 59.5% NDF, 13.7% NFC. Berzins et al. (2015) remarked that the forage quality of first-cut green mass of *Festuca pratensis* cultivars was 10.66-12.50% CP, 5.46-6.79% ash and RFV= 89.49-104.53, but – of second-cut– 15.27-17.13% CP, 6.97-7.49% ash and RFV=101.28-114.43, respectively. Staniak (2016) found that the tested *Festuca pratensis* cultivars, in the second year of growth, contained 146-156 g/kg CP, 286-296 g/kg CF, 73.4-75.2% DDM, 86.6-94.5% PDIE, 32.9-38.1% PDIF, 0.90-0.91 UFL, 0.84-0.86 UFV. Coblentz et al. (2020) found that the biochemical composition and nutritive value of meadow fescue fresh mass was 71 g/kg CP, 90 g/kg ash, 98.3 g/kg WSC, 609 g/kg NDF, 364 g/kg ADF, 27.5 g/kg ADL, 1.40 Mcal/kg NEl, but tall fescue – 75 g/kg CP, 82 g/kg ash, 107.3 g/kg WSC, 627 g/kg NDF, 356 g/kg ADF, 27.9 g/kg ADL, 1.40 Mcal/kg NEl, respectively. De Boer et al. (2020), mentioned that herbage mass of meadow fescue contained 11% CP and 59% NDF. Pabón-Pereira et al. (2020) reported that *Festuca pratensis* green leaves contained 482.9 g/kg NDF, 279.2 g/kg ADF, 15.2 g/kg ADL, but *Zea mays* green leaves contained 588.6 g/kg NDF, 277.3 g/kg ADF, 5.8 g/kg ADL.

Grass hay plays an important role in the diet of herbivores, represents a low-cost and abundant source of nutrients and is vital to keep animals healthy and productive. We would like to mention that the hay prepared from *Festuca pratensis* 'Tâmpa' (Table 1) contained 113-125g/kg CP, 378-386 g/kg CF, 88-91 g/kg ash, 407-418 g/kg ADF, 651-673 g/kg NDF, 41-43 g/kg ADL, 366-375 g/kg Cel and 244-255 g/kg HC. The nutritive value and the energy value of the hay were 59.0-61.5% DMD, 54.2-58.1% DOM, RFV=88-89, 9.63-9.71 MJ/kg ME and 5.65-5.73 MJ/kg NEl. The amounts of minerals, crude fibre, cellulose, hemicellulose and lignin increased substantially in the hay obtained in the third year, which contributed to the reduction of dry matter digestibility, relative feed value and energy content. During the process of preparing hay, we observed an increase in the concentration of structural carbohydrates, lignin, minerals and a decrease in the total soluble sugars content, dry matter digestibility and relative feed value and energy concentration as compared to green mass.

Some authors mentioned various findings about the quality of fescue hay. Burlacu et al. (2002) reported that *Festuca pratensis* hay contained: 8.5-11.5% ash, 10.0-18.2% CP, 2.8-4.1% fats, 22.5-32.0% CF, 43.7-46.7% NFE, 18.1-18.2 MJ/kg GE. Akdeniz et al. (2019), reported that the dry biomass yield and nutritional quality of tall fescue hay, in the second year, were: 10.23 t/ha, 9.54% ash, 9.86% CP, 1.15% fats, 44.85% CF, 64.05% NDF, 47.64% ADF, RFV=75.22 and of creeping red fescue hay – 4.34 t/ha, 8.94% ash, 8.85% CP, 1.44% fats, 43.61% CF, 69.25% NDF, 44.26% ADF, RFV=71.11.

The management of forage as silage provides the opportunity to harvest the crop at a desired level of digestibility for subsequent feeding. Silage production minimizes the risk associated with field losses, which can be incurred under rainy conditions during hay making. Wilting herbage prior to ensiling has many advantages including reducing effluent production and fuel consumption, improved characteristics of ensiling, reduced quantities of silage for transport during feed out and reduced straw requirement for bedding livestock. Grass, when harvested and stored as silage and haylage, is an important source of nutrients for livestock, is a great way to preserve nutrients for autumn - middle spring, a period when grasslands are less productive. When opening the glass vessels with fermented fodder, silage and haylages, prepared from *Festuca pratensis* cv. 'Tâmpa' in the third growing season, there was no gas or juice leakage from the preserved mass. The fodder had agreeable colour and aroma, the consistency was retained, in comparison with the initial green mass, without mould and mucus. During the sensorial assessment, it was found that the colour of the silage was dark green leaves and light green stems with pleasant smell specific to pickled vegetables, but haylage – homogeneous yellow-olive colour with pleasant smell like pickled fruits. The fermentation quality, biochemical composition and nutritive value of silage and haylage from *Festuca pratensis* cv. 'Tâmpa' is shown in Table 2. It has been determined that the pH index was 4.06-4.23, the concentrations of organic acids reached 37.6-39.2 g/kg, and most amounts of organic acids were in fixed form. The content of lactic acid increased in haylage. Butyric acid not was detected in the fermented fodder.

Table 2. The fermentation quality, biochemical composition and nutritive value of silage and haylage from meadow fescue, *Festuca pratensis* cv. 'Tâmpa'

Indices	Silage	Haylage
pH index	4.06	4.23
Content of organic acids, g/kg	37.6	39.2
Free acetic acid, g/kg	3.7	3.9
Free butyric acid, g/kg	0	0
Free lactic acid, g/kg	8.5	6.9
Fixed acetic acid, g/kg	4.4	4.1
Fixed butyric acid, g/kg	0	0
Fixed lactic acid, g/kg	21.0	24.3
Crude protein, g/kg DM	119	106
Crude fibre, g/kg DM	380	399
Minerals, g/kg DM	96	89
Acid detergent fibre, g/kg DM	402	414
Neutral detergent fibre, g/kg DM	679	677
Acid detergent lignin, g/kg DM	28	31
Total soluble sugars, g/kg DM	76	68
Cellulose, g/kg DM	374	383
Hemicellulose, g/kg DM	277	263
Digestible dry matter, g/kg DM Digestible	622	569
organic matter, g/kg DM	535	492
Relative feed value	79	78
Digestible energy, MJ/ kg	11.45	11.27
Metabolizable energy, MJ/ kg	9.40	9.25
Net energy for lactation, MJ/ kg	5.41	5.28

Analyzing the results regarding the quality of fermented fodder from *Festuca pratensis* cv. 'Tâmpa', in the third growing season, Table 2, we found that the dry matter of fermented fodder was characterized by 106-119 g/kg CP, 380-399 g/kg CF, 89-96 g/kg ash, 402-414 g/kg ADF, 677-679 g/kg NDF, 28-31 g/kg ADL, 68-76 g/kg TSS, 374-383 g/kg Cel, 402-414 g/kg HC, with nutritive and energy value 56.9-62.2% DMD, 49.2-53.5% DOM, RFV=78-79, 11.27-11.45 MJ/kg DE, 9.63-9.71 MJ/kg ME and 5.65-5.73 MJ/kg NEl. There was a significantly higher content of crude protein, total soluble sugars, hemicellulose and energy concentration in the prepared silage.

According to Smith et al. (1987), the nutrient concentration and fermentation characteristics of fescue haylage were 479 g/kg DM, 10.5% ash, 15.5% CP, 3.8% fats, 44.7% nitrogen-free extract, 45.7% NDF, 25.5% ADF, 3.2% ADL, 3.1% WSC with 69% DMD, pH=4.3, 3.22 % lactic acid, 1.52 % acetic acid, 0.10% butyric acid. Burlacu et al. (2002), reported that *Festuca pratensis* silages contained: 18.5-24.5% DM, 8.0-10.5% ash, 13.5-17.5% CP, 4.7-5.7% fats, 23.8-27.9% CF, 43.0-45.3% NFE, 18.6-18.8 MJ/kg GE, 11.59-12.99 MJ/kg DE, 9.09-10.40 MJ/kg ME, but haylage (wilting silage) 35.0% DM, 8.4-10.9% ash, 11.5-15.7% CP, 3.5-5.4% fats, 25.0-30.6% CF, 43.0-46.0% NFE, 18.3-18.4 MJ/kg GE, 11.55-12.84 MJ/kg DE, 9.45-10.46 MJ/kg ME. Pozdíšek et al. (2003) mentioned that the chemical composition and energy value of silage from *Festuca arundinacea* were 118.5 g/kg CP, 33.8 g/kg fat, 261.9 g/kg CF, 477.4 g/kg NFE, 528.9 g/kg NDF, 308.6 g/kg ADF, 108.4 g/kg ash, 9.54 MJ/kg ME, 5.65 MJ/kg NEl. Muller& Uden (2007) compared the quality of conserved grass from permanent grassland consisting of timothy grass, meadow fescue, and a small proportion (0.1%) of couch grass, *Agropyron repens*, found that the prepared silage had pH=4.3 and contained 31.8 g/kg lactic acid, 6.6 g/kg acetic acid, 1.0 g/kg butyric acid, 309 g/kg DM, 11.3% CP, 6.6% ash, 58.5% NDF, 2.6% WSC, 77% DMD, 9.7 MJ/kg ME for horses; the haylage had pH=5.63 and contained 2.6 g/kg lactic acid, 1.4 g/kg acetic acid, 0.4 g/kg butyric acid, 577 g/kg DM, 10.8% CP, 6.4% ash, 60.8% NDF, 6.9% WSC, 74% DMD, 9.4 MJ/kg ME for horses; the hay contained 884 g/kg DM, 11.3 % CP, 6.6% ash, 60.5% NDF, 10.1% WSC, 77% DMD, 9.8 MJ/kg ME for horses. Kuoppala et al. (2009) remarked that the chemical composition and the feed value of silage prepared from mixed timothy grass and meadow fescue were: 283 g/kg DM, 6.8-8.2% ash, 12.7-15.5% CP, 49.8-58.9% NDF, 5.0-9.7% iNDF, 44.8-49.2 pdNDF, 2.3-2.7% lignin, pH=3.97-4.22, 6.84% lactic acid, 1.31-1.74% acetic acid, 0.13-0.48% butyric acid with 64.4-70.4% DOM and 10.3-11.3 MJ/kg ME. Guo et al. (2013) mentioned that ensiled fescue first-cut fresh mass contained 178 g/kg DM, 18.7 % CP, 47.2% NDF, 22.8% NFC, pH=4.84, 5.59% lactic acid, 4.59% acetic acid, but ensiled fescue wilted forages contained 26.4-44.7 g/kg DM, 19.8-20.2% CP, 46.6-47.4% NDF, 21.4-22.4% NFC, pH=4.93-5.10, 2.95-4.25% lactic acid, 1.23-2.54% acetic acid. Purwin et al. (2014) mentioned that red fescue silage from wilted mass contained: 437-485 g/kg DM, 16.5-17.0 g/kg total nitrogen, 598-617 g/kg NDF, 408-410 g/kg ADF, 62-65 g/kg ADL, 345-346 g/kg Cel, 190-207 g/kg HC. Kupryś-Caruk & Kołodziejski (2016) reported that the dry matter content and the chemical composition of silages from *Festuca arundinacea* were 214 g/kg DM, pH=5.2, 89.7 g/kg lactic acid, 2.3 g/kg acetic acid, 10.8% CP, 11.7% ash, 2.3% fats, 5.0% sugars, 3.0% ADL, 30.4% Cel, 5.7% HC. Coblentz et al. (2020) compared the feed quality and the energy value of silage from different species of fescue and mentioned that *Festuca pratensis* silage contained 77 g/kg CP, 97 g/kg ash, 51.4 g/kg WSC, 644 g/kg NDF, 391 g/kg ADF, 30.1 g/kg ADL, 1.34 Mcal/ kg NEl, 21.7 g/kg lactic acid, 7.6 g/kg acetic acid, 3.0 g/kg butyric acid, but *Festuca arundinacea* – 85 g/kg CP, 91 g/kg ash, 74.7 g/kg WSC, 649 g/kg NDF, 366 g/kg ADF, 26.9 g/kg ADL, 1.37 Mcal/ kg NEl, 16 g/kg lactic acid, 8.2 g/kg acetic acid, 2.4 g/kg butyric acid. Müller et al. (2020) remarked the nutritive value of a grass mixture, consisting of *Phleum pratense*, *Lolium perenne* and *Festuca pratensis* conserved in big round bales, which was as follows: 12.6% CP, 9.6% ash, 10.7% WSC and 9.9 MJ/kg ME for horses.

Replacing fossil fuels with renewable energy alternatives has become a major global issue of the 21th century and a key to sustainable development. Anaerobic digestion is dedicated to the use of organic waste as a source for renewable energy, though often combined with energy crops as input material. However, in order to consolidate the role of biogas production via anaerobic digestion as a renewable energy production technology, it is important to ensure the availability of sustainable biomass sources, because installations do not only produce energy but also digestate, which is rich in organic matter and plant nutrients such as nitrogen, phosphate and potash, and could serve as a replacement for fossil based fertilizers. The biodegradation of different types of lignocellulosic biomass feedstock depends on the chemical structure, primarily on the content of cellulose, hemicellulose, lignin and the C/N ratio. The carbon nitrogen ratio (C/N) of biomass plays a crucial role in the process of decomposition of organic matter by microorganisms. Mowed grass can be processed conveniently into an attractive feedstock for biogas production. Good ensiling, where lactic acid production dominates the fermentation, will efficiently conserve grass as a feedstock for anaerobic digestion. Ensiled grass may provide a year-round supply of feedstock for biogas production facilities.

The results regarding of the substrate quality and its biochemical methane potential are illustrated in Table 3. We found that the investigated substrates from *Festuca pratensis* cv. 'Tâmpa', according to the C/N ratio, which constituted 26-30, met the established standards. The essential differences were observed between the content of cellulose, hemicellulose and lignin. The silage and haylage substrates contained high amount of hemicellulose and reduced lignin content, compared with green mass substrates. The biochemical methane potential of investigated green mass substrates was 346-352 l/kg ODM, but – from silage and haylage substrates – 355-362 l/kg ODM. According to Mähnert et al. (2002), *Festuca pratensis* 'Cosmos11' fresh mass substrate contained 176 g/kg DM, 91.5% OM, pH= 6.4 with biogas yield 708 l/kg and silage substrate – 274 g/kg DM, 89.9% OM, pH=4.7, 887 l/kg, but *Festuca arundinacea* 'Elfina' fresh mass substrate contained 139 g/kg DM, 89.1% OM, pH=6.4 with biogas yield 688 l/kg and silage substrate – 173 g/kg DM, 89.6% OM, pH=4.0 with biogas yield 887 l/kg, respectively. Jagadabhi et al. (2010) found that the ensiled grass mixture 75% *Phleum pratense* and 25% *Festuca pratensis* biomethane yield achieved 400 l/kg VS. Kaiser & Gronauer (2007) mentioned that the methane potential of studied red fescue varieties was 280–335 l/kg or 3800–5300 m^3/ha. Kupryś-Caruk & Kołodziejski (2016) indicated that the biogas potential of fescue silage was 734.1 m^3/t with 55% methane content.

Table 3. The biochemical composition and biomethane production potential of *Festuca pratensis* cv. 'Tâmpa' substrates

Indices	Second growing season	Third growing season		
	green mass	green mass	silage	haylage
Crude protein, g/kg DM	112	120	119	106
Minerals, g/kg DM	78	79	96	89
Nitrogen, g/kg DM	17.92	19.20	19.04	16.96
Carbon, g/kg DM	512.22	511.67	502.22	506.11
Carbon/nitrogen ratio	28.58	26.65	26.38	29.84
Cellulose, g/kg DM	340	343	374	383
Hemicellulose, g/kg DM	256	241	277	263
Acid detergent lignin, g/kg DM	34	38	28	31
Biomethane potential, L/kg VS	352	346	363	355

CONCLUSIONS

The green mass yield of *Festuca pratensis* cv. 'Tâmpa' reached 3.10-4.07 kg/m^2, the dry matter of harvested green mass contained 112-120 g/kg CP, 361-394 g/kg CF, 78-79 g/kg ash, 374-381 g/kg ADF, 622-630 g/kg NDF, 34-38 g/kg ADL, 340-343 g/kg Cel, 241-256 g/kg HC, with nutritive and energy value 59.0-61.5% DMD, 54.2-58.1% DOM, RFV=88-89, 9.63-9.71 MJ/kg ME and 5.65-5.73 MJ/kg NEl. The biochemical composition, the nutritive and energy value of the prepared hay has the following indices: 113-125 g/kg CP, 378-386 g/kg CF, 88-91 g/kg ash, 407-418 g/kg ADF, 651-673 g/kg NDF, 41-43 g/kg ADL, 366-375 g/kg Cel and 244-255 g/kg HC, 9.0-61.5% DMD, 54.2-58.1% DOM, RFV=88-89, 9.63-9.71 MJ/kg ME and 5.65-5.73 MJ/kg NEl.
The fermented fodder, silage and haylage, from *Festuca pratensis* 'Tâmpa', is characterized by pH = 4.06-4.23, 8.0-8.1 g/kg acetic acid, 29.5-31.2 g/kg lactic acid, 106-119 g/kg CP, 380-399 g/kg CF, 89-96 g/kg ash, 402-414 g/kg ADF, 677-679 g/kg NDF, 28-31 g/kg ADL, 68-76 g/kg TSS, 374-383 g/kg Cel, 402-414 g/kg HC, with nutritive and energy value 56.9-62.2% DMD, 49.2-53.5% DOM, RFV=78-79, 11.27-11.45 MJ/kg DE, 9.63-9.71 MJ/kg ME, 5.65-5.73 MJ/kg NEl.
The biochemical methane potential of green mass substrates achieved 346-352 l/kg ODM, silage substrate – 362 l/kg ODM and haylage substrates – 355 l/kg ODM.
Under the conditions of the Republic of Moldova, cv. 'Tâmpa' of *Festuca pratensis* produces optimal yield and nutrient content, can be used to re-cultivate permanent grasslands and to establish temporary grasslands in order to prevent soil erosion, in monoculture or associated with other grasses and forage legume, and the harvested mass can be used as green mass, hay, silage and haylage for livestock, or as a substrate for the production of biomethane and renewable energy.

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NUTRITIONAL PROFILE OF SOME ROMANIAN WINTER BARLEY GENOTYPES

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Abstract

This paper aims to describe the grains chemical composition of some winter barley genotypes performed in two experiments at NARDI Fundulea and ARDS Turda during two years (in four environmental conditions). The obtained results (i.e., ADF, NDF, ash, hemicellulose, protein, and starch content) were used to assess the nutritional profile and value of each genotype using specific formulas and correlations of the studied quality indices. These indices have been assessed separately among six-row and two-row winter barley grown and harvested during the 2017-2018 and 2018-2019 years. The nutritional profile of the analysed samples has varied due to genotype and environment and has been clearly influenced by their interactions. These outcomes are important to highlight the differences between genotypes and elaborate on several recommendations regarding their uses.

Key words: *ADF, NDF, protein content, starch content, winter barley genotypes.*

INTRODUCTION

Among the crops worldwide, barley (*Hordeum vulgare* L.) is one of the oldest cereals and it is used in malt and brewing industry, for feeding cattle and food (human nutrition) due to many end-uses, for instance as pearled grain for soup, bread, biscuits, muffins, pasta, breakfast cereals (Velebna et al., 2012; AEGIC, 2016), shochu - traditional Japanese spirit, whiskey, distilled spirits, malt extract, malt vinegar, flavored sweet drinks (GRDC, 2017).

The changes in climatic conditions could lead to a significant demand regarding the quantity and quality of barley yield in correspondence with those three uses. According to Food and Agriculture Organization (FAO)), rice, wheat, barley, and rye (small grain cereal) are the main contributors to the world's calorific intake (more than 50%) (Awika, 2011). Increasing interest for barley used in human nutrition was noticed because the consumption of different products obtained from barley grains is associated with benefits for human body health.

This interest is related to barley superior nutritional qualities due to the presence of soluble fiber namely beta-glucan with a role in decreasing the level of cholesterol (Newman et al., 1989), and the glycemic index stability (Klopfenstein, 1988), phenolic compounds with free radical action scavenger (Siebenhandl-Ehn et al., 2011) acetylcholine carbohydrate substance which nourishes the human nervous system and helps to recovers memory loss, easy digestibility due to low gluten and high lysine content (Behall et al., 2004; Kumar et al., 2013).

Another mention about barley nutritional quality was made by Březinová et al. (2009), where barley grain is identified as a source of soluble fiber (the most important are β-glucans and arabinoxylans) and enzymes (SOD - superoxide dismutase).

Also, barley represents one of the most significant sources of anti-oxidants due to the vitamin E which can range from 16 to 24 mg kg^{-1} (Pryma et al., 2007) and with exception of B12 vitamin it is a source of B vitamins (Newman & Newman, 1992).

Regarding the barley grain chemical composition, the main component of this is starch (which constitutes the source of energy), protein (being important for nutritional value and technological quality in malt), and non-starch polysaccharides called dietary fiber, (determining a suitable activity of the human body). In order to describe the dietary fiber, two indicators can be used, namely Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF).

The amount of hemicellulose or cell sugar can be obtained through the difference between NDF and ADF values (Hindrichsen et al., 2006). Newman and Newman (1992) stated that both types of fiber, NDF and ADF, are practically indigestible in the gastrointestinal tract of humans.

MATERIALS AND METHODS

Two winter barley field experiments in two seasons (2017-2018 and 2018-2019) and two locations were carried out to investigate the nutritional barley profile of 19 winter genotypes (varieties and lines).

The first location was National Agricultural Research and Development Institute Fundulea (NARDI Fundulea) considered location 1 (L1), situated in the south-east of Romania and the second location was Agricultural and Research Development Station Turda (ARDS Turda) situated in the Transylvanian Plateau (Turda), considered location 2 (L2). The L1 is located at 44°26`N latitude and 26°31`E longitude at the elevation level of 68 meters and L2 is located at 46°35`N latitude and 23°47`E longitude at the elevation level between 345-493 meters.

These locations were chosen due to the pedoclimatic differences and suitability of the area to growing winter barley versus spring barley (the L1 zone is recommended to grow winter barley and the L2 zone are very favorable or thermally favorable areas for spring barley growing) and also how the nutritional value can be influenced by the variety and environmental conditions (Ehrenbergerová et al., 2008; Benkova et al., 2012; Alijošius et al., 2016; Loskutov & Khlestkina, 2021).

Whole grain flour obtained by grinding barley samples with a laboratory mill (Mill WZ-1) was used to determine the chemical composition of seeds.

After grinding the barley samples in triplicate, the whole grain flour was analyzed by Tango FT-NIR spectrometer. In order to assess the chemical traits, no additional reagents were necessary. ADF (%), NDF (%), ash (%), protein (%), and starch content (%) were determined and the content of cell wall structural carbohydrates namely hemi-cellulose was calculated as the differences:

$$\text{Hemicellulose} = \text{NDF} - \text{ADF}$$

All the data obtained were expressed in percent on a dry weight basis.

The results obtained from the analyzes were statistically processed with MS EXCEL 2016 and the average, minim and maxim values, the standard deviation and the coefficient of variability were calculated according to Ardelean (2008). Data are expressed as two years mean for each location.

The simple correlation coefficients (r) were calculated for the analyzed traits (separately for six row genotypes and two row genotypes) in order to establish the degree of association between each trait with all the others studied.

A value of “r” close to 1 could be interpreted as an indicator for a trait with high heritability.

The L1 zonal climate is continental temperate with an average annual temperature of 10°C and the coldest month is January with an average of -3.0°C. The warmest month is July, with an average temperature of 22°C and an absolute maximum temperature of 41°C, which is very higher. The annual average rainfall is 571 mm and the quantity of 72% occurring during the growing season of winter barley, mainly in May-June.

The L2 zonal climate is generally a typical continental boreal climate, the rainfall during the year have only one maximum at the beginning of summer, hot summers and winters are quite harsh. From a thermal point of view, the annual average temperature is around 8.6°C, the July month having the highest average temperature of 19.3°C and January the coldest with an average temperature of -4.4°C. The main purpose of this research was to investigate few representative barley grain chemical parameters of some Romanian winter barley genotypes (six and two rows) tested in two locations.

RESULTS AND DISCUSSIONS

The analysis of variance was performed separately on six row and two row winter barley to determine how each trait is influenced by location. The source of variation is presented in Table 1, L1 (a and b) and L2 (c and d). The analysis of variance showed for the L1 that location (L), genotype (G) and their interaction (L x G) influenced significantly all the traits in the case of six rows and two rows winter barley (a and b), except the influence of location on ash (ASH) and starch content (SC) of two rows winter barley which was insignificant (b). For the L2, location, genotype and the interaction between L x G (c and d) had a significant influence but the F test showed an inverse reaction of six rows barley genotypes (c) regarding the influence of location on ASH and SC comparing with two rows winter barley from L1 (b). The behavior of two rows winter barley in L2 was different (d) comparing with L1, where the location had an insignificant influence on acid detergent fiber (ADF), neutral detergent fiber (NDF) and hemicellulose content (HC). This analysis revealed a different pattern depending on the type of winter barley (six or two rows).

Table 1. ANOVA for ADF (acid detergent fiber), NDF (neutral detergent fiber), ASH (ash content), HC (hemicellulose content), PC (protein content), SC (starch content), L1 (location 1) and L2 (location 2)

ANOVA table - F factor for six row winter barley, L1 (a)							
Source of variation	DF	ADF	NDF	ASH	HC	PC	SC
Location	1	1115.553**	59.646**	147.075**	48.554**	5605.809**	61.828**
Genotype	11	6.913**	8.795**	14.334**	10.214**	123.960**	3.132**
L x G	11	7.308**	3.571**	4.791**	4.028**	85.825**	3.799**
Error	48						

ANOVA table - F factor for two row winter barley, L1 (b)							
Source of variation	DF	ADF	NDF	ASH	HC	PC	SC
Location	1	17.254**	10.782**	2.846^{ns}	50.628**	335.463**	0.654^{ns}
Genotype	6	12.115**	3.231**	2.910**	4.951**	42.622**	3.467**
L x G	6	5.987**	4.096**	4.753**	6.630**	18.216**	3.623**
Error	28						

ANOVA table - F factor for six row winter barley, L2 (c)							
Source of variation	DF	ADF	NDF	ASH	HC	PC	SC
Location	1	17.254**	10.782**	2.846^{ns}	50.628**	335.463**	0.654^{ns}
Genotype	11	12.115**	3.231**	2.910**	4.951**	42.622**	3.467**
L x G	11	5.987**	4.096**	4.753**	6.630**	18.216**	3.623**
Error	48						

ANOVA table - F factor for two row winter barley, L2 (d)							
Source of variation	DF	ADF	NDF	ASH	HC	PC	SC
Location	1	1.519^{ns}	3.597^{ns}	22.519**	3.787^{ns}	19.505**	5.492*
Genotype	6	26.824**	11.103**	15.583**	5.106**	93.019**	18.860**
L x G	6	15.252**	5.927**	9.104**	3.497*	92.648**	9.086**
Error	28						

** significant at 0.01 level; * significant at 0.05 level, ^{ns} – insignificant.

Among L1 different studied six row winter barley genotypes (Table 2), ADF content varied from 5.05% (F 8-5-13 line) to 6.19% (F 8-4-12 line), NDF content from 10.93% (Onix variety) to 13.62% (F8-6-12 line), ASH content from 1.55% (Simbol and Onix varieties) to 1.82% (F 8-4-12 line), HC content from 5.58% (Onix variety) to 8.22% (F8-6-12 line), protein content from minim value of 9.29% (Simbol variety) to maxim value of 11.54% (F 8-3-2001 line) and SC from 54.11% (F 8-6-12 line) to 58.18% (Onix variety). It can be noticed a low coefficient of variation (good stability) for all chemical traits (<10%), except HC where a CV>10% shows a medium stability of this. The two rows winter barley genotypes (Table 3) values of chemical parameters, ranged for ADF from 3.59% (F 8-114-10 line) to 5.99% (Andreea variety), the NDF content from 9.03 (F 8-114-10 line) and 13.99% (Artemis variety), ASH content from 1.51% (F 8-114-10 line) to 1.90% (Artemis variety), HC content from

5.44% (F 8-114-10 line) to 8.29% (Artemis variety), PC content from 10.56% (Gabriela variety) to 13.09% (F 8-114-10 line) and for the SC content the lowest value was 52.29% (Artemis variety) and the higher 58.42% (Gabriela variety). The most stable parameters were ASH, PC and SC content with 7.53%, 7.37% and 3.84% values respectively, for the coefficient of variation. ADF, NDF and HC content had medium stability according to their CV%.

Table 2. Chemical grain composition of six rows winter barley, L1

Genotypes	ADF	NDF	ASH	HC	PC	SC
Dana	6.00	12.79	1.71	6.78	10.66	56.88
Cardinal	5.58	12.17	1.61	6.59	10.21	56.95
Univers	5.82	12.82	1.67	7.01	10.72	56.76
Ametist	5.74	11.68	1.64	5.95	10.80	57.19
Smarald	5.60	11.62	1.59	6.03	9.58	57.12
Simbol	5.46	11.59	1.55	6.14	9.29	56.50
Onix	5.34	10.93	1.55	5.58	10.61	58.18
Lucian	5.60	11.87	1.67	6.27	10.39	57.13
F8-3-01	5.65	11.31	1.69	5.67	11.54	56.55
F8-4-12	6.19	12.57	1.82	6.38	11.16	55.87
F8-5-13	5.05	12.64	1.69	7.59	10.13	55.93
F8-6-12	5.40	13.62	1.81	8.22	10.85	54.11
Average	**5.62**	**12.13**	**1.67**	**6.52**	**10.49**	**56.60**
Min	**5.05**	**10.93**	**1.55**	**5.58**	**9.29**	**54.11**
Max	**6.19**	**13.62**	**1.82**	**8.22**	**11.54**	**58.18**
CV (%)	**5.39**	**6.34**	**5.18**	**12.04**	**5.99**	**1.75**
STDEV (%)	**0.30**	**0.77**	**0.09**	**0.78**	**0.63**	**0.99**

Table 3. Chemical grain composition of two rows winter barley, L1

Genotypes	ADF	NDF	ASH	HC	PC	SC
Andreea	5.99	13.34	1.83	7.35	11.14	55.06
Artemis	5.70	13.99	1.90	8.29	11.46	52.29
Gabriela	4.79	11.06	1.65	6.27	10.56	58.42
DH 375-4	5.23	12.80	1.82	7.56	11.89	57.16
F8-106-10	5.37	12.75	1.79	7.38	12.70	55.28
F8-114-10	3.59	9.03	1.51	5.44	13.09	58.30
DH 267-66	5.43	12.33	1.71	6.90	11.95	56.55
Average	**5.16**	**12.19**	**1.74**	**7.03**	**11.83**	**56.15**
Min	**3.59**	**9.03**	**1.51**	**5.44**	**10.56**	**52.29**
Max	**5.99**	**13.99**	**1.90**	**8.29**	**13.09**	**58.42**
CV (%)	**15.23**	**13.61**	**7.53**	**13.25**	**7.37**	**3.84**
STDEV (%)	**0.79**	**1.66**	**0.13**	**0.93**	**0.87**	**2.15**

Under the L2 conditions (Table 4), the tested genotypes had a different behavior regarding the chemical studied parameters. Therefore, the six rows winter barley average values for ADF, NDF and HC content were a little bit higher than in L1 (5.86% ADF, 12.98% NDF, 7.13% HC comparing with 5.62% ADF, 12.13% NDF, 6.52% HC under L1 environment). All the traits of six rows winter barley were noted by the low CV which means good stability of them.

Regarding two rows winter barley different values were registered (Table 5) and the coefficient of variation was lower than 10%, except ADF content value.

Table 4. Chemical grain composition of six rows winter barley, L2

Genotypes	ADF	NDF	ASH	HC	PC	SC
Dana	6.36	13.61	1.67	7.25	9.71	53.57
Cardinal	6.10	12.80	1.59	6.70	9.13	54.84
Univers	6.45	13.99	1.70	7.55	9.48	53.18
Ametist	6.53	13.01	1.73	6.48	10.24	53.08
Smarald	5.93	13.03	1.62	7.10	9.14	54.54
Simbol	5.95	12.65	1.59	6.70	8.99	54.42
Onix	5.55	13.08	1.62	7.53	9.92	55.37
Lucian	5.87	13.31	1.70	7.44	10.24	54.33
F8-3-01	5.24	12.82	1.64	7.58	9.76	54.51
F8-4-12	5.30	13.20	1.68	7.90	10.18	54.97
F8-5-13	5.38	11.86	1.66	6.49	10.18	55.72
F8-6-12	5.61	12.44	1.66	6.84	9.77	54.76
Average	**5.86**	**12.98**	**1.65**	**7.13**	**9.73**	**54.44**
Min	**5.24**	**11.86**	**1.59**	**6.48**	**8.99**	**53.08**
Max	**6.53**	**13.99**	**1.73**	**7.90**	**10.24**	**55.72**
CV (%)	**7.63**	**4.22**	**2.68**	**6.73**	**4.69**	**1.49**
STDEV (%)	**0.45**	**0.55**	**0.04**	**0.48**	**0.46**	**0.81**

Table 5. Chemical grain composition of two rows winter barley, L2

Genotypes	ADF	NDF	ASH	HC	PC	SC
Andreea	6.07	14.80	1.83	8.74	10.73	53.91
Artemis	5.70	13.52	1.68	7.83	10.18	52.89
Gabriela	5.58	13.75	1.65	8.17	10.06	54.81
DH 375-4	4.83	11.99	1.61	7.16	10.71	56.77
F8-106-10	6.06	14.27	1.76	8.21	10.26	54.09
F8-114-10	4.31	11.15	1.56	6.84	10.97	55.80
DH 267-66	5.56	12.94	1.66	7.39	10.04	55.31
Average	**5.44**	**13.20**	**1.68**	**7.76**	**10.42**	**54.80**
Min	**4.31**	**11.15**	**1.56**	**6.84**	**10.04**	**52.89**
Max	**6.07**	**14.80**	**1.83**	**8.74**	**10.97**	**56.77**
CV (%)	**11.91**	**9.71**	**5.40**	**8.61**	**3.59**	**2.36**
STDEV (%)	**0.65**	**1.28**	**0.09**	**0.67**	**0.37**	**1.30**

If are compared the results obtained under both L1 and L2 environmental conditions, in general, better stability of chemical parameters was assessed under the L2 condition.

Analyzing as a whole the behavior of six rows winter genotypes (in L1 and L2) can be clearly observed the influence of environmental conditions on the chemical parameters, a statement attested by the oscillation of chemical parameters. No patterns were observed with reference to any genotype but on average, an increase or decrease of some parameters was observed if we compare the locations between them. Concerning two rows winter barley genotypes, the obtained results, somehow revealed a pattern, two of them had the opposite behavior (Andreea and Artemis varieties). Andreea variety registered the highest value of ADF content under the L1 environment (only one) and the highest value of ADF, NDF, ASH and HC content under the L2 environment.

The maxim value of NDF, ASH and HC was presented by Artemis variety under L1 environment and the SC was similar comparing with L2. It noticed that F 8-114-10 line had a gradually increase of ADF, NDF, ASH and HC under L2 environment while PC and SC decreased.

Statistically significant strong positive and negative correlations were observed between six chemical barley grain parameters under both environmental conditions and separately for six and two rows barley.

According to correlation coefficients, the same number of positive strong corelations was confirmed for two rows winter barley on both environmental conditions for NDF, HC and ASH (Table 6a and 6b, blue colour). There was a significant negative correlation between starch and NDF, ASH and HC in L1 and only between starch and ADF in L2 (-0.743).

According to Campbell et al. (1995), genotypes barley grain with a high-test weight had a low NDF or cell wall content, and it was the statement that NDF weighed less per unit volume than did starch (r=-0.811, r=-0.818 and r=-0.845).

In the case of six rows winter barley (green colour, Table 6a), a strong positive correlation between ASH, HC and NDF was found (r=0.760, r=0.924) and between HC, PC and ASH moderate correlations (r=0.599 and r=0.618) under the environment conditions of the L1. Significant negative correlations were observed between SC and NDF, ASH and HC (r=-0.778, r=-0.759 and r=-0.794).

Under environment conditions of the L2 (green colour, Table 6b) positive correlations were found only in three cases: HC and PC with NDF and SC with ASH (r=0.629, r=0.743 and r=0.892). Also, significant negative correlation was confirmed between SC and ADF (r=-0.827), respectively SC and NDF (r=-0.552). SC and ASH was strong positive correlated, and SC and HC were uncorrelated comparing with six rows winter barley tested under L1 conditions.

Table 6a. Simple correlations between six chemical barley grain parameters, L1 (green colour - six rows winter barley; blue colour - two rows winter barley)

Variables	ADF	NDF	ASH	HC	PC	SC
ADF	**1**	**0.960****	**0.907****	**0.866****	-0.500	-0.711
NDF	0.149	**1**	**0.985****	**0.972****	-0.399	**-0.811^{0}**
ASH	0.380	**0.760****	**1**	**0.989****	-0.355	**-0.818^{00}**
HC	-0.241	**0.924****	**0.599***	**1**	-0.288	**-0.845^{00}**
PC	0.421	0.170	**0.618***	0.005	**1**	0.120
SC	0.080	**-0.778^{00}**	**-0.759^{00}**	**-0.794^{0}**	-0.183	**1**

Table 6b. Simple correlations between six chemical barley grain parameters, L2 (green colour - six rows winter barley; blue colour - two rows winter barley)

Variables	ADF	NDF	ASH	HC	PC	SC
ADF	**1**	**0.973****	**0.897****	**0.896****	-0.590	**-0.743^{0}**
NDF	0.551	**1**	**0.932****	**0.974****	-0.463	-0.737
ASH	0.304	0.385	**1**	**0.917****	-0.176	-0.661
HC	-0.302	**0.629***	0.157	**1**	-0.316	-0.693
PC	-0.276	**0.743****	0.414	0.184	**1**	0.420
SC	**-0.827^{00}**	**-0.552^{0}**	**0.892****	-0.012	0.073	**1**

** or ^{00} significant at 0.01 level; * or ^{0} significant at 0.05 level; ADF - acid detergent fiber, NDF - neutral detergent fiber, ASH - ash content, HC - hemicellulose content, PC - protein, SC - starch content, L1 - location 1, L2 - location 2.

CONCLUSIONS

Variation for acid detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose content (HC), ash content (ASH), protein content (PC) and starch content (SC) in the winter grain barley were due to differences among genotypes, the effect of environment (location) and their interaction.

An analysis of six chemical grain parameters in 19 winter barley genotypes showed variations between the type of barley, six or two rows, and different level of chemical parameters which can help to choose the proper genotype for a specific end-use.

To create, assess and promote new barley cultivars with specific nutritional profile in order to obtain barley food product, which can add benefits to the human body has to be one of the priorities in the barley breeding programme (winter and spring barley) due to beneficial influence of human health.

Furthermore, investigations will be made to determine the beta-glucan content of the analyzed varieties and lines from this study for genotype end-use assessment.

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A STUDY OF SOME FOREIGN WHEAT CULTIVARS IN BULGARIA (*Triticum aestivum* L.)

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Abstract

The purpose of the study is to investigate four winter wheat cultivars (Triticum aestivum L.). The areas were found to be heavily weeded with Lamb's (Chenopodium album), Amaranth (Amaranthus retroflexus L.), Field mustard (Sinapis arvensis L.) and Wild oats (Avena fatua L.). The studied cultivars are characterized by good productivity as the average yield for the group is 773.7 kg/da. Avenue cultivar showed the highest grain yield - 861 kg/da. It was found that the tested cultivars are characterized by good baking qualities. The average wet gluten content is 27.6%. It has been found that foreign cultivars are significantly attacked by Powdery mildew (Erysiphe graminis De candolle), Brown (Puccinia recondita Rob. Et Desm. f. sp. tritici) and wheat stripe (yellow) rust (Puccinia striiformis West.) and Septoria tritici leaf bloth (Septoria tritici Rob. ax Desm.).

***Key words**: wheat, cultivars, weeds, disease, yield.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the main crop in Bulgaria, and it occupies about 60 % of the area of cereals in the country and in the last decade provides nearly three quarters of the grain for the local economy. Wheat is a major cereal crop. It is grown exclusively for grain, which serves as food for most people in the world. Its wide distribution is due to the high nutritional value of the grain. The protein content of wheat grain is 13-15% and carbohydrates – 70-78 %. Bread made from soft wheat flour is porous and large in volume. In addition to food, wheat grain is used as a raw material for alcohol and other derivatives, and in the food industry the grain of the cultivars with low baking qualities is used for fodder. The area of wheat in the world is over 2 billion decares with an average yield of 230-240 kg/da. In our country it occupies 11-12 million decares, and the average yields are 450 – 550 kg/da.

Wheat forms its leaf mass in different phases. The leaf consists of a petiole and a leaf base and has a structure typical for cereals. The leaf surface of a plant varies from 70 to 140 square centimeters. The larger the leaf surface and the longer it stays fresh, the higher the expected yield will be. The upper leaves have a great importance for the formation of the yield (Tsenov et al., 2002., Yankov et al. 2009).

Wheat grain contains an average of 12 – 14 % protein, 60 % nitrogen-free extracts, 2% fat, 3 % cellulose and 1.6 % ash. Carbohydrates (starch) occupy the largest share, with the most significant amount in the middle of the grain. However, protein substancesth are the most important ones. The quality of bakery products depends on the quality and quantity of proteins and especially on the water insoluble ones-gluten (Panayotov & Todorov, 2008., Penchev et al. 2007). Soft wheat, has the ability to swell strongly, absorb gases, form crusts, as a result this kind of bread is highly valued. In durum wheat, gluten is highly stretchable, does not break and the flour is used to obtain quality pasta, noodles, semolina and other pasta products (Nikolova & Panayotov, 2006). In Bulgaria, as one of the countries of the temperate climate zone, the cultivation of wheat is carried out mainly through winter type of development cultivars. Modern winter wheat cultivars need to have a balanced complex set of features and properties to ensure consistently high and stable grain yields with appropriate technological properties, suitable for the production of a quality product (Stefcheva, 2005; Chavdarov & Kolev, 2008). Many pests attack the wheat crops in the country (Raykov et al., 2010). Rust, Wheat powder head, Septoria and others are economically significant diseases for the crops

(Gospodinova, 1973; Townley-Smith, 2001; Todorova, 2008; Patrick & Mills, 2008; Wolf, 2008; Chungu et al. 2001; Pavlova & Grigorova, 2011).

MATERIALS AND METHODS

The study was conducted in a Limagrain Company experimental region in the area of Madara village. The climate of Shumen municipality is characterized by a temperate – continental features. The main factors determining this type of climate are the location of the municipality in the southeastern part of the Danube plain and the possibility of free flow of northwestern, northern and northeastern air masses.

The temperature regime of the municipality is typical for the temperate – continental climate - with hot summers and cold winters.

The average monthly temperature of the coldest month (January) is – 1.1°C (Table 1).

Table 1. Meteorological data for the study period

Month	Norm/ Year					
	t° C	2019	2020	mm/m²	2019	2020
I	- 0.6	0.2	1.7	45	36.8	36.2
II	1.3	2.0	2.3	49	44.3	42.4
III	5.6	7.1	7.8	47	48.1	67.5
IV	10.1	12.1	11.7	55	24.1	19.8
V	16.3	17.3	15.9	70	63.5	72.5
VI	19.0	20.8	19.4	82	79.9	123.4
VII	21.6	22.5	21.6	65	32.4	68.4
VIII	21.0	21.1	22.5	51	19.9	57.6
IX	17.4	17.5	15.7	40	44.4	110.9
X	10.6	11.3	11.6	40	96.3	63.6
XI	6.9	9.8	5.7	55	66.3	99.2
XII	3.2	2.3	3.8	53	25.4	86.6

The average monthly temperature of the warmest month (July) is + 22° C, thus forming a significant annual temperature amplitude of 23 - 24°C. The average annual temperature is 16,9°C. The average annual rainfall is 606 mm. The soil type in the area is calcic chernozems (FAO-UNESCO), with a strong humus horizon. The mechanical composition of the soil is heavy sandy-clay containing 30 – 60 % physical clay. It is considered to be well stocked with mobile forms of molybdenum (Mo), magnesium (Mg) and others.

The study included four cultivars with a sown area of 60 decares for each one. According to the manufacturer, the most common characteristics of the cultivars are the following Alcantara cultivar was presented, as a highly productive cultivar of the second strongest wheat group. It has good tillering and very good resistance to lodging. This is an awned head type cultivar with a height of 80 – 90 cm. under normal cultivation. Airbus cultivar has an excellent complex assessment of the quality of grain for the production of bakery products. Quality group: Strong wheat.

Annapurna – a representative of the new wave of wheat, perfectly adapted to local growing conditions. Superior qualities and excellent yield potential. Registered in Bulgaria in 2018.

Avenue cultivar is a low stemmed, awnless cultivar with reduced leaf mass and a well – defined waxy coating, with a high tillering ratio. It has good resistance to lodging. It shows good resistance to some fungal diseases. A route method (by placing meters) was carried out to determine the species composition of weeds. It was done diagonally across the field. Five meters of each cultivar were reported in detail. Weeding was established by meters before the introduction of herbicides and the assessment was grouped as follows:

Degree of weed distribution
+ - weak - up to 25%
++ - average – 25% to 50%
+++ - strong – 50% to 75%
++++ - massive – 75% to 100%.

Visual diagnosis of the presence of diseases was performed according to generally accepted methods (Bobev, 2009). A precise general assessment of 50 – 100 plants was performed. A symptomatic picture of randomly taken plants was analyzed, and the data from the general assessment was recorded in a journal of route reviews. The same evaluation criteria were used, on a five-point scale from zero to 100%. During the inspections, the development of diseases that appeared in the crops was reported and samples were taken from diseased plants with well-defined symptoms of the diseases.

RESULTS AND DISCUSSIONS

Table 2 presents the data on weeding of the experimental area planted with wheat. The data shows that there is a wide variety of weeds. It is necessary to point out that the weeding with annual deciduous weeds is relatively stronger. Relatively weak is the weeding with annual and perennial wheat weeds. Our survey found that the areas are heavily weeded with Lamb's quarters (*Chenopodium album* L.), Amaranth (*Amaranthus retroflexus* L.) and Field mustard (*Sinapis arvensis* L.). We can point out a significant weeding with Creeping thistle (*Cirsium arvense* (L). Scop.) and Field bindweed (*Convolvulus arvensis* L.). We definitely consider a significant problem the severe weeding with Wild oats (*Avena fatua* L.).

Table 2. Weed species and their distribution in wheat average for the period (2019 – 2020)

Weed species	Degrree
1.Annual weeds 1.1 Annual cereal weeds	
Echinochloa crus – galli L. *Avena fatua* L.	+ +++
1.2 Annual deciduous weeds *Chenopodium album* L. *Solanum nigrum* L. *Galium tricorne* L. *Papaver rhoeas* L. *Amaranthus retroflexus* L. *Anthemis arvensis* L. *Consolida regalis* – Gray. *Xanthium strumarium* L. *Sinapis arvensis* L. *Polygonum convolvulus* L.	 +++ ++ ++ + ++++ ++ + + ++++ ++
2. Perennial weeds 2.1 Pernnial root and shoot weeds Sonchus arvensis L. *Cirsium arvense* (L). Scop. *Convolvulus arvensis* L. *Aristolochia clematitis* L.	 + +++ ++++ +

For the agricultural manufactures, the average yields obtained from wheat are essential (Figure 1). Avenue cultivar was reported with the highest yield of grain of the tested cultivars – 861 kg/da, where the excess over the average of the four cultivars is 10.1%. The second and the third places are occupied by the Airbus cultivar – 795 kg/da and Alcantara cultivar 752 kg/da. Overall an average yield of 773.7 kg/da was obtained from the tested cultivars.

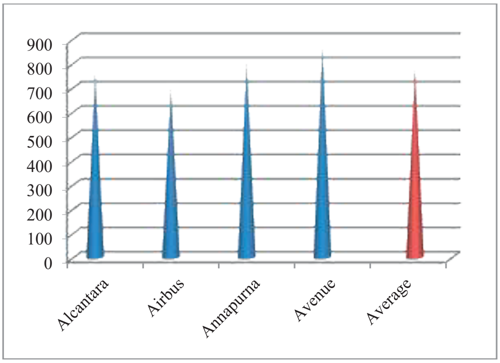

Figure 1. Wheat cultivars average yields in kg/da

The hectoliter mass is an indicator of the milling qualities of wheat. This shows how much flour can be provided by different batches of wheat. The effective minimum of total flour yield in wheat processing for the three groups is 76 kg. This is a limit value below which the efficiency of the technological process falls.

Figure 2 presents the data for the reported hectoliter weight of the tested cultivars. The average hectoliter mass for the group was 77.3%. The highest value was reported for Avenue cultivar – 80.1%. These results give us reason to conclude that the tested cultivars have good milling qualities.

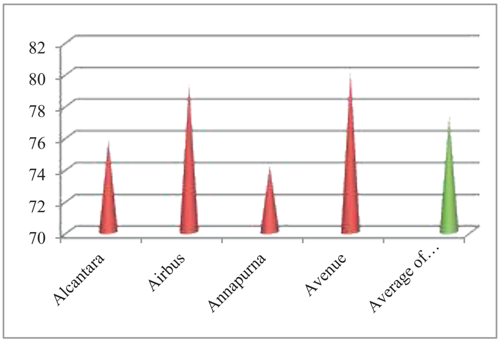

Figure 2. Hectoliter weight of wheat cultivars in kg

In practice, special attention is paid to the indicator of wet gluten, which is shown a percentage. On the one hand, the yield of wet gluten in the grain provides, the protein content and nutritional value. On the other hand, it guarantees a corresponding amount of gluten in the flour (Figure 3). According to this indicator, the values of Alcantara (23.4%) and Airbus cultivars (24.8%) are approximately the same. The content of wet gluten is higher in the cultivars Annapurna (30.1%) and Avenue (31.2%).

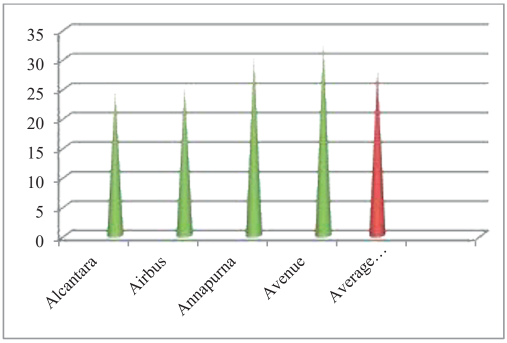

Figure 3. Wet gluten content in wheat cultivars in %

The average wet gluten content is 27.6%. The mass of 1000 seeds (in the older nomenclature it is known as absolute weight), shows the weight of 1000 absolutely dry seeds. This feature guides us about the nutrition of wheat seeds (Figure 4). It is necessary to point out that in all cultivars the nutrition of the seeds is good (37.6 g). The highest value – 43.1 g. was reported for Avenue cultivar, where the excess compared to the average for the group was 14.5 %.

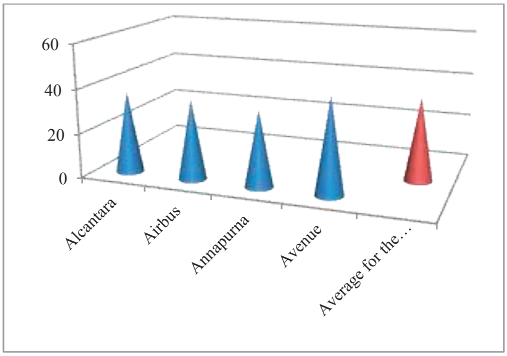

Figure 4. Mass per 1000 seeds of wheat cultivars in g.

The grain hyaliness as an indicator of the milling properties provides a certain minimum yield of light flours. The range for the three groups of wheat regarding their strength is from 40% to 50%. These values are limit values, below which the yield of light (white) flours significantly decreases (Figure 5).

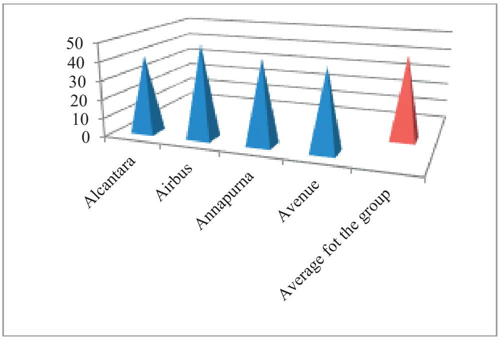

Figure 5. Grain hyaliness of wheat cultivars in %

From the data review on the grain hyaliness it was found that the studied cultivars show an average value of 44.7%, from which we can conclude that the studied cultivars guarantee a high percentage of white flours.
Wheat as a crop provides about 20% of human energy (carbohydrates). At the same time, wheat grain is a basic protein food for humans and in this respect it is in the first place. According to literature data (Panayotov & Todorov, 2008, Yankov et al. 2002), the protein content in wheat cultivars in our country - varies from 15.1 to 18.8%. The studied cultivars (Figure 6) show an average grain protein content of 17.2%. The highest protein content in the grain was reported in Avenue cultivar 18.3%. Alcantara (15.8%) is characterized by a lower protein content. The other cultivars occupy position between these two.

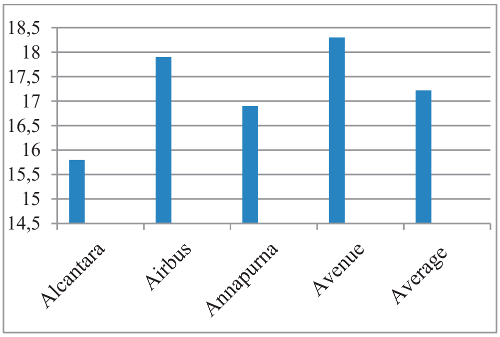

Figure 6. Protein content in the grain of wheat cultivars in %

A higher degree of damage from these diseases was found in Airbus cultivar. Avenue cultivar is presented with good resistance to these diseases.

Wheat diseases are one of the most important factors for grain production and if the control over them is not adequate, the danger of losing millions of tons of production and deteriorating its quality is very high. The most important are the diseases that cause damage to the leaves and stems of cereals during the growing season – Powdery mildew, Brown rust, Yellow rust, Septoria under certain conditions Fusarium on wheat ear and others. The crop loss can reach 25 – 30% of the yield for each disease separately and is mainly due to a reduction in the number of wheat ears and seeds.
Table 3 presents the reported data of economically important diseases attacks in the studied foreign wheat cultivars.

Table 3. Wheat diseases

Pathogen	Cultivars			
	Alcan tara	Airbus	Annap urna	Avenue
Powdery mildew Erysiphe graminis De candolle	++	+++	++	++
Brown rust Puccinia recondita Rob. Et Desm. f. sp. tritici	++	+++	+++	+
Septoria tritici leaf blotch Septoria tritici Rob. ax Desm.	+	++	++	+
Tilletia foetida Tilletia foetida (Wall.) Liro (syn.T.levis*)*	0	0	+	0
Tilletia contraversa Tilletia contraversa Kuhn	+	+	0	0
Ustilago tritici Ustilago tritici (Pers) Jens	+	0	+	0
Fusarium head/ear blight/scab Fusarium graminearum Schwabe.	0	0	+	+
Early leaf blight Septoria tritici Rob. at Desmazieres	0	0	+	0
Wheat stripe(yellow) rust Puccinia striiformis West f. sp. tritici	++	+++	++	+

In general, the cultivars are significantly attacked by Powdery mildew, Brown rust, Yellow rust and Septoria.
A higher degree of damage from these diseases was found in Airbus cultivar. Avenue cultivar is presented with good resistance to these diseases.

CONCLUSIONS

The areas were found to be heavily weeded with Lamb's quarters (*Chenopodium album* L.), Amaranth (*Amaranthus retroflexus* L.) and Field mustard (*Sinapis arvensis* L.). As significant we can point out the weeding with Creeping thistle (*Cirsium arvense* (L). Scop.) and Field bindweed (Convolvulus arvensis L.).
The studied cultivars show a good productivity, as the average yield for the group is 773.7 kg/da. Avenue presented the highest grain yield of the tested cultivars 861 kg/da where the excess compared to the average of the four cultivars is 10.1%.
It was found that the tested cultivars have good milling qualities. The average hectoliter mass for the group was 77.3% The highest value was reported for Avenue cultivar – 80.1%.
In terms of wet gluten, the values are approximately the same for the cultivars Alcantara (24.3%) and Airbus (24.8%). The content in Annapurna (30.1%) and Avenue (31.2%) cultivars is higher. The average content of wet gluten is 27.6 %.
The studied cultivars guarantee the production of a high percentage of white flours. The data for the grain hyaliness index shows an average value of 44.7% for the studied cultivars.
It has been found that foreign wheat cultivars are significantly attacked by Powdery mildew, Brown, Wheat stripe (yellow) rust and Septoria tritici leaf blotch. A higher degree of damage from these diseases was found in Airbus cultivar. Avenue cultivar is characterrized by good resistance to the presented diseases.

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POSSIBILITIES FOR HERBICIDAL CONTROL OF MIXED WEED INFESTATION IN MAIZE (*Zea mays* L.)

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Abstract

In 2018-2019, on the experimental field of the Agricultural University of Plovdiv, Bulgaria, a field experiment with maize hybrid P1114 was performed. The experiment included the following herbicide treatments: Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1}), Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}), Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1}), Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}), Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) and Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}). The application was done in 4th - 6th true leaf of maize (BBCH 14-16). The dominant weeds on the field were Sorghum halepense (L.) Pers. developed from seeds and rhyzomes, Setaria viridis (L.) P. Beauv., Chenopodium album L., Amaranthus retroflexus L., Xanthium strumarium L., Abutilon theophrasti Medik., Datura stramonium L. and Solanum nigrum L. The nicosulfuron-containing herbicide in combination with other products showed good to excellent efficacy depending on the weed species. For all variants treated whit herbicides 1000 grain weight, hectolitre mass and the yield, were higher and with statistically proved differences in comparison to the untreated control.

Key words: *maize, weeds, herbicides, efficacy, yield.*

INTRODUCTION

Providing an abundance of food for the ever-growing population of Earth, of technical and medicinal raw materials, of fodder for farm animals, of building materials, etc. is an inalienable concern for humanity (Tonev et al., 2019). All branches of biological science work intensively and purposefully to solve a large number of problems and tasks (Georgiev et al., 2019; Nenova, 2019; Nenova et al., 2019; Petrova et al., 2019; Georgiev et al., 2017; Nenova, 2017; Shopova & Cholakov, 2015; Shopova & Cholakov, 2014; Tonev et al., 2010a; Tonev et al., 2009a).

Maize *(Zea mays* L.) is one of the most widely grown plants in the world and is a major food crop in many developing countries (Ram et al., 2017). It is grown for both grain and green fodder and silage for animals (Iken & Amusa, 2004). Its grain is a source of starch and cooking oils, and is used as a raw material for fermentation. Due to its productive potential and wider adaptability, maize is also known as the queen of cereals.

According to Aldrich et al. (1975) *Zea mays* L. has the greatest potential for carbohydrates per unit area per day.

Weed vegetation is a major limiting factor, providing serious competition to maize in terms of vegetation factors, leading to lower yields and quality of the production (Tonev et al., 2019; Saleem et al., 2015; Arnold et al., 2013). In addition, weeds deplete 30-40% of the applied nutrients from the soil. They hinder the efficiency of the use of fertilizers from cultivated plants, as a significant part of the fertilizer added to the soil is used by the weeds (Mundra et al., 2002).

Studies by a number of authors show that depending on the type and degree of weed infestation, as well as period of crop-weed competition and its intensity (Rai et al., 2018) the maize grain yield can be decreased from 24.0% to 96.7% (Dimitrova et al., 2018; Ehsas et al., 2016; Jagadish et al., 2016; Kakade et al., 2016; Yakadri et al., 2015; Mukherjee & Puspajit, 2013; Jat et al., 2012; Oerke & Dehne, 2004; Khan et al., 2003; Zhalnov & Raikov, 1996). According to Hall et al. (1992), maize is highly sensitive to weed competition in the early development stages.

In addition to weeds, the lack of nutrients, which is often caused by them, also has a negative effect on the formation of crop yields (Ivanov et al., 2019; Manolov & Neshev, 2017;

Neshev & Manolov, 2016; Kostadinova et al., 2015; Manolov et al., 2015; Neshev & Manolov, 2014; Neshev et al., 2014; Goranovska et al., 2014).

Depending on the latitudes and the presence of weed seeds in the soil, different weed associations develop in maize. In Bulgaria the weeds economically important in this crop are *Amaranthus retroflexus* L., *Datura stramonium* L., *Xanthium strumarium* L., *Solanum nigrum* L., *Chenopodium album*, *Abutilon theophrasti* L., *Sinapis arvensis* L., *Echinochloa crus-gali* L., *Setaria glauca* L., *Sorghum halepense* L., *Convolvulus arvensis* L, *Cinodon dactilon* L. and *Cirsium arvense* L. (Mitkov et al., 2019; Hristova et al., 2012; Kalinova et al., 2012; Tonev et al., 2010b).

According to Vizantinopoulos and Katranis (1998) in Greece, in areas with intensive maize cultivation the weed species *Amaranthus* spp. dominates.

In Slovakia studies showed that the most common weeds in maize are *Chenopodium album* L., *Amaranthus* spp., *Echinichloa crus-galli* (L.) P. Beauv, *Datura stramonium* (L.), *Fallopia convolvulus* (L.) A. Lôve, *Persicaria* spp., *Cirsium arvense* (L.) Scop, *Elytrigia repens* (L.) P. Beauv, *Avena fatua* (L.) and *Abutilon theophrasti* Medik (Týr and Vereš, 2012). Smatana et al. (2015) reported that in maize the weeds *Atriplex* spp. and *Setaria viridis* (L.) P. Beauv. are widely distributed.

In India, the most aggressive weeds growing in the early stages of maize development are *Polygonum* (*P. pensylvanicum, P. persicaria, P. orientale*), *Stellaria media, Stellaria aquatica, Oldelandia diffusa, Oldenlandia umbellate, Physalis minima, Solanum nigrum*. In Belgaum district of Karnataka, India, the most distributed weeds are *Cynodon dactylon, Dinebra retroflexa, Echinochloa colonum, Eleusine indica, Cyperus rotundus, Parthenium hysterophorus, Commelina benghalensis, Portulaca oleracea, Cynotis cuculata, Phyllanthus niruri* and *Amaranthus viridis* (Soren et al., 2018; Mukherjee & Puspajit, 2013; Haji et al., 2012).

In maize fields in Mashhad, Iran the most often observed broadleaf weed species are *Amaranthus retroflexus* L., *Chenopodium album* L., *Portulaca oleracea* L. and *Solanum nigrum* L. (Baghestani et al., 2007).

One of the main weed control methods in maze is the usage of herbicides (Mitkov, 2020; Goranovska & Kalinova, 2018; Goranovska et al., 2017; Janak & James, 2016; Sevov et al., 2015; Umesha & Sridhara, 2015; Goranovska & Kalinova, 2014; Dimitrova et al., 2013b; Skrzypczak et al., 2011; Pannacci & Covarelli, 2009, Tonev et al., 2009b).

Against the annual grass and broadleaf weeds high efficacy for the application of Gardoprim plus gold 500 SK – 4.00 l ha^{-1} (99%), Lumax 538 SK – 4.00 l ha^{-1} (97%), Wing – 4.00 l ha^{-1} (97%) and Merlin flex – 0.42 l ha^{-1} (94.6%) was recorded (Dimitrova et al., 2013a).

The application of Merlin Duo in rates of 1.00 l ha^{-1} to 2.00 l ha^{-1} after sowing before germination of maize controls *Abuthilon theophrasti* L. and *Solanum nigrum* L. The highest efficacy against *Chenopodium album* L. after the application of Merlin Duo in the rates of 1.25, 1.5 and 2.00 l ha^{-1} was reported (Mitkov et al., 2018).

It was found that the application of foramsulfuron in dose of 20.3 g ai ha^{-1} showed 95% efficacy against *Amaranthus retroflexus* L., *Setaria viridis* (L.) Beauv., *Sinapis arvensis* L. and *Solanum nigrum* L. Against *Abutilon theophrasti* Medik., *Chenopodium album* L. and *Echinochloa crus-galli* (L.) Beauv. the same efficacy results are obtained, but for the rates of 20 до 50 g ai ha^{-1} of foramsulfuron (Pannacci, 2016).

For control of *Sorghum halepense* L., *Convolvulus arvensis* L., *Echinochloa crus-gali* L., *Chenopodium album* L., *Amaranthus retroflexus* L. and *Abutilon theophrasti* L. in maize it is recommended to apply Stomp 33 EK + Mistral 4 SK in rates of 3.00 l ha^{-1} + 1.30 l ha^{-1} (Kalinova et al., 2000). It is important to note that the use of pendimethalin has a lower risk of groundwater contamination compared to herbicides as alachlor (Brahushi et al., 2011).

Acording to Kierzek et al. (2012), the best control of mixed weed infestation in maize after the application of the mixture s-metolachlor + terbuthylazine + mesotrione, followed by foliar application of nicosulfuron with adjuvant Atpolan Bio 80 SL. In the maize fields Tonev et al. (2016) recorded high efficacy against broadleaf and grass weed species as *Sorghum halepense* L. *Convolvulus arvensis* L. and *Cirsium arvense* L. after application of the herbicide combination of Flurostar 200 EK + Nishin 4 OD in rates of 0.70 l ha^{-1} + 1.30 l ha^{-1}. If there is high infestation with *Chenopodium album* L. the combination of Mustang 306.25 SK and Nishin 4 OD in doses of 0.60 l ha^{-1} + 1.30 l ha^{-1} in tank mixture is recommendable.

For control of *Xanthium strumarium, Amaranthus retroflexus, Datura stramonium* and *Chenopodium album* in maize Damalas et al. (2018) established efficacy from 92 to 100% after the alone treatment of tembotrione at 100 g ai ha^{-1} and three mixtures of tembotrione with: rimsulfuron at 10 g ai ha^{-1}, nicosulfuron at 40 g ai ha^{-1} and foramsulfuron at 60 g ai ha^{-1}. After the application of foramsulfuron and nicosulfuron (sulfonylurea herbicides) applied in tank-mix with 2.4 D + MCPA the weeds *Amaranthus retroflexus* L. and *Chenopodium album* L. can be successfully controlled - from 78 to 100%, depending on the herbicide rates (Sarabi et al., 2018).

The aim of the current research is to study some possibilities for herbicidal control of mixed weed infestation in maize (Z*ea mays* L.).

MATERIALS AND METHODS

In the period of 2018 – 2019 a field plot trial with the maize hybrid P1114 (590 FAO) was conducted. The experiment was situated on the experimental field of the department of "Agriculture and herbology" at the Agricultural University of Plovdiv, Bulgaria.

The experiment included the following tretments: 1. Untreated control; 2. Callam (600 g/kg dicamba + 125 g/kg tritosulfuron) + Kelvin OD (40 g/l nicosulfuron) + Dash (adjuvant) in rates of 0.40 kg ha^{-1} + 1.00 l ha^{-1} + 1.00 l ha^{-1}; 3. Callam + Kelvin OD + Dash in rates of 0.50 kg ha^{-1} + 1.25 l ha^{-1} + 1.25 l ha^{-1}; 4. Callam + Kelvin OD + Dash in rates of 0.30 kg ha^{-1} + 1.25 l ha^{-1} + 0.625 l ha^{-1}; 5. Callam + Kelvin OD in rates of 0.30 kg ha^{-1} + 1.25 l ha^{-1}; 6. Cambio SL (320 g/l bentazone + 90 g/l dicamba) + Kelvin OD in rates of 2.00 l ha^{-1} + 1.25 l ha^{-1}; 7. Arat (500 g/kg dicamba + 250 g/kg tritosulfuron) + Kelvin OD in rates of 0.20 kg ha^{-1} + 1.25 l ha^{-1}. The trial was performed by the randomized block design in 4 replications with size pf the experimental plot 28 m². The herbicide application was done in 4th – 6th true leaf of maize (BBCH 14-16) via electrical backpack sprayer SOLO (model 417) with size of the working solution 250 l ha^{-1}.

During the two years of the research the maize was grown as monoculture. Also, the plants were grown under conditions of drip irrigation. On the trial field deep ploughing, two times disc harrowing and two times cultivation before sowing were done. On the whole experimental area basic combine fertilization with 250 kg ha^{-1} NPK (15:15:15) and spring dressing with 200 kg ha^{-1} NH_4NO_3 was performed. The sowing was performed in the optimal period. The sowing distance between the rows was 70 cm, with sowing density of 78 000 germinating seeds ha^{-1}. After sowing rolling was done.

The experimental area was naturally infested with *Sorghum halepense* (L.) Pers. developed from seeds and rhyzomes, *Setaria viridis* (L.) P. Beauv., *Chenopodium album* L., *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Abutilon theophrasti* Medik., *Datura stramonium* L. and *Solanum nigrum* L.

The biological efficacy was reported on 14th, 28th and 56th day after the herbicide application. The efficacy against the weeds was evaluated by the 10-score visual scale of EWRS. The efficacy results were compared with the untreated control.

The selectivity of the studied herbicides was evaluated on the 7th, 14th, 28th and 56th day after the treatments by the 9-score visual scale of EWRS (at score 0 - there is no damage on the crop, and at score 9 there is complete death of the crop).

The following indicators are evaluated and analyzed: absolute seed mass of 1000 air-dry seeds (g), hectolitre seed mass (kg) and maize grain seed yield (t ha^{-1}).

The reported biometric indicators were processed with the software package SPSS 17 - module two-factor analysis of variance for Windows 8. The difference between the evaluated treatments was statistically analysed by ONE WAY ANOVA by using Duncan's multiple rage test. Statistical differences were considered proved at $p<0.05$.

RESULTS AND DISCUSSIONS

During both experimental years the weed infestation was presented by weeds belonging to two biological groups. The presenters of the late-spring weeds were *Setaria viridis* (L.) P.Beauv., *Chenopodium album* L., *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Abutilon theophrasti* Medik., *Datura stramonium* L. and *Solanum nigrum* L. Presenter from the perennial group of weeds was *Sorghum halepense* (L.) Pers. developed from seeds and rhyzomes.

On the 14th day after application the highest herbicidal efficacy against *S. halepense* developed from seeds for the treatment with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) – 90.0% average for both year was found (Table 1). Similar to those efficacy for the treatment with Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1}) was reported - 86.7% in 2018 and 90.0% in 2019. For the other treatments, the efficacy against this weed varied from 83.4% - 87.5%.

The average results on the 28th day after treatments showed increasing values regarding the efficacy against *S. halepense* developed from seeds. For treatments 2, 3, 4 and 7 the efficacy was 100%.

On the 56th day after treatments, the reported efficacy reched 100% for all tretments. This can be explained by the sufficient time of action of the herbicides, especially nicosulfuron, on the weeds and their ability to show their maximum potential. Hernández-Labrador et. al. (2000) also reported that nicosulfuron at 30 g a.i. ha^{-1} showed excellent control of *Sorghum halepense*.

Table 1. Efficacy of the studied herbicides against *Sorghum halepense* (L.) Pers. developed from seeds, %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	86.7	100	100	90.0	100	100	88.4	100	100
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	90.0	100	100	90.0	100	100	90.0	100	100
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	85.0	100	100	83.3	100	100	84.2	100	100
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	81.7	100	100	85.0	98.3	100	83.4	99.2	100
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	83.3	100	100	85.0	98.3	100	84.2	99.2	100
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	88.3	100	100	86.7	100	100	87.5	100	100

Compared with the efficacy of the studied herbicides against *S. halepense* developed from seeds, the efficacy against *S. halepense* developed rhyzomes was lower.

Table 2. Efficacy of the studied herbicides against *Sorghum halepense* (L.) Pers. developed rhyzomes, %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	60.0	85.0	88.3	70.0	85.0	90.0	65.0	85.0	89.2
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	70.0	88.3	93.3	78.3	88.3	93.3	74.2	88.3	93.3
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	58.3	85.0	88.3	58.3	80.0	90.0	58.3	82.5	89.2
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	43.3	80.0	90.0	60.0	76.7	86.7	51.7	78.4	88.4
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	60.0	78.3	90.0	70.0	76.7	85.0	65.0	77.5	87.5
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	65.0	80.0	91.7	68.3	86.7	90.0	66.7	83.4	90.9

During the experimental period, an increase in the herbicidal effect from the 14th to the 56th day after application of the products was observed.

On the 14th day the highest efficacy reported in 2018 was 70.0% for the treatment wit Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}). In 2019 the efficacy of this treatment was similar - 78.3%.
On average the efficacy for the concrete treatment reached 88.3% on the 28th day.
The average results for the efficacy of the studied products on the 56th day were similar. The highest efficacy was found to be for the treatment with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}0 – 93.3%. The lowest herbicide efficacy for the treatment with Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) was recorded – 87.5% (Table 2). In experiments carried by Eleftherohorinos and Kotoula-Syka (1995), the authors observed satisfactory efficacy against *S. halepense* from rhizomes after application of nicosulfuron.
Regarding the control of *Setaria viridis* (L.) P. Beauv, the obtained results were close to those recorded for the control of *S. halepense* developed rhyzomes. In 2018, on the 14th day after application of Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) and Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1}) the efficacy against the weed was 83.3%. In 2019 the highest efficacy against *S. viridis* was for Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) – 86.7% (Table 3).
On the 28th day after treatments, average for the period, the highest herbicide efficacy for the treatment with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) was observed – 95.0%.
The results obtained on the 56th day did not show significant increase when compared to those recorded on the 28th day. The highest efficacy against *S. viridis* for Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) – 95.9% and Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) – 95.8% average for both years was found.
From the herbicides studied, the lowest efficacy against *S. viridis* was reported for the treatment with Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) – 90.9% (Table 3).
Dobbels and Kapusta (1993) reported that the application of nicosulfuron in maize showed 98-100% efficacy against *S. viridis*. Control of *S. viridis* was reduced when nicosulfuron at 24 g was applied as a tank-mix with atrazine alone or in combination with bentazone, bromoxynil or dicamba. Also, bentazone + atrazine in combination with nicosulfuron at 35 g reduced control of the weed.

Table 3. Efficacy of the studied herbicides against *Setaria viridis* (L.) P.Beauv., %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	83.3	91.7	93.3	85.0	93.3	95.0	84.2	92.5	94.2
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	76.7	93.3	93.3	86.7	96.7	98.3	81.7	95.0	95.8
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	75.0	91.7	93.3	78.3	86.7	95.0	76.7	89.2	94.2
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	70.0	90.0	91.7	75.0	85.0	90.0	72.5	87.5	90.9
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	73.3	90.0	91.7	81.7	93.3	95.0	77.5	91.7	93.4
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	83.3	95.0	96.7	85.0	93.3	95.0	84.2	94.2	95.9

The efficacy obtained after the treatments against the *Chenopodium album* L. is presented on table 4. In 2018, on the first reporting date, the efficacy of treatment 6 (Cambio SL - 2.00 l ha^{-1} + Kelvin OD 1.25 l ha$^{-1)}$ was the highest – 88.3%. In 2019 for treatment 3 and 2 the efficacy was – 91.7%. The average efficacy on the second reporting date was for the treatment with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) - 95.9%.
In 2018, on the third reporting date 100% efficacy after the treatments with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) and Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}). At the other treatments, the herbicide efficacy varied between 96.7% and

98.3%. In 2019, on the 56th day after application, the efficacy for all treatments achieved 100% control of the weed.

Mitkov et al. (2019) reported 100% control of *Ch. Album* after the application of Arigo WG (330 g ha^{-1}) + Trend 90 (1000 ml ha^{-1}) and Principal Plus WG (440 g ha^{-1}) + Trend 90 (1000 ml ha^{-1}). Sarabi et al. (2018) established that the combine application of foramsulfuron and nicosulfuron in tank mixture with 2.4-D + MCPA successfully controlled *Ch. Album*.

Table 4. Efficacy of the studied herbicides against *Chenopodium album L.*, %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	78.3	93.3	96.7	91.7	93.3	100	85.0	93.3	98.4
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	85.0	95.0	100	91.7	96.7	100	88.4	95.9	100
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	78.3	90.0	98.3	85.0	93.3	100	81.7	91.7	99.2
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	78.3	93.3	98.3	80.0	95.0	100	79.2	94.2	99.2
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	88.3	93.3	96.7	88.3	93.3	100	88.3	93.3	98.4
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	83.3	91.7	100	85.0	93.3	100	84.2	92.5	100

As well as *S. halepense* developed from seeds, during the trial, very easy to control with the evaluated herbicides were the weed species *Amaranthus retroflexus* L. and *Xanthium strumarium* L.

The highest herbicide efficacy against *A. retroflexus* on the 14th day for the application of Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) average for the years of the study was reported – 93.3%. On the 28th day the efficacy was increased and reached 100% on the last reporting date (Table 5). Dobbels and Kapusta (1993) also recorded 100% control of *A. retroflexus* after the alone application of nicosulfuron or in combinations with other herbicides.

Table 5. Efficacy of the studied herbicides against *Amaranthus retroflexus* L., %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	88.3	100	100	95.0	100	100	91.7	100	100
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	93.3	100	100	91.7	100	100	92.5	100	100
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	90.0	100	100	88.3	98.3	100	89.2	99.2	100
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	90.0	100	100	88.3	98.3	100	89.2	99.2	100
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	91.7	100	100	93.3	100	100	92.5	100	100
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	93.3	100	100	93.3	100	100	93.3	100	100

Regarding the control of the weed *Xanthium strumarium* L., the studied herbicide treatments showed approximately equal efficacy as those for the weed *A. retroflexus*.

The efficacy of all herbicide treatments varied within narrow extends, ranging from 83.3% to 91.7% on the 14th day after application in the first year and from 88.3% to 93.3% in the second year. The average results on the 28th day after application was 100% for the treatments with Callam (0.50 kg ha^{-1}) + Kelvin

OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) and Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}).

On the 56th day the efficacy reached 100% for all treatments (Table 6).

Table 6. Efficacy of the studied herbicides against *Xanthium strumarium L.*, %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	83.3	98.3	100	90.0	98,3	100	86.7	98.3	100
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	88.3	100	100	91.7	100	100	90.0	100	100
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	83.3	100	100	90.0	96,7	100	86.7	98.4	100
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	85.0	100	100	88.3	96,7	100	86.7	98.4	100
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	85.0	95.0	100	93.3	100	100	89.2	97.5	100
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	91.7	100	100	91.7	100	100	91.7	100	100

According to Dobbels and Kapusta (1993) the control of *Abutilon theophrasti* depends on the nicosulfuron content, on the other herbicide when applied as a tank mix and growing conditions. In the current study, the most efficient control of *Abutilon theophrasti* Medik., average for the period, on the 14th day after the treatment with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) – 74.2%, followed by Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) – 73.4% was acieved.

On the second reporting date, the efficacy was the highest for the variant treated with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) – 96.7%.

On the last reporting date the highest efficacy of 96.7% against *A. theophrasti* was found to be for treatment 3 (Table 7).

Table 7. Efficacy of the studied herbicides against *Abutilon theophrasti* Medik., %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	58.3	88.3	90.0	73.3	98.3	100	65.8	93.3	95.0
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	66.7	93.3	93.3	81.7	100	100	74.2	96.7	96.7
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	58.3	85.0	91.7	73.3	96.7	100	65.8	90.9	95.9
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	61.7	83.3	86.7	71.7	88.3	96,7	66.7	85.8	91.7
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	70.0	80.0	91.7	76.7	91.7	100	73.4	85.9	95.9
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	58.3	86.7	88.3	71.7	91.7	100	65.0	89.2	94.2

The application of nicosulfuron + dicamba + bentazon; nicosulfuron + dicamba + bentazon; nicosulfuron + tritosulfuron + dicamba; nicosulfuron + mesotrione; nicosulfuron + mesotrione + atrazine; etc. can have very good results for controlling *D. stramonium* (Torma et al., 2006). In the present research the efficacy of the studied herbicides against *Datura stramonium L.* is presented on table 8.

On the first reporting date in 2018, the highest results were for the treatment with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) – 58.3%, followed by Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1}) – 56.7%. In 2019 the highest efficacy was for Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1}) – 76.7% and Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) – 75.0%. On the second date the highest results were also for the treatments with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})– 96.7% and Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1}) – 93.4% average for the period.

On the 56th day, the highest efficacy for the following treatments was reported: Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) – 99.2%, Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1}) – 99.2% and Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1}) – 98.4%.

Table 8. Efficacy of the studied herbicides against *Datura stramonium* L., %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	56.7	90.0	96.7	76.7	96.7	100	66.7	93.4	98.4
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	58.3	95.0	98.3	75.0	98.3	100	66.7	96.7	99.2
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	55.0	88.3	98.3	71.7	90.0	100	63.4	89.2	99.2
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	51.7	85.0	91.7	63.3	90.0	100	57.5	87.5	95.9
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	50.0	83.3	93.3	61.7	96.7	100	55.9	90.0	96.7
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	38.3	88.3	96.7	35.0	81.7	100	36.7	85.0	98.4

The weed *S. nigrum* is one of the difficult-to-conrol weeds. In 2018, on the 14th day, the highest efficiency for the variant with Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) - 73.3% was reported. The lowest for Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) - 43.3%.

Table 9. Efficacy of the studied herbicides against *Solanum nigrum* L., %

Variants	2018			2019			Average		
	14th	28th	56th	14th	28th	56th	14th	28th	56th
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	63.3	75.0	90.0	75.0	100	100	69.2	87.5	95.0
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	63.3	66.7	78.3	71.7	76.7	83.3	67.5	71.7	80.8
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	60.0	66.7	85.0	71.7	80.0	86.7	65.9	73.4	85.9
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	58.3	75.0	83.3	66.7	85.0	91.7	62.5	80.0	87.5
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	73.3	78.3	91.7	85.0	96.7	100	79.2	87.5	95.9
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	43.3	55.0	83.3	51.7	68.3	90.0	47.5	61.7	86.7

In 2019, these results were repeated as the efficiency in treatment 6 was 85%, and in treatment 7 - 51.7%. On average for the two-year of the study period, the best control against *S. nigrum* on the 28th day after treatment in variants 6 and 2 was obtained - 87.5%. The lowest in this reporting date was the efficiency for variant 7 - 61.7%.

The average results for the biological efficacy of the herbicides on the 56th day after treatment showed that the best control against *S.nigrum* was for the treatment with Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) - 95.9 % and Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1}) - 95.0% (Table 9).

The visual observations of phytotoxicity in both experimental years showed that all studied herbicides in the respective doses showed excellent selectivity for the hybrid P1114.

The indicator absolute seed mass of 1000 air-dry seeds gives an idea of the size and nutritional status of the seeds. The obtained results showed that there is a statistically proven difference between the control and the treated variants. The best results were reported after the treatment with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}), where in 2018 the absolute seed mass was 330.06 g, and in 2019 - 328.97 g.

In the other variants treated with herbicides, on average for the two years, the values of the indicator vary from 311.45 g to 322.85 g. It is worth noting that there is no mathematically proven difference between the variants treated with herbicide.

By using Duncan's multiple range test with proved differences and the lowest values (at a level of significance gD = 5%) was the mass of 1000 seeds obtained from the untreated control. On average for the period it was 272.25 g (Table 10).

Table 10. Absolute seed mass of 1000 air-dry maize seeds, g

Variants	2018	2019	Average
1. Untreated control	269.71 b	274.79 b	272.25 b
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	318.73 a	326.96 a	322.85 a
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	330.06 a	328.97 a	329.52 a
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	306.33 a	322.80 a	314.57 a
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	305.90 a	317.00 a	311.45 a
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	315.49 a	325.58 a	320.54 a
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	310.89 a	323.19 a	317.04 a

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

Regarding the indicator hectolitre seed mass, the highest values on average for the period - 77.66 kg was obtained after the application of Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}).

The evaluated herbicide combinations did not show significant influence to this indicator. This fact is confirmed by the mathematically unproven differences between the herbicide-treated variants. The lowest was the hectoliter mass of the untreated control - 75.67 kg, proven with the other treatments at the level of significance gD = 5% (Table 11).

Table 11. Hectolitre seed mass (kg)

Variants	2018	2019	Average
1. Untreated control	76.33 b	76.01 b	75.67 b
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	76.95 a	77.78 a	77.37 a
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	77.25 a	78.06 a	77.66 a
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	76.83 a	77.56 a	77.20 a
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	76.57 a	77.39 a	76.98 a
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	76.93 a	77.75 a	77.34 a
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	76.91 a	77.68 a	77.30 a

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The data for the maize seed yield of hybrid P1114 confirm that there is a positive correlation between the effect of herbicides against the available weeds and the mass of 1000 seeds, hectolitre mass and seed yield. The maize seed yield was the lowest for the untreated control where the weed density was severe and led to yield losses. For the untreated control the yield in 2018 was 6.99 t ha^{-1}, and in 2019 - 7.11 t ha^{-1}. The highest grain seed yield after the treatment with Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) was obtained. For the concrete treatment the yield in 2018 was 11.84 t ha^{-1}, and in 2019 - 12.48 t ha^{-1}. For the other treatments, average for the period, the maize seed yield varied from 11.88 to 12.02 t ha^{-1}. Only the difference between the untreated control and the variants with herbicide combinations was mathematically proven (Table 12).

Table 12. Maize grain seed yield (t ha^{-1})

Variants	2018	2019	Average
1. Untreated control	6.99 b	7.11 b	7.03 b
2. Callam (0.40 kg ha^{-1}) + Kelvin OD (1.00 l ha^{-1}) + Dash (1.00 l ha^{-1})	11.77 a	12.27 a	12.02 a
3. Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1})	11.84 a	12.48 a	12.14 a
4. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (0.625 l ha^{-1})	11.60 a	12.18 a	11.93 a
5. Callam (0.30 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	11.54 a	12.13 a	11.88 a
6. Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	11.74 a	12.24 a	11.99 a
7. Arat (0.20 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1})	11.68 a	12.20 a	11.95 a

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

CONCLUSIONS

On the 56th day after application the studied herbicide combinations showed 100% efficacy against *Sorghum halepense* (L.) Pers. developed from seeds, *Amaranthus retroflexus* L. and *Xanthium strumarium* L.

The most efficient control of *Sorghum halepense* (L.) Pers. developed rhyzomes, *Setaria viridis* (L.) P. Beauv., *Chenopodium album* L.*,* *Abutilon theophrasti* Medik. and *Datura stramonium* L. after the application of Callam (0.50 kg ha^{-1}) + Kelvin OD (1.25 l ha^{-1}) + Dash (1.25 l ha^{-1}) was accomplished.

The highest efficacy against the weed *Solanum nigrum* L. was for the treatment of Cambio SL (2.00 l ha^{-1}) + Kelvin OD (1.25 l ha^{-1}).

Absolute seed mass of 1000 air-dry maize seeds, hectolitre seed mass and maize grain seed yield of hybrid P1114 were higher and with statistically proved differences for all variants treated with herbicides in comparison to the untreated control.

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FIRST REPORT OF LOOSE SMUT - *Ustilago syntherismae* (Schweinitz) Peck ON *Digitaria sanguinalis* (L.) Scop. IN BUCHAREST - ROMANIA

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Abstract

There are only two previous citations since the 20th Century of this smut species for Romania. However, this relates to herbarium material collected for the first time in 1957 in Târgu Mureș recorded by K. Vánky (according dataset: The Fungal Collection at the Botanische Staatssammlung München) and to the second collecting in 1978 in Valea lui Mihai-Șimian, Brașov by Negrean G. (according dataset: The Fungal Collection at the Senckenberg Museum für Naturkunde Görlitz). In the present paper the interaction between the phytopathogenic fungus Ustilago syntherismae and the plant at which it infects, Digitaria sanguinalis, has been studied. Aspects related to the phenotypic expression (symptoms) manifested by Digitaria sanguinalis under field conditions and to the morphological characteristics of the spores of the fungus Ustilago syntherismae are presented. The observations took place in the period 2018-2020.Further research is required to investigate the current distribution of the loose smut - Ustilago syntherismae (Schweinitz) Peck on Digitaria sanguinalis (L.) Scop.in Romania.

***Key words**: smut, fungus, chlamydospores, Ustilago syntherismae, Digitaria sanguinalis.*

INTRODUCTION

There are about more than 230 species of *Digitaria*, all superficially similar with digitate or sub-digitate inflorescences, in tropical and warm temperate regions, particularly in the Old World (Clayton and Renvoize, 1986).
Digitaria sanguinalis (L.) Scop. is an annual, late spring-and summer-germinating mono-cotyledonous plant (Chirilă, 1989), and the species is a terophyte (Ciocârlan et al., 2004). Tillering initiates after emergence of the fourth leaf. Mature plants cover extensive areas developing a “mulch” or “tuft” 40-60 cm deep (Kissman and Groth, 1993).
During the European Middle Ages, *D. sanguinalis* was cultivated by Slavic peoples in Eastern Europe, where it was cooked in soups and porridges (Nesbitt, 2005).
D. sanguinalis (large crabgrass) is highly nutritious, compared to other grasses, it has a relatively high protein percentage, especially before the plant exhausts itself producing seed. For this reason, it is frequently sown in fields to provide graze for animals. Often large crabgrass are considered as weed when encroaching into newly planted forages (Rouquette et al., 2020), it is also a noxious weed particularly common in maize, in early-sown rice, beet, vegetable crops, orchards and vineyards; it is also found on waste ground, railway embankments, neglected lawns and grassy ridges (Behrendt and Hanf, 1979; Choi et al., 1998).
Originally native to Europe, *D. sanguinalis* is now found in about 45 countries, including Romania.
Reporting pathogens in different areas of Romania is a permanent goal for phytopathological scientific activity in our country (Cristea & Manole, 2014; Cristea & Jurcoane, 2016; Paraschivu et al., 2009; 2010; Zălă, 2012). Some pathogens can develop high epidemics in Romania depending on its virulence and the susceptibility of the host plant Mirela Paraschivu et al., 2015). Correct diagnosis of pathogens is the primary requirement in any sound disease management practice (Sundar et al., 2012).
Digitaria sanguinalis is an intermediate host for various microorganisms, large crabgrass itself can be attacked by various pathogens, in this case the *Ustilago syntherismae* fungus. The genus *Ustilago* (Pers.) Roussel (1806 is the

largest genus in the *Ustilaginales*, with approximately 300 species, and most species parasitize monocotyledonous hosts (Boekhout et. a., 2011). The genus *Ustilago* contain about 170 described species of fungi that cause smut on cultivated or spontaneous grasses (Vánky, 2012). Some smut fungi may attack only the stems, flowers, anthers, or ovules of their hosts and no other part and no other host. Smut fungi are important pathogens of grasses. Typically, smut fungi infect the inflorescence of their host plant (Otilia Cotuna et al., 2018). The main symptom of loose smut is the "smutted" grasses heads, which contain masses of black spores where the grain would normally be.

Loose smut of *Digitaria sanguinalis* (L.) Scop.is caused by *Ustilago syntherismae* (Schweinitz) Peck (1874) synonyms *Caeoma* (*Uredo*) *syntherismae* Schwein; *Sorosporium syntherismae* (Schwein.) Farlow; *Sphacelotheca digitariae-pedicellaris* Mishra; *Ustilago belgiana* Zundel; *Ustilago destruens* var. *digitariae* Sacc.; *Ustilago digitariicola* Speg.; *Ustilago rabenhorstiana* Kühn.

Caeoma syntherismae is a fungal species that was first discovered by botanist and mycologist Lewis David von Schweinitz (1832), now known as *Ustilago syntherismae* due to Peck (1874).

Although Europe is the continent of which the smut fungus mycota is the best known of all continents (Vánky, 2005), *U. syntherismae* is a specie was rarely been collected in Romania.

The presence of *U. syntherismae* has been reported for the first time, most likely in Pennsylvania-USA; since 1875 in Malaga, Spain (Thuemen, 1880); since 1985 in Switzerland; since 1992 in India; since 1999 in Zimbabwe; since 2001 in Zambia, and more recently, this year in Slovenia (according dataset: University of Minnesota Bell Museum; Brown University Herbarium; Botanische Staatssammlung München; The New York Botanical Garden; Zogg, 1985; Ogris, 2021).There are only two previous citations since the 20th Century of this smut species for Romania. However, this refers to the herbarium material collected for the first time in 1957 in Târgu Mureș by K. Vánky (according dataset: The Fungal Collection at the Botanische Staatssammlung München) and to the second collecting in 1978 in Valea lui Mihai-Șimian, Brașov by G. Negrean (according dataset: The Fungal Collection at the Senckenberg Museum für Naturkunde Görlitz).

There is approximate 1450 known "classical" smut fungi (those possessing ustilospores) which are classified into two classes, eight orders, 18 families and 73 genera (Vánky, 2001).

Systematic classification of the fungus *Ustilago syntherismae*: superkingdom clade *Eukaryota*; *Opisthokonta*; kingdom *Fungi*; subkingdom *Dikarya*; phylum *Basidiomycota*; subphylum *Ustilagino-mycotina*; class *Ustilaginomycetes*; order *Ustilaginales*; family *Ustilaginaceae*; genus *Ustilago* (Schoch et al., 2020)

MATERIALS AND METHODS

The research was carried out on a spontaneous population of *Digitaria sanguinalis* in the area adjacent to Drumul Cooperativei Street, Bucharest.

Visual observation is the fastest method to identify loose smut based on symptoms shown by infected large crabgrass plants. This method involves a high degree of subjectivity, depending largely on the diagnosing person's level of knowledge.

Scouting for *Ustilago syntherismae* attack has a particular importance in large crabgrass to establishing the attack value during the vegetation season.

The attack value is represented by frequency (F%), intensity (I%) and attack degree (AD%).

Frequency is the percentage of plant attacked out of 100 examined large crabgrass plants.

Intensity indicates the degree to which the large crabgrass inflorescence plant is attacked under examination. Intensity was noted directly in percentage.

The attack degree present severity of loose smut in the crop and was calculated using the frequency (disease incidence) and intensity(severity).

Attack degree was calculated using the formula:

$$\mathrm{AD}\ (\%) = \frac{\mathrm{F}\ (\%) \mathrm{x}\ \mathrm{I}\ (\%)}{100}$$

The period analysed in this study was 2018-2020. Observations were made under natural contamination. For scouting optimization and for the observation of the climatic conditions necessary for the appearance and development of the disease, precipitation, wind speed and temperatures were taken into account (Mirela Paraschivu et al., 2020).

The simple microscopic preparation (Figure 1) was performed with the help of a scalpel and consisted in detaching a small amount of the powdery, blackish mass of chlamydospores from the attacked inflorescences, placing it in the drop of water on the blade and lamella coating (Constantinescu, 1974).

Figure 1. Performing the microscopic preparation

Examination of the preparation under a microscope was first done with a small lens (with a magnification of 10x) viewing the entire slide, using the macrometric visa. Next, I chose the most characteristic portion of the preparation, which, after fixing in the center of the microscopic field, will be viewed with a large lens (with a magnification of 40x). To clarify the image I used the micrometric visa. The microscopic preparation was visualized under the Zeiss Primo Star microscope, and to determine the dimensions of the chlamydospores we used the Zen software.

To determine the number of chlamydospores in the soil (at a depth of 1 cm) we used decimal dilutions (Figure 2). In order to prepare soil dilutions, weigh 1 g of soil. The soil is then placed in a test tube with 9 ml of distilled water. Then, the mixture of 1 g of soil + 9 ml of distilled water is stirred well for 5 minutes. By stirring the dilution $1/10=10^{-1}$ is obtained. From the 10^{-1} dilution, take 1 ml of liquid with a sterile graduated pipette and place in another test tube with 9 ml of distilled water. We obtain, by gentle stirring, the dilution $1/100=10^{-2}$. Then proceed as in the previous dilution and obtain dilutions $10^{-3}…10^{-7}$ (Waksman, 1927).

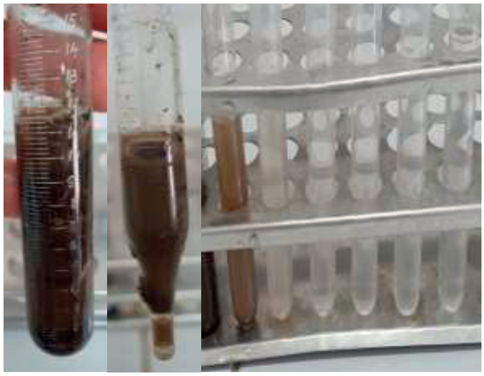

Figure 2. Decimal dilution until 10^{-1} to 10^{-7}

RESULTS AND DISCUSSIONS

Loose smut of large crabgrass are generally characterized by black, dusty masses of spores. These spores are, in reality, teliospores but are frequently and incorrectly called chlamydospores (Barnes, 1979). However, I prefer the term chlamydospores. *Ustilago syntherismae* is a fungus parasite of meristematic tissues that infects plants by chlamydospores and the infection appear in the plant when spores sprout and infectious hyphae penetrate the base layers of the bud and reach meristematic tissue (Ferreira and Comstock, 1989). For this reason, when the inflorescence appears it is largely affected by loose smut.

The first appearance of symptoms was found to end of May, every year.

Clamydospores in the inflorescence replacing the spikelets with a blackish semi-agglutinated to dusty spore mass, at first hidden by enveloping leaf sheaths.

The intensity of the inflorescence attack varies from one large crabgrass plant to another. If in some large crabgrass plants only a few spikelet are affected, in others finally, only the rachises of spiciform panicle remain intact (Figure 3). This can be attributed to the number of chlamydospores that have infected a plant.

During the three years of research, the main climatic parameters (average monthly temperature and amount of precipitation), from March (the time of the emergence of large crabgrass) to June (appearance of diseased inflorescences), were between 5.3°C (the lowest temperature in March, 2018) and 24.0°C (highest temperature in June 2019).

Figure 3. Typical symptoms of loose smut

The amount of precipitation is important in June, in terms of dislocation of chlamydospores from inflorescences (Figure 4).

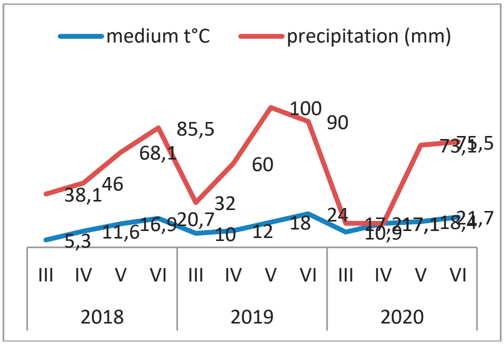

Figure 4. Average monthly temperatures and the volume of precipitation from the emergence of the large crabgrass to the appearance of loose smut (source: https://insse.ro/; http://www.meteoromania.ro; meteoblue.com/ro)

Also wind speed is important in June, in terms of chlamydospore dissemination. In June, the wind speed in Bucharest is, on certain days, between a minimum of 4 km/h and a maximum of 35 km/h, which means that we are dealing with a perceptible to significant wind according to the Beaufort wind force scale. The scale was devised in 1805 by the Irish hydrographer Francis Beaufort (Mather J.R., 2005).

Chlamydospores are globose, with a diameter of about 5-9 µm, unicellular, brown, finely equinulate, with a thick wall about 1 µm (Figure 5).

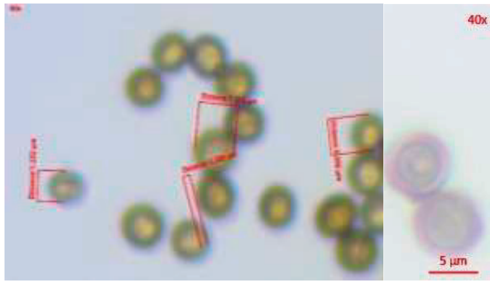

Figure 5. Measurement of some chlamydospores

The number of large crabgrass inflorescences attacked by loose smut increased compared to 2018 (when the average frequency was 47 attacked inflorescences) by 29.79% (61 attacked inflorescences) in 2019 (61 attacked inflorescences) and by 61.71% in 2020 (76 attacked inflorescences). The intensity of the inflorescence attack varied between 74.0% and 83.0. The average degree of attack increased progressively in the three years of research, from 39.01% in 2018 to 61.56% in 2020. Expressed as a percentage, the increase in the degree of attack was 15.72% higher in 2019 and with 57.81% in 2020 (Table 1).

Table 1. Scoring of the loose smut attack on the inflorescences of large crabgrass (%)

Scoring the attack → / Year ↓	F (%)	I (%)	AD (%)
2018	47.0	83.0	39.01
2019	61.0 (+29.79%)	74.0	45.14 (+15,72%)
2020	76.0 (+61.71%)	81.0	61.56 (+57.81%)

The spread of chlamydospores over short distances is achieved with the help of insects and humans. Chlamydospores adhere to the feet of insects and the soles of human footwear. Over long distances the spread is done with the help of the wind. Raindrops have the role of dislocating the chlamydospores from the attacked inflorescences, moving them to the ground, fixing the spores to the soil surface and circulating them in the surface layer of the soil. Chlamydospores also ensure the transmission over the winter, from one vegetative cycle to another, of the *Ustilago synterismae* fungus.

The average number of chlamydospores of *Ustilago synterismae* determined in the soil layer 1 cm deep, at the beginning of the vegetation start of the large crabgrass plants, was 2046 x 10^{-7}/ml.

I wanted to highlight the changes induced by the loose smut attack in terms of the height of the large crabgrass plants with attacked inflorescences, the number of internodes of these plants and the length of the inflorescences, compared to healthy plants (Figure 6).

Figure 6. Large crabgrass plants (healthy and with inflorescences attacked by loose smut) intended for different measurements

The fungus *Ustilago synterismae* caused a reduction in the height of plants with attacked inflorescences by 16.04% compared to healthy plants; the number of internodes in diseased plants decreased by 20.83% and the length of the attacked inflorescences decreased by 45.08% compared to the length of healthy inflorescences (Table 2).

Table 2. Changes caused by loose smut (%)

Large crabgrass plants	plant height (cm)	number of internodes	inflorescence length (cm)
healthy	21,25	4,8	6,1
with inflorescences attacked by loose smut	17,84 (-16.04%)	3,8 (-20,83%)	3,35 (-45,08)

CONCLUSIONS

Ustilago syntherismae (Schweinitz) Peck (1874) was rarely collected in Romania.

The attack of the loose smut manifested itself only at the level of the inflorescences of large crabgrass.

The average temperatures recorded during the vegetation period of the large crabgrass plants, which favored the appearance of loose smut, were between 5.3 and 24.0°C.

A high reserve of chlamydospores favors the increase of the frequency and intensity of the loose smut attack at *Digitaria sanguinalis*.

The evolution of loose smut disease in hearths explains the increase in the degree of attack from one year to another.

Further research is required to investigate the current distribution of the loose smut - *Ustilago syntherismae* (Schweinitz) Peck on *Digitaria sanguinalis* (L.) Scop.in Romania.

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BACTERIAL STRAINS INVOLVED IN SOILBORNE PHYTOPATHOGENS INHIBITION

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Abstract

Soil borne phytopathogens are a continuous threat to plant health. Most soil borne pathogens have a broad spectrum of plant hosts being capable to infect cereals, oil crops and legumes of various botanical families. Biological measurements capable to reduce plant pathogens growth and development are a sustainable way to prevent crop infections with minimum risks for farmers, consumers and environment. In the present study, several bacterial strains isolated from different sources were evaluated for their potential to reduce the growth of Fusarium oxysporum, Fusarium graminearum, Rhizoctonia solani, Sclerotinia sclerotiorum and Sclerotium bataticola. Tested strains revealed clear aspects of fungal cell wall and cell membrane alteration.

Key words: *biocontrol bacteria, soil borne phytopathogens.*

INTRODUCTION

Plants are exposed to a large spectrum of phytopathogens, and many of them are found in the soil. Such soil-borne disease complexes are especially difficult to control. Once established, they significantly reduce microbial diversity and consequently affect rhizosphere and endosphere of plants, increasing the phytosanitary risks for the crops (Wolfgang et al., 2019).

Among soil-borne pathogens, this study is focused on two *Fusarium* species, *F. oxysporum* and *F. graminearum*, on *Rhizoctonia solani, Sclerotinia sclerotiorum* and *Sclerotium bataticola*.

Fusarium oxysporum causes vascular wilts in more than 100 host plants (Joshi, 2018), having many *formae speciales* which are host specific.

Fusarium graminearum is an important pathogen of cereals, also involved in mycotoxin contamination. Although it produces *Fusarium* head blight of cereal crops, it can survive in the soil, on plant debris (Leplat et al., 2013).

Rhizoctonia solani is a ubiquitous soil-borne necrotroph, able to damage a wide range of economically important crops, from *Poaceae, Fabaceae, Solanaceae, Amaranthaceae, Brassicaceae, Rubiaceae, Malvaceae, Asteraceae, Araceae, Moraceae* and *Linaceae* families. Depending on the host plant and infection points, symptoms can include seed, root, hypocotyl, crown and stem rot, stem canker, black scurf, seedling blight, or damping off (Ajayi-Oyetundea & Bradley, 2018).

Sclerotinia sclerotiorum is a devastating soil-borne fungal pathogen causing stem rot of a wide range of plant species, such as oilseed rape, sunflower, soybean, and numerous vegetable crops (Willbur et al., 2019).

Sclerotium bataticola, also known as *Macrophomina phaseolina*, is a soil borne fungus causing charcoal rot to various plants, such as soybean, sunflower, corn, potato, or sweet potato (Lodha & Mawar, 2019).

The aim of this study is to characterize various plant-associated bacterial strains with potential biocontrol qualities, able to inhibit soil borne phytopathogenic fungi.

MATERIALS AND METHODS

Plant associated bacteria

A total of 30 bacterial strains isolated from different vegetal sources were used in this study (Table 1), one of which is a reference strain, *Bacillus subtilis* ATCC 6633, from the American Type Culture Collection. Some of these strains were previously identified at specie level. BW, OS15, OS17, BIR and BPA are *Bacillus amyloliquefaciens* strains (Sicuia et al., 2017) and 1T2 strain is affiliated to *Bacillus endophyticus* (Boiu-Sicuia et al., 2017).

Table 1. Plant associated bacterial strains

Bacterial strains	Isolation source	Bacterial strains	Isolation source
ATCC6633	reference strain	LT MYM1	endophytes of various plant species
BN7	agricultural wastes	LFF MYM1	
B7.2		LFF MYM5	
E1Ps	pea endophyte	E1Pv	
BVFs3	fava endophyte	E2Pv	
B4	compost tea	1T2	
B5		c	
B6		BAHs1	seed endophytes
BIR	plant pathogen antagonists	BPVs2	
BPA		BTAs3	
BW	soil	E2Ms	root nodules endophytes
OS15	onion rhizosphere	E1Ml	
OS17		E2Ml	
FL400	root nodules	E2Vh	
T2	plant pest	MC2	momordica root

Routinely, these bacteria were grown on Luria Bertani agar medium at 28°C. However, they were also able to grow on Potato-Dextrose-Agar (PDA).

Fungal plant pathogens

Five fungal species of plant pathogens were used in this study: *Fusarium graminearum* DSM4527, *Fusarium oxysporum* ZUM2407, *Rhizoctonia solani* DSM63002, *Sclerotinia sclerotiorum* and *Sclerotium bataticola*. The first three are reference strains from international microbial collections. The fourth strain is a Romanian isolate from USAMV collection, and the fifth belongs to RDIPP microbial collection. All of these fungi were routinely grown on PDA medium.

Antifungal activity evaluation

All 30 bacterial strains were analysed for their antifungal activity against previously mentioned soil-borne fungi. The test was performed *in vitro*, on PDA medium, similar to the dual culture technique (Soria et al., 2012).

The antagonism test was performed in 9 cm Petri dishes, using fresh fungal and bacterial biomass. Mycelia plugs of 5 mm in diameter were inoculated on PDA, in the centre of the plates. Against each fungus, at 2.5 cm distance from the centre of test plates, five bacterial strains were co-inoculated per dish, in equidistant distributed spots. Control plates were also prepared for each fungal strain.

Cultures were incubated at 28°C for 10 days, and antifungal activity was periodically evaluated according to Dinu et al. (2012). Bacterial efficacy to inhibit fungal growth (E%) was calculated using the following equation:

$$E (\%) = (Rc-Ri)/Rc * 100$$

where Rc is the radius of fungal growth in control plates, and Ri is the radius of fungal growth influenced by the bacterial strain.

Clear inhibition zones between fungal and bacterial colonies were also measured, and the mycelia edge was microscopically analysed.

RESULTS AND DISCUSSIONS

The antifungal potential of several plant associated bacteria was evaluated *in vitro*, using direct confrontation method. A total of 30 strains of plant associated bacteria were evaluated against five important phytopathogens.

Among all bacterial strains tested, 60% were endophytic isolates.

Taking into account the antagonistic activity towards the tested fungi, only half of the strains were able to inhibit all five pathogens, with at least 50% efficacy (Figure 1).

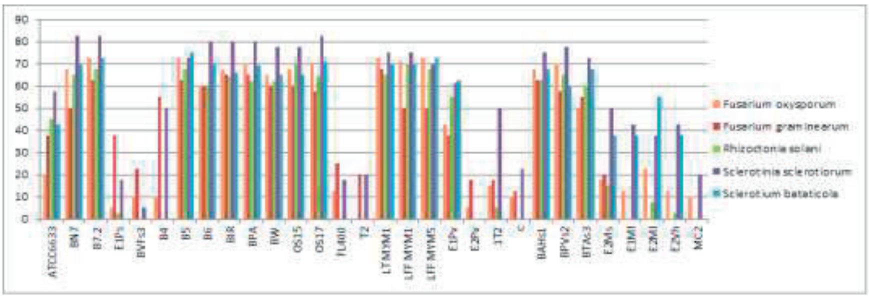

Figure 1. Bacterial efficacy (%) in fungal growth inhibition (after one week of co-cultivation)

Between the tested fungi, *S. sclerotiorum* was most vulnerable to the studied bacteria, where 66.7% of the strains reduced fungal growth with at least 50% and up to 82.5% efficacy (Table 2). *S. bataticola* was inhibited with at least 50% efficacy by 56.7% of the bacterial strains. *R. solani* and *F. graminearum* were inhibited by almost the same strains, representing 53.3% of the total bacteria tested, and *F. oxysporum* by 50% of the tested bacteria.

Table 2. Bacterial potential to inhibit fungal growth (after one week of co-cultivation)

Plant pathogenic fungi	Efficacy interval	Percentage of antagonistic bacteria	Antagonistic bacterial strains
F. oxy	50-72.5%	50 %	BN7, B7.2, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5
F. gram	50-67.5%	53.3%	BN7, B7.2, B4, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5
R. s.	50-70%	53.3%	BN7, B7.2, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5, E1Pv
S. s.	50-82.5%	66.7%	ATCC6633, BN7, B7.2, B4, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5, E1Pv, E2Ms
S. b.	50-75%	56.7%	BN7, B7.2, B5, B6, BIR, BPA, BW, OS15, OS17, BAHs1, LT.MYM1, BPVs2, LFF.MYM1, BTAs3, LFF.MYM5, E1Pv, E2Ml

where: F. oxy = *Fusarium oxysporum*, F. gram = *Fusarium graminearum*, R. s. = *Rhizoctonia solani*, S. s = *Sclerotinia sclerotiorum*, S. b. = *Sclerotium*

Clear zones (CZs), between plant associated bacteria and each plant pathogenic fungi, were considered an antagonistic effect of the beneficial strains, and were also measured (Table 3).

Table 3. Clear zones of microbial inhibition (after one week of co-cultivation)

Bacterial strain	*F. oxy*	*F. gram*	*R. s.*	*S. s.*	*S. b.*
	Clear zone (mm)				
ATCC 6633	0	0	0	1	0
BN7	2	0	1	8	2
B7.2	3	0	2	9	3
B5	4	2	1	7	3
B6	1	1	1	9	2
BIR	3	1	1	11	2
BPA	5	2	0	9	3
LT MYM 1	5	2	1	7	3
LFF MYM 1	5	0	1	8	3
LFF MYM5	4	0	2	6	3
E1Pv	0	0	0	3	1
BAHs1	4	1	2	9	2
BPVs2	4	1	2	9	2
BTAs3	0	0	2	7	2
BW	3	0	2	9	3
OS15	2	0	3	7	1
OS17	4	0	2	10	3

where: F. oxy = *Fusarium oxysporum*, F. gram = *Fusarium graminearum*, R. s. = *Rhizoctonia solani*, S. s = *Sclerotinia sclerotiorum*, S. b. = *Sclerotium bataticola*

Among the evaluated bacteria, 17 strains maintained a clear inhibition zone, unable to be colonized by the fungi. The wider CZs were noticed against *S. sclerotiorum*, the same pathogen with most severely inhibited mycelia growth. According to the biometric evaluation of the CZs, the bacterial strains inducing wider inhibition zones were the same expressing higher antifungal activity: BN7, B7.2, B5, B6, BIR, BPA, BW, OS15, OS17, LT.MYM1, LFF.MYM1, LFF.MYM5, BAHs1, BPVs2, and BTAs3 (Figure 2).

Figure 2. Bacterial antagonistic activity against five fungal phytopathogens

In order to evaluate more accurately the antifungal activity and understand fungal growth alterations, mycelia was analysed under the microscope.

The inhibited mycelia growth of *F. oxysporum* revealed fungal cells ulceration and lysis, with cytoplasmic content leaks (Figure 3). Such aspects were previously described (Boiu-Sicuia et al., 2017, 2018a, 2018b) and similar results were also mentioned in other several studies (Giorgio et al., 2015).

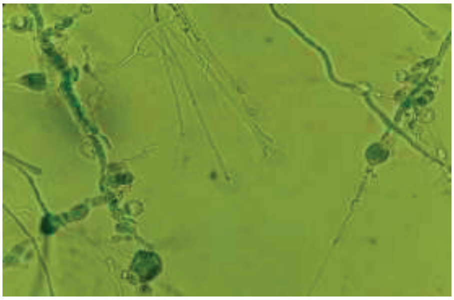

Figure 3. *F. oxysporum* cells swelling and lysis caused by antagonistic plant associated bacteria.

F. graminearum mycelia modifications also revealed cells ulceration and lysis (Figure 4), leakage and/or inactivation of *F. graminearum* cellular contents.

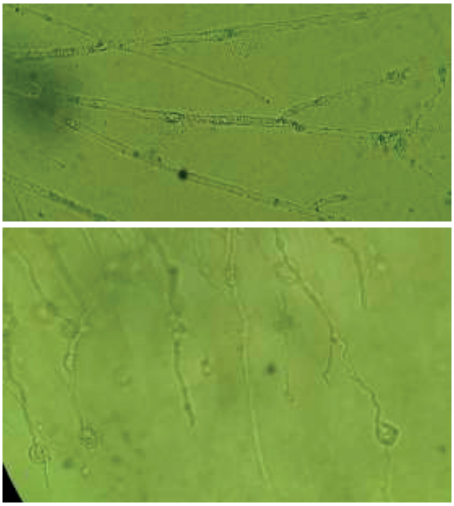

Figure 4. Cells ulceration and lysis of *F. graminearum* due to antagonistic bacteria

Ntushelo et al. (2019) suggest that iturin and other lipopeptides cause leakage of cellular contents and/or inactivation of *F. graminearum* conidia. Morphological distortions in conidia and hyphae, and severe damage of the cell coat, were already associated with iturin producers (Gong et al, 2015).

Mycelia growth and cell morphology modification were also seen on *R. solani* in presence of antagonistic bacteria. Similar aspects were previously described in similar studies mentioning *Rhizoctonia* growth inhibition (Boiu-Sicuia et al., 2017, 2018b).

Antifungal activity against *S. sclerotiorum* induced cell wall and plasma membrane damage, which lead to cell contents' leakage (Figure 5).

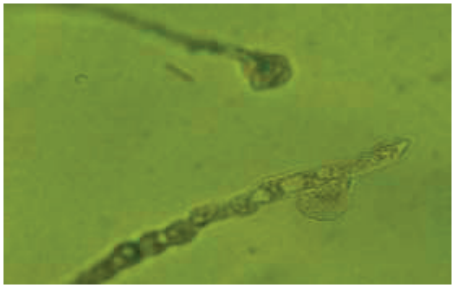

Figure 5. *Sclerotinia sclerotiorum* cells damage caused by antagonistic plant associated bacteria

Reviewed studies on *S. sclerotiorum* biocontrol (Kamal et al., 2016; Smolińska & Kowalska, 2018) describe bacterial antagonists involved in hyphal disintegration, cytoplasm leakage, delayed formation of infection cushion, weakening or killing of sclerotia as well as germination restriction (Saharan & Mehta, 2008; Gao et al., 2013; Chen et al., 2014). Among bacterial antagonists several endophytic strains were listed, best described being *B. subtilis* EDR4 strain (Chen et al., 2014).

The microscopic analysis of *S.bataticola* mycelia inhibited growth revealed cells ulcerations, fungal perforation and leaks of cytoplasmic content (Figure 6). Similar aspects were previously described against this pathogen (Singh et al., 2008; Boiu-Sicuia et al., 2018a, 2018b)

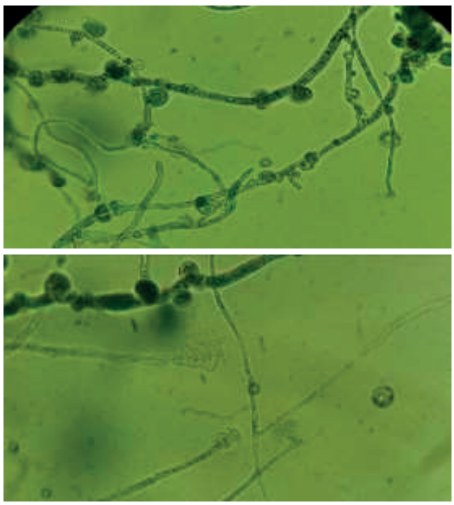

Figure 6. Cells ulceration, hyphal disintegration and cytoplasm leakage of *Sclerotium bataticola*

The mycelia growth modifications and cell alteration are probably due to the antifungal metabolites delivered by the biocontrol bacteria (Calderón et al., 2014). Similar perturbations of the fungal growth were also described in the presence of various volatile organic compounds released by *Pseudomonas* spp. and *Bacillus* spp. strains (Giorgio et al., 2015). Cell wall and plasma membrane damage could also be caused by the lytic enzymes released by the biocontrol bacteria (Boiu-Sicuia et al., 2018c).

CONCLUSIONS

Plant associated bacteria have an important role in plant protection against phytopathogens. Among the bacterial strains used in this study, the *Bacillus* spp. express a moderate to high biocontrol activity against important soil-borne phytopatogens, such as *Fusarium oxysporum, Fusarium graminearum, Rhizoctonia solani, Sclerotinia sclerotiorum and Sclerotium bataticola*. Antifungal properties were seen in both rhizospheric and endophytic bacteria. However, rhizobia-like strains did not reveal antifungal potential.

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ASPECTS REGARDING RARE PLANT SPECIES IN THE BASIN OF THE OLTET RIVER, ROMANIA

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Abstract

The habitats in the Basin of the Oltet River are characterized by a great plant diversity which is constantly changing due to natural and anthropogenic factors. The present study shows aspects regarding the existence of rare plant species in this river basin. We have focused on finding the presence of these species, with indication of location and date, to monitor the continuity of their existence in the habitats. We have identified 74 rare plant species, vulnerable or critically endangered. For these 16 species we have got the sozology status (protected species) given by the International Union for Nature Conservation (IUCN Red List Category, Europe 2011). Some of the data about the rare plant species are over 50 years old and, consequently, new aspects have been noticed, with the habitat having undergone natural changes or having acquired a different use. It has been observed that some species have a small number of populations in the basin's habitats, and the populations are made up of a small number of individuals.
This situation, as well as the indication of the existence of some new rare plant speciesin the studied area, shows the necessity to establish the conservation status.

***Key words**: Basin of the Olteț river, spontaneous plants, rare plants, critically endangered plants, vulnerable plants.*

INTRODUCTION

The flora of the Olteț river comprises species of rare plants, vulnerable or critically endangered, which we have found in the area of the gorge separating the Parâng Mountains from the Căpățânii Mountains, in the Polovragi Depression, the Getic Plateau and the Romanian Plain, where the Olteț river flows into the Olt river.
The habitats are highly varied, offering natural conditions to these species.
Data about some rare species of plants were collected by Păun (1957, 1966), Păun & Popescu (1971), Popova-Cucu & Muică (1993), Răduţoiu (2006, 2014), Popescu & Ciortan (2009).
Our research work has been correlated with the existing data.
The aspects we had in view were the conservation of these species in their habitats, the reduction of their area or their extinction.
The identified species are found on the red lists or are described sozologically, chorologically, cenologically, biologically (Dihoru & Negrean 2009). The European Red List of Vascular Plants (Bitz M. et al., 2011) comprises some of these species included in the IUCN categories which no longer use the rare plant term (R).
Our data of this river basin consisted in the identification of the taxa, the indication of the location and the estimation of the influence of the natural and anthropogenic factors.
We consider that a survey of their present situation, especially with regard to the factors which have led to a decrease in the number of these specimens or their extinction, can contribute to establishing such protection measures which would ensure the conservation and restoration of these species' populations.

MATERIALS AND METHODS

The sozological status of these plant species has been identified following the study of the bibliography and the national and European red lists.
For plant observation we have made field trips, from 2007 to 2020, March to November. For location we have used the actual name of the localities, as well as the placename given by the local people.
The identified taxa are presented in a list (Table 1). For each taxon we have indicated their

distribution in the investigated area as well as several ascertained sozological aspects.

For taxa distribution we have introduced two abbreviations, one for the distribution taken from the specialty literature (D1) and another one for our findings (D2). For the data from the specialty literature we have made mention of the bibliographical references, whereas our findings were correlated with the data of their identification and the aspects observed in the field.

For these species of plants we have made up a table (Table 1) of their sozological status in the three National Red Lists (Boşcaiu and collab., 1994; Dihoru Gh. & Dihoru Alexandrina, 1994; Oltean et al., 1994; Negrean, 2001), the Red Book (Dihoru & Negrean, 2009) and the European Red List of the Vascular Plants (Bitz M. et al., 2011).

For the nomenclature we have used the work Vascular Plants of Romania (Sârbu and collab., 2013), Euro+Med. (2007-).

RESULTS AND DISCUSSIONS

The inventory work of the plant species in the river basin gave us the possibility to identify 74 taxa with an important sozological status for protection and conservation activities. The research performed on the field trips points to some sozological aspects in the taxa found in the areas. For the taxa indicated many years before our research, which we did not find out, we have tried to identify the changes in the habitats caused by the natural or anthropogenic factors.

1. ***Anacamptis pyramidalis*** (L.) Rich.

D2: Polovragi, 12 July 2009, 19 June 2016, 18 July 2018, 14 June 2019, 21 June 2020.

Populations with a small number of specimens exposed to mowing and grazing.

2. ***Angelica arhangelica*** L.

D2: Polovragi, North of the Olteţ Gorges, 21 July 2014, 5 July 2016, 20 July 2018, 30 July 2019, 2 August 2020. A very small number of specimens, some of them endangered by the forest logging, the mechanical action of animal herds, the upstream floods.

3. ***Asplenium cuneifolium*** Viv.

D2: The Olteţ Gorges, the rocky areas, 3 June 2007, 10 September 2007, 10 July 2011, 20 July 2018, 30 July 2019, 2 August 2020.

Most often covered with a thich layer of dust raised by vehicles.

4. ***Asplenium lepidium*** C. Presl

D1: The Olteţ Gorges (Popova-Cucu & Muică 1993);

D2: The Olteţ Gorges and North of the Gorges, 10 September 2007, 14 June 2010, 20 July 2018, 30 July 2019, 2 August 2020.

Most often covered with a thich layer of dust raised by vehicles**.**

5. ***Athamanta turbith*** (L.) Brot. subsp. *hungarica* (Borbás) Tutin (*A. hungarica* Borbás)

D2: Polovragi, in the Olteţ Gorges, 27 June 2009, 14 June 2010, 21 June 2018, 30 July 2019.

A small number of specimens exposed to the mechanical destruction caused by the passage of cattle.

6. ***Beckmannia eruciformis*** (L.) Horst

D1: Petriș (Păun 1963); Between Greci and Osica de Sus (Răvăruț 1972); the Olteţ meadow, between Vlăduleni and Greci, Osica de Sus, 44°15′687-700″N, 24°20′356-347″E, alt. circa 92-99 m, 13 VI 2010 [cra 60.113-136] (Răduţoiu 2013).

D2: Cioroiu, In the Olteț meadow, 5 July 2019.

Several specimens found in an area inaccessible for grazing.

7. ***Botrychium matricariifolium*** (Retz.) A. Braun ex W.D.J. Koch (*B. ramosum* (Roth) Asch.)

D1: The Olteţ Canyon (Popescu & Ciortan 2009).

8. ***Campanula grossekii*** Heuff.

D1: The Olteț Gorges (Păun & Popescu 1971);

D2: The Olteț Gorges, 25 June 2008, 12 July 2011, 20 July 2018, 30 July 2019, 2 August 2020.

Specimens exposed to picking by tourists and todust raised by vehicles.

9. ***Camphorosma monspeliaca*** L. subsp. *monspeliaca*

D1: Osica de Sus (Păun 1965); Osica de Sus NE (Vlăduleni) - Greci S, Tabonu Mic, 29 VII 2017, G. Negrean (N: 25.962).

D2: Peretu, Tabonul Mic, drainage channel, 5 July 2018, 18 July 2019, 7 August 2020.

Specimens exposed to mechanical action of domestic animals.

10. ***Centaurea affinis*** Friv.
D1: Oltet Valley above the Cave (Grecescu 1898); The Olteţ Gorges (Păun & Popescu 1971); The Olteţ Gorges (Negrean & Dihoru 2009);
D2: The Olteţ Gorges, 11 September 2007, 30 June 2017, 18 June 2018, 29 July 2019.
Specimens exposed to dust raised by vehicles.
11. ***Centaurea atropurpurea*** Waldst. et Kit.
D1: The Olteţ Gorges (Păun & Popescu 1971);
D2:The Olteţ Gorges, 11 July 2007, 30 June 2017, 18 July 2018, 30 July 2019, 2 August 2020.
Specimens exposed to picking by tourists and to dust raised by vehicles, and the ones in the Grohotiș area are exposed to the mechanical action of the cattle grazing unattended.
12. ***Cephalanthera rubra*** (L.) Rich.
D1: The Olteţ Gorges(Sârbu A. 2007).
D2: The Olteţ Gorges, 25 June 2008, 2 June 2016, 2 July 2019.
Specimens exposed to picking by tourists and to dust raised by vehicles.
13. ***Cephalaria laevigata*** (Waldst. et Kit.) Schrad.
D1: The Olteţ Gorges (Păun, & Popescu 1971); The Olteţ Gorges (Păun & Popescu 1975);
D2: The Olteţ Gorges, The Olteţ Canyon, 30 June 2017, 18 July 2018, 30 July 2019.
Specimens exposed to mechanical action of domestic animals.
14. ***Cerastium banaticum*** (Rochel) Heuffel
D1: Stony, rocky and grassy areas, the Polovragi Cave (Prodan 1953);
D2: Polovragi in the Olteț Gorges, 16 June 2008, 13 June 2012, 29 June 2013; 21 July 2018, 21 June 2020.
Specimens exposed to picking by tourists and to dust raised by vehicles.
15. ***Corydalis solida*** (L.) Clairv. subsp. *slivenensis* (Velen.) Hayek
D1: The Olteț Gorges, between Izmona and Mount Negovanu (Păun & Popescu 1971);
D2: Călui în Pădurea Cerăt - Călui in the Cerăt Forest, 40°26′06.70″N, 24°04′30.77″ E, alt. cca 160 m, 2 April 2009, 19 April 2012, 25 April 2013, 18 April 2016, 21 April 2018, 25 April 2020.
A small number of specimens exposed to destruction during logging activities.
16. ***Cyperus serotinus*** Rottb. (*Juncellus serotinus* (Rottb.) C. B. Clarke;
D2: Fălcoiu, the dammed area of the river Olteţ, 14 August 2014, 18 July 2019, 8 August 2020.
A small number of specimens exposed to destruction from grazing and burning of vegetation.
17. ***Delphinium fissum*** Waldst. et Kit.
D1: The Olteț Gorges (Păun & Popescu 1971);
D2: The Olteț Gorges, 27 June 2008, 20 July 2009, 25 July 2015, 11 July 2017, 22 June 2018, 14 June 2019, 21 June 2020.
Specimens exposed to picking by tourists and to dust raised by vehicles.
18. ***Dianthus spiculifolius***Schur
D1: The Olteț Gorges (Păun & Popescu 1971);
D2: Polovragi in the Olteț Gorges and the Olteţ Canyon, 16 June 2008, 29 June 2013, 21 July 2018, 30 July 2019, 21 June 2020.
Specimens exposed to picking by tourists and to dust raised by vehicles.
19. ***Dianthus trifasciculatus*** subsp. *trifasciculatus* Kit.
D1: Osica de Sus (Păun 1965); Dobrosovleni-Chili (the "Sociologia Militans" Professional Association: 2014).
20. ***Dictamnus albus*** L.
D1: The Olteț Gorges (Păun & Popescu 1971);
D2: The Olteț Gorges, 16 May 2008, 14 May 2018, 14 June 2019.
Specimens exposed to dust raised by vehicles.
21. ***Elatine alsinastrum*** L.
D1: In the forest pool between Greci and Cioroiu (Păun 1963);
D2: Balș, the Saru Forest, North of the national highway, East of the forest road, 30 June 2017, 22 July 2018, 5 June 2020.
A small number of specimens exposed to destruction during logging activities.
22. ***Elodea canadensis*** Michx.
D2: Cioroiu, Fălcoiu in the dammed area of the river Olteţ, 3 July 2011, 29 July 20015, 1 July 2017, 19 July 2019, 8 August 2020.
Specimens exposed to drainage, pollution with household organic waste and plastic bottles.
23. ***Erythronium dens-canis*** L. subsp. *niveum* Baumg.
D1: The Curtișoara Forest, Vulpeni (r. Balș) (Zahariadi 1966); Sârbeşti, Polovragi (Păun & Popescu 1971);

D2: Tetoiu, at Piscul lui Ţâpan (Ţâpan Peak), at Cetate, in Chirca, in Vălceaua Şetrarului, 10 March 2007, 27 February 2014, 15 March 2018, 20 April 2020; Zătreni, in Făget, 5 April 2009; Săscioara, in Crin, Piscul Ioanei (Ioana's Peak), 18 April 2011; Bălceşti, in the forest of Aninoasa, 30 March 2014; Călui, in the forest of Cerăt and Petriş Forest, 5 April 2016, 3 April 2017, 21 April 2018, 25 April 2020.
The specimen is picked as an ornamental plant and forsuperstitions – to bring good luck, more chicks from the brooding hen.
24. *Fimbristylis bisumbellata* (Forssk.) Bubani
D1: the Olteț meadow, North of Balș (Păun 1966).
25. *Fritillaria montana* Hoppe
D1: Romula in the Prapor Forest (r. Balș) (Zahariadi 1966);
D2: The Olteț Gorges, 14 April 2010,21 April 2017, 8 April 2018, 21 April 2019.
A very small number of specimens, exposed to picking by tourists.
26. *Genista tinctoria* L. subsp. *oligosperma* (Andrae) Borza (*G. oligosperma* Andrae).
D1: Curmătura Oltețului – the Olteț Pass; Mount Negovanu (Păun & Popescu 1971);
D2: The Olteț Gorges, 21 June 2006, 17 July 2017, 28 July 2019.
Small number of specimens exposed to mechanical action of domestic animals and dust raised by vehicles.
27. *Gladiolus imbricatus* L.
D2: Ciuperceni de Olteţ, 29 V 2013; Polovragi in the fenced grasslands, 20 June 2009, 28 June 2019;
The grasslands are exploited by mowing/scything.
28. *Jovibarba heuffelii* (Schott) Á. Löve et D. Löve (*Sempervivum heuffelii* Schott)
D1: At Peștera-Polovraci Cave (Grecescu 1898); The Olteț Gorges (Păun & Popescu 1971); The Olteț Gorges (Păun & Popescu 1975);
D2: Polovragi in The Olteț Gorges and the Olteț Canyon, 25 June 2008, 12 June 2009, 16 July 2012, 20 July 2103, 12 July 2017, 14 June 2019, 21 June 2020.
Specimens exposed to dust raised by vehicles, and the ones in the Grohotiș area are exposed to the mechanical action of the cattle.
29. *Lactuca viminea* (L.) J. Presl et C. Presl.
D2: Tetoiu, in the grassland Dealul Viilor (Vineyard Hill), 4 July 2008; the Olteț Canyon, 29 July 2019; Ciupercenii de Olteț, 31 July 2019.
Specimens exposed to scything and the mechanical action of domestic animals.
30. *Lamium garganicum* L. subsp. *laevigatum* Arcang. (*L. bithynicum* Benth.)
D1: La Peștera Polovraci (Polovraci Cave) on the banks of the Olteț river (Grecescu 1898); Peștera Polovragi (Polovraci Cave) on the banks of the Olteț river (Grințescu 1961); the Olteț Gorges (Păun & Popescu 1971);
D2: Polovragi in the Olteț Gorges, 16 May 2008, 3 June 2016, 2 June 2018, 27 July 2019.
Specimens exposed to the dust raised by vehicles.
31. *Lathyrus sphaericus* Retz.
D2: Irimeşti in the meadow, 21 June 2015, 18 May 2018, 16 May 2019.
A small number of specimens on the reduced areas of grassland unexploited by scything, but used for grazing. Risk for these areas to be turned into farmland.
32. *Leontopodium alpinum* Cass.
D2: Polovragi in the Olteţ Gorges, at Grotă (the Grotto), 24 July 2011, 7 July 2017, 27 June 2019. A very small number of specimens on the rocky areas near the Grohotiș-Grotă.
33. *Leucojum vernum* L.
D1: Between Izmona and Mount Negovanu (Păun & Popescu 1971);
D2: The Olteţ Gorges, 14 April 2010, 20 April 2017, 21 April 2019, 7 April 2020.
Specimens exposed to picking by tourists and local people for sale and to the mechanical action of domestic animals and logging equipment.
34. *Limodorum abortivum* (L.) Sw.
D1: between Horezu and Iancu Jianu (Dobricior Forest), between Morunglav and Morunești (Paucă 1972).
D2: Iancu Jianu, 12 June 2018, 9 May 2019, 4 June 2020.
A small number of specimens exposed to logging activities.
35. *Limonium tomentellum*(Boiss.) Kuntze (*Statice tomentella* Boiss.)
D1: The meadow of the Olteţ river, between Vlăduleni and Greci, Osica de Sus, 44°15′687-700″N, 24°20′356-347″E, alt. cca 92-99 m, 13

June 2010, 15 August 2011, leg. et det. I. Costache et D. Răduţoiu [CRA60.113, 60.133-136], (Răduţoiu 2014);
D2: Between Vlăduleni and Greci, 5 July 2016, 11 July 2019, 7 August 2020, 44°15′37,09″N, 24°20′42,68″E.
A small number of specimens exposed to intensive grazing.

36. ***Linum uninerve*** (Rochel) Jav.
D1: The Olteţ Gorges (Păun & Popescu 1971);
D2: The Olteţ Gorges, 16 May 2008, 2 June 2016, 14 May 2018, 21 June 2020.
Specimens exposed to picking by tourists, to crushing and to dust raised by vehicles.

37. ***Marsilea quadrifolia*** L.
D1: South-East of the ROSCI0266 area the Olteț Valley at the confluence withthe river Olt ("Sociologia Militans" Professional Association: 2014);
D2: Fălcoiu, 12 August 2019.
A very small number of specimens exposed to the competition with other aquatic species and to the pollution of the water with wastes.

38. ***Medicago polymorpha*** L. (*M hispida* Gaertn.)
D1: Greci in the Olteț meadow, Găvănești in the Geamartalău meadow, Vârtina (Păun 1963); Găvăneasa to Gropșani, Gropșani to Mardare in the Horezu meadow, Horezu, the Horezu meadow, Greci to Osica Mare, Tomeni, the Geamartalău meadowbetween Băleasa and Găvănești, Găvănești at Balta Borească (Borească Pond), between Greci and Osica de Sus, Greci at Balta Stejarului (Stejarul Pond), between Greci and Tomeni, Horezu (Păun 1966);
D2: North of the Olteţ Gorges, 25 June 2010, 2 June 2018; Gropșani, 10 June 2019.
A small number of specimens exposed to intensive grazing.

39. ***Mercurialis annua*** L.
D2: The Olteţ Gorges, 14 May 2018, 28 July 2019.
A small number of specimens exposed to the mechanical action of domestic animals.

40. ***Nasturtium officinale*** (L.) R. Br.
D1: North Polovragi, The Olteţ Gorges, in aqua, 45°12′39,08″N, 23°46′34,79″E, alt. cca 670 m, 2 June 2013, G. Negrean (GN 20.662);
D2: The Olteţ Gorges, 22 May 2006; 14 June 2010; North of the Olteţ Gorges, 16 July 2016, 12 July 2017, 29 July 2019.
Specimens exposed to the dust raised by vehicles, to draught and the mechanical action of animal herds.

41. ***Neottia nidus-avis*** (L.) Rich.
D1: Between The Olteţ Gorgesand Strîmtori (Păun & Popescu 1971);
D2: Tetoiu in the forest of Chirca and the forest at Piscul lui Țâpan (Țâpan Peak) 3 May 2009, 10 May 2011, 18 June 2014, 5 June 2017, 26 May 2019.
Specimens exposed to the mechanical action of animal herds.

42. ***Oenanthe aquatica*** (L.) Poir
D1*:* Balș, Greci the pond area (Păun 1966);
D2: Irimeşti, Tetoiu, Stanomiru, 12 June 2007, 20 July 2017; Tabonu Mic, Osica de Sus, 30 June 2017, 5 June 2020.
Specimens exposed to the mechanical action of animal herds and to the dumping of household waste.

43. ***Orchis elegans*** Heuff. (*Anacamptis palustris* subsp. *elegans* (Heuff.) R. M. Bateman, Pridgeaon & M. W. Chase).
D1: Baldovinești, Buzduc (Păun 1966);
D2: Tetoiu, in the wet grasslands of Dealul Viilor (Vineyard Hill), to Piscul lui Ţâpan (Ţâpan's Peak), 12 May 2008, 19 June 2013; 26 May 2019.
Specimens exposed to the mechanical action of animal herds.

44. ***Orchis ustulata*** L.
D1: The Olteţ Gorges (Sârbu A. 2007).
D2 Polovragi in the grasslands, the chestnut tree area, 25 June 2008. 14 May 2018, 14 June 2019.
Specimens exposed to picking by tourists and the mechanical action of domestic animals.

45. ***Peltaria alliacea***Jacq. (*P. perennis* (Ard.) Markgraf).
D1: The Polovragi Gorges (Nyárády 1953); The Olteţ Gorges (Păun & Popescu 1971);
D2: The Olteţ Gorges, 25 June 2008, 11 July 2010, 21 July 2014, 5 July 2016, 20 July 2018, 30 July 2019, 21 June 2020.
Specimens exposed to destruction by tourists and to dust raised by vehicles.

46. ***Peucedanum rochelianum*** Heuff.
D1: Dumbrava Forest between Sîrbești and Poenari (Păun & Popescu 1971);
D2: Sîrbești Forest 6 May 2018, 7 June 2019.
Specimens exposed to the mechanical action of animal herds.
47. ***Platanthera bifolia*** (L.) Rich.
D1: Lunca Olteţului(Olteț Meadow), in Călui Forest (Păun 1966);
D2: Tetoiu at the edge of Traian Doctorul Forest, 10 June 2006, 5 June 2017, 18 June 2019; Călui Forest, 30 June 2017, 5 June 2020.
Specimens exposed to the mechanical action of animal herds.
48. ***Ranunculus constantinopolitanus*** (DC) D'Urv.
D1: Roșieni Forest, Dobrun Forest, the forest between Greci and Cioroi (Păun 1963);
D2: Călui in Cerăt Forest, 16 April 2009, 20 April 2011, 25 April 2020; Fălcoiu, 2 April 2016, 20 April 2017, 5 May 2018; East of Pietriș, 9 May 2019.
Specimens exposed to the mechanical action of domestic animals and the forest logging activities.
49. ***Sedum cepaea*** L.
D2: Tetoiu, at Pricu lângă Groapă (Pricu by the Pit), 6 July 2008, 10 July 2010, 5 June 2017, 18 June 2019.
Specimens exposed to the mechanical action of domestic animals.
50. ***Sedum rubens*** L.
D2: Polovragi, in the vicinity of the power dam on the Olteț river, 2 June 2019.
Specimens exposed to the mechanical action of domestic animals and the vehicles carrying gravel.
51. ***Seseli rigidum*** Waldst. & Kit.
D1: Steep limestonecliffs, rock crevices on the precipices, Polovraci (Grecescu 1898); Polovraci (Todor 1958); the Olteț Gorges (Păun & Popescu 1971);
D2: Polovragi in the Olteț Gorges 9 July 2007, 20 July 2016, 14 August 2018, 30 July 2019.
Specimens exposed to the dust raised by vehicles and those at Grohotiș are exposed to the mechanical action of cattle.
52. ***Silene flavescens*** Waldst. et Kit.
D1: Polovragi Monastery on the Olteț Valley (Gușuleac 1953); the Olteț Gorges (Păun & Popescu 1971);
D2: Polovragi in the Olteț Gorges,10 July 2007, 26 June 2008, 13 June 2012, 29 June 2013, 21 July 2018.
Specimens exposed to the dust raised by vehicles.
53. ***Silene gallinyi*** Rchb. (*Silene trinervia* Sebast. et Mauri).
D1 Balş, on road sides, by the fences, on sandy soil (Guşuleac 1953).
54. ***Sorbus graeca*** (Spach) Schauer (*S. cretica* Fristsch et Rech.).
D1: The Olteț Gorges (Păun & Popescu 1971);
D2: The Olteţ Gorges to the East, 25 May 2007, 11 July 2009, 16 June 2016, 30 July 2019.
Few specimens, at high altitudes.
55. ***Spiranthes spiralis*** (L.) Cheval
D1: The Olteț Gorges (Păun & Popescu 1971);
D2: Polovragi, South-Westof the Olteț Gorges, to Baia de Fier (the Iron Mine) 10 September 2007, 18 July 2018.
Populations with a small number of specimens exposed to scything and grazing.
56. ***Stipa eriocaulis*** Borbás.
D1: The Olteț Gorges, 45°11′44,49″N, 23°46′1159,17″E, alt. cca 676 m, 2 July 2004, (G. Negrean) [bucm 69.625*];
D2: The Olteț Gorges, on chalk rocks, at the Grotă (Grotto) 25 June 2008, 14 July 2010, 3 June 2016, 21 June 2018, 14 June 2019, 21 June 2020.
Specimens exposed to the mechanical action of cattle.
57. ***Symphyandra wanneri*** (Rochel) Heuff.
D1: Polovragi (Ghișă, Gușuleac, Morariu 1964); The Olteț Gorges (Păun & Popescu 1971);
D2: The Olteț Gorges, to the North, 17 July 2007; The Olteţ Canyon 20 July 2018, 27 July 2019.
Specimens exposed to the picking by tourists and the dust raised by vehicles.
58. ***Taeniantherum caput-medusae*** (L.) Nevski.
D2: Tetoiu in the grasslands at Pricu, 27 June 2008, 11 June 2018; Dobriceni, 29 June 2009;Balș, Saru Forest, North of the national highway, edge of wheat field, 44°22′50,92″N, 24°11′43,57″E, alt. cca 191 m, 1 July 2017, 22 July 2018, 11 July 2019.

Specimens exposed to destruction by farming equipment.

59. ***Tanacetum macrophyllum*** (Waldst. & Kit.) Sch. Bip. (*Crysanthemum macrophyllum* Waldst. & Kit.)

D2:The Olteț Gorges, 25 June 2008, 18 July 2019.

Specimens exposed to the mechanical action of domestic animals.

60. ***Teucrium montanum*** L.

D1: The Olteț Gorges(Păun & Popescu 1971); the upstream Olteț river (Păun & Popescu 1975);

D2: Polovragi la Grotă (at the Grotto), 2 June 2010, 6 July 2016, 12 July 2017, 18 June 2018, 21 June 2020.

Specimens exposed to the mechanical action of cattle.

61. ***Thlaspi alliaceum*** L.

D1: Oteteliș (Răduţoiu 2006);

D2: Tetoiu, 15 May 2008, 5 June 2017, 17 May 2019; Polovragi in the Olteț Gorges, 2 May 2010.

Specimens exposed to scything and the mechanical action of domestic animals.

62. ***Thlaspi dacicum*** subsp. *banaticum* (R. Uechtr.) Jáv (*Th. banaticum* R. Uechtr.).

D2: The Olteț Gorges to the ruins of Cetatea (the Citadel), 20 July 2010, 12 July 2017, 29 July 2019.

Specimens exposed to the picking by tourists and the dust raised by vehicles.

63. ***Thymus comosus*** Heuff. ex Grisb. et Schenk.

D1: The Olteț Gorges (Păun & Popescu 1971);

D2: The Olteț Gorges and the Olteț Canyon, 14 July 2008, 19 June 2009, 26 June 2012, 11 July 2016, 30 July 2019, 2 August 2020.

Specimens exposed to the mechanical action of domestic animals and the dust raised by vehicles.

64. ***Trapa natans*** L.

D2: Cioroiu, at the confluence of therivers Olteț and the Olt, 16 July 2010, 29 June 2017; 8 August 2020.

Specimens exposed to competition with other aquatic species and to pollution of the waters of river Olteț.

65. ***Trifolium michelianum*** Savi

D1: In the meadow of the river Geamărtălui, at Balgovinești, Petriș, Găvănești, Gropșani (Păun 1963); Petriș, Spineni, Balș (Păun 1957).

66. ***Trifolium scabrum*** L.

D1: Vlăduleni, on the slope between the road going to Piatra Olt and the meadow of the Olt river, as well as at the entrance to Vlăduleni from Piatra Olt, on the slopes along the road; Baldovinești, on the slope in the communal pasture land, at the exit to Găvănești (Păun 1957); Vlăduleni, Baldovinești to Găvănești, Gubandru, (Păun 1963); the outskirts of Balșului (Păun 1969);

D2: Balș, Saru Forest, North ofthe national highway, a degraded grassland, 44°22′50,52″N, 24°11′40,90″E, alt. cca 188 m, 1 July 2017, 18 July 2019, 5 June 2020.

Specimens exposed to excessive grazing.

67. ***Trifolium squamosum*** L.

D2: Saru Forest, the meadow of Pârâul Bârlui (the Bârlui Brook), 44°21′36,30″N, 24°11′20,40″E, alt. cca 140 m, 29 June 2017, 18 July 2019, 5 June 2020.

Specimens exposed to excessive grazing.

68. ***Typha minima*** Funk

D1: The Olteț Meadow at Mîinești, Osica de Jos (Păun 1963); Oteteliș (Răduţoiu 2006);

D2: Ghioroiu, 26 July 2015; Cioroiu, the drainage channel outside the Olteț river dam, 22 July 2018, 18 July 2019; Dobrețu at the bridge over Bulzești river, 20 July 2019.

Specimens exposed to pollution of the waters of the Olteț river.

69. ***Typha shuttleworthii*** Koch & Sonder.

D1: Polovragi (Păun & Popescu 1971);

D2: Polovragi, 18 June 2018, 14 June 2019.

Specimens exposed to the mechanical action of domestic animals.

70. ***Vallisneria spiralis*** L.

D2: Fălcoiu in the dammed area of the Olteţ river, 3 July 2011, 29 July 2015, 1 July 2017, 19 July 2019.

Specimens exposed to pollution of the waters of Olteț river.

71. ***Veronica bachofenii*** Heuff.

D1: Between Izmona and Mount Negovanu (Păun & Popescu 1971); the Olteț Gorges (Păun & Popescu 1975);

D2: The Olteț Gorges, 23 July 2008, 17 July 2017, 30 July 2019.

Specimens exposed to the mechanical action of domestic animals and the dust raised by vehicles

72. ***Vicia sparsiflora*** Ten.
D1: Vulpeni, to Horezu through the forests (Păun 1963); the forests at Văleni and Horezu (Păun 1964); Vulpeni, in the Pescărești Forest, Cârtișoara Forest, Horezu, in Tăstine Forest, Făgețel Forestat Horezu (Păun 1965); east of Balș, Saru Forest (Negrean:1973).
73. ***Vicia tenuissima*** (M. Bieb.) Schinz & Thell.
D2: Tetoiu, 16 May 2008, 5 June 2017, 17 May 2019; Osica de Sus NE (Vlăduleni) - Greci S, Tabonu Mic, 44°16′07,83″N, 24°20′15,88″E, alt. cca 88 m, 13 July 2016, 9 May 2019, 5 June 2020.
Specimens exposed to excessive grazing.
74. ***Zingeria pisidica*** (Boiss.) Tutin
D1: Surrounding area of Balș (Păun 1969); Olari, Găvănești, Baldovinești, Pârșcoveni, Pietrișu (Beldie 1972);
D2: Balș, Saru Forest, North of the national highway, East of the forest road, 19 July 2016, 22 July 2018, 17 July 2019.
Specimens exposed to excessive grazing.
A number of 55 taxa were quoted by researchers, in some cases the data being older than 50 years. Out of these we have found a number of 49 taxa in other habitats in the river basin or the places they were mentioned at.
For the 6 taxa which we have not found we searched to identify the causes that generated this situation.
Botrychium matricarii folium has not been found, the possible cause being the modification of the area due to traffic, the falling rocks or the mechanical action of the domestic animals. Species such as *Dianthus trifasciculatus* subsp. *trifasciculatus, Trifolium michelianum, Vicia sparsiflora* are affected by the excessive grazing which begins as early as March.
The Olteț Meadow North of Balș is occupied by a ballast machine and large landfills of household waste.
The search for the species *Fimbristylis bisumbellata* in this area did not have positive results. The species *Silene gallinyi* may not have been found in the mentioned type of areadue to the changes caused by farming, grazing or road maintainance.
The 19 taxa with special sozological status which we are further mentioning, are proof of the value of the floristic heritage of the river basin: *Angelica arhangelica, Asplenium cuneifolium, Athamanta turbith* subsp. *hungarica, Cyperus serotinus, Elodea canadensis, Gladiolus imbricatus, Lactuca viminea, Lathyrus sphaericus, Leontopodium alpinum, Mercurialis annua, Sedum cepaea, Sedum rubens, Taeniantherum caput-medusae, Tanacetum macrophyllum, Thlaspi dacicum* subsp. *banaticum*, *Trapa natans, Trifolium squamosum, Vallisneria spiralis, Vicia tenuissima.*
We have analysed the sozological status in the three National Red Lists (Dihoru Gh. & Dihoru Alexandrina 1994; Oltean & al. 1994; Negrean 2001), The Red Book (Dihoru & Negrean 2009) andthe European Red List of Vascular Plants (Bitz M. & al. 2011). We have made a comparative table which shows the status of these rare, endangered and vulnerable taxa in the Basin of the Olteț river.

Table 1. Species from the National Red Lists and the IUCN List

Nr. crt.	Taxoni	Statut LR (Boşcaiu et al., 1994)	Statut LR (Oltean et al., 1994)	Statut LR (Dihoru et al., 1994)	Statut LR (Negrean, 2001)	IUCN Red List Category (Europe) 2011
1.	*Anacamptis pyramidalis* (L.) Rich.		V/R		V/R	LC
2.	*Angelica arhangelica* L.	V	V	V	V	
3.	*Asplenium cuneifolium* Viv.	R		R		
4.	*Asplenium lepidum* C. Presl subsp. *lepidum*	R	R	R		DD
5.	*Athamanta turbith* (L.) Brot. subsp. *hungarica* (Borbás) Tutin			R	R	
6.	*Beckmannia eruciformis* (L.) Horst				R	
7.	*Botrychium matricariifolium* (Retz.) A. Braun ex Koch	I	R	E	E	NT
8.	*Campanula grossekii* Heuffel		R	R	R	
9.	*Camphorosma monspeliaca* L. subsp. *monspeliaca*	I	V/R	E	V/R	
10.	*Centaurea affinis* Friv. subsp. *affinis*		R	R	R	
11.	*Centautrea atropurpurea* Waldst. & Kit.		R	R	R	
12.	*Cephalanthera rubra* (L.) L. C. M. Richard		R			
13.	*Cephalaria laevigata* (Waldst. et Kit.) Schrad.		R	R	R	
14.	*Cerastium banaticum* (Rochel) Heuffel		R	R	R	LC
15.	*Coydalis solida* (L.) Clairv. subsp. *slivenensis* (Velen.) Hayek		R			
16.	*Cyperus serotinus* Rottb.		R		R	
17.	*Delphinium fissum* Waldst. & Kit.		R		R	
18.	*Dianthus spiculifolius* Schur		R	V	R	
19.	*Dianthus trifasciculatus* subsp. *trifasciculatus* Kit.		R		R/CR	
20.	*Dictamnus albus* L.		V/R			
21.	*Elatine alsinastrum* L.	I	E/R	R (V)		
22.	*Elodea canadensis* Michx.	R				
23.	*Erythronium dens-canis* (incl. subsp. *niveum*)			R		
24.	*Fimbristylis bisumbellata* (Forssk.) Bubani	I	R	V	V	
25.	*Fritillaria montana* Hoppe		V/R	V	V	LC
26.	*Genista tinctoria* L. subsp. *oligosperma* (Andrae) Borza			nt		
27.	*Gladiolus imbricatus* L.			R		
28.	*Jovibarba heuffelii* (Schott) A. & D. Löve		R			DD
29.	*Lactuca viminea* (L.) J. & C. Presl s		V/R		R	
30.	*Lamium garganicum* L. subsp. *laevigatum* Arcang.		R	R	R	LC
31.	*Lathyrus sphaericus* Retz.	R		R	R	
32.	*Leontopodium alpinum* Cass.	E	V/R	V	V	
33.	*Leucojum vernum* L.			R	R	
34.	*Limodorum abortivum* (L.) Swartz	R	R	V		LC
35.	*Limonium tomentellum* (Boiss) O. Kuntze		V/R	R	V	LC
36.	*Linum uninerve* (Rochel) Jav. R		R	V	R	
37.	*Marsilea quadrifolia* L.	E	V	V	R	
38.	*Medicago polymorpha* L. (*M. hispida* Gaertn.)		R	V	R	NT
39.	*Mercurialis annua* L.			R		

40.	*Nasturtium officinale* (L.) R. Br.	R				
41.	*Neottia nidus-avis* (L.) L. C. M. Richard		R			LC
42.	*Oenanthe aquatica* (L.) Poir	R				
43.	*Orchis elegans* Heuff.		R		R	
44.	*Orchis ustulata* L.		R		R	LC
45.	*Peltaria alliacea* Jacq.		R	R		
46.	*Peucedanum rochelianum* Heuff.	R	R	R(V)	R	
47.	*Platanthera bifolia* (L.) Rich.		R			
48.	*Ranunculus constantinopolitanus* (DC.) D'Urv.		R	V		
49.	*Sedum cepaea* L.		R	R	R	
50.	*Sedum rubens* L.			V	R	
51.	*Seseli rigidum* Waldst. & Kit. s. l.		R			
52.	*Silene flavescens* Waldst. et Kit.		R	R	R	
53.	*Silene gallinyi* Rchb.	R				
54.	*Sorbus graeca* (Spach) Schauer		R			
55.	*Spiranthes spiralis* (L.) Chevall.		R		E	LC
56.	*Stipa eriocaulis* Borb.		K	R	R	
57.	*Symphyandra wanneri* (Rochel) Heuffel		R	R		
58.	*Taeniantherum caput-medusae* (L.) Nevski			R		
59.	*Tanacetum macrophyllum* (Waldst. & Kit.) Schultz Bip.		R		R	
60.	*Teucrium montanum* L.			R		
61	*Thlaspi alliaceum* L.			R		
62	*Thlaspi dacicum* Heuff. subsp. *banaticum* (Uechtr.) Jáv.		R	R		
63	*Thymus comosus* Heuff. ex Grisb. et Schenk		A nt	nt	R	
64	*Trapa natans* L.		V		R	NT
65	*Trifolium michelianum* Savi	R	R	V	R	
66	*Trifolium* scabrum L.	R	R	R	R	
67	*Trifolium squamosum* L.	R	R	E	R	
68	*Typha minima* Funck	R	R	R(V)	R	DD
69	*Typha shuttleworthii* Koch & Sonder		V/R	R	R	DD
70	*Vallisneria spiralis* L.	V	V/R			
71	*Veronica bachofenii* Heuff.		R	R	R	
72	*Vicia sparsiflora* Ten.	R	V/R	E		
73	*Vicia tenuissima* (M. Bieb.) Schinz & Thell.		K	R	R	
74	*Zingeria pisidica* (Boiss.) Tutin		R	V	R	

CONCLUSIONS

The results of our study consist in the identification of 74 rare, endangered and vulnerable taxa from the Basin of the Olteț river. We are signalling the existence in the area of a number of 68 taxa which prove a great adaptation and resistance to limiting factors.

The status of some species quoted in the bibliographic sources has changed over the years, consequently this presentation is made comparatively, using information from the specialized works and our field data.

The 6 taxa (*Botrychium matricariifolium, Dianthus trifasciculatus* subsp. *trifasciculatus, Trifolium michelianum, Vicia sparsiflora,* *Fimbristylis bisumbellata, Silene gallinyi*) on which we do not have field data have determined us to consider necessary to reduce limiting factors, a condition that could favour the recovery of their populations.

The floristic inventory of the river basin increases by the 19 taxa (*Angelica arhangelica, Asplenium cuneifolium, Athamanta turbith* subsp. *hungarica, Cyperus serotinus, Elodea canadensis, Gladiolus imbricatus, Lactuca viminea, Lathyrus sphaericus, Leontopodium alpinum, Mercurialis annua, Sedum cepaea, Sedum rubens, Taeniantherum caput-medusae, Tanacetum macrophyllum, Thlaspi dacicum* subsp. *banaticum*, *Trapa natans, Trifolium squamosum, Vallisneria spiralis, Vicia tenuissima*) which we have added.

Our study brings about contributions to the knowledge of the present-day situation of the species with special status in the basin area and of the variation of the factors directly or indirectly influencing these species.

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Abbreviations

Distributions:

D1 - data from specialized literature

D2 - personal data

Sozological categories:

V - vulnerable, R - rare, E - endangered, K - insufficiently known, CR - critically endangered with extinction, NT - nearly threatened, LC - least concern, DD - insufficient data.

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STUDY ON THE USE OF LAND SCANNING IN SOIL EROSION INVENTORY WORKS FOR SUSTAINABLE AGRICULTURE IN AGRITOURISTIC FARMS

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Abstract

The paper presents a modern, precise and fast method of lifting land areas affected by deep soil erosion, from an agritourism farm, by using land scanning technology. The method allows to take as much information as possible about the object to be scanned, and based on the records made is obtained the so-called "point cloud", which is a collection of points, defined as position by the coordinates X, Y, Z in a common reference system, which reveals to the observer information on the shape, position, and spatial distribution of an object or group of objects. From the point cloud with the help of the specialized program, based on the coordinates you can easily and accurately calculate the size and shape of land areas affected by erosion, thus creating a complex database of land that may be affected by this phenomenon. Also, from the database obtained, thematic maps can be created that can be used in other works on agricultural farms, in order to practice a modern agriculture such as precision agriculture, which would effectively contribute to soil conservation and protection, so to practicing sustainable agriculture.

Key words*: coordinates, inventory, land scan, point cloud, sustainable agriculture.*

INTRODUCTION

It is known from the literature that the soil is subjected to a series of degradation processes. Some of these processes are closely related to agriculture: water erosion, wind erosion and agricultural soil preparation works; compaction; decrease in the amount of organic carbon in the soil and soil biodiversity (Răduţoiu et al., 2013, 2018). In the factsheets, special attention is paid at European level to water erosion and compaction, the reduction of soil organic matter, as well as salinisation and sodisation. Soil degradation processes involve the need to protect, maintain and improve soil quality (Burghilă et al., 2016). Soil properties, as well as soil formation factors such as climate, land use or soil management determine the degree of soil degradation (Călina & Călina, 2019).
Cross-compliance is part of the common agricultural policy, with many implications for soil conservation and refers to the requirement to keep land in good agricultural and environmental condition (BCAM). This applies to direct income support payments, as well as to most environmental payments applied in rural development. The BCAM requirement refers to a number of standards related to protection against soil erosion, conservation of organic matter and soil structure, avoidance of habitat damage and water management. Reducing the area of barren soil and terracing the land for consolidation directly contributes to preventing soil erosion (Iagăru et al., 2016).
Given the above and the fact that erosion processes tend to have a negative impact on ecosystems and in land use decision-making, the team of researchers set out to conduct this study, which will assess the erosion process using old and new data, state-of-the-art topo-geodetic methods and data, such as land scanning that have been introduced into a complex but easy-to-use mathematical system to calculate affected agricultural land areas.
This new laser scanning measurement method involves sampling or scanning a field surface using laser technology. It analyzes a real world environment or an object in order to collect information from its surface and possibly from its appearance (eg color). The collected information can then be used to construct two-dimensional representations or three-dimensional models, usable in a wide variety of applications (Pop et al., 2019).
Through this study we want to highlight the large areas of land that are removed from the

agricultural circuit by soil erosion, due to irrational exploitation by practicing a super-intensified agriculture. From the previous studies we found that this phenomenon is also manifested in agritourism farms, because so far and here the aim has been to obtain high yields at the expense of sustainable agriculture (Adamov et al., 2020) with a crop technology environmentally friendly, which will preserve the soil and even improve some agroproductive properties (Călina & Călina, 2019).

MATERIALS AND METHODS

In solving the approached topic, the technology of Terrestrial Laser Scanning (S.L.T.) was used, which tends to "revolutionize" the measurement techniques in Topography and Engineering Topography (Calinovici & Călina, 2008). The use of this technology allowed the rapid and accurate determination of the surface of a formation that appeared on an agricultural surface, following deep erosions. As with any measurement process encountered in the technique of topographic measurements and in this case the planning or preliminary design is an extremely important step, decisive in obtaining the results, respectively the information needed to describe the object to be scanned (Kolbe et al., 2011).

The design stage is indispensable for the measurement process and due to the fact that in this stage the shape and size of the object are balanced, its position in the environment and last but not least, the requirements of the beneficiary regarding the accuracy to be obtained in the end at the object reprezentation (Li et al., 2009).

After a rigorous design, it was concluded that the following phases must be completed: - definition of the area to be scanned and preliminary investigations; - determining the resolution and accuracy required for the points that make up the point clouds, depending on the beneficiary's sorting; - selecting the type of laser scanner to be used, depending on the specifics of the work we intend to perform; - designing the optimal positions of the station points for scanning, starting from the premises of providing the necessary coverages to ensure the accuracy and the need to scan the entire object; - the choice of the type of targets to be used in the georeferencing registration operations and of the positions in which they will be located, in such a way as to ensure the premises of an optimal geometric configuration for georeferencing; - estimating the volume of data that will be acquired during the scanning process (Remondino et al., 2010).

In the case of the relief form studied, it was considered that the use of terrestrial laser scanning is the most optimal due to the following reasons: - very complex surface structure; - presentation of the final 3D product; - measuring the surface instead of measuring the individual points; - the recorded data can be used by multidisciplinary teams, for different purposes; - archiving data without having a priori knowledge, regarding their future use (Mihai et al., 2015).

Stage I. *Analysis of the area to be scanned*

Retrieving as much information as possible about the object to be scanned can provide information about the complexity and time required for such an operation. Field information, reports, existing maps, photographs or video images of the location of the object to be scanned can greatly help determine the possible risks when scanning the object (Paunescu et al., 2020). However, it is also very important to analyze the area, the surroundings of the respective location. Possible obstructions determine the choice of station point positions. Possible time constraints are also decisive in choosing the methods and timing of the scan. Indirectly, the positions of the station points determine the minimum and maximum distances that the scanner could record.

Stage II. *Determining the optimal positions for scanning*

Once the site documentation has been analyzed and laser scanning has been chosen as the most effective recording technique, the scanning positions and those in which the aiming targets will be placed must be designed (planned). The choice of the optimal positions of the station points must guarantee maximum coverage and accuracy but also a minimum number of station points.

When designing the optimal positions of the station points, the following basic rules must be observed: - the positions are chosen that offer a good (wide) coverage of the scanning area, without obstructions on the line of sight, which could produce the shading effect; - it is checked whether the distance limits are met, in order to increase the accuracy; - decrease in the number of scanning stations; - choosing scanning positions in places that ensure comfortable measuring conditions, free from vibrations and the influence of wind; - ensuring a convenient height of the device and ensuring visibility to natural and artificial targets.

Of particular importance in the measurement design phase, in addition to the optimal scanning positions, is the choice of target types, their position and / or geometric configuration. An important remark regarding the use of aiming targets is that they, placed in position, must have a large opening in all three directions of the axes (X, Y, Z).

Stage III. *Data management*

Given the very large amount of data that is collected during the scanning process, it is very important to scan the data so that it is ensured throughout a working day. The positioning accuracy of the image points, defined in the reference system of the station by spatial coordinates X, Y, Z is accredited at ± 6mm / 50m, considering the fact that at this distance the laser spot maintains its point diameter of 6 mm. Currently, there is no standard procedure for scheduling the terrestrial laser scanning session. Based on the records made, the so-called "point cloud" is obtained, which is a collection of points, defined as position by the coordinates X, Y, Z in a common reference system, which reveals to the observer information on the shape, position, and the spatial distribution of an object or group of objects (Călina et al., 2020). It may contain additional information, such as intensity. It can be concluded that the point cloud contains two types of information: - metrics, which describe the geometry of the object and its spatial relationships with the environment; - thematic, which are used to describe the surface properties of the scanned objects and to estimate the confidence given to the acquired data.

RESULTS AND DISCUSSIONS

The actual fieldwork began with the preparation phase of the measurements which includes the decision regarding the recording technique to be used. These techniques fall into three categories: free station, using the 3D intersection of visas to scanned targets, station at known coordinate points, and recording using constraint points from different point clouds.

The first operation performed in the field involved the placement of the scanner in the station, which generally follows the same procedures as in the case of any topogeodesic device: - placement of the tripod at a convenient height; - placing the scanner on a tripod; - centering, if necessary, using the optical centering device; - leveling the instrument.

Before setting up the scanner, it must be connected to the computer (laptop) that receives and stores all the data from the scanner and conducts virtually all scanning operations. Turns on the scanner and waits for it to acclimatize. The software component on the laptop starts. The connection is established between the computer and the scanner and between the controller and the scanner (using the IP address, USB cable, wireless connection, etc.).

After that, scan parameters were set by defining the 3D sections to be scanned using the scanner control options in the software component. This procedure involves taking a picture of the entire space (scan scene), which then allows you to select the scan area.

Choosing the right resolution is the key issue in carrying out a terrestrial laser scanning project. The resolution is defined as the distance between the points to be measured later, which ultimately determines the density of points in the point cloud. It should be noted that by choosing a high resolution it is necessary to scan more points and - consequently - increase the scanning time. Subsequently, the primary filtering was performed to ensure that the collected data falls within the accuracy limits of distance measurement, characteristic of the scanner. The other points, considered not to fall within the limits of accuracy, will be eliminated, due to the low values of the reflectance. After completing these steps, the scanning operation is conducted entirely by the specialized software of the instrument, without the need for operator intervention. Scanning in progress can be viewed on the computer screen. After the section is fully scanned, the data is saved in files created for that job.

Data acquisition, once the scanning area and the corresponding resolution have been established, can start the scanning operation. This process, as mentioned before, takes place completely automatically, led by the software component. After starting the scanning process, the scanner automatically goes to the starting point, starts purchasing points and - via the laptop - stores the data in the internal memory. Depending on the resolution chosen and the target area, the scanning process can take from a few minutes to hundreds of minutes. During this time, observations, descriptions and sketches of the area to be scanned can be made, which were not performed when designing the measurements.

Data collection and processing (3D point cloud) - modeling and visualization

Following the georeferencing recording operations, the resulting common point cloud enters the modeling process. The final product of this operation is represented by the 3D model of the scanned object (Călina et al., 2018). Point cloud processing involves the transformation of the raw point cloud into a final product, according to design requirements. These final products can be presented in a multitude of forms consisting of: cloud of points cleaned of noise, standard 2D representations (plans, elevations, profiles), complete 3D models suitable for various purposes.

In general, point cloud processing can be divided into two categories:-extracting the final products directly from the point clouds, without further processing;-first creating the 3D model of the surface from the point clouds and extracting the final products from this model (Vosselman & Maas, 2010).

The choice of one of the two methods depends largely on the final products required. For example, if a limited number of profiles is required, it is preferable that they be extracted directly from the point cloud. However, if a larger number of profiles is required (over 50, for example), the second method is more efficient, as there are options for automatically generating multiple profiles from a processed model. In addition, a surface pattern contains more information than a simple raw point cloud.

Representation of the point cloud

The result of the scan is an impressive number of points in space, each being characterized as a position by the X, Y, Z coordinates and - usually - by the value of the reflectance of the laser beam. A number of scanning systems even provide color information in the form of RGB values (Red, Green, Blue). The point cloud can be represented by projecting all these points on the screen (display), but this creates a first impression of chaotic image and the user finds it very difficult to recognize the structures or shapes in the point cloud. If each point is additionally characterized by reflective or color, the entire structure of the point cloud becomes virtually incomprehensible (Figure 1). Since the vast majority of measurement systems scan object space (the so-called scan scene) in columns and rows, one way to represent the point cloud could be - in the simplest possible way - as a projective view or as a map in depthmap (Sala et al., 2020).

Due to the fact that this type of representation also incorporates a lot of information about the neighboring environment / objects, it is useful to use point cloud processing algorithms that lead to its so-called organization. By using the complex triangulation algorithm (meshes), the neighboring points can be connected to the surfaces of the shape. It provides a closer representation of reality, because the structures of the surface or mesh are not transparent, therefore, the points behind others cannot be seen. By calculating the normal local directions of the surface, the artificial shadow can be used to highlight the details of the surface (Figure 1).

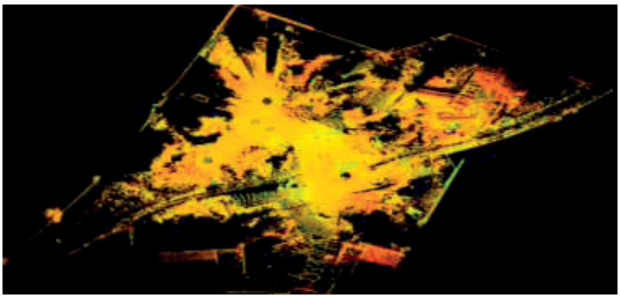

Figure 1. The point cloud of the surface

Improving data

The first step in the geometric modeling process using a polygonal network is to remove noise-containing data from the point cloud. If the noises were generated by the influence of the wind, poor quality reflection on the surface, etc., the polygonal network will contain triangles that connect the points affected by the noises with the correct ones. This results in the appearance of irregularities (peaks) in the 3D geometric model of the surface. It is very important to eliminate these point noises in the first steps of processing. Currently, an operator can easily identify portions of the scanned area that are not needed in the final product. Therefore, it is recommended that the operator make a first analysis of the point cloud and manually remove all unnecessary points from the data set (Chiabrando et al., 2009).

The automatic algorithm for eliminating points that are affected by noise is based on two principles: the first principle is based on the fact that points that have few or no other points in the immediate vicinity are considered erroneous (useless) (Rosca et al., 2020). These algorithms try to match (adjust) plans locally, on the points in the point cloud. If the center point is very far from the right plane, it is moved to the plane so as to ensure greater consistency of neighboring points. There are other noise filters, some specialized depending on the scanning system or others that eliminate systematic errors. It is of course necessary to consider the precautions when eliminating the points affected by noise, as the characteristics of the details may be lost in the event of over-uniformity of data or too many points may be eliminated.

Opening the point cloud

After the actual scan, the files are saved in the control unit (in our case, laptop) so that they can be opened and modified later.

To start working with scanned data in software such as Leica Cyclone, make a connection between the scan database containing the scanned information and Cyclone, - open Cyclone; - right-click on the (unshared) icon in the server list and select **Databases**. In the dialog box, click on the **Add** button, then on the **Database File name** and search to scan the database named: Ravine - Tutorial Inside –start - Reduced. imp or– Ravine Tutorial Inside - start.imp (full dataset). Select the "* .imp" file and press the **Open** button. Back in the previous dialog, just press the **OK** button and then click **Close** in the dialog. This takes us back to the Cyclone Navigator (Figure 2).

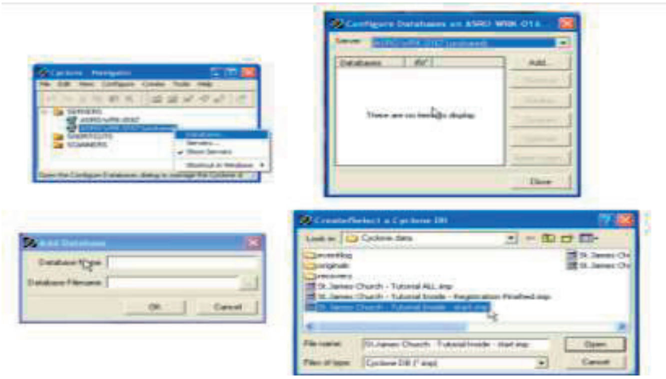

Figure 2. Uploading a database file to Cyclone

The Scans file contains each scan separately as raw information. Here, even scans made from the same position, but with different settings, are separated. The two most important files are: ControlSpace and ModelSpace. These files contain the data you are working with. Controlspace is the space that actually contains the data from the raw scan. Controlspace is used in all calculations, for example in recording. However, a security measure must be taken. A user cannot directly modify ControlSpace. It must modify ModelSpace and then copy the changes to ControlSpace. Modelspace is actually a photo of Controlspace. This gives 'View' on the control space at a certain time. It is impossible to have multiple Modelspaces performed at different times from a single Controlspace, (www.3driskmapping.eu).

Referencing scans from different angles

In most cases, scans are performed from multiple angles to obtain a complete scan of the object. Referencing consists of "gluing" scans from various angles to obtain the complete point cloud of the object, so that it can be used further (Figures 1 and 2). To combine (assemble) different scanner positions, the orientation and positions of the coordinate system relative to a local / global coordinate system in the area must be known.

In principle, each scan operation generates a cloud of points whose positions are characterized by coordinates (X, Z, Y) in an internal system of the scanner (Doneus et al., 2005). The scan was performed from several positions, it was necessary to represent it in a unique coordinate system (local system). Aiming targets are ideal accessories for overlapping images taken from different stations and quality assurance.

They are used for accurate georeferencing of scanning at checkpoints. Using the Create - Registration command, we will be able to reference the 2 scans.

We open the newly created referencing and add the 2 scans to the referencing. After adding the 2 scans to the referencing, we need to define a minimum of 3 common points of the 2 scans (targets) (Figures 3 and 4).

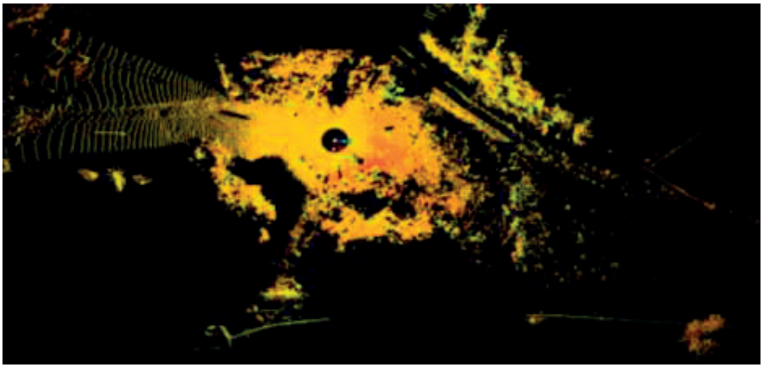

Figure 3. Scanning view from station 1

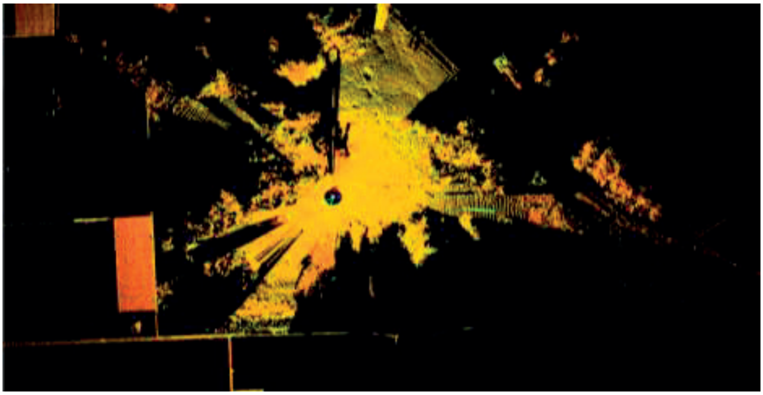

Figure 4. Scanning view from station 2

The common points of the scans must meet an important condition: they must be well defined in the point cloud, so that they can be easily identified in both scans (Figure 5). Generally, the corners or tips of the object are chosen. After choosing the common points and checking the object through the preview option, we can compile the two point clouds into one, to be used more easily.

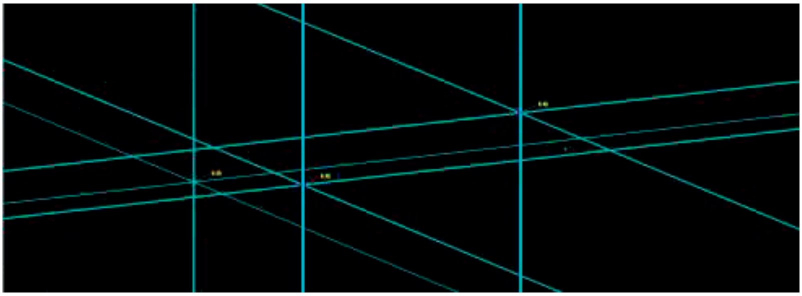

Figure 5. Viewing targets from 2 stations

When registering / georeferencing, there must be certainty that the error values of the whole process are at least equal to the required geometric accuracy of the final products. When targets are scanned at a very sharp angle, automatic target recognition features should not be used because they generate poor quality results.
The distribution of control points must correspond to an optimal geometry, their location must be as uniform as possible in the scanning space, otherwise situations of incompatibility may occur during the transformation.
The quality of the point clouds can influence the final result of the recording, for this reason it is necessary to filter through a preprocessing of the data sets, of the noise and of the gross errors. Finally, the 2 scans were 'glued', obtaining the overall image of the relief form as in Figures 6 and 7.

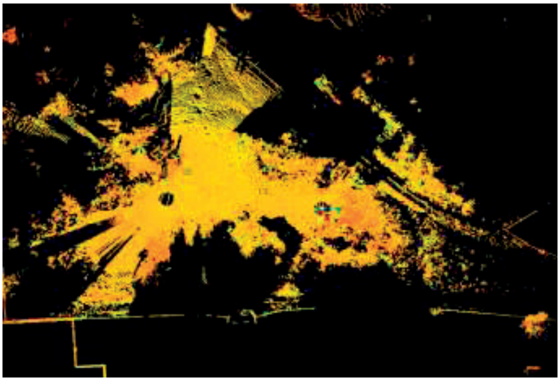

Figure 6. Recording of 2 scans: the point cloud in station 1, referenced with the one in station 2

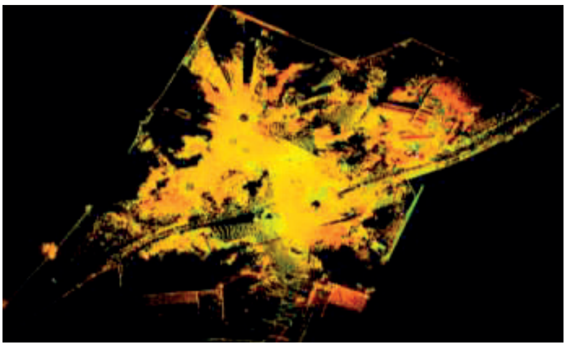

Figure 7. The point cloud of the relief form

Calculation of point coordinates in the local system

We worked in the local reference system, this explains the negative coordinates of the points. It was decided to work in this system because the purpose of the work was to determine the surface of the entire form of relief, and this is done equally regardless of the chosen reference system. It should be specified that due to the very large volume of points it was chosen to present only a small part of them, in facsimile (Tables 1 and 2).

Table 1. Target coordinates in relation to the local reference system X, Y, Z = 0.000 m

Target	Coordinates (m)		
	X	Y	Z
T1	-55.562	-30.563	-1.803
T2	-50.615	-14.576	-1.579
T3	-550.291	-31.294	-1.599
T4	-0.0168	-25.997	-1.087
T5	-0.144	-26.800	-0.680
T6	-1.575	-28.350	-0.980

Table 2. Scan point coordinates

Point no.	Point coordinates (m)			Point no.	Point coordinates (m)			Point no.	Point coordinates (m)		
	X	Y	Z		X	Y	Z		X	Y	Z
1	-60.0518	-46.0209	-2.5567	83	-60.1324	-46.0257	-2.5631	165	-60.030	-46.1105	-2.4730
2	-60.0721	-46.0165	-2.5596	84	-60.1395	-46.0352	-2.5656	166	-60.0410	-46.1086	-2.4705
3	-60.0956	-46.0115	-2.5631	85	-60.1027	-46.0537	-2.5624	167	-60.0453	-46.0971	-2.4736
4	-60.1063	-46.0092	-2.5598	86	-60.1165	-46.0509	-2.5606	168	-60.0559	-46.1058	-2.4703
5	-60.1179	-45.9957	-2.5667	87	-60.1324	-46.0477	-2.5610	169	-60.0340	-46.1205	-2.4738
6	-60.1240	-46.0054	-2.5610	88	-60.1453	-46.0451	-2.5596	170	-60.0500	-46.1176	-2.4665
7	-60.1629	-45.9970	-2.5642	89	-60.1515	-46.0328	-2.5639	171	-60.0640	-46.1150	-2.4647
8	-60.1601	-45.9754	-2.5651	90	-60.1448	-46.0231	-2.5616	172	-60.0589	-46.0945	-2.4727
....	....	....	....	....	....	....	....	....	....	....	....
....	....	....	....	....	....	....	....	....	....	....	....
35	-60.0483	-46.1180	-2.5455	117	-60.1371	-46.0466	-2.5056	199	-60.1346	-46.1019	-2.4749
36	-60.0867	-46.1109	-2.5577	118	-60.1335	-46.0584	-2.5027	200	-60.1847	-46.1259	-2.4725
37	-60.0670	-46.0931	-2.5571	119	-60.1776	-46.0273	-2.51059	201	-60.1799	-46.0935	-2.4730
38	-60.0410	-46.1300	-2.5495	120	-60.1537	-46.0322	-2.5068	202	-60.1793	-46.0825	-2.4736
39	-60.0411	-46.1405	-2.5399	121	-60.2021	-46.0335	-2.4963	203	-60.1792	-46.0826	-2.4646
40	-60.0542	-46.1276	-2.5476	122	-60.1496	-46.0662	-2.5018	204	-60.1899	-46.0917	-2.4607
....	....	....	....	....	....	....	....	....	....	....	....
....	....	....	....	....	....	....	....	....	....	....	....
75	-60.0794	-46.0475	-2.5600	157	-60.0186	-46.1128	-2.4755	239	-60.5266	-45.8072	-2.5417
76	-60.0980	-46.0328	-2.5623	158	-60.0284	-46.1321	-2.4701	240	-60.5407	-45.8037	-2.5391
77	-60.0974	-46.0438	-2.5607	159	-60.0186	-46.1339	-2.4736	241	-60.5832	-45.8061	-2.5567
78	-60.0776	-46.0262	-2.5527	160	-60.0185	-46.1444	-2.4727	242	-60.5955	-45.7904	-2.5545
79	-60.1203	-46.0392	-2.5636	161	-60.0310	-46.1422	-2.4704	243	-60.5998	-45.7766	-2.5487
80	-60.1161	-46.0181	-2.5637	162	-60.0461	-46.1502	-2.4696	244	-60.5946	-45.8033	-2.5529
81	-60.1182	-46.0286	-2.5637	163	-60.0386	-46.1196	-2.4686	245	-60.6141	-45.7858	-2.5538
82	-60.1186	-46.0176	-2.5572	164	-60.0336	-46.0993	-2.4754	246	-60.6156	-45.7727	-2.5465
								247	-60.6391	-45.7927	-2.5634

Area calculation

Due to the fact that the relief form is made up of several valleys, in order to arrive at the total calculation of the area, it was necessary to first calculate the area of each valley separately. In order to achieve this, each valley was divided into elementary geometric shapes (cylinders). Thus, the elementary cylinders and the base areas of these valleys resulted, the data being processed in the Cyclone program (Barazzetti et al., 2010). Having the areas of the bases of the elementary cylinders in each valley, the surface area of each valley was calculated, and finally that of the negative relief form, which appeared on the agricultural land of the agrotourism farm, on whose territory this study was conducted (Table 3, Figure 8).

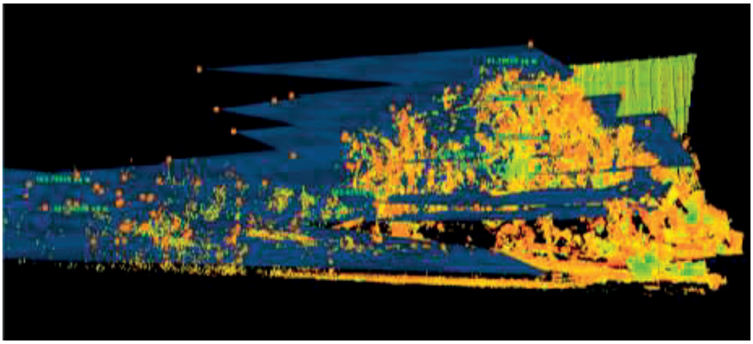

Figure 8. Values elementary cylinder base areas (image superimposed with the point cloud)

As can be seen from Table 3, the area of agricultural land affected by erosion is very significant, as it has over 1.13 ha. Therefore, it is very important that through specific topo-geodetic methods we permanently monitor the situation of lands degraded by surface and deep erosion, in order to be able to intervene in time to stop or fully combat it.

Table 3. Calculation of partial and total areas of the negative relief form

Plan no.	Surface 1 (m^2)	Surface 2 (m^2)	Surface 3 (m^2)	Surface 4 (m^2)	Surface 5 (m^2)	Surface 6 (m^2)	Surface 7 (m^2)	**TOTAL SURFACE**
1	103.795	3.5	99.86	174.04	11.69	3.86	8.04	
2	179.087	17.17	135.81	199.49	17.53	21.33	119.63	
3	241.092	41.99	6.79	237.46	128.48	104.51	134.38	
4	762.625	45.66	17.02	341.22	246.23	153.91	229.32	
5	313.227	49.3	27.03	516.08	409.9	198.18	300.17	
6	236.31	296.62	53.87	587.11			494.8	
7	152.63	298.37					566.34	
8	117.026	12.044						
9	75.711	569.84						
10	91.299	635.93						
11		674.93						
12		763.77						
Total	2.272,802	3.409,124	340,38	2.055,4	813,83	481,79	1.852,68	**11.226,006**

CONCLUSIONS

Combating soil erosion is very important in the practice of sustainable agriculture because it removes large areas of land from the agricultural circuit and removes a large amount of fertile soil and with it significant amounts of organic matter and thus carbon. In order to be able to take timely measures to stop or combat this extremely negative phenomenon, agricultural landowners must constantly carry out an inventory study of areas prone to such phenomena.

In order to meet the needs of farmers who also own land affected by this phenomenon, we have developed a very precise, fast and suggestive method for determining the size and shape of land areas affected by erosion. The method involves the use of terrestrial or 3D scanning that provides accurate and complete data about the scanned objects, allowing the visualization of real field conditions and design according to these conditions, using specialized software. The use of laser scanning systems requires minimal intervention on the part of the operator, focusing on the instrument, setting and setting the scanning parameters.

The data recording time is considerably reduced, which is also found in the costs of the survey work, and the remote data recording contributes to increasing the efficiency and safety of topographic surveys. The high density of points ensures the complexity of topographic surveys and helps to interpret very correctly the scanned objects even if they are small. Operation of such a system is easy and very accurate because it has processing software, both for registration, but also for analysis, interpretation and evaluation, being usually modular without requiring special resources.

The information gathered from the field by terrestrial laser scanning is very complex and diverse and can be accessed from a computer or laptop, thus allowing a greater freedom of view than in reality. Based on them, a series of optimizations of the point cloud processing process can be made, as well as of the 3D object, which can lead to the improvement of the transformation efficiency from point cloud to 3D object and obtaining small files, with the possibility automatic determination of the areas of the scanned areas and finally the creation of thematic digital maps, which can be used for various purposes in sustainable agriculture.

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BIOCHEMICAL CHANGES, INDUCED BY LED LIGHT, IN TOMATO PLANTS, GROWN IN THE INTEGRATED MANAGEMENT SYSTEM (SMI) OF AGROECOSYSTEM RESISTANCE

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Abstract

In this research, biochemical analyses were monitored by determination at the level of tomato plants and fruits, within a protected growing environment (vegetation house), before and after the application of light provided by LEDs in red, blue and monochrome white, in addition to natural light, during the summer (June, 3rd decade -August 3rd decade, 2019). In parallel with the analysis of tomato plants, the substrates from the pots corresponding to the plants were analyzed. Values obtained for tomato plants of the analyzed varieties (Solanum lycopersicum L. cv. 'Sonia de Buzau', cv. 'Coralina' and cv. 'Hera') after 2 months of cultivation, regarding the content in mineral elements of non-metabolized forms (nitrates N-NO_3, ppm (phosphates P-PO_4, ppm, and K + ppm), showed a good supply of them, fact that determined their normal development in the protected cultivation area. The obtained biochemical results were situated within the limits accepted by the scientific literature, but were differentiated in correlation with the tomato genotype, LED light and daily exposure time, compared to control plants (unexposed to additional LED light). The fruits formed at the level of the first fruiting stage, toward the end of the experimental period, were analyzed together with the mineral elements and their contents in acidity %, soluble carbohydrates (%) and vitamin C (mg/100 g fresh product). The response to the applied treatment ensured uniform fruiting yield, after exposure to the treatment for 45 minutes/day with monochrome LEDs for all three colors, in the case of 'Coralina' tomato assortment, compared to the other 2 study varieties.

Key words: *monochrome LEDs, biochemical changes, tomatoes protected culture.*

INTRODUCTION

The main aim of an integrated management system in agriculture is to develop nonpolluting agricultural management practices, that are reduced the danger of infestation with various pathogens of agricultural crops. This can be achieved with methods that raise soil fertility, increase and conserve biodiversity, reducing the vulnerability of plants to abiotic and biotic stress factors andreducing the danger of polluting the environment with pesticides, by decreasing the number of chemical phytosanitary treatments and using ecological methods instead (STANDARD ICS: 65.020.20, AGRO 2–1).
Supplementation of natural light in protected environments is essential, especially in the areas located in the northern hemisphere, where light intensity and day length are reduced, particularly during autumn, winter and spring (Palmitessa el al., 2020).
A major limitation for tomato-seedling growth is represented by their high light demands, especially in temperate areas, where the days are short and cloudy during the time the seedling production starts. Supplementation of natural light with red and blue LED light can promote better growth.
Results obtained by Gomez and Michel (2015), demonstrated that supplementing the natural light with a combination of red and blue light induced better growth in tomato seedlings, by increasing the hypocotyl diameter, epicotyl length, dry mass, and the number and size of leaves.
To prevent light deficit in protected growing environments, different illumination technologies can be used to increase plant productivity: fluorescent lamps, high-pressure sodium lamps (HPS), metal halide lamps (MH) or light emitting diodes (LED) (Paucek et al., 2020). Although in the past, HPS lamps were the most used source of artificial lightning in

greenhouses, they present numerous inconveniences: low energy efficiency, variable spectral distribution and release of a large amount of radiant heat (Bantis et al., 2018). Recent studies have focused on using light emitting diodes (LED) to increase the light level in protected culture environments. They present multiple advantages, such as low energy usage, better operating life and they do not release radiant heat (Lu et al., 2012). Even though high-pressure sodium lamps (HPS) are an excellent light source, they are not the most efficient in obtaining high yield. LED lamps can be adjusted to emit only certain wavelengths, to maximize plant yield (Deram et al., 2014).

Paucek et al. (2020), demonstrated that supplementing natural light with LED in 'Siranzo' tomatoes grown in hydroponic system in the Mediterranean climate, accelerated fruit maturation with one week during the spring and two weeks during summer. Also, supplementing natural light with LED light increased cumulated productivity with 16%, compared to control plants. Palmitessa et al. (2020), obtained an increase of 21.7% in production by using LED during winter, on 3 cultivars of tomatoes (Juanita, Sorentyno and Salarino) cultivated in greenhouses in southern Italy.

The idea of a planned vegetable production that uses a factory has been suggested for years as a technology to extend horticulture under greenhouse conditions. The enclosed environment of the greenhouse has its own unique requirements, compared to cultivating crops in the field. Pests, diseases and the extreme values of heat and humidity must be controlled, and irrigation is necessary to supply water permanently (Sugimura et al., 2015).

Worldwide and in Romania, commercial seedling producers are facing inconsistencies in seedling morphology, possibly because of the change in light quality in the afternoon (Delian, 2008). In this situation, using supplementary sources of light and heat is necessary, especially during winter for vegetable production (Massa et al., 2008; Olle & Virsile, 2013; Sugimura el al., 2015). Because greenhouse temperature and humidity must be constantly monitored to ensure optimal growing conditions, a wireless network of sensors may be used for remote data collection. Information is transmitted to a control location and it is used to control the heating system, cooling and irrigation and to inervine in real time and at optimal values (Delian, 2008; Smith, 1982).

Light emitting diodes present a narrow light spectrum, which allows them to focalize a specific wavelength necessary for plant growth. Even more, it is possible to completely reduce infrared radiation. This means that the light source can be positioned at a short distance from the plants. Moreover, the absence of infrared radiation represents an advantage regarding the cost of temperature management in an enclosed growing system (Massa el al., 2008; Olle & Virsile, 2013; Sugimura el al., 2015).

Light influences the metabolic processes by its duration and intensity, but also by its spectral composition (Smith H., 1982; Delian, 2008; Dănăilă-Guidea & Delian, 2020a). Dănăila-Guidea & Delian, (2020a), in a review article, mentioned the scientific results of numerous researchers, results that showed the general effects that blue light has on the functioning of plants during the vegetation period, but also the influences on harvested fruits and their storage capacity. The use of blue light emitted by LED diodes was proved to be a good technology to modify the light spectrum, to regulate the growth process of obtaining high horticultural crops with high nutritional and nutraceutical content (Ballester et al., 2018; Jensen et al., 2018; Manivannan et al., 2017; Burescu et al., 2015; Kopsell et al., 2013; Brazaitytė et al., 2010; Morrow, 2008).

Red light is the most efficient from the light spectrum to stimulate de process of photosynthesis. According to Goto E. (Gotto, 2003), the growing and development plant processes are obstructed in monochromatic red light conditions. Growing plants in monochromatic red light reduces chloroplast number, thin cell walls, mesophyll tissues with abnormal structures, aspects that can be prevented by supplementing the light spectrum with blue light (Miao et al., 2019).

In this paper, the results obtained on tomato plants regarding the applied treatment with monochrome light emitted by LEDs were analysed, from the experimental scheme developed.

By using light emitting diodes (LEDs) characterized by a narrow spectrum of light, which allows them to focus light with the wavelength necessary for plant growth, the aim was to develop nonpolluting management technology for agro-systems, in a protected area, for growing vegetables.

MATERIALS AND METHODS

Biological material and experimental scheme

Experiments occurred at the Vegetation House within UASVM Bucharest during June – August 2019. The experimental scheme was composed of three factors arranged using the block design, in 3 repetitions: Factor **A** was represented by the tomato cultivar (Sonia de Buzau, Hera, Carolina); Factor **B** was represented by the monochromatic light emitted by LEDs (red, blue and white) and Factor **C** was represented by the illumination period in three times (15 minutes/day, 30 min/day, and 45 minutes/day) supplemented daily to natural light, compared to the control group which was not exposed with supplementary LED-produced light;

Figure 1 illustrates the devices represented by LED diodes panels (Factor C), during the daily application of monochromatic light treatment on the selected tomato seedlings (Factor A), cultivated in the Vegetation House of UASVM Bucharest.

The biological material used for this study was represented by seedlings of the three tomato cultivars registered at S.C.D.L. Buzau, two of them with cherry type fruit (Sonia de Buzau and Carolina) [General Catalog Vegetable Varieties And Hybrids SCDL BUZĂU (1957 – 2015)], and the third one, Hera, with kapia pepper type fruit (https://agrointel.ro/80100/hera-soiul-de-rosii-cu-forma-de-ardei-capia-creat-la-statiunea-de-la-buzau). Details about the morphological characteristics and productivity of the biological material (Sonia de Buzau, Coralina and Hera) and the characteristics of the experimental devices were described and detailed by this research team, in two previous publications published in 2020. (Dănăilă-Guidea et al., 2020b; Dănăilă-Guidea et al., 2020c).

During the growing period of tomato plants, two fertilizers were used: Florovit (NPK 7-5-6) and organic fertilizer Lumbreco based on biohummus, that were administered by alternative leaf treatments, to supply the plants with the necessary nutrients and to stimulate the growth of the seedlings. Also, to increase the resistance of the plants to specific pests (whitefly, the common red mite and thrips) and to counter the attack of *Operophtera brumata,* that was identified in the growing environment, two nontoxic and ecological products were used: Rock Effect (Natura products, AGRO CS) and PIPER-CIP (AMIA International) insecticide.

The results obtained from the biochemical determination performed at the level of young plants from the Romanian tomato varieties, before and after the additional treatment of short-term exposure to monochrome LED light, administered daily, were analyzed using statistical correlations.

Figure 1. Location of the experiments regarding the supplementary illumination with monochromatic LED devices on Sonia de Buzau, Coralina and Hera tomatoes in the Vegetation House of UASVM Bucharest (original photo – June 2019)

Biochemical analysis was performed at the plant level (nitrates N-NO3, ppm; phosphates P-PO4, ppm, and K+, ppm), at fruit level (nitrates N-NO3, ppm; phosphates P-PO4, ppm; K+ ppm; soluble carbohydrates % and vitamic C content mg/100 g fresh fruit) and at substrate level (soluble salts content - N, P, K, expressed in % and ppm).

Methods used for biochemical parameters determination

Before and after the application of supplementary monochromatic red, blue and white LED light during the summer (June, 3^{rd} decade and August, 3^{rd} decade, 2019), plants were harvested and analyzed regarding the supply of nutritive elements necessary for their growth and development.

Analyses on tomatoes in their juvenile stage (seedlings) and mature stage (during flowering and fruiting), were done accordingly to the INCDAP Bucharest methodology (ICPA Bucharest, 1980) and included the following: N-NO3- determination, in CH3COOH 2% extraction and colorimetric analysis with AFDS (Griess Analysis – STAS 3048–77), P-PO4 determination, in CH3COOH 2% extraction and colorimetric analysis with Duval reagent, as well as K+ determination in CH3COOH 2% extraction 1:2 and flame fotometric analysis.

Three plants of each variety and experimental variant were used, and the results were compared with control plants grown only in natural light in the same protected area represented by the vegetation house of UASVM Bucharest.

Fruit analysis included acidity (%) determination, extracted în distilled water, 1:2,5 and titrimetric determination with NaOH 0,1 N, soluble carbohydrates (%) determination using the Abbe refractometric method; volumetric determination of vitamin C (mg/100 g fresh fruit), using 2,6-diclorohenoldiphenol titration.

Simultaneously, plant nutrients and agrochemical indices of the growing substrate were analyzed for each experimental variant. Analyses covered pH în aqueous extract 1:2,5 and potentiometric analysis (SR ISO 10523–2012); soluble salt content în water extraction 1:5 and conductometric analysis with AFDS for nitrates, Nessler reagent colorimetric analysis for N-NH4+, Duval reagent colorimetric analysis for P-PO4 3 – and flame fotometric analysis for K + (STAS 7184/19-82).

The analysis methodology and interpretation of the results was done accordingly to norms and standards existent în Romania (ICPA Bucharest, 1987). Polynomial function was selected to interpret the results. Linear regression (R) was calculated using Microsoft Excel software.

RESULTS AND DISCUSSIONS

This study presents the variability of the response to the factors represented by 3 monochromatic colors, emitted by the LED panels (Factor B: red, blue, white) and 3 illumination durations (Factor C: 15 min/day, 30 min/day, 45 min/day) on plants from 3 genotypes represented by Romanian tomato varieties registered by the Vegetable Research and Development Station in Buzău (Factor A: Sonia de Buzău. Hera, and Coralina).

The determinations were performed using agrochemical analysis at the level of plants, crop substrate and fruits.

A. Results of the agrochemical analysis recorded before the treatment with monochromatic LEDs

A.1. Agrochemical analysis performed on plant samples. The first biochemical determinations were initially performed at the beginning of the experiment on the tomato seedlings of the three varieties studied (Sonia de Buzău, Coralina, and Hera), as well as on the potting substrate used during their development period. The results, obtained from the biochemical analysis of the supply of tomato seedlings in N, P, K (ppm) before starting the treatment with additional light emitted by the monochrome LEDs (average wave times determined at 3 repetitions / whole plant) are graphically represented in Figure 2.

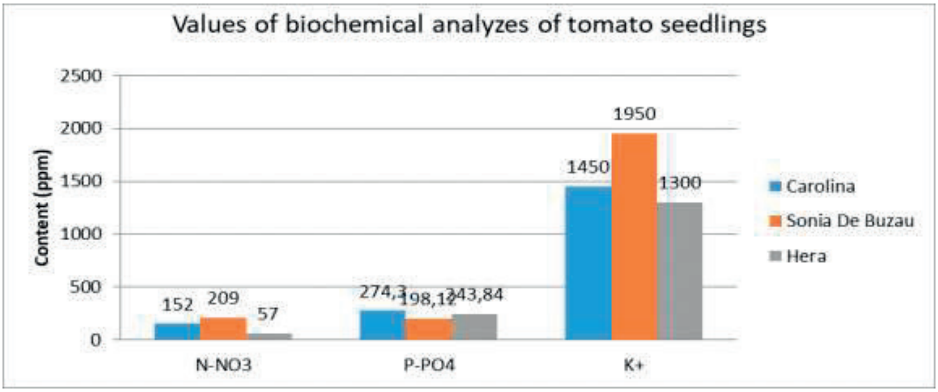

Figure 2. Analysis of tomato seedlings in the supply of N, P, K, before applying the LED treatment

The values obtained from seedlings analisys present a good supply of nutrients, which ensures their normal development in the culture environment. Depending on the tomato genotype, the values indicated an average nitrate content ranging from 57 ppm (for Hera) to 209 ppmN-NO3 (for Sonia de Buzău), high phosphorus content between 198 ppm (for Sonia de Buzău) and 274 ppm P-PO43 (for Coralina), and high potassium content, respectively 1300 ppm K (for Hera) and 1950 ppm K (for Sonia de Buzău).

A.2. Agrochemical analysis performed on substrate samples

Before starting treatment with additional light emitted by the monochrome LEDs, the nutrients and agrochemical indices were analyzed simultaneously, such as: pH, soluble salt content (%) N, P, K, as well as the dosages for N-NH4 +, N-NO3, P-PO_4^{3-} and K +, from the remaining culture substrates, after harvesting the tomato plants, from the experimental variants whose results are previously presented in Figure 2.

From the analysis, of the obtained results and presented in Table 1, resulted the fact that the substrate for the three tomato varieties shows a weak acid pH range between 6.3 and 6.4; the content of soluble salts characterizes poorly salinized soils that do not pose problems to crops; the nitrogen content presented medium values, the phosphorus in the substrate is high, as well as the potassium; so we concluded that the substrates can provide at that time, suitable conditions for plant growth and development

Table 1. Analysis of the substrate cultivated with tomato seedlings, before applying the LED treatment

Experimental Variant	pH	Soluble salts (%)	Content (ppm) N-NH4,	N-NO3	P-PO4	K+
Substrate from **Coralina** seedling	6.4	0.176	43.65	9.5	30.48	67
Substrate from **Sonia de Buzău** seedlings	6.4	0.196	41.22	9.5	38.1	72
Substrate from **Hera** seedlings	6.3	0.274	41.22	19.0	49.53	68

B. Resultes of agrochemical analysis performed <u>after</u> treatment with monochromatic LEDs

B.1. Agrochemical analysis performed on plant samples. After 50 days of daily application of additional treatments analyzed by the experimental Factor **B** (lighting duration in 3 times: 15 minutes/day, 30 minutes/day, 45 minutes/day) and the experimental Factor **C** (monochrome color of the emitted light spectrum of LEDs, in 3 times: red, blue, white), the biochemical determinations were repeated, at the level of the developed plants, of the cultivated soil and of the fruits, compared to the

tomato plants from Control, which were not influenced by supplementary lighting with light emitting diodes (LEDs).
To perform them, plant samples were collected, weighed and subjected to many analyses: dry matter and mineral elements in non-metabolized forms. Time simultaneously, the substrate corresponding to the harvested plants was taken and biochemical determinations were performed by the same methods applied at the beginning of the experiment. The results obtained after 50 days of cultivation of tomato plants from the three varieties studied (Sonia de Buzău, Coralina, and Hera), are presented in Table 2 and Table 3, compared to the tomato plants from Control.

Table 2. Biochemical parameters determined for tomato plants, after intervals of 50 days of culture

Sample	Tomatoes Variety	Exposure time to LED light	N-NO3, ppm	P-PO4, ppm	K, ppm
CONTROL	Sonia de Buzău	-	684	202	215
	Hera	-	862	246	250
	Coralina	-	646	240	215
Red LED	Hera	15 min	513	200	215
		30 min	665	282	252
		45 min	646	270	262
	Sonia de Buzău	15 min	285	260	255
		45 min	190	200	225
	Coralina	15 min	266	185	215
		30 min	589	242	315
		45 min	266	215	205
White LED	Coralina	15 min	532	242	215
		30 min	570	252	225
		45 min	266	200	210
	Sonia de Buzău	15 min	304	242	240
	Hera	30 min	912	257	225
Blue LED	Coralina	15 min	608	242	245
		30 min	361	215	250
	Sonia de Buzău	15 min	456	205	220
		30 min	570	220	250
		45 min	494	229	245
	Hera	15 min	380	189	225
		30 min	456	215	240
		45 min	760	260	256

The cantity of nitrates **(N-NO3)** ensures the growth, development and flowering capacity for plants. The performed analysis of the **Sonia de Buzău** variety (Table 2), in terms of the influence of the exposure color, we can reveal that the blue color determined the highest absorption (values between 456 and 570 ppmN-NO3), and in terms of illumination the highest value of nitrates was recorded at 30 min exposure/day of 570 ppmN-NO3.
Regarding **Hera** variety, nitrates accumulated the most when using white LED treatment on 30 min/day, with the value of 912 ppm N-NO3, followed by the next high value (760 ppm N-NO3) after the exposure for 45 min/day to blue LED. (Table 2)
Concerning **Coralina** variety, the values of absorbed nitrates (Table 2) were higher when using blue LED lighting, at 608 ppm N-NO3, followed by one with red LED lighting (589 ppm N-NO3) and white LED lighting (570 ppm N-NO3). The highest value in the accumulation of nitrates was recorded in the plants exposed for 15 min to blue LED.
Regarding the phosphorus analysis **(P-PO4, ppm)**, the biochemical results presented in Table 2 for the 3 varieties of plants analyzed indicate that they do not show significant differences.Values range from 189 ppm (15 min/day exposure/Blue LED) to 282 ppm (30 min/day exposure/Red LED) for the HERA variety. For the other 2 varieties, the values are in the range of 200 and 260 ppm for Sonia de Buzău samples and in the range of 185 ppm and 252 ppm for the Carolina variety samples. The values of P-PO4 (ppm) obtained in the experiments are within the limits set out in the literature.
Potassium **(K+,ppm),** as an element of nutrition, determines a vigorous growth and development of plants, especially for the quality of plants. Potassium analysis in the case of Carolina

variety plants showed a minimum limit of 210 ppm when the exposure was in 45 min/day with white LED lighting, up to high values of 315 ppm when the exposure was in 30 min/day with red LED lighting. An explication for the lower accumulations of the K+ element (ppm), would be a possible blockage of the absorption due to the higher temperatures manifested in the culture space during the summer period. The flowering capacity of the tested tomato samples was negatively influenced by the lower supply of potassium, the Hera variety being the most sensible to this aspect.

Along with the analysis of tomato plants, the soils corresponding to them were also analyzed, the results are presented in Table 3.

Table 3. Biochemical determinations performed on the substrate cultivated with tomato plants, after intervals of 50 days of culture

Sample	Tomatoes Variety	Exposure time to LED light	pH	Soluble salts, %	N-NH4, ppm	N-NO3, ppm	P-PO4, ppm	K, ppm
CONTROL	Sonia de Buzău	-	6,9	0.1445	27.62	65.25	92.87	25.71
	Hera	-	6.4	1.0115	31.87	859.5	891.37	352.4
	Coralina	-	6.3	1.1993	114.75	544.5	659.25	781.5
Red LED	Hera	15 min	6.4	0.5924	14.87	216	188.5	25
		30 min	6.5	0.4479	14.87	157.5	154.3	26
		45 min	6.3	0.8525	63.75	256.5	24.76	28
	Sonia de Buzău	15 min	6.5	0.4046	17	42.75	116.2	39
		45 min	6.4	0.5202	34	171	150.49	30
	Coralina	15 min	6.5	0.4479	14.87	157.5	154.3	26
		30 min	6.5	0.4046	17	42.75	116.2	39
		45 min	6.6	13.438	61.62	859.5	661.03	40
White LED	Coralina	15 min	6.3	0.6358	19.125	225	192.4	41
		30 min	6.5	0.8236	14.87	477	230.5	42
		45 min	6.6	10.837	108.37	306	737.23	44
	Sonia de Buzău	15 min	6.5	0.5491	14.87	22.5	249.55	37
		30 min	6.4	0.4624	8.5	207	112.4	25
		45 min	6.5	0.7369	12.75	454.5	188.6	28
	Hera	30 min	6.6	0.4624	8.5	20.25	173.35	30
Blue LED	Coralina	15 min	6.5	0.5202	14.87	225	192.4	35
		30 min	6.4	0.5491	25.5	463.5	229.4	40
	Sonia de Buzău	15 min	6.5	0.6213	21.25	306	153.92	45
		30 min	6.6	0.5924	19.125	315	24.76	51
		45 min	6.7	0.5725	8.5	22.5	36.2	60
	Hera	15 min	6.5	0.5202	8.5	171	173.35	41
		30 min	6.6	0.5491	19.125	157.5	191.3	45
		45 min	6.5	0.5924	14.87	42.75	192.4	37

B.2. Agrochemical analysis performed on substrate samples. Based on the results of the analysis regarding the tomato plants and the analysis of the corresponding soils, polynomial regressions of degree 2 were performed on nitrogen (N), phosphorus (P) and potassium (K) levels (Figure 3, Figure 4 and Figure 5).

The regression of nitrogen (N) from the plant and soil showed a regression coefficient R = 0.1791 which is distinctly statistically significant (Figure 3).

In the case of phosphorus (P), the regression coefficient between soil phosphorus and phosphorus absorbed in plants was R = 0.1466, which is also classified as significant distinct (Figure 4).

Statistical analysis in the case of potassium (K), has a regression coefficient of R = 0.2347, which is also characterized as a distinguished significant (Figure 5).

From this statistical analysis, we can say that the presence of the three nutrients in the soil N, P, K have properly ensured the growth and development of plants through a sustainable absorption in tomato plants, taking into account scientific publications in the field (Davidescu &Davidescu, 1999).

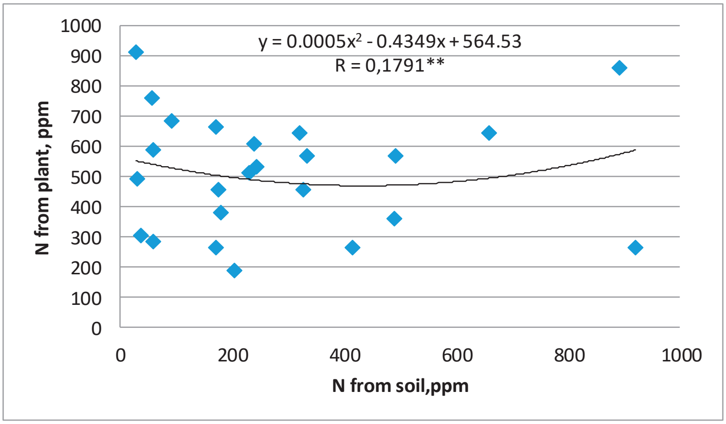

Figure 3. Statistical analysis of the influence of soil nitrogen content and nitrogen content absorbed by tomato plants

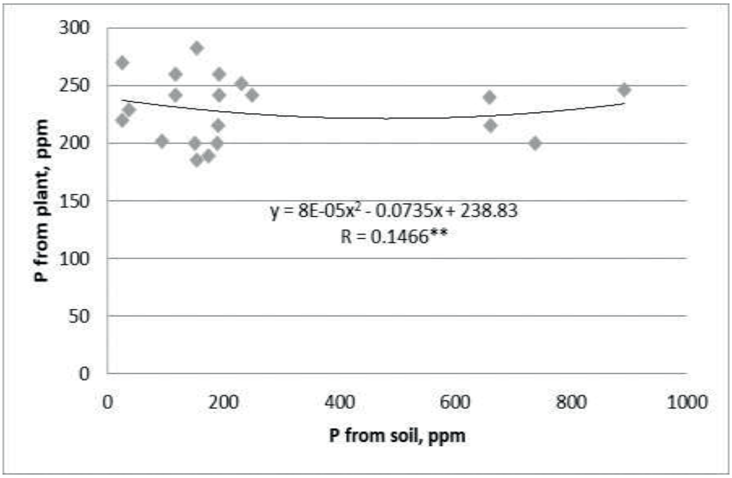

Figure 4. Statistical analysis of the influence of soil content on phosphorus and phosphorus content absorbed by tomato plants

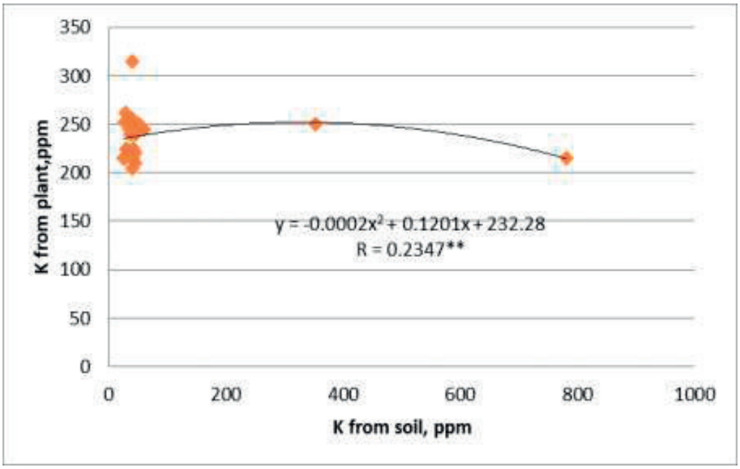

Figure 5. Statistical analysis of the influence of soil potassium content and potassium content absorbed by tomato plants

B.3 Agrochemical analysis performed on fruit samples. The flowering and fruiting period of the analyzed tomato varieties spread over a period of three months. The results obtained from the fruit quality analysis are presented in Table 4.

Table 4. Biochemical determinations performed on tomato fruits; average values.

Sample type	Tomatoes Variety	Exposure time to LED light	N-NO3, ppm	P-PO4, ppm	K, ppm	Carbohydrates, %	Acidty, %	Vit. C, mg/ 100g p.p.
Control	Coralina	-	273	282	1200	4.57	0.44	5.35
	Sonia de Buzău	-	200	225	1100	4.67	0.34	5.60
	Hera	-	210	215	1150	3.90	0.40	5.20
White LED	Coralina	15 min	283	262	950	4.65	0.42	5.37
		45 min	297	278	1020	4.70	0.45	5.40
		30 min	245	250	980	4.75	0.42	5.62
Red LED	Coralina	15 min	330	292	1150	4.60	0.40	5.40
		30 min	95.0	139.4	1050	4.75	0.40	5.25
		45 min	370	297	1120	4.65	0.42	5.45
	Sonia de Buzău	15 min	275	257	1150	4.70	0.40	5.50
		30 min	152.0	380	1150	4.80	0.40	5.37
	Hera	15 min	114.0	122.7	850	5.20	0.38	5.45
		30 min	171.0	179.0	825	5.15	0.39	5.42
		45 min	114.0	142.5	792	5.25	0.37	5.40
Blue LED	Coralina	15 min	270	270	1010	4.52	0.43	5.40
		45 min	275	280	1015	4.60	0.46	5.55
	Sonia de Buzău	15 min	237	252	970	4.70	0.45	5.40
	Hera	15 min	38.0	180.6	850	5.10	0.40	5.45

From the data presented in Table 4, it can be observed that the Hera variety did not register any fruits when exposed to white LED light and also to blue LED light except for the variant of exposure of 15 minutes/day.

The analysis of the fruit samples regarding the biochemical composition were also recorded in Sonia de Buzău with the exposure to white LED light of 15 minutes/day and 45 minutes/day. Also, we consider that Sonia de Buzău is a sensitive variety to blue light, fruiting being recorded only at exposure to 15 minutes of blue LED light.

Regarding the nitrate levels, N-NO3- it is observed that for all fruits the registered values are qualitatively good below the limit imposed by the Romanian legislation (Order no. 1050 / 12.10.2005) regarding the quality of fresh vegetables.

The phosphorus absorbed by the tomato samples is in the normal limits between 100 ppm and 200 ppm P-PO4, as specified by the scientific literature (Davidescu V and Davidescu D, 1999), values that ensure the good quality of the fruits for consumption.

The soluble carbohydrates (%) of the tested samples (Table 4) present values between 3.90% (for Hera Control samples) and 5.25% (for Hera samples, with exposure to red LED light for 45 minutes). The accumulation of carbohydrates was good in all varieties, ensuring the sweet taste of tomatoes (Gherghi, 1999). Acidity (%) varies between 0.34% for Sonia de Buzău control sample and 0.45% for Sonia de Buzău samples that were exposed to 15 minutes/day of blue LED lighting and Carolina samples that were exposed to 15 minutes/day white LED lighting (Table 4). The values of acidity are within the limits specified by the literature for tomatoes, the variation of values depends on the variety (Gherghi, 1999), ensuring a good balance between carbohydrates and acidity.

Vitamin C (mg/100g fresh product) levels varies between 5.20 mg/ 100g fresh product in the Hera control sample and 5.62 mg/ 100g fresh product in Sonia de Buzău sample exposed to 30 minutes/day at white LED lighting (Table 4). This indicator is within the limits provided by the literature for assessing the quality of tomatoes. (Gherghi, 1999; Gherghi, 1994; Davidescu D, and Davidescu V., 1981)

CONCLUSIONS

Agrochemical analysis of tomato plants, performed after 50 days of daily application of additional treatments with monochromatic LED light, analyzed experimentally, showed that they accumulated a large amount of P (P-PO4, ppm) and K salts. (K +, ppm), when exposed for 15 minutes/day with red LED light, in the case of plants of Sonia de Buzău (260 ppm P-PO4; 255 ppm K) and at the exposure of 30 minutes/day, at the plants of Hera (282 ppm P-PO4; 252 ppm K), while the values of N (N-NO3, ppm) from all experimental samples were below those of the Control, exceptions being registered in the plants of Hera treated with white LED (30 minutes/day) and blue LED (45 minutes/day).

Agrochemical analysis of substrates in the experimental versions showed lower pH values; The content of soluble salts in the tested substrates compared to the Control variants was the highest (13.438%) in the substrates of the red LED variant (45 minutes/day), provided to Coralina plants.

The nitrogen supply (N-NH4, ppm) of the substrates was determined at the low value of 8.5 ppm N-NH4, for the experimental variants of the plants from the Sonia de Buzău variety (exposure 30 minutes/day, with white LED and respectively after exposure 45 minutes/day at the blue LED) and Hera (exposure 15 minutes/day). Phosphorus in the substrates was best absorbed (24.76 ppm P-PO4) in Hera variety, the red LED variant (45 minutes/day) and in the samples from the variety Sonia de Buzău version with blue LED (30 minutes/day); the same aspect was found in potassium which was well absorbed from the substrates by all plants in the experimental variants.

Biochemical determinations performed on tomato fruits harvested at the end of 3 months of crop maintenance, indicated among the indices of maturity appreciation at harvest, differences recorded for carbohydrates (%) in Hera fruits, from 2 experimental variants exposed at red LED, which exceeded by 13.46% (45 minutes/day) and by 13.33% (15 minutes/ day) the values of the control. The correlation of the coefficients was established, and allowed the comparison of the variation for the data set and the regression coefficients between the NPK supply of the substrates and that of the absorption at the plant level. The database was established in electronic system, and the data were analyzed through the statistical program "Data Analysis" from Microsoft Office - Excel.

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MORPHOLOGICAL AND ANATOMICAL CHARACTERIZATION OF SAFFLOWER (*Carthamus tinctorius* L.) HYPSOPHYLS AND LEAVES

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Abstract

Safflower (Carthamus tinctorius L.) is an annual plant that belongs to the Asteraceae family. It is drought resistant and both seeds and flowers have multiple uses in food, pharmaceutical, dermato-cosmetic and textile industries. Safflower can also be grown as an ornamental plant. The main objective of this study is to identify the morphological and anatomical differentiation of safflower hypsophylls and leaves. The study was conducted on two varieties of C. tinctorius grown in the Botanical Garden of the U.S.A.M.V. Bucharest. The samples were examined with Leica stereomicroscope, Leica optical microscope and scanning electron microscope. The results showed differences between the two varieties regarding the shape of the blade, the arrangement of the vein, the presence and arrangement of the secretory channels. This result brings new elements in the identification of bioactive compounds and for the differentiation of the varieties.

Key words*: safflower, secretory channels, leaves, morphology.*

INTRODUCTION

Oilseed crops have an important role not only for their nutritional value and therapeutic properties but also for industrial uses.

With global demand, there is a need to develop varieties that have higher oil content, higher productivity, and are resistant to different challenging environments (Weselake, 2016; Zafar et al., 2019; Kotecka-Majchrzak et al., 2020; Hassani et al., 2020).

In general, most lipids are stored in the seeds of oil crops in the form of high-energy-density triacylglyceride (TAG), but there are studies that show their presence in important quantities in non-seed organs, including mesocarp, flower petals, pollen, stems, tubers, and seed pods, husks and also in the leaves (Alameldin et al., 2017; Nikiforidis, 2019; Vanhercke et al., 2019; Zafar et al., 2019).

In the leaves, TAG synthesis and oil bodies formation can be influenced by various stress factors like darkness, extreme temperature (heat, cold, freezing) and senescence (Weber et al., 2018; Nikiforidis, 2019; Vries & Ischebeck, 2020).

Fungal infections of the leaves can also affect TAG content by increasing linolenoyl content. In senescent leaves, oil bodies have antifungal properties, producing the antifungal compound 2-HOT. Thus, leaf oil cavities may have an intercellular role to produce lipid compounds for plant defence responses (Vanhercke et al., 2019; ENV/JM/MONO 2020).

Nikiforidis et al. (2019) have shown that oil bodies are more present in senescent leaves than mature leaves and the genes involved in the biosynthesis of oleosin are not found in the leaves, so they suggested that the components of oil bodies from the seeds are different than those from the leaves.

Different accumulation of lipids can lead to some clues that can be useful in strategies for increasing oil content in plant stems and leaves. Different extraction methods can be applied for the extraction of oil from vegetative tissues with importance in biodiesel and pharmaceutical industry (Vanhercke et al., 2019; Singh et al., 2021).

Secretory structures of oil bodies can also be found as secretory cavities and ducts in leaves, stems, roots and flower. They are important taxonomic characters for many plant families (Cury & Appezzato-da-Glória, 2009; Fernandes et al., 2018)

Asteraceae family has secretory cavities formed in a schizogenic way that elongate and give the impression of secretory channels (Bartoli et al., 2011; Cury & Appezzato-da-Glória, 2009; Russin et al., 1992)

A well-known oilseed and medicinal plant belonging to *Asteraceae* family is *Chartamus tinctorius.*

Leaf essential oil such as methyl eugenol, α-pinene, cinnamyl acetate, flavonoids like luteolin and its glucopyranosides, carotenoids, dehydroabietylamine, have also been found in the leaves, having antibacterial, antifungal, antiinflamatory, antioxidative, hepatoprotective activity (Lee 2002; Asgarpanah, 2013; Salem et al., 2014). The leaves can be also used in: vitiligo, psoriasis, pain relief according to Delshad et al. (2018). Safflower young leaves can be used as a vegetable food, and leaves and stems can be used as green fodder, hay or silage (Heuzé & Tran, 2015; Dobrin et al., 2016).

In the growth and development stages, young leaves form a rosette, followed by rapid stem elongation, branching, then flowering, with alternate leaves on each side of the stem (Dajue & Mündel, 1996). Leaf size may vary according to variety and position on the plant, ranging between 2.5-5 cm wide and 10-15 cm long. The leaf can be sessile and ovate-lanceolate. In some varieties, upper leaves often develop spines, while lower on the stem are usually spineless (Dobrin & Marin, 2015).

Morphological characteristics regarding the presence or absence of leaf spines are related to the fertility of the plant so the leaves of fertile plants have spines, and non-spiny traits are associated with sterile plants. Knowing safflower leaves botanical and morphological characteristics is important in the selection and production of pure hybrid seeds and the faster breeding of elite varieties (ENV/JM/MONO 2020).

The main objective of this study is to identify the botanical and morphological aspects of distiction of the senescent hypsophylls and leaves of two varieties of *Carthamus tinctorius* L., and characterisation of the secretory structures of oils, in order to differentiate the varieties and for the possible standardization with importance in the botany, agriculture and pharmaceutical industry.

MATERIALS AND METHODS

The safflower varieties used in the study were: a spineless variety (Zanzibar) with a high content in linoleic acid (Dobrin & Marin, 2015) and a variety with spines (S2). The experiment was carried out in the Botanical Garden of the U.A.S.V.M. Bucharest. The morphological and anatomical characterisation of safflower hypsophylls and leaves was performed in the Research Center for Studies of Food Quality and Agricultural Products, U.A.S.V.M. Bucharest. The hypsophylls and leaf samples were harvested in September. The samples were rehydrated and stored in 70% ethanol (Talbot & White, 2013) before being analysed with a Leica S8 APO stereomicroscope, optic microscope Leica DM 1000 LED and with the Scanning Electron Microscope (SEM) FEI Inspect S50.

RESULTS AND DISCUSSIONS

Hypsophylls anatomy and morphology

The presence of hypsophylls was observed in both varieties. For the anatomy and morphology characterisation the hypsophylls were examined using stereo-microscope, optic microscope and SEM.

Figure 1. Stereo microscope images of hypsophylls of *C. tinctorius* var. Zanzibar and S2

Zanzibar variety has hypsophylls lamina ovate to obovate shape, with round apex, slightly narrowed to the base, cross-venulate; the margins are whole, with a smooth margin, the blade is smooth and shiny, without spines (Figure 1 A).
The hypsophylls from variety with spines (S2) have lanceolate lamina shape, with acuminate apex; cross-venulate, slightly denticulate, with mate blade and few spines (Figure 1B).

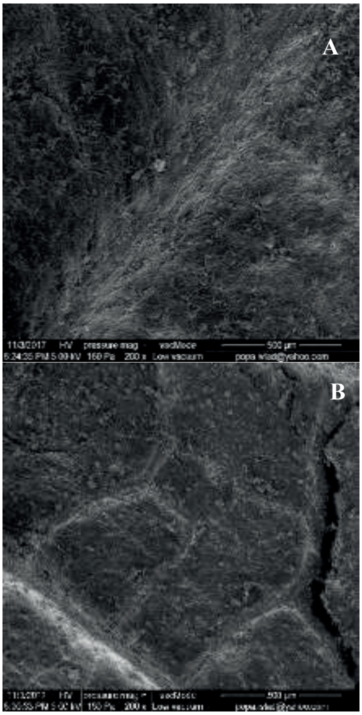

Figure 2. Hypsophylls surface from *C. tinctorius* var. Zanzibar (A) and S2 (B) examined with SEM

We found open and closed stomata on senescence hypsophylls surfaces of both safflower varieties, on low vacuum SEM, at 500 µm (Figure 2 A and B).
In the Figure 3 and 4 it can be seen that the structures identified do not have the regular shape of secretory channels, but are spaces that develop in the mesophyll that accompany the secondary or tertiary conducting vessels. Also, it can be seen that the colour of the Zanzibar variety`s structures are orange to red (Figure 3), and of S2 variety are yellow (Figure 4).
The presence of oil cells in *Asteraceae* has been identified in the foliar structure also by Rikisahedew & Naidoo (2018).

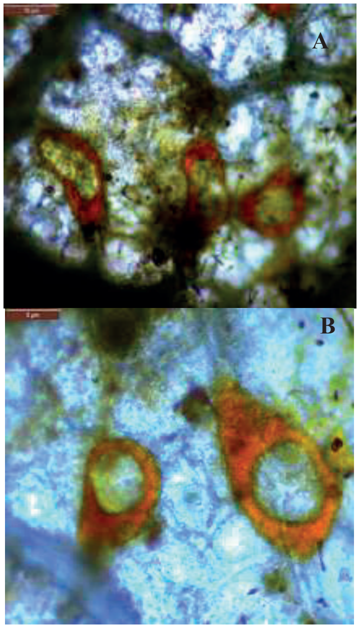

Figure 3. Hypsophylls from *C. tinctorius* var. Zanzibar-middle area 10x, scale bar =10 µm (A), hypsophyll detail 20x, scale bar =5 µm (B)

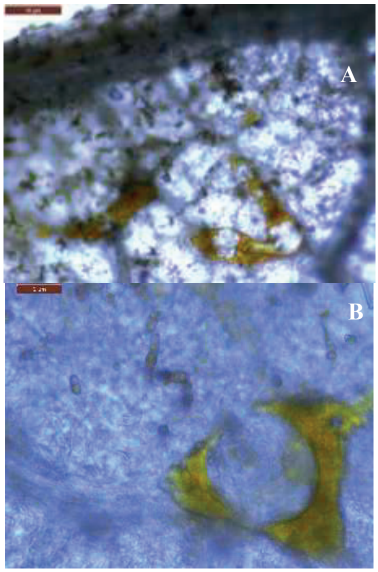

Figure 4. Hypsophylls from *C. tinctorius* var. S2 -middle area 10x, scale bar =10 µm (A), hypsophyll detail 40x, scale bar =2 µm (B)

Leaves anatomy and morphology

Leaves anatomy and morphology were examined with stereo-microscope, optic microscope and SEM.

Figure 5. *C. tinctorius* var. Zanzibar leaf apex (A) and detail of leaf (B) -stereo microscope images

In the Figure 5 A and B it can be seen that Zanzibar variety`s leaves, like hypsophylls, has elliptic-cuneate shape, and are cross-venulate; the margins are entire, with a smooth margin, smooth and shiny blade, without spines, like hypsophylls.

The leaves from variety with spines have lanceolate lamina shape, with acuminate apex, and cross-venulate, slightly denticulate, mate blade, with spines, like hypsophylls (Figure 6 A and B).

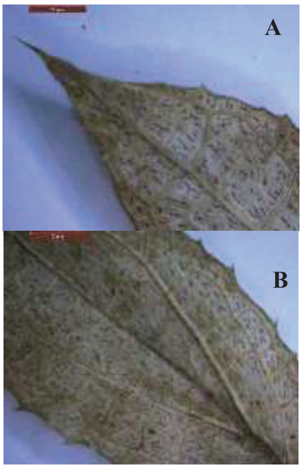

Figure 6. *C. tinctorius* var.S2 leaf apex (A) and detail of leaf (B) -stereo microscope images

On low vacuum SEM at 300 µm and 500 µm images of the senescence leaves surfaces, open and closed stomata (Figure 7 A and B) were found on both safflower varieties, like in hypsophylls.

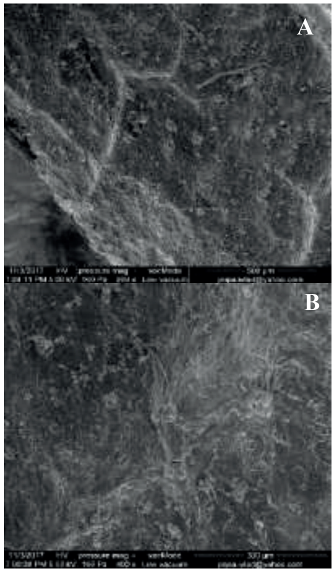

Figure 7. Leave surface from *C. tinctorius* var. Zanzibar (A) and S2 (B) examined with SEM

It can be seen that in the leaf, the colour of the Zanzibar variety`s structures (Figure 8 A and B), is similar with that of S2 variety (Figure 9 A and B).

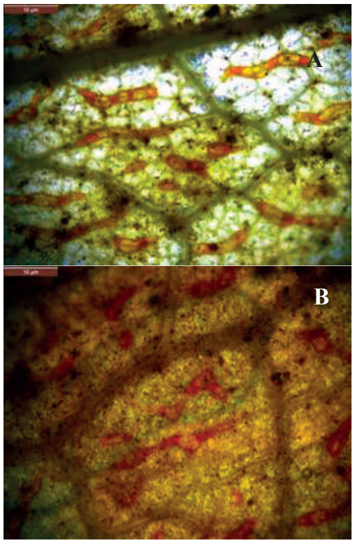

Figure 8. Leaf lamina from *C. tinctorius* var. Zanzibar-middle area 10x, scale bar =10 µm (A), leaf lamina base detail 10x, scale bar =10 µm (B)

In figures 8 and 9 it can be noticed that the structures identified in the leaves do not have the regular shape of secretory channels, but are spaces that develop in the mesophyll that accompany the secondary or tertiary conducting vessels, like in hypsophylls (Figure 3 and 4).

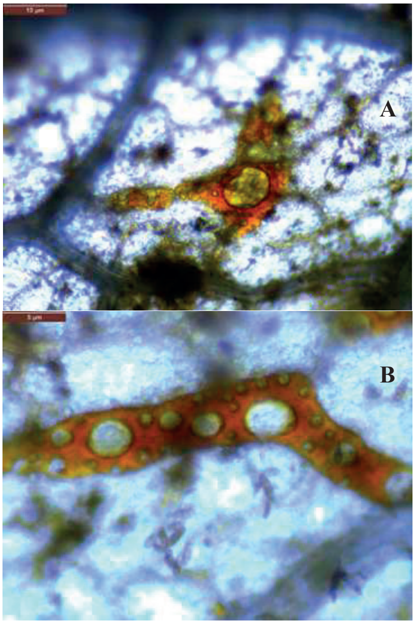

Figure 9. Leaf lamina from *C. tinctorius* var. S2 -middle area 10x, scale bar =10 μm (A), middle area 20x, scale bar =5 μm (B)

CONCLUSIONS

The originality of the paper presented, in terms of anatomical descriptions, is that it comes and confirms the following observation: plants in the *Asteraceae* family have secretory cavities formed in a schizogenic way that elongate and give the impression of secretory channels.

The description of these cavities should not be based only on cross sections (according to Lestern & Curtis, 1989).

In the presented images it can be seen that these structures do not have the regular shape of secretory channels, but are spaces that develop in the mesophyll that accompany the secondary or tertiary conducting vessels.

There are differences between the two varieties of *Carthamus tictorius* L. regarding the shape of hypsophylls and leaves blade, the arrangement of the vein, the presence, arrangement and irregular shape of the secretory cavities.

Macro and microscopic identification of the morphological and anatomical characteristics of safflower senescence hypsophylls and leaves brings new elements for the differentiation of varieties, for the possible standardization and in the identification of bioactive compounds, with importance in the botany, agriculture and pharmaceutical industry.

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SOME AGRO-BIOLOGICAL FEATURES AND POTENTIAL USES OF VIRGINIA MALLOW, *Sida hermaphrodita* IN MOLDOVA

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Abstract

Plant biomass is a promising alternative to conventional, non-renewable sources of energy. The aim of this paper was to evaluate some biological peculiarities and the quality of green and dry biomass of Virginia mallow, Sida hermaphrodita cv. Energo, which has been cultivated in the experimental field of the National Botanical Garden (Institute), Chişinău. Corn, Zea mays, was considered as control. The results of our research revealed that the dry matter of the harvested Virginia mallow green mass contained 160 g/kg CP, 84 g/kg ash, 371 g/kg ADF, 529 g/kg NDF, 60 g/kg ADL, 311 g/kg cellulose and 158 g/kg hemicellulose; the biochemical biomethane potential of green mass substrates was 272 l/kg ODM. The dry stalks of Virginia mallow contained 46.03% carbon, 5.94% hydrogen, 0.32% nitrogen, 0.08% sulphur, 1.87% ash, 556 g/kg cellulose, 241 g/kg hemicellulose and 131 g/kg acid detergent lignin, but corn stalks –44.06% carbon, 5.65% hydrogen, 0.95% nitrogen, 0.21% sulphur, 4.40% ash, 417 g/kg cellulose, 250 g/kg hemicelluloses and 82 g/kg acid detergent lignin. The theoretical ethanol yield from structural carbohydrates averaged 578 L/t in Virginia mallow substrate, as compared with 485 L/t in corn substrate. The gross calorific value of biomass from Virginia mallow stalks averages 19.73 MJ/kg, but – from corn stalks – 17.8 MJ/kg. Sida hermaphrodita cv. Energo may serve as multi-purpose feedstock for renewable energy production.

***Key words**: biomethane potential, calorific value, Sida hermaphrodita cv. Energo, pellets, theoretical ethanol yield.*

INTRODUCTION

In the past few years, major challenges such as climate change, population growth, food and energy security and intensive exploitation of natural resources have caused increasing concerns worldwide. The total energy consumption and pollutant emissions of the world have doubled in the last 40 years. Replacing fossil fuels with renewable energy alternatives has become a major global issue of the XXI century and a key to sustainable development. Biomass is one of the biggest renewable resources of energy, which may be utilized in different ways: directly by burning and indirectly by converting it into solid, liquid or gaseous fuels. Its use for energy purposes generates less pollution to the natural environment and climate. Lignocellulosic biomass, or plant cell walls, is the most abundantly available renewable resource on Earth and it has the potential to be converted to second-generation biofuels, chemicals, or materials without compromising global food security (Zoghlami&Paës, 2019).

Due to the high interest in biomass, the plantation of energy crops has gained more interest lately. The species selected as potential energy crops should be characterized by a high annual growth and productivity, resistance to diseases and pests, low habitat requirements and adaptation to climatic conditions (Ţîţei& Roşca, 2021).

Sida hermaphrodita (L.) Rusby syn. *Ripariosida hermaphrodita* (L.) Weakley & D.B. Poind., *Napaea hermaphrodita* L. is a perennial herbaceous species belonging to the *Malvaceae* family, and its common names include Virginia mallow and Virginia fanpetals. It is indigenous to North America, where it occurs in or near wetlands, floodplains and on river banks. It is a polycarpic perennial herb, looks like a dense bush with a few dozens of stems of 400 cm in height and of 5 to 35 mm in diameter. The leaves are simple, but palmately cleft into 3 to 7 elongated lobe tips, 10 to 20 centimeters long and borne on petioles. The inflorescences produce clusters of white flowers. The fruit is capsule with four brownish-grey triangular seeds. The weight of 1000 seeds is 3.30-3.90 g (Ţîţei, 2015).

Sida hermaphrodita is tolerant to almost any quality of soil; the pH of these soils varies between 5.4 and 7.5, with a medium to high organic matter content. It can withstand temperatures down to -35 °C without any problems, and it is considered resistant to temporary droughts. It is deemed rather insusceptible to pests and diseases, although it can be affected by *Sclerotinia sclerotiorum.* Virginia mallow plantations can be established using seeds, seedlings or root cuttings. It is sown in late autumn or early spring with layered seeds at a depth of 2-3 cm, in quantities of 2-3 kg/ha, the distance between rows – 45 cm or 70 cm, with soil compaction before and after sowing. The vegetative propagation is done by planting root cuttings, 3-7cm long, in the middle of March-April and – by seedlings – in the middle of May, at a density of 25-30 thousand plants per hectare. Once planted, *Sida hermaphrodita* can be productive over 10 years or more, and some authors have suggested that it can remain highly productive for 15–20 years. The crop biomass can be harvested with conventional harvesting techniques and stored without problems. In particular, in the last decade, an increasing number of researchers has admitted the potential of *Sida hermaphrodita* as an ecologically valuable raw material for fodder, fibre and energy production, as well as soil stabilization (Howaniec&Smoliński, 2011; Dezbowski et al., 2012; Jasinskas et al., 2014; Siaudinis et al., 2015; Jablonowski et al., 2017; Fijalkowska et al., 2017; Bilandžija et al., 2018; Wróbel et al., 2018; Jankowski et al., 2019; Szwaja et al., 2019; Cumplido-Marin et al., 2020).

The main objective of this research has been to evaluate the biomass quality of Virginia mallow, *Sida hermaphrodita*, as feedstock for the production of biomethane, cellulosic ethanol and solid biofuel – pellets.

MATERIALS AND METHODS

The cultivar '*Energo*' of Virginia mallow, *Sida hermaphrodita,* created in the National Botanical Garden (Institute), registered in 2014, in the Catalogue of Plant Varieties** and patented in 2016, by the State Agency on Intellectual Property (AGEPI) of the Republic of Moldova, patent no. 207/31.05.2016*, has been cultivated in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chişinău, latitude 46°58′25.7″N and longitude 28°52′57.8″E. It served as subject of the research, and corn, *Zea mays*, was used as control.

The green mass of *Sida hermaphrodita* cv. 'Energo', in the 7th year of growth, was cut for the first time in the end of May, but corn, *Zea mays* – in kernel milk-wax stage, in early August. The harvested green mass was chopped with a stationary forage chopping unit. The fractional composition of the chopped green mass was determined using a vibrating screen device. It used 400 mm diameter sieves, where sieves with round pores were put one on other (in the order from the top sieve): diameter of 63 mm, 31.5 mm, 16 mm, 8 mm, 3.15 mm and 1 mm. For chemical analyses, green mass samples were dried in a forced air oven at 60 °C, milled in a beater mill equipped with a sieve with diameter of holes of 1 mm and some assessments of the main parameters, such as crude protein (CP), crude fibre (CF), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL) and total soluble sugars (TSS), have been determined by near infrared spectroscopy (NIRS) technique using PERTEN DA 7200. The concentrations of hemicellulose (HC) and cellulose (Cel) were calculated according to standard procedures. The carbon content of the substrates was obtained using an empirical equation according to Badger et al. (1979). The biochemical biogas potential (Yb) and the methane potential (Ym) were calculated according to the equations of Dandikas et al. (2014), based on the concentration of acid detergent lignin (ADL) and hemicellulose (HC):

$$Yb=727+0.25\ HC-3.93\ ADL$$
$$Ym=371+0.13HC-2.00ADL$$

The Virginia mallow and corn dry stalks were harvested manually in February. For the production of solid biofuel, the harvested stalks were chopped into chaff using a stationary forage chopping unit. The chopped biomass of Virginia mallow and corn stalks were milled in a beater mill equipped with a sieve with diameter of holes of 6 mm.

To perform the analyses of the content of cell walls, the biomass samples were dried in an oven at 85 °C and then milled (<1 mm) and homogenized. After that, the total carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) amounts were determined by dry combustion in a Vario Macro CHNS analyzer (Elemental Analyzer, GmbH, Langenselbold, Germany), according to standard protocols at the State Agrarian University of Moldova. The pelleting was carried by the equipment developed at the Institute of Agricultural Technique "Mecagro", Chişinău. The physical and mechanical properties of dry biomass and pellets were determined according to the standards: the moisture content of the plant material was determined by SM EN ISO 18134 in an automatic hot air oven MEMMERT100-800; the content of ash was determined at 550 ºC in a muffle furnace HT40AL according to SM EN ISO 18122; the automatic calorimeter LAGET MS-10A with accessories was used for the determination of the calorific value, according to SM EN ISO 18125; the particle size distribution was determined according to SM EN ISO 17827 using standard sieves, the collected particles in each sieve were weighed; the cylindrical containers were used for the determination of the bulk density, calculated by dividing the mass over the container volume according to SM EN ISO 17828, SM EN ISO 18847. The mean compressed (specific) density of the pellets was determined immediately after removal from the mould as a ratio of measured mass over calculated volume.

To determine the cell wall components in the dry mass of tested species, the amounts of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were assessed using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200. The amount of cellulose was calculated as ADF minus ADL and hemicelluloses – NDF minus ADF. The Theoretical Ethanol Potential (TEP) was calculated according to the equations of Goff et al. (2010) based on conversion of hexose (H) and pentose (P):

$$H = [\%Cel+(\%HC \times 0.07)] \times 172.82$$
$$P = [\%HC \times 0.93] \times 176.87$$
$$TEP = [H + P] \times 4.17$$

RESULTS AND DISCUSSIONS

As we mentioned in our previous study, in the first growing season, *Sida hermaphrodita* cv. '*Energo*' stopped growing in the flower bud stage, when the stems were about 171 cm tall and 6-13 mm thick at base. In the second year and in the further years, in spring, when the air temperature exceeded 6 °C, plant development started from the generative buds. A high growth rate (5-6 cm/day) was observed during May and June (Ţîţei, 2015). The studied plants of *Sida hermaphrodita* cv. 'Energo', in the 7th year, were cut for the first time at the end of May, when the shoots with 37-40 leaves were about 235-265 cm tall, the green mass yield reached 70.5 t/ha. A high aerial biomass productivity of *Sida hermaphrodita* plants was also mentioned in other studies. So, Rakhmetov (2011) stated that, under the climatic conditions of Ukraine, *Sida hermaphrodita* could have a productivity of 123.9-187.7 t/ha natural fodder, depending on the genotype.

The sustainable management of biogas production via anaerobic digestion involves the use of alternative biomass sources that are not competitive with food production. Energy crops have been largely used as lignocellulosic biomass feedstock in the production of biogas via anaerobic digestion in recent years. Particle size distribution of chopped green mass influenced the costs of transport and particle size reduction has been reported as one of the major effects of different substrate pre-treatments of lignocellulosic biomasses. The results, Table 1, show that a higher amount of particles (57.7%) with a size < 8 mm was found in *Sida hermaphrodita* '*Energo*' green mass, but in *Zea mays* green mass, on average 66.8% of the particles were larger than 8 mm.

Table 1. Particle size distribution of chopped green mass from the studied species, %

Particle size	*Sida hermaphrodita*	*Zea mays*
3.15 mm	4.9	5.5
3.15-8.00 mm	52.6	27.2
8.00-16.00mm	24.7	48.4
16.00-31.50 mm	12.7	18.4

The biodegradation of different types of lignocellulosic biomass feedstock depends on the chemical structure, primarily on the content of cellulose, hemicellulose, lignin and the C/N ratio. The results regarding the quality of the investigated substrates and the potential biomethane are illustrated in Table 2. We found that the dry matter of the harvested green mass of *Sida hermaphrodita* '*Energo*' contained 160 g/kg CP, 369 g/kg CF, 60 g/kg ash, 371 g/kg ADF, 529 g/kg NDF, 60 g/kg ADL, 60 g/kg TSS, 311 g/kg Cel, 158 HC g/kg TSS, but the corn green mass substrates – 88 g/kg CP, 303 g/kg CF, 60 g/kg ash, 310 g/kg ADF, 520 g/kg NDF, 31 g/kg ADL, 294 g/kg TSS, 279 g/kg Cel, 210 g/kg HC, respectively.

Table 2. The biochemical composition and biomethane production potential of the investigated green mass substrates

Indices	*Sida hermaphrodita*	*Zea mays*
Crude protein, g/kg DM	160	88
Crude fibre, g/kg DM	369	303
Ash, g/kg DM	60	60
Acid detergent fibre, g/kg DM	371	310
Neutral detergent fibre, g/kg	529	520
Acid detergent lignin, g/kg DM	60	31
Total soluble sugars, g/kg DM	60	294
Cellulose, g/kg DM	311	279
Hemicellulose, g/kg DM	158	210
Nitrogen, g/kg DM	25.60	14.08
Carbon, g/kg DM	522.22	522.22
Ratio carbon/nitrogen	20.40	35.43
Biogas potential, L/kg VS	525	591
Biomethane potential, L/kg VS	272	302

The nitrogen content in the studied substrates ranged from 1.41% in corn substrates to 2.56% in *Sida hermaphrodita* '*Energo*' substrates, the estimated content of carbon was the same – 52.2%, the C/N ratio varied from 35 to 20, respectively. Essential differences were observed between the protein, fibre and lignin contents, which played an important role in degradation and biomethane production. The biochemical methane potential of *Sida hermaphrodita* green mass substrate reached 272 l/kg ODM and corn substrate - 302 l/kg ODM.

Several literature sources describe the biochemical composition and biomethane potential of *Sida hermaphrodita* green mass. In Poland, in the herbage harvested in the bud formation stage, the dry matter content was 197 g/kg, its biochemical composition – 199 g/kg CP, 8.35 % ash, 73 g/kg WSC and structural carbohydrates – 403 g/kg NDF, 308 g/kg ADF and 38 g/kg ADL (Fijalkowska et al. 2017). The anaerobic digestion batch tests on *Sida hermaphrodita* biomass harvested in July revealed the following results: 435 l/kg ODM biogas or 220 l/kg ODM methane (Oleszek et al. 2013). According to Hartmann & Haller (2014), the methane yields varied from 280 to 293 l/kg ODM. Siwek et al. (2019) reported that the specific biogas yield of *Sida hermaphrodita* ranged from 505 to 514 l/kg, and for *Silphium perfoliatum* biomass – from 483 to 504 l/kg, methane concentrations from 50.8 to 52.8 % and from 51.1 to 52.9 %, respectively.

The elemental composition of dry biomass is a significant asset that defines the amount of energy and evaluates the clean and efficient use of biomass materials, provides significant parameters used in the design of almost all energy conversion systems and projects, for the assessment of the complete process of any thermochemical conversion techniques (Lawal et al., 2021).

Table 3. Elemental composition of dry biomass from the studied species, %

Indices	*Sida hermaphrodita*	*Zea mays*
Carbon	46.03	44.06
Hydrogen	5.94	5.65
Nitrogen	0.32	0.95
Sulphur	0.08	0.21

The main constituents of dry biomass are carbon (C), oxygen (O) and hydrogen (H). As carbon and hydrogen are oxidised in the combustion process, they release energy. Carbon is obviously representing foremost contributions to overall heating value. Furthermore, higher hydrogen content determines and leads to a higher net caloric value (Demirbaş, 2007). Nitrogen (N), sulphur (S) and chlorine (Cl) contents are some of the main causes of air pollution from biomass combustion. A higher percentage of these elements generally results in a higher level of air contaminants being released. The average elemental composition of studied species biomass is presented in Table 3. We found that *Sida hermaphrodita* '*Energo*' biomass is characterized by an optimal content of carbon (46.03%) and hydrogen (5.94%), and very low content of nitrogen (0.32 %) and sulphur (0.08%), as compared with *Zea mays* stalks.

Stolarski et al. (2014) found that *Sida hermaphrodita* biomass contained 46.08-48.89% C, 5.46-5.80% H, 0.023-0.038% S. Howaniec and Smolinski (2011) reported the nitrogen and sulphur contents in the biomass of *Sida hermaphrodita* to be at rather low levels, i.e. 0.01% N and 0.04% S, while Šiaudinis et al. (2015) and Krička et al. (2017) determined nitrogen to be 0.75% and sulphur 0.17-0.29%, respectively. Kron et al. (2017) remarked that harvested biomass contained 40.94-43.74% C, 5.68-6.44% H, 0.77-1.62% N, 0.03-0.10 % S and 49.14-51.26% O. Bilandžija et al. (2018) mentioned that the *Sida hermaphrodita* biomass harvested in March contained 18.64% moisture, 1.94% ash, 50.08% C, 6.10% H, 0.65% N, 0.10% S.

Majlingova et al. (2021) reported that *Sida hermaphrodita* contained 43.74% C, 5.68% H, 0.77% N, 0.24 % S and 50.96% O.

Voicea et al., 2013, mentioned that corn stalks contained 1.8% ash, 50% C, 6.2% H, 0.6% N, 43.10% O, 0.9% S, but Antonenko et al. (2018) - 6.37% ash, 42.76% C, 5.36% H, 1.17% N, 39.83% O, 0.23% S, respectively.

Particle size distribution is one of the most important physical properties of solid biomass that is used in biofuel processing. Therefore, biomass size reduction and related particle physical property quantification are indispensible in investigating the biomass feedstock supply-conversion chain. Fractional composition of chopped dry stem mass is presented in Table 4. Having evaluated the fractional composition of chopped mass, we may see that the highest fraction of *Sida hermaphrodita* '*Energo*' mass accumulated on a sieve with holes of 3.15-8.00 mm diameter – 54.1%, and the lowest amount accumulated on a sieve with holes of 16.00-31.50 mm diameter – 13.0%. At the same time, the highest fraction of *Zea mays* – 50.3% –accumulated on a sieve with holes of 8.00-16.00 mm diameter.

Table 4. Particle size distribution of chopped dry stem mass from the studied species, %

Particle size	*Sida hermaphrodita*	*Zea mays*
<3.15mm	14.1	5.0
3.15-8.00 mm	54.1	24.2
8.00-16.00mm	18.7	50.3
16.00-31.50 mm	13.1	20.5

To enhance packing density of biomass and produce pellets and briquettes, for instance, biomass feedstock has to be ground into 3-8 mm particles before compacting the material into a denser product (Mani et al., 2006). Particle size reduction and its distribution is an important parameter used for handling, storage, conversion, dust control systems and the combustion of biomass solid fuels. In the case of Virginia mallow milled chaffs, the highest percentage of particles obtained by us was larger than 3 mm (30.1%), and the lowest – particles with a size of 1 mm (13.3%). Moreover, the proportion of 1-3 mm particles was relatively high in the case of milled corn chaffs (Table 5). This is probably an effect of the anatomical structure of *Sida hermaphrodita* stalks; the high level of pith microstructures and the fibre content in biomass influences the passage of particles through the sieve meshes.

Table 5. Particle size distribution of milled stem chaffs from the studied species, %

Particle size	*Sida hermaphrodita*	*Zea mays*
<5mm	3.0	0.5
4-5mm	8.9	1.4
3-4mm	18.2	10.4
2-3mm	34.2	34.3
1-2mm	21.9	32.0
1mm	13.3	21.4

One of the most economically advantageous methods of densification is pelletization. Thus, raw biomass can be converted into pellets with improved fuel quality, such as increased bulk density, and even shape and size. Pelletized fuels are also more consistent in their structure, which is beneficial for the automated fuel system in the corporate and individual boilers.

The physical and mechanical properties of biomass and pellets from *Sida hermaphrodita* '*Energo*' is illustrated in Table 5. Ash content is one of the main factors of biomass quality, since higher amounts of ash decrease the quality of fuels, especially solid ones. The Virginia mallow biomass is characterized by very low ash content (2.1%), lower than corn stalks (4.4%), excellent gross calorific value (19.73 MJ/kg), which is higher than that of corn stalks (17.84 MJ/kg), due to the total stem defoliation and higher concentrations of cellulose, hemicellulose and lignin. The bulk density of the *Sida hermaphrodita* '*Energo*' chopped chaffs reached 130 kg/m^3 and milled chaffs – 204 kg/m^3. The specific density of the pellets made from Virginia mallow milled chaffs reached 870 kg/m^3 and the bulk density – 570 kg/m^3, so, they had still lower density than corn pellets, perhaps because of the anatomical structure of this plant. Thus, most of the particles of *Sida hermaphrodita* milled chaffs were larger than 3 mm.

Table 6. Some physical and mechanical properties of biomass and pellets

Indices	*Sida hermaphrodita*	*Zea mays*
Ash content of biomass, %	1.87	4.40
Gross calorific value, MJ/kg	19.73	17.84
Density of chopped mass, kg/m^3	130	87
Density of milled chaffs, kg/m^3	204	165
Specific density of pellets, kg/m^3	870	1174
Bulk density of pellets, kg/m^3	570	701

Some authors mentioned various findings about the physical and mechanical properties of *Sida hermaphrodita* biomass and pellets. Nahm & Morhart (2018) reported average high heating values of 18.4 MJ/kg and low heating values of 16.1 MJ/kg. According the review of Cumplido-Marin et al. (2020), the energy value of stems ranged from 15.0 MJ/kg low heating values to 19.4 MJ/kg high heating values. Jankowski et al. (2019) noted an increase of the energy value with the age of the *Sida hermaphrodita* plantation, from 18.5 to 19.4 MJ/kg HHV. Jablonowski et al. (2017) mentioned that calculated values for the energy yield of biomass used as solid fuel varied from 19.2 to 19.6 MJ/kg HHV and 16.62 to 16.96 MJ/kg LHV. Siaudinis et al. (2015) reported that Virginia mallow pellets had an average density of 969.3 kg/m^3. Zajac, et al. (2017) remarked that *Sida* pellets contained 7.1% moisture, 2.9% ash, 82.69% volatile materials, 48.1 % C, 5.78% H, 0.42% N, 0.07% S, 19.08 MJ/kg HHV and 16.80 MJ/kg LHV. Jablonowski et al. (2020) mentioned that *Sida hermaphrodita* pellets contained 7.1% water, 3.59% ashes, 1.1% N, 0.04% S, and reached a density of 662 kg/m^3 and low heating values of 16.13–17.21 MJ/kg. Styks et al. (2020) reported that specific density of pellets from *Sida* raw material in grain size below 1 mm varied from 860 to 980 kg/m^3 depending on the compaction pressure and moisture content. Majlingova et al. (2021) reported that *Sida hermaphrodita* had 18.75 MJ/kg HHV and 13.35 MJ/kg LHV. Antonenko et al. (2018) remarked that corn stalks had 18.4 MJ/kg HHV or 17.1 MJ/kg LHV.

Biofuels such as bioethanol are an alternative to fossil fuels that is gaining popularity. Bioethanol is produced through sugar fermentation by some microorganisms. Second generation bioethanol produced from lignocellulosic biomass is attracting attention as an alternative energy source and it is currently a topic of great interest for researchers around the world. From structural point of view, lignocellulose is basically composed of secondary cell walls of structural and parenchymatous plant tissues. The structure of lignocellulose consists of cellulose, the skeleton of which is surrounded by hemicellulose and lignin. The contents of these components vary significantly depending on the plant species, type of biomass and harvesting period. The effective decomposition of lignocellulose biomass should be preceded by conversions of these polysaccharides into monosaccharides.

Table 7. The composition of cell walls and the theoretical ethanol potential of Sida hermaphrodita 'Energo' dry matter

Indices	*Sida hermaphrodita*	*Zea mays*
Acid detergent fibre, g/kg	687	499
Neutral detergent fibre, g/kg	928	749
Acid detergent lignin, g/kg	131	87
Cellulose, g/kg	556	417
Hemicellulose, g/kg	241	250
Hexose sugars, g/kg	99.0	75.1
Pentose sugars, g/kg	39.6	41.1
Theoretical ethanol potential, L/t	578	485

Analyzing the cell wall composition of dehydrated stems, Table 7, we could mention that the concentrations of structural carbohydrates in *Sida hermaphrodita* '*Energo*' substrate are much higher in comparison with *Zea mays* substrate. The analysis of lignocellulose composition suggested that the dry matter of the whole *Sida hermaphrodita*

'*Energo*' plants contained 556 g/kg cellulose, 241 g/kg hemicellulose and 131 g/kg acid detergent lignin, but corn stalks – 417 g/kg cellulose, 250 g/kg hemicellulose and 82 g/kg acid detergent lignin. The estimated content of monosaccharides in Virginia mallow biomass was 99.0 g/kg pentose sugars and 39.6 g/kg hexose sugars, but in corn stalks – 75.1 g/kg and 41.1 g/kg, respectively. The estimated theoretical ethanol yield from cell wall carbohydrates averaged 578 L/t in *Sida hermaphrodita* '*Energo*' substrate, as compared with 485 L/t in corn substrates.

Several literature sources describe the composition of cell walls in energy biomass and ethanol potential. Kricka et al. (2017) remarked that the contents of cellulose, hemicellulose, and lignin in *Sida hermaphrodita* biomass were about 40.1%, 27.2% and 26.4%, respectively. Majlingova et al. (2021) reported that *Sida hermaphrodita* contained 17.92% lignin, 36.30% cellulose and 44.00% hemicellulose. For sorghum crop, the theoretical ethanol potential ranged from 560 to 610 L/t of dry biomass (Goff et. al., 2010).

CONCLUSIONS

The substrate prepared from green mass of *Sida hermaphrodita* '*Energo*' contains about 160 g/kg CP, 84 g/kg ash, 371 g/kg ADF, 529 g/kg NDF, 60 g/kg ADL, 311 g/kg cellulose and 158 g/kg hemicellulose, and its biochemical biomethane potential averages 272 l/kg ODM.

The harvested Virginia mallow dry stalks contain 46.03% carbon, 5.94% hydrogen, 0.32% nitrogen, 0.08% sulphur, 1.87% ash, 556 g/kg cellulose, 241 g/kg hemicellulose and 131 g/kg acid detergent lignin.

The theoretical ethanol yield from structural carbohydrates averages 578 L/t in Virginia mallow substrate and 485 L/t – in corn substrate.

The gross calorific value of the biomass consisting of Virginia mallow stalks is on average 19.73 MJ/kg, but – of corn stalks 17.8 MJ/kg.

The specific density of densified solid fuel – pellets – made from *Sida hermaphrodita* '*Energo*' milled chaffs reached 870 kg/m^3 and the bulk density – 570 kg/m^3.

Sida hermaphrodita cv. '*Energo*' can serve as multi-purpose feedstock for renewable energy production.

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MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *Longidorus distinctus* (Nematoda: Longidoridae) FROM ROMANIA

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Abstract

Genus Longidorus Micoletzky, 1922 comprises ectoparasitic nematode species feeding on plant roots. Some of them have considerable economic importance directly as plant pests or indirectly as vectors of nepo-viruses. During an extensive study on longidorids in Romania Longidorus distinctus Lamberti, Choleva and Agostinelli, 1983 was recovered from vineyards (Vitis vinifera L.) in Alba county, alfalfa (Medicago sativa L.) in Prahova county and fruiting shrubs in Arges county. The two populations from Prahova and Arges counties were characterised morphometrically. An integrative approach (morphology, morphometrical and DNA sequencing data) was used to identify and characterise the population from Alba county. The phylogenetic position of the species and the D2-D3 28S rDNA sequence dissimilarity with the closest species were discussed. All studied populations have been compared with other European populations; described from Bulgaria, hitherto L. distinctus has been reported from Serbia, Slovakia, Ukraine, Russia and Poland from arable land and natural habitats. It represents a new geographical record for Romania.

Key words*: 18S rDNA, 28S rDNA D2-D3, COI mtDNA.*

INTRODUCTION

Genus *Longidorus* Micoletzky, 1922 includes a great number of ectoparasitic nematode species that have a broad host range of herbaceous and woody plants within agriculture, horticulture and forestry. Over 180 species belonging to the genus *Longidorus* are considered valid (EPPO, 2020). Currently only three species have been reported from Romania: *Longidorus elongatus* (de Man, 1876) (Popovici, 1973); *Longidorus euonymus* Mali & Hooper, 1974 (Groza et al. 2014) from the rhizosphere of barley (*Hordeum vulgare* L.), strawberry (*Fragaria* x *ananasa* Duch), blackberry (*Rubus fruticosus* L.) cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.) and plum (*Prunus domestica* L.); *Longidorus piceicola* Lišková, Robbins & Brown, 1997 (Groza et al., 2017) from the rhizosphere of *Larix decidua* Mill. and deciduous trees (*Quercus* sp., *Tilia* sp., and *Fraxinus* sp.).

Longidorus distinctus Lamberti, Choleva and Agostinelli, 1983 was originally described from Bulgaria (Lamberti et al., 1983) and recorded from other European countries: Serbia (Barsi, 1989, Krnjaic et al., 1999, Barsi & Lamberti, 2003), Poland (Szczygiel & Zepp, 2004, Kornobis & Sobczyńska, 2017), Russia (Subbotin et al., 2014), Slovakia (Lišková, 2007), Ukraine (Romanenko, 1998).

MATERIALS AND METHODS

Soil samples were collected from the rhizosphere of vine (*Vitis vinifera* L.) from Crăciunelul de Jos, Alba county, (46°10'26.46"N, 23°51'0.05"E); alfalfa (*Medicago sativa* L.), Teleajen, Prahova county, 45°56'4.09"N, 27°18'1.30"E and fruiting shrubs from Mărăcineni, Argeș county, (44°54'5.72"N, 24°48'25.52"E).
Nematodes were isolated from soil samples using Oostenbrink elutriator and Baermann

funnels. Recovered specimens were heat killed at 55°C for two minutes, fixed in a 4% formalin/1% glycerol mixture, processed to anhydrous glycerol (Seinhorst, 1959), and mounted on microscope slides. Photographs were taken using an Axio Imager.M2-Carl Zeiss compound microscope with a digital camera (ProgRes C7) and specialised software (CapturePro Software 2.8). Measurements were made using an Olympus BX41 light microscope, a digitising tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale, AZ, USA), and computer Digitrak 1.0f programme, (Philip Smith, Scottish Crop Research Institute, Dundee, UK).

DNA extraction, amplification and sequencing

DNA extraction of six juvenile specimens from Crăciunelul de Jos population was performed according to the mammalian protocol of the High Pure PCR template preparation kit (Roche, Switzerland). Nematodes were crushed in Tissue Lysis Buffer using a micro-pestle prior to DNA extraction. Three gene regions, the D2-D3 expansion segments of 28S rRNA gene (large ribosomal subunit), partial 18S rRNA gene (small ribosomal subunit) and the partial mitochondrial cytochrome c oxidase subunit 1 gene (COI) were amplified following the nematode section in the European and Mediterranean Plant Protection Organization (EPPO) DNA barcoding standard (EPPO, 2016). PCR products were cycle sequenced in both directions with the BigDye terminator kit 3.1 (Applied Biosystems, United States) and sequenced on a 3500 genetic analyzer (Applied Biosystems, United States). Trace data were analysed using Geneious v6.1 (BioMatters, New Zealand). Consensus sequences were constructed from the sequence reads, and amplification primers and terminal low quality sequence data were trimmed from the consensus sequence. BLASTn similarity search tool was used to compare each gene fragment with those of other nematode species deposited in the GenBank database. The homologous sequences of D2-D3 28S rDNA nearest to those of *L. distinctus* were aligned using MAFFT algorithm at the GUIDANCE2 Server (http://guidance.tau.ac.il/ (Sela et al., 2015) and manually trimmed and edited using Mega 6 (Tamura et al., 2013). Pairwise sequence identities/similarities were computed using the Sequence Manipulation Suite online (http://www.bioinformatics.org/sms2/) (Stothard, 2000). The analysis involved 17 nucleotide sequences with a total of 638 positions in the final dataset including gaps. The sequences have been deposited in GenBank with the following accession numbers: MW701430 (28S rDNA); MW699618 (18S rDNA), and MW699614 (COI).

RESULTS AND DISCUSSIONS

Longidorus distinctus Lamberti, Choleva and Agostinelli, 1983
Figures 1- 3
Morphometric data of specimens are presented in Table 1 and 2.
Description
Female (Crăciunelul de Jos population). Body posture ventrally curved in the shape of a "C" to a spiral shape, tapering strongly toward posterior end. Head region expanded, slightly set off, anteriorly somewhat flattened. Cuticle thin, 2–2.5 μm thick at postlabial region, 2 μm along the body and 3 μm on tail posterior to anus. Amphidial fovea hardly visible, pouch like, asymmetrically bilobed, with code E3 according to Chen et al. (1997) and type 4 according to Decraemer & Coomans (2007), amphidial aperture assumed to be a minute pore. Lateral chord with glandular bodies. Guiding ring 4 μm wide, odontostyle very slender, 1 μm wide at base, odonthophore slightly swolen at base. Nerve ring behind odontophore base, situated at a distance of 161-183 μm, from anterior end. Pharyngo-intestinal valve heart-shaped to bluntly rounded, 7.5-11 μm long and 9-10 μm wide. Normal arrangement of pharyngeal glands: dorsal opening (DO) and dorsal nucleus (DN) situated at 13.3±1.0 (12.0-14.8)% and 26.8±2.6 (24.7-30.5)% of pharyngeal bulb length, respectively (n=5), nuclei of the left and right ventrosublateral glands situated at 51.2±1.3 (48.5-52.7)% and 50.5±1.2 (48.4-52.2)% (n=7), respectively; opening of the ventrosublateral glands at 85.8±1.5 (82.7-87.0)% of the same distance (terminology following Loof and

Coomans, 1972). Nucleus of the dorsal gland slightly smaller than nuclei of the ventrosublateral glands 1.5-2 and 2.5-3 µm diam. respectively. Vagina extending to *ca.* half the corresponding body width. *Pars distalis vaginae* 11-12 µm long; *pars proximalis vaginae* 12-17 µm long. Uteri of almost equal length, bipartite, the distal part narrower and with granular structure; anterior uterus 151.8±6.5 (140-163), posterior uterus 148.2±6.3 (141-161) µm long, respectively;

Table 1. Measurements of *Longidorus distinctus,* Crăciunelul de Jos population (mean ± standard deviation, with range), in micrometers, except body length (in mm)

Character	**Females**	**J1**	**J2**	**J3**	**J4**
N	n=9	n=9	n=2	n=6	n=6
L	4.8±0.34 4.2-5.3	1.0.2±0.072 0.9-1.1	1.5, 1.7	2.4±0.23 2.1-2.7	3.4±0.478 2.9-4.0
A	109.5±5.6 98.6-117.5	56.2±4.4 51.0-65.8	64.8, 61.9	74.8±8.4 62.1-82.8	87.7±7.5 77.2-94.7
B	13.0±1.1 11.6-14.7	4.9±1.0 3.6-6.1	6.1, -	9.6±1.8 7.8-12.6	12.7±3.1 8.9-17.1
C	78.1±9.4 64.6-91.3	22.9±3.4 19.6-29.6	27.7, 32.1	38.5±2.9 35.1-43.9	55.2±6.0 48.9-63.5
c’	2.1±0.2 1.8-2.4	3.6±0.3 2.9-4.0	- , 2.8	2.9±0.1 2.7-3.0	2.5±0.3 2.1-2.9
V (%)	48.9±2.7 44.9-52.9	-	-	-	-
G1 (%)	6.8±0.6 6.1-8.0	-	-	-	-
G2 (%)	6.9±0.9 5.4-8.0	-	-	-	-
D	2.5±0.1 2.3-2.6	2.6±0.2 2.4-3.0	2.3, 2.3	2.4±0.3 2.1-2.7	2.3±0.1 2.2-2.5
d’	1.6±0.1 1.5-1.7	1.8±0.2 1.5-2.1	1.6, 1.6	1.6±0.2 1.4-1.9	1.6±0.1 1.5-1.8
Anterior end to guiding ring	29.0±1.1 27.5-30.5	17.4±0.9 16-19	19, 20	22.1±1.7 20-24	24.6±1.0 23-25
Odontostyle	75.7±2.0 72-79	46.7±1.4 45-48	50.5, 50	59.0±0.7 58-60	65.9±2.0 64-69
Replacement odontostyle	-	49.6±1.7 47.5-53	57, 58	65.3±1.4 64-67	75.3±1.2 74-77
Odontophore	55.7±4.5 50-64	29.6±3.6 24-36	43.5, 41	43.9±2.0 41-46	53.6±7.0 47-63
Pharynx length	370±23.4 327-409	212.9±38.9 167.5-281.5	246, -	253.4±34.6 204-306.5	277.4±39.4 236-335
Tail	61.9±4.1 56-68	45.0±6.0 33-50	54.5, 54	61.9±5.2 54-68	62.3±7.4 54-74
Length of hyaline part	13.6±1.8 11-16	3.8±1.0 3-6	5.5, 6	6.8±1.0 5-8	10.5±1.8 8-12
Body diameter at: - lip region	11.6±0.5 11-12	6.7±0.3 6-7	8, 9	9.4±0.7 8.5-10	10.8±0.3 10-11
- guiding ring	18.5±0.6 17.5-19	11.9±0.6 11-13	13.5, 14	15.3±0.9 14-16.5	17.2±1.1 16-19
- base of pharynx	37.4±2.1 33.5-41	17.4±1.5 14-20	22, 25	28.4±3.1 24-32	32.5±2.6 29-37
- mid-body/at vulva	43.9±2.7 38-47	18.1±1.4 15-20	23, 28	32.3±5.7 25-41	39.5±7.6 31-53
- anus	29.8±1.8 27-32	12.5±1.0 10-13.5	- , 19	21.6±2.1 18-23.5	25.4±2.4 22-29
- hyaline part	10.5±0.6 10-11	4.0±0.5 3-5	5, 5.5	5.6±0.5 5-6	7.3±1.2 6-9

Table 2. Measurements of *Longidorus distinctus* (mean ± standard deviation, with range), in micrometers, except body length (mm)

Localities	Teleajen					**Mărăcineni**
Character	**Females**	**J1**	**J2**	**J3**	**J4**	**Females**
N	18	10	10	6	23	3
L	4.3±0.34 3.6-4.8	1.1±0.05 0.9-1.1	1.4±0.11 1.3-1.7	2.06±0.12 1.9-2.2	3.0±0.31 2.5-3.6	4.4, 4.8, 4.1
A	102.2±10 77.5-114.8	60.0±2.5 54.6-63.6	65.3±4.1 59.9-73.1	76.6±3.8 71.7-82.5	90.6±7.5 74.3-103.8	105, 112, -
B	11.4±1.2 9.1-13.4	5.1±1.0 2.3-5.8	6.1±0.5 5.5-7.4	7.3±1.0 6.1-9.1	9.5±1.0 7.3-11.9	11, 12, -
C	80.0±13 64.5-120.5	24.3±1.2 21.7-25.5	30.2±3.0 26.1-36.0	38.9±2.7 34.7-41.8	54.6±6.6 44.2-67.9	74, 92, 79
c’	2.0±0.2 1.4-2.3	3.5±0.2 3.3-3.8	3.0±0.2 2.7-3.3	2.7±0.2 2.5-3.0	2.3±0.2 1.9-2.6	2, 1.7, 1.8
V (%)	45.1±1.6 42.5-47.5	-	-	-	-	48.4, 45.2, 51.8
G1 (%)	6.8±0.8 5.5-8.4	-	-	-	-	7.1, 6.2, 6.8
G2 (%)	6.4±0.7 5.0-8.0	-	-	-	-	7.3, 5.5, 6.6
D	2.6±0.2 2.4-3.0	2.2±1.0 2.8	2.5±0.2 2.2-2.9	2.4±0.1 2.3-2.4	2.1±0.2 1.8-2.5	2.5, 2.5, 2.3
d'	1.7±0.2 1.5-2.4	1.5±0.8 2.1	2.0±0.2 1.6-2.4	2.0±0.3 1.7-2.6	1.9±0.1 1.6-2.2	1.6, 1.5, 1.5
Anterior end to guiding ring	29.9±1.1 28-31	17.3±0.8 16-19	19.1±0.9 18-21	22.5±1.0 21-24	26.4±1.1 24.0-28.5	30, 30, 28
Odontostyle	77.6±5.4 68-86	49.5±3.1 44-54	51.0±2.745- 54.5	62.4±1.8 60-65	69.9±3.4 63-78	83, 84, 81
Replacement odontostyle	-	52.1±2.3 47-55	61.6±3.1 57-67	70.7±2.0 67-72	79.2±2.7 75-86	
Odontophore	52.6±5.5 41-63	32.1±3.9 24-36	37.5±3.7 31-44	44.9±6.9 37-57	48.9±3.4 43-58	56, 60, -
Pharynx length	382.2±25.6 342.8-423	227.2±76.8 184-443	238.6±17.9 209.4-264.3	288.9±35.7 226.5-326	323.4±29.9 279-408	
Tail	54.7±5.6 38-63	44.7±1.3 42-46	48.4±3.2 43-53	53.3±1.1 52-55	55.7±4.6 46-66	60, 52, 53
Length of hyaline part	14.3±1.8 11-18	4.4±0.8 3-5	5.2±0.8 4-7	5.3±0.8 4-7	8.9±2.8 4-15	12, 11, 12
Body diameter at: - lip region	11.4±0.9 10-13	7.1±0.5 7-8	8.6±0.7 8-10	9.5±0.6 9-10	10.4±0.8 9-12	12, 12, 12
- guiding ring	19.3±2.7 18-30	11.9±0.5 11-13	13.8±0.5 13-14	15.2±0.4 15-16	17.4±2.0 16-26	20, 19, 19
- base of pharynx	35.2±2.5 32-41	18.4±0.7 17-19	22.5±1.3 21-25	26.0±1.5 24-28	29.9±2.9 19-34	36, 33
- mid-body/at vulva	42.5±4.2 38-55	18.1±1.1 16-20	22.4±1.8 19.5-26	27.1±2.2 24-30	33.5±3.1 29.5-38.7	42, 43
- anus	27.5±2.2 23-33	13.2±0.8 12-15	16.1±0.8 15-17.5	19.4±0.8 19-21	24.6±1.7 21-28	29, 30, 28

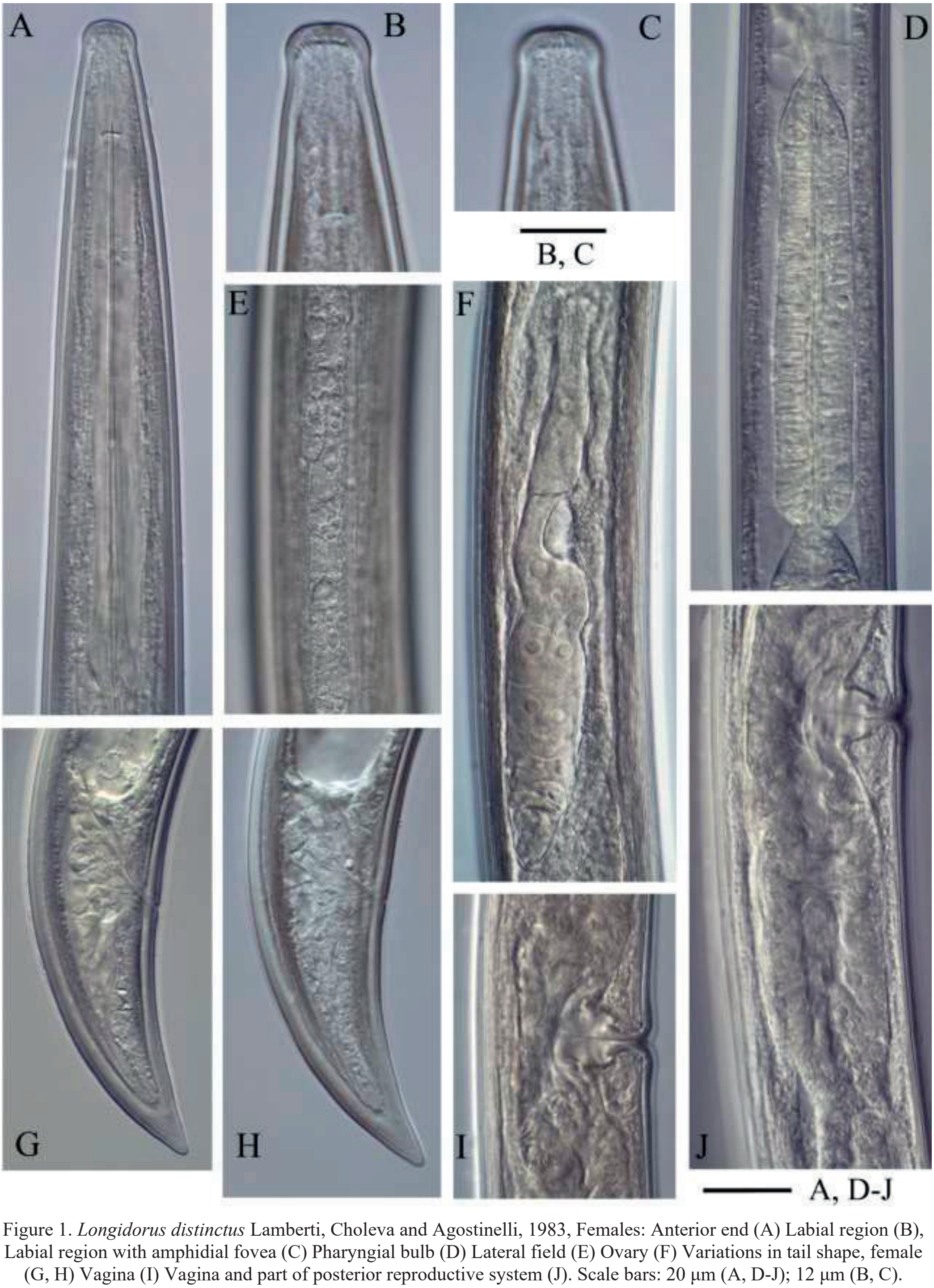

Figure 1. *Longidorus distinctus* Lamberti, Choleva and Agostinelli, 1983, Females: Anterior end (A) Labial region (B), Labial region with amphidial fovea (C) Pharyngial bulb (D) Lateral field (E) Ovary (F) Variations in tail shape, female (G, H) Vagina (I) Vagina and part of posterior reproductive system (J). Scale bars: 20 µm (A, D-J); 12 µm (B, C). (Crăciunelul de Jos population)

Figure 2. *Longidorus distinctus* Lamberti, Choleva and Agostinelli, 1983, Anterior end of first (J1) to fourth (J4) stage juveniles (A–D), Anterior end of female (E) Tail of first to fourth juvenile stages (F–I) Tail of female (J). Scale bars: 20 μm. (Crăciunelul de Jos population)

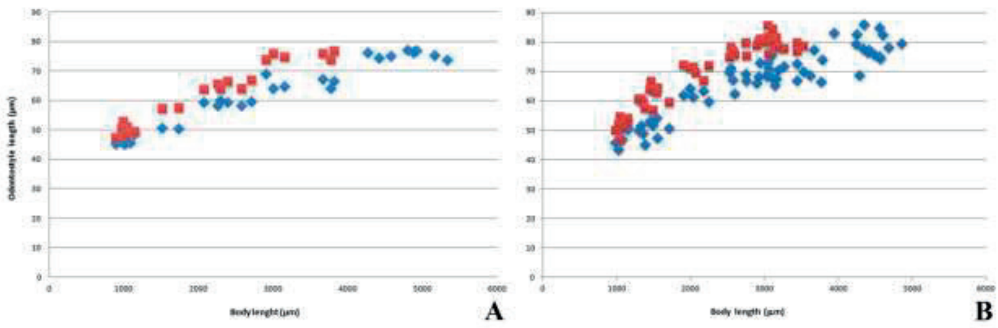

Figure 3. Scatter plot of odontostyle and replacement odontostyle against body length of *Longidorus distinctus,* A) Crăciunelul de Jos population and B) Teleajen population.

well-developed sphincter (8-10 µm) between uterus and weakly developed *pars dilatata oviductus*, *pars dilatata* and uteri not containing sperms. Rectum 26-27 µm or about 0.8-1.0 of body diameter at anus. Tail conical, dorsally convex, ventrally slightly concave, terminus narrowly rounded. Two pairs of lateral pores.

Male. Not found

Juveniles. Morphometrics obtained from juvenile specimens, and of the relationship between the lengths of their functional and replacement odontostyles, and body lengths revealed the presence of four juvenile stages (Figure 3). Habitus does not change significantly during the juvenile development being J shaped to ventrally curved, tail of the first stage juvenile conoid with rounded terminus, often subdigitate; body width at anus level gradually becoming greater and **c'** values becoming progressively lower in subsequent stages.

The codes for identifying *L. distinctus* from Romania when using the polytomous key by Chen et al. (1997) and Peneva et al. (2013) are: A23, B12, C23, D3, E3, F23, G2, H56, I2, J1, K6. Thus, we propose an amendment in the code D originally introduced as D2, since the shape of anterior region of *L. distincus* fits more the code D3 (see Figure 2 I in Chen et al. 1997 as well as the illustrations and descriptions provided by the different authors mention above).

The morphometrics of *L. distinctus* specimens from Romania in general agree with the original (Lamberti et al. 1983) and subsequent descriptions of the species from Bulgaria, Serbia, Slovakia and Poland (Barsi, 1989; Peneva & Choleva, 1992; Krnjaic et al., 1999, Lišková, 2007; Kornobis & Sobczyńska, 2017). Specimens from Slovakia had somewhat longer body compared to the average (av.) body lengths of the type population and our data (av. 5.1 (4.3-5.6) mm *vs* av. 4.6 (3.6-5.3) mm and av.4.8 (4.2-5.3) mm, av. 4.3 (3.6-4.8) mm, while those from Serbia were with a shorter body (L=av. 4.1 (4.09-5.35) mm). Also, the specimens from Slovakia were with highest **a** value when compared with the type population and materials from Romania (av. 127.5 (108-137) *vs* av. 100 (85-127) and av. 109.5 (98.6-117.5), av. 102.2 (75.5-114.8). Studied populations differed slightly in odontostyle length when compared to the type population (av. 75.7 (74-79) and 75.7 (68-86) *vs* av. 80 (71-84) µm; specimens from Kolarovo (Bulgaria) had longer (84-103 µm) while those from Serbia - shorter (av. 74.8 (70-81) odontostyles (Lamberti et al., 1997; Krnjaic et al., 1999). Further, specimens from Romania had long and slender pharyngeal bulb (93-112x16.5-19 µm) *vs* 80-90x20 µm in the type population and materials from Serbia (85-106x18-21) and Poland (85-96x18-20 µm). Some of the populations of *L. distinctus* are characterised with slightly longer tail length (52-68 µm): the type and the population from Petrich, Bulgaria (Lamberti et al., 1997), Crăciunelul de Jos specimens and those from, Serbia (Barsi & Lamberti, 2003) and Poland (Kornobis & Sobczyńska), av. 58 in type population and avs. 59, 61.9, 61.4, 57 and 57.2 µm, respectively. The tail length in specimens from Kolarovo (Bulgaria) (Lamberti et al., 1997), Serbia (Krnjaic et al., 1999), Slovakia (Lišková) and Telejean (Romania) was somewhat shorter, avs. 52.6, 52.9, 52, 54.7 µm, respectively, and varied from 38 to 65 µm.

Sequence analyses and phylogenetic relationships

The sequencing of the D2-D3 expansion segments of 28S rDNA, the near complete 18S rDNA and the partial COI mtDNA of *L. distinctus* yielded single fragments of 1678, 879 and 393 bp, respectively. The BLASTn search using D2-D3 rDNA sequence revealed 100% identity to sequences of *L. distinctus* from three populations: Slovakia (EF654539, Liskova, 2007), Russia (KF242317, Subbotin et al., 2014) and Poland (KY513282, Kornobis & Sobczyńska, 2017) and 99.86% similarity to another population from Poland (KY513283, Kornobis & Sobczyńska, 2017). In two previous D2-D3 28S rDNA phylogenetic reconstructions (Groza et al., 2017, Cai et al., 2020), the sequence of *L. distinctus* from Slovakia grouped in a well-supported clade (PP=0.9–1.0) with two sequences of *L. juvenilis* Dalmasso, 1969 from Slovakia and Slovenia (AY601579 and DQ364599) and showed close relationships with populations of *L. aetnaeus* Roca, Lamberti, Agostinelli & Vinciguerra, 1986 from Russia and USA and *L. leptocephalus* Hooper, 1961 from Slovenia and UK (Figure 8, Groza et al., 2017). The D2-D3 rDNA sequence dissimilarity between *L. distinctus* and the phylogenetically closest species was 5.3-5.8% (*L. juvenilis*), 5.9-6.5% (*L. aetnaeus*) and 6.21% (*L. leptocephalus*). One nucleotide difference was revealed when comparing 18S rDNA and COI mtDNA sequences of the Romanian population with the corresponding sequences of the Russian population (KF242290, 823 nb length and KY81667, 317 nb length, respectively). The phylogenetic reconstruction based on 18S rRNA gene region showed different phylogenetic relationships of *L. distinctus* (Cai et al., 2020) probably due to the absence of 18S rDNA sequences of *L. juvenilis* and *L. leptocephalus*, and shorter sequence length of *L. aetnaeus*. BLASTn search of COI sequences revealed much lower similarity (≤80%) to sequences with other *Longidorus* species.

CONCLUSIONS

Longidorus distinctus is distributed in Central and south-eastern Europe, reaching Poland to the north, being the most widespread in Bulgaria. It has been recovered more often in agrosystems associated with various crops (tobacco, alfalfa, maize, wheat, grapevine, orchard trees, small fruits, rose, tulip, grapevine, forest nursery seedlings) (Lamberti et al., 1983; Barsi, 1989, Barsi & Lamberti, 2003 etc.) and rarely with natural vegetation (Subbotin et al., 2014; Kornobis & Sobczyńska, 2017).
This species is reported and characterised for the first time from Romania in association with cultivated plants. The studied populations did not differ significantly in morphology and genetically from other materials obtained in different parts of its range. Phylogenetically, *L. distinctus* is close to other three species - *L. juvenilis*, *L. aetnaeus* and *L. leptocephalus*, which are also similar in their morphology. Among them only *L. juvenilis* develops through 3 juvenile stages.

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CHARACTERIZATION OF LEAF GEOMETRY AT *Datura stramonium* L. BY IMAGING ANALYSIS

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Abstract

Datura stramonium L. is a species with different active principles, it has toxicity, but it has found certain uses in the pharmaceutical, medicinal, or food field. In certain pedoclimatic conditions it is present in agricultural crops, as a weed. The study analyzed the geometry of the leaves in the species Datura stramonium L. A set of leaves was taken randomly from different specimens of mature plants. The leaves were scanned and analyzed in terms of dimensional parameters (L - length, w - width, Per - perimeter), leaf surface (SLA - scanned leaf area; MLA - measured leaf area), leaf geometry (D - fractal dimension). The experimental data set presented statistical safety, according to the ANOVA test (F> Fcrit, p <0.001, for alpha = 0.001). Very strong and strong correlations were recorded between foliar parameters and SLA, MLA and D respectively (eg r = 0.930 between MLA and L; r = 0.969 between MLA and w; r = 0.946 between Per and SLA; r = 0.933 between D and SLA). Polynomial equations described the interdependence relations between foliar parameters and SLA, MLA and D, in statistical safety conditions (p<0.001).

Key words: *Datura stramonium, fractal geometry, imaging, leaves.*

INTRODUCTION

Datura stramonium L. is an annual species in the Solanaceae family. The species is widespread on the globe, in different ecoclimates (Akbar, 2020). The whole plant (root, stem, leaves, flowers) is toxic, with variations in the level of toxicity depending on the organs of the plant (Trancă et al., 2017).

The herb of *Datura stramonium* L. presents a series of active principles with potential for use in pharmacy and medicine (Li et al., 2012; Soni et al., 2012; Aboluwodi et al., 2017).

The content of active principles in *Datura* was analyzed in the conditions of the plant's relationship with influencing factors, soil, water, nutrients, stress factors etc. (Ali, 1991; Moreno-Pedraza et al., 2019).

Based on the content of bioactive compounds, *Datura stramonium* has been studied as a potential allelopathic effect, in relation to different plant species from spontaneous flora and agricultural crops (Pacanoski et al., 2014).

At the same time *Datura stramonium* L. occurs in different crops, and is approached as a weed (Saayman-duToit, 2000).

The ecological plasticity and the relation of *Datura* plants with ecophysiological factors, with other natural plant species, with nutrients and composition in minerals were studied (Benvenuti and Macchia, 1997; Bhattacharjee et al., 2004; Castillo et al., 2014; Camargi et al., 2017). Studies have been conducted on competition relations between *Datura* plants and different cultivated plant species (Cavero et al., 2002; Karimmojeni et al., 2010).

The effect of different herbicides on *Datura* plants was also analyzed, in order to control weeds in agricultural crops (Sakadzo et al., 2018).

The present study analyzed the species *Datura stramonium* L. to describe models of leaf surface determination and to describe the fractal geometry of the leaves.

MATERIALS AND METHODS

Biological material was represented by the species *Datura stramonium* L. Leaf samples were taken from various mature plants, from the agricultural area adjacent to Timisoara, Timis County.

To obtain the values of dimensional parameters, such as length (L) and width (w), the leaves were measured with an accuracy of ± 0.5 mm.

The leaves were scanned in a 1:1 size ratio. Leaf images were analyzed to obtain datas regarding perimeter (Per) and scanned leaf area (SLA).
The fractal geometry of the leaves was analyzed on binarized images, Figure 1, by the box-counting method (Voss, 1985).

Figure 1. *Datura stramonium* L. leaf sample (binarized image representation)

Experimental data were analyzed to assess the presence of variance and data safety. Also, correlations between determined foliar parameters, interdependence relations, and prediction relations were analyzed. ANOVA test, correlation analysis, regression analysis were used. Correlation and regression coefficients (r, R^2), parameter p and RMSEP were used as parameters for estimating the statistical safety of the results.
ImageJ (Rasband, 1997), PAST (Hammer et al., 2001), and Wolfram Alpha (2020) softwares were used for image analysis and statistical data processing.

RESULTS AND DISCUSSIONS

From the determinations made at the leaf samples, *Datura stramonium* L. species, different values were registered for the leaf length L = 6.90-12.40 ± 0.147 cm, and leaf width w = 4.90-9.70 ± 0.109 cm, for the leaf perimeter Per = 23.273-51.978 ± 0.640 cm, for the scanned leaf area SLA = 17.21-67.38 ± 1.088 cm^2 and for the measured leaf area MLA = 17.25 - 60.14 ± 0.998 cm^2 respectively. The graphic distribution is presented in Figure 2.

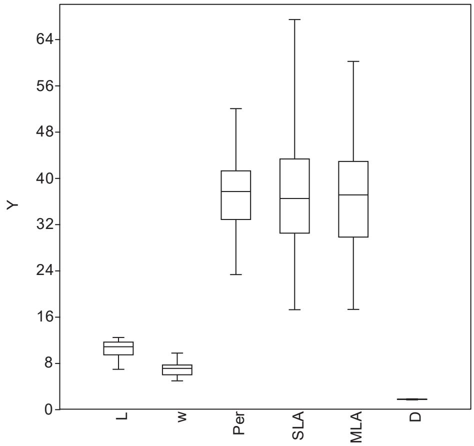

Figure 2. Graphical representation as box-plot type, of the values of the studied foliar parameters of the species *Datura stramonium* L.

The statistical safety of the experimental data and the presence of variance in the data set were evaluated by the ANOVA Test (F> Fcrit; p <0.001), for Alpha = 0.001. The experimental data, in the case of the studied leaf parameters, had a normal distribution.
A non-destructive method, very accessible for determining the leaf area is based on the parameters of the leaves (length - L, width - w). The relationship between L and w leads, in the case of leaves, most frequently to a rectangular geometric surface. The leaf falls within the respective geometric surface, but the relationship requires a correction to render only the actual surface of the leaf. In this sense, a specific correction factor (CF) is required. In order to find out the optimal value of the CF, the model proposed by the Sala et al. (2015) was used. The correction factor has subunit values (CV<1). In the present study, a narrow range (0.45, 0.55) was identified by preliminary calculations. During this interval, calculations were made with a variation of 0.1 units of CF to find the MLA (measured leaf area), relation (1).

$$MLA = L \cdot w \cdot CF \qquad (1)$$

The MLA values obtained by calculation were compared with the SLA values, and at CF = 0.50 the minimal errors means (MEM) was registered. This confirmed that the value 0.50 represented the optimal value for CF, Table 1, Figure 3.

Table 1. MLA values in relation to CF for *Datura stramonium* L. leaves

CF values	SLA	MLA	MEM	RMSEP
0.45		32.887	-3.334	4.55600
0.46		33.618	-2.603	3.98023
0.47		34.349	-1.872	3.47484
0.48		35.080	-1.141	3.07473
0.49		35.811	-0.410	2.82497
0.5	**36.221**	**36.542**	**0.321**	**2.76661**
0.51		37.272	1.051	2.91116
0.52		38.003	1.782	3.23153
0.53		38.734	2.513	3.68209
0.54		39.465	3.244	4.22136
0.55		40.196	3.975	4.81966

The RMSEP statistical parameter, relation (3), also confirmed that the value CF = 0.50 represented the optimal value for the correction factor (RMSEP = 2.76661, for CF = 0.50). The RMSEP values for the series of tested CF values, are presented in Table 1.

$$\mathrm{RMSEP} = \sqrt{\frac{1}{n}\sum_{j=1}^{n}\left(y_j - \hat{y}_j\right)^2} \qquad (2)$$

Corresponding to CF = 0.50 value found, and of the measured leaf parameters (L and w), was calculated the value of the MLA (measured leaf area), Table 2. For CF = 0.50 was found MLA = 36.542 cm^2, with MEM = 0.321.

The leaves of the species *Datura stramonium* L. can be described as simple leaves, elliptical in shape, with toothed edges, with alternating arrangement on the plant. Fractal analysis, the box-counting method, was used to describe the geometry of the leaves (Voss, 1985).

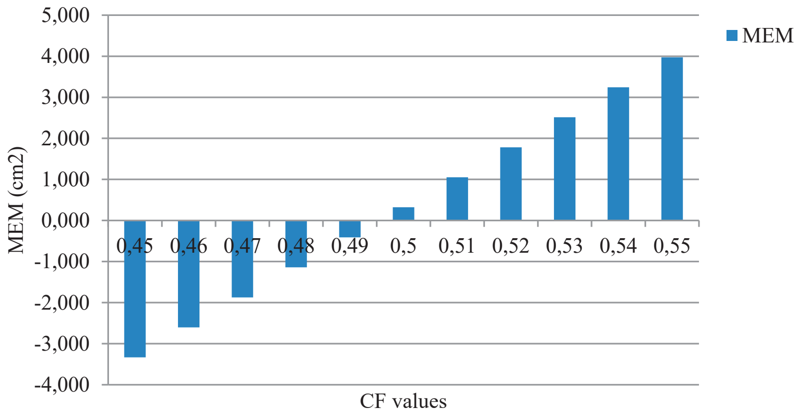

Figure 3. Graphic distribution of MEM, in the calculation of MLA based on CF values, leaf samples *Datura stramonium* L.

The binarized images of the leaf samples were analyzed, in conditions of high statistical safety, R^2 for D = 0.997, SE = 0.004, Table 1.

The average value of the fractal dimension D that described the geometry of the leaves at *Datura stramonium* L. was D = 1.707.

Very strong and strong correlations were recorded between foliar parameters and SLA, MLA and D respectively (eg. r = 0.930 between MLA and L; r = 0.969 between MLA and w; r = 0.946 between Per and SLA; r = 0.933 between D and SLA).

The regression analysis highlighted the close relationship between the leaf parameters L, w and SLA in the variation of the fractal geometry of the leaves, respectively in the definition of the fractal dimension (D).

The fractal dimension D, depending on the foliar parameters L and SLA, was described by equation (3), in conditions of statistical safety of the equation, according to R^2 = 0.999, p <0.001. According to the ANOVA test and the values of the coefficients of equation (3) presented statistical certainty (p << 0.001 for a; p = 0.0408 for b; p << 0.001 for c; p <0.001 for d; p = 0.0011 for e). The 3D graphic distribution is presented in figure 4, and the distribution in the form of isoquants is shown in figure 5.

$$D_{(L,SLA)} = ax^2 + by^2 + cx + dy + exy + f \qquad (3)$$

where: $D_{(L,SLA)}$ - fractal dimension, in relation to L and SLA

x – L; y – SLA;
a, b, c, d, e, f – coefficients of the equation (3);
a= -0.0298384699529701;
b= -0.000190689869023275;
c= 0.445520607743716;
d= -0.0353362346248073;
e= 0.00490581157233495;
f= 0.

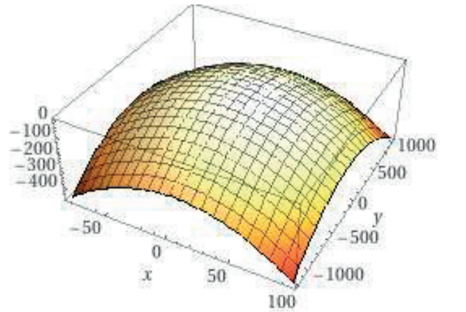

Figure 4. 3D graphical representation of the distribution of the fractal dimension D according to L and SLA, *Datura stramonium* L.

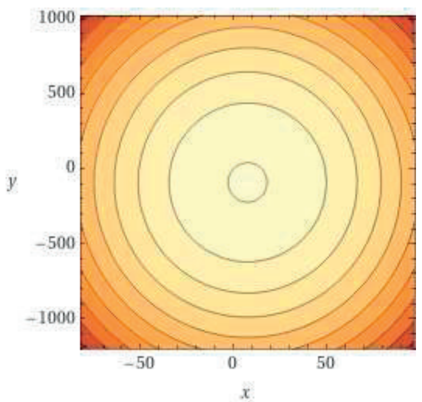

Figure 5. Graphic representation as isoquant of the distribution of the fractal dimension D according to L and SLA, *Datura stramonium* L.

The variation of the fractal dimension D depending on the foliar parameters w and SLA, was described by equation (4), in conditions of general statistical safety of the equation, according to $R^2 = 0.999$, $p < 0.001$. According to the ANOVA test and the values of the coefficients of equation (4) presented statistical safety ($p \ll 0.001$ for a; $p = 0.00379$ for b; $p \ll 0.001$ for c; $p < 0.001$ for d; $p < 0.001$ for e). The 3D graphic distribution is presented in figure 6, and the distribution in the form of isoquants is presented in Figure 7.

$$D_{(w,SLA)} = ax^2 + by^2 + cx + dy + exy + f \qquad (4)$$

where: $D_{(w,SLA)}$ - fractal dimension in relation to w and SLA

x – w; y – SLA;
a, b, c, d, e, f – coefficients of the equation (4);
a= -0.0993837006585064;
b= -0.000777662962919886;
c= 0.768644952854588;
d= -0.0549306446216847;
e= 0.016679744981095;
f= 0.

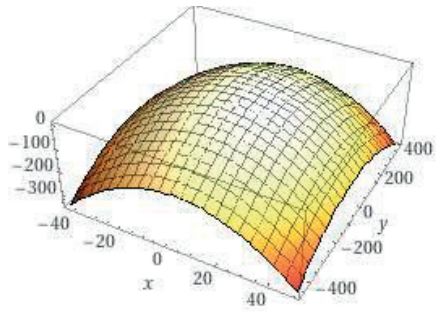

Figure 6. 3D graphical representation of the distribution of the fractal dimension D according to w and SLA, *Datura stramonium* L.

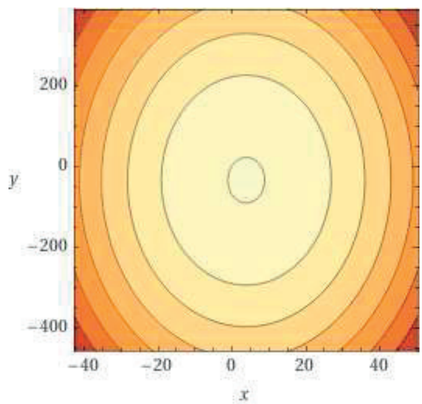

Figure 7. Graphic representation as isoquant of the distribution of the fractal dimension D according to w and SLA, *Datura stramonium* L.

The variation of the fractal dimension D in relation to the foliar parameters Per and SLA, was described by equation (5), in conditions of general statistical safety of the equation, according to $R^2 = 0.999$, $p < 0.001$. According to the ANOVA test and the values of the coefficients of equation (5) presented statistical certainty ($p << 0.001$ for a; $p < 0.001$ for b; $p << 0.001$ for c; $p << 0.001$ for d; $p < 0.001$ for e). The 3D graphic distribution is presented in figure 8, and the distribution in the form of isoquants is presented in Figure 9.

$$D_{(Per,SLA)} = ax^2 + by^2 + cx + dy + exy + f \qquad (5)$$

where: $D_{(Per,SLA)}$ - fractal dimension in relation to Per and SLA

x – Per; y – SLA;
a, b, c, d, e, f – coefficients of the equation (5);
a= -0.00477548971090102;
b= -0.00133694153031225;
c= 0.170424058654503;
d= -0.0793952105658202;
e= 0.00492200264906732;
f= 0.

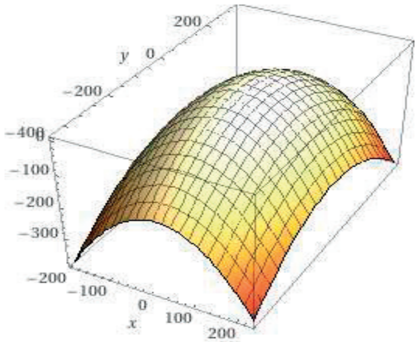

Figure 8. 3D graphical representation of the distribution of the fractal dimension D according to Per and SLA, *Datura stramonium* L.

Analyzing the foliar parameters studied in terms of coefficient of variation (CV), high values were found for SLA ($CV_{SLA} = 30.0438$) and MLA ($CV_{MLA} = 27.3120$), moderate values in the case of L ($CV_L = 14.2455$), w ($CV_w = 15.6528$) and Per ($CV_{Per} = 17.5079$). Low value of the coefficient of variation were recorded in the case of fractal dimension D ($CV_D = 2.4234$).

Variations in plant leaves are generated by complex causes, such as the relationship of plants with natural and anthropogenic environmental factors (Datcu et al., 2017; Li et al., 2018; Liu et al., 2019), by foliar treatments, cultivation and growth conditions (Rawashdeh and Sala, 2014; del Pazo et al., 2020; Ren et al., 2020), by stress factors (Poorter et al., 2009) etc.

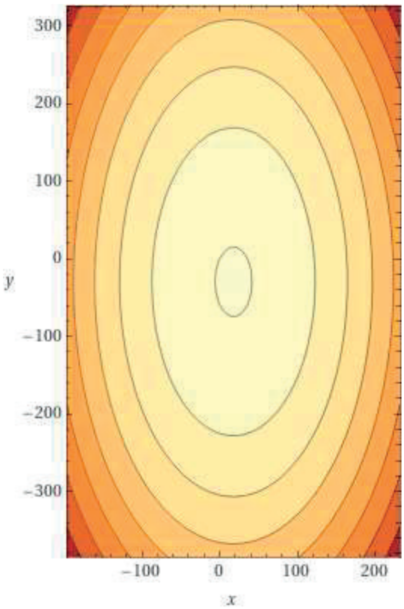

Figure 9. Graphic representation as isoquant of the distribution of the fractal dimension D according to Per and SLA, *Datura stramonium* L.

Imaging analysis is a very useful method for the study of plants at the foliar level, the whole plant, or plant associations (natural or anthropogenic ecosystems) due to the various approaches and facilities it offers; obtaining results in real time (or very short compared to classical methods), has applicability on mobile devices, high portability etc. It also allows integration into complex analysis schemes, and association with various image capture devices (Li et al., 2014; Komis et al., 2018; Beć et al., 2020).

Imaging analysis has been used for studies of leaf structure and morphology (Mathers et al., 2018), plant phenotyping (Li et al., 2014; Rousseau et al., 2015), analysis of physiological indices (Drienovsky et al., 2017a; Constantinescu et al., 2018), detection of symptoms at the foliar level and plant diseases (Drienovsky et al., 2017b; Veys et al., 2019; Singh et al., 2020), characterization of biomass in agricultural crops (Pandey et al., 2017) etc.

In the present study, based on the CV values resulting from the analysis of the leaf samples for the species *Datura stramonium* L., it can be appreciated that for the description of the leaf geometry the greatest stability was presented by the fractal dimension (D). Similar results were reported by Sala et al. (2020a) for the black alder.

Similar studies have been conducted for the analysis of leaf fractal geometry and characterization of some plant species (Backes et al., 2009; Du et al., 2013; Gazda, 2013; Da Silva et al., 2015), and for the analysis and classification of five varieties of apple, based on the fractal geometry of the leaves (Sala et al., 2017).

Fractal analysis was used to describe the geometry of the ash tree rhytidome, and based on the fractal dimensions, a variation model was performed in relation to the shooting distance (Nicolin et al., 2019).

The geometry of some wheat plant associations, at the level of wheat crops, was studied by fractal analysis, in order to classify 10 wheat varieties (Rujescu et al., 2020). The method used facilitated the analysis and classification of the varieties studied, in conditions of statistical safety.

In the analysis of the degree of spatial variability of agricultural lands, fractal analysis led to the development of variation models based on the values of fractal dimensions D resulting from imaging analysis (Sala et al., 2020b).

The results obtained in the present study fall within the general and specific context of the studied bibliographic sources for the scientific substantiation of this research and bring information on the particular geometry of the leaves to the analysed specie, *Datura stramonium* L.

CONCLUSIONS

To find the leaf area at *Datura stramonium* L. by non-destructive methods, based on the leaf parameters L and w (measured leaf area - MLA) the value of the correction factor was found, CF = 0.50. This facilitated the finding of MLA values with high accuracy and in statistical safety conditions.

The description of the leaf geometry was possible by fractal analysis (D = 1.707), of the leaf samples taken in the study.

Very strong correlations were identified between foliar parameters of the leaves and elements of the geometry of the studied leaves (SLA, MLA, D). Mathematical models were found that described the interdependence relationships between stdiati foliar parameters and fractal dimension D.

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CORRELATION BETWEEN SOME FOLIAR FERTILIZERS USED IN ORGANIC AGRICULTURE AND NITROGEN UPTAKE IN DIFFERENT CROPS AND GROWTH STAGES

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Abstract

The use of organic fertilizers is becoming more widespread, especially since an increasing number of farmers are reorienting to organic farming. However, on the market there is a relatively limited offer of these treatments compared to conventional fertilizers. Studying their efficiency is a domain still at early stages in our country. This study aims to determine the influence of a chelated complex fertilizer of micronutrients (Codamix) and an organic fertilizer based on proteins and seaweed (Ecoaminoalga), on the total nitrogen content of some crops. These foliar treatments were applied to wheat, maize, sunflower and soybean crops and the total nitrogen content of both leaves and seeds was determined. A control lot where no foliar fertilizers were used was also analysed. Both plant and fertilizer samples were analysed with CHNS Elemental Analyzer, after dehydration. Multiple differences were observed between the control group and the lots where foliar treatments were used, as well as correlations between the type of fertilizer and the nitrogen content for certain crops.

Key words: *crop nitrogen status, foliar fertilization, organic, CHNS elemental analysis.*

INTRODUCTION

One of the main concerns of fertilizer producers is to find a way to provide the nutrients needed by the plants at an optimal level. Nitrogen, as a vital element for plants, can be delivered through special nitrogen fertilizers, in different forms. In addition, some other nutrients can improve nitrogen use efficiency, so the key of a good fertilization is to find a balance between elements. In a detailed review, Rietra et al. (2017) describes the interaction between macro and micro nutrients for different agricultural crops, with the effect on yield quality and quantity, providing good knowledge for fertilizer design and optimization of fertilization strategies. Foliar fertilization has a series of advantages over conventional soil fertilization, especially when it comes to micronutrients. These elements are often inaccessible although their quantity in soil is sufficient. Micronutrients availability from soil to plants is influenced by a lot of factors such as the organic matter, soil minerals, redox potential, pH, soil microorganisms, enzymes and many more (Kurešová et al., 2019).

Many times, micronutrients are immobilized in soil, so using foliar fertilizers can improve the nutrients uptake (Haytova, 2013).

Brown et al. (2012) presents the perspectives of foliar fertilization as modern crop management and with lower environmental risk in contrast to soil applications. Also, foliar fertilizers can be mixed with many types of water-soluble pesticides, reducing the time and costs of crop production. (Kuepper, 2003).

Micronutrients have an important role in plant nutrition as they are essential for a normal development and for regulating many plant functions. Some studies showed that micronutrient foliar application increases the yield and quality of crops especially when used in addition to macronutrient classic fertilization (Tariq & Khalid, 2020; Dinu et al., 2019).

In another experiment on wheat crops, Mikos-Szymańska et al. (2018) showed that micronutrient foliar fertilisation combined with standard fertilisation significantly improved thousand grain weight (TGW) unlike standard soil fertilisation alone. No significant effects were mentioned on other yield indicators.

Stepien and Wojtkowiak (2016) reported that micronutrient foliar spraying contributed to a high proportion of proteins in wheat grain due to plant metabolism stimulation, but no significant difference was observed.

On sunflower crops, some studies also revealed that foliar micronutrient treatments improved some characteristics. Tegegnework el al. (2015) observed a significant efficacy of foliar applications on physical characteristics: height of plants, number of leaves per plant, leaf area, stem girth, minimum days to flowering, head diameter and total dry matter accumulation. Similar studies showed an improvement of the same physical indicators (Baraich et al., 2016; Keerio et al., 2020), but regarding the chemical composition, especially oil content, other studies found no significant differences when foliar micronutrients were applied (Škarpa et al., 2013; Rao et al., 2020).

The effects on maize crops were also studied, but the conclusions are unclear. Some works reported no differences on yield when foliar micronutrients were used (Mueller & Diaz, 2011; Sharma et al., 2018).

Another study by Yousefi (2012) pointed out that a foliar spraying with Zn, Fe and Mn improved the quality and quantity of the harvest in spite of the fact that TGW and cob weight increase was not significant. Also, a study by Stewart (2016) concluded that the foliar micronutrient applications have an unpredictable response on grain yield on some maize cultures in Nebraska, USA. The results showed both positive and negative response, in cases without visual signs of micronutrient deficiency on plants. He also recommends the foliar micronutrient fertilization only when evidence of mineral deficiency is observed. Similar conclusions were highlighted for soybean crops. Some works have concluded that foliar applications of micronutrients has no significant effect (Sutradhar et al., 2017; Lilley, 2020). Moreover, a study by Staton (2019), conducted in Michigan (USA) over the past 10 years, showed that foliar fertilizers application to soybeans is not recommended since the unfertilized control in 109 of the 117 trials was more profitable. But at the same time, other studies showed that some foliar fertilizers improved some crop characteristics as bean size, TGW and yield (Kolesar et al., 2020; Heidarzade et al, 2016). As Dimkpa and Bindraban (2016) concluded in a review about fortification of micronutrients for efficient agronomic production, is impossible to have a single product with a balanced composition of micronutrients, due the variation of soil composition, the different needs of the crops and the negative interaction that can occur between some nutrients.

This study aims to determine the influence of a chelated complex fertilizer of micronutrients (Codamix) and an organic fertilizer based on proteins and seaweed (Ecoaminoalga), on the total nitrogen content of four crops: wheat, maize, sunflower and soybean.

MATERIALS AND METHODS

A field experiment was conducted in 2020 at the Pitești Agricultural Development Research Station, Romania. The field is part of the West Romanian Plain situated on a terrace of Arges River at an altitude of 334 m (Răducu et al., 2009). The plant material was represented by four crops: wheat (Trivale variety), maize (F.376 hybrid), sunflower (Puntasol hybrid), and soybean (Florina F variety). For each culture, a one-factor experiment was conducted, consisting by three variants of treatments: V1 – Control variant (no fertilization); V2 – Foliar fertilization with Codamix; V3 – Foliar fertilization with Ecoaminoalga. Codamix is a water-soluble fertilizer which contains trace elements chelated by citric acid, lignosulphonic acids and EDTA. It is used often a supplement to NPK fertilising schedules (Sustainable Agro Solutions, Codamix producer, 2021). Ecoaminoalga is an organic fertilizer obtained from soy and seaweed protein hydrolysis with over 40% organic matter and peptides content. It is recommended for use in organic farming .

The treatments were applied on 25 June 2020, using 2.5 L/ha for both fertilizers. For each culture, two types of samples were taken: leaf samples from medium stage of development and grain samples from the moment of harvest. The plant samples and the sampling moments are presented in Table 1. For each sample, three replicates were taken.

Table 1. The plant samples used for analyzes

Crop	Plant part	Date of reception
Wheat	Leaves	04.06.2020
	Grains	28.07.2020
Maize	Leaves	02.07.2020
	Grains	07.09.2020
Sunflower	Leaves	02.07.2020
	Grains	07.09.2020
Soybean	Leaves	02.07.2020
	Grains	20.10.2020

All plant samples were dried to constant mass, ground with the laboratory grinder into fine powder and kept in desiccators until analysed.
An amount of 1-3 mg of sample was used to determine the total nitrogen content. The analysis was performed using the CHNS elemental analyzer (EuroVector EA3100 Elemental Analyzer). Cystine was used as standard reference material. All determinations were performed in three repetitions. The fertilizers were also analyzed for total nitrogen content. The values represent the mean of three determinations (Table 2).

Table 2. Nitrogen content of used fertilizers

Fertilizer	Nitrogen content (%)
Codamix	0.230
Ecoaminoalga	3.520

All the analyses for this study were made using the infrastructure of Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest.
The obtained data were processed using IBM SPSS statistical software. Duncan's Multiple Range Test at P≤0.05 level was used for significance determination between groups of means of the three variants used in the experiment. The bars in the charts represent the means±SE of each variant. The same letters above each bar means that they are not significantly different.

RESULTS AND DISCUSSIONS

This study follows only the changes of nitrogen content in mentioned crops when the mentioned foliar fertilizers are used. Although the fertilization effects usually can be observed on quantitative measurements of crops, also qualitative improvement may occur (as the content of proteins, minerals, fats, etc.).

The effect on wheat crop

The wheat leaves from both fertilized variants showed a significant higher content of nitrogen compared with the control variant (Figure 1). An increase of 21.55% and 29.87% over control variant was observed in case of Codamix fertilization and Ecoaminoalga, respectively.

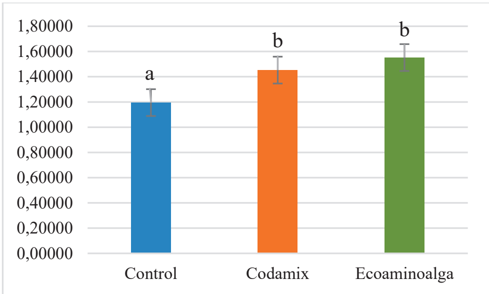

Figure 1. Nitrogen content in wheat leaves (% in DW) .

The wheat grain also showed a significant difference on nitrogen content between control and fertilized variants. The nitrogen increased with 29.83% and 41.84% using Codamix and respectively, Ecoaminoalga fertilizer (Figure 2).

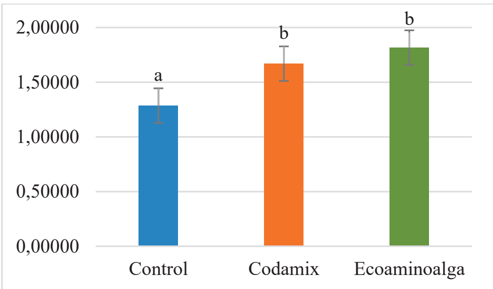

Figure 2. Nitrogen content in wheat grains (% in DW).

Even these fertilizers have a small quantity of nitrogen compared with special nitrogen fertilizers, it can be observed that both the leaves and the grains have accumulated a significant amount of nitrogen compared to the control. Also, the content of nitrogen of V3 samples (Ecominoalga) slightly exceeds the V2 samples (Codamix), but not significantly. This difference may occur due the higher nitrogen content on Ecoaminoalga fertilizer.

The effect on maize crop

Unlike the wheat, maize crop had a different response to micronutrient foliar applications regarding the nitrogen uptake. There are no significant difference between the control and Ecoaminoalga fertilization on maize leaves. On the contrary, a slight decrease in nitrogen can be observed on Ecoaminoalga variant. This direction was noticed by another study on maize crops when foliar micronutrients were used (Stewart, 2016). This insignificant decrease was attributed to the toxic effects of some micronutrients to leaves. However, the Codamix variant recorded a significant increase by 14.41%, compared to the control (Figure. 3).

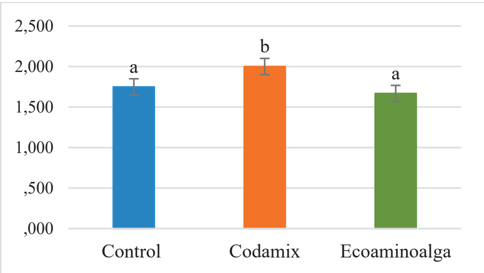

Figure 3. Nitrogen content in maize leaves (% in DW).

Regarding the nitrogen content in maize grains, there are no important modification between control and both the fertilized variants (Figure 4).

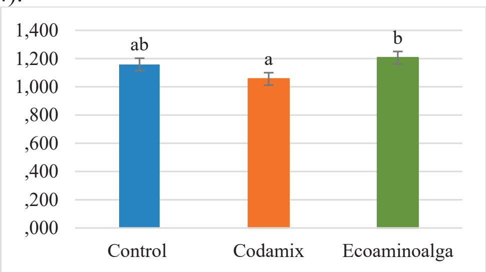

Figure 4. Nitrogen content in maize grains (% in DW).

Still, a difference between fertilized variants can be noticed. An interesting fact is that the nitrogen content in leaves was 20% higher on Codamix variant in summer, but the grains harvested in autumn had a bigger nitrogen content (14.2%) on Ecoaminoalga variant. This may be due to different micronutrient content in fertilizers, which can change the nitrogen use efficiency.

The effect on sunflower crop

The results on sunflower were almost invisible regarding the nitrogen uptake. No notable difference was observed neither to the leaves (Figure. 5), nor to the seeds (Figure. 6).

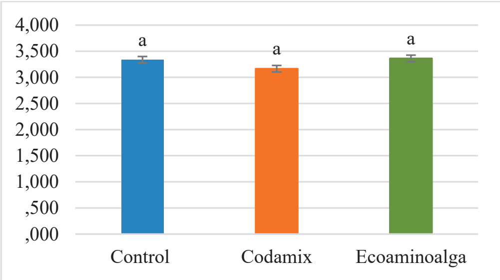

Figure 5. Nitrogen content in sunflower leaves (% in DW)

This does not mean that foliar applications are not useful. On the contrary, as pointed out earlier, a lot of physical characteristics were improved using this type of fertilization. But the advantages over the chemical composition are still unclear.

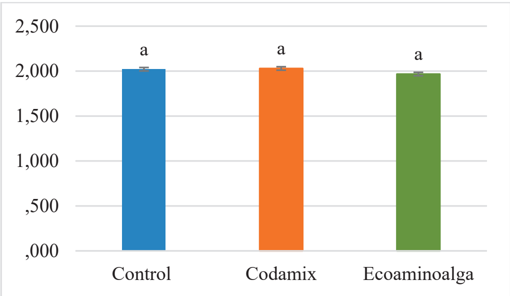

Figure 6. Nitrogen content in sunflower seeds (% in DW)

The effect on soybean crop

Regarding soybean, although the nitrogen uptake of leaves was significantly higher on fertilized crops (by 13.12% on Codamix variant and 22.36% on Ecoaminoalga variant) compared both control crop (Figure. 7), the nitrogen content did not differ much in soy beans from all variants (Figure. 8).

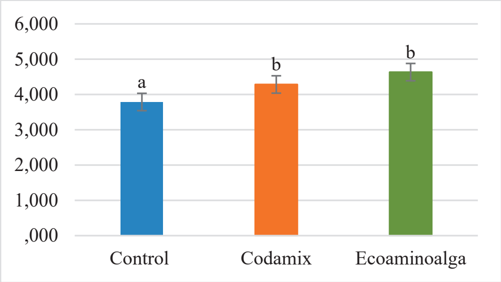

Figure 7. Nitrogen content in soybean leaves (% in DW)

Moreover, a slightly decrease was observed both on Codamix variant and on Ecoaminoalga variant, the same as observed in the case of maize crops. As in related studies, the foliar fertilizers did not produce promising results regarding nitrogen content. But also, some other characteristics may be improved using these type of application.

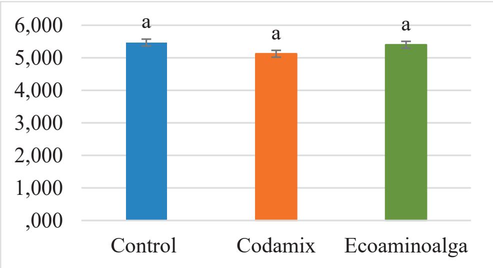

Figure 8. Nitrogen content in soy beans (% in DW)

CONCLUSIONS

The foliar micronutrient fertilization have an unpredictable response to nitrogen uptake on different crop types.

On wheat, the nitrogen uptake was clearly superior when foliar fertilizers were used, especially when the nitrogen content of fertilizer was higher.

On maize crops, the foliar fertilization has no noticeable effect on grain nitrogen content, but may increase the yield. This aspect was not followed in this paper.

Also, the same response was achieved for sunflower crop, with no increase in nitrogen content on fertilized variants.

On soybean, the nitrogen uptake was well observed on leaves collected in summer, but on the beans harvested in autumn the nitrogen content was not significantly different from the control crop.

Concluding, the type of crop has an important influence on fertilizer efficiency. An optimal foliar fertilizer must take into account the needs of the plant but also the water and nutrient uptake capacity of the leaves. These aspects, together with other factors such as soil characteristics, climate are responsible for the efficiency of different fertilizers.

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Lindernia dubia (L.) Pennell: A NEW INVASIVE IN THE ROMANIAN BANAT AREA

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Abstract

In this paper we reporte the presence of the species Lindernia dubia (L.) Pennell in the flora of Banat (SW Romania) and at the same time we present a new place for this species in Romania. We found the species on the lake Surduc area. This is a 362 ha artificial lake, a protected area (nature reserve of national interest). We found Lindernia dubia here for the first time in 2005, in flooded areas periodically (on clay soils), on the shore of the lake as well as in the nearby arable fields. We suppose he arrived here with the fish he brought to populate his lake or by seeds brought through the birds. The size of the population depends on the fluctuation of water level. Being an invasive species, we believe it is necessary to monitor it; some authors say it extends into characteristic habitats while others consider it one of the most abundant invasive species from the Danube Delta.

Key words*: Lindernia dubia (L.) Pennell, Romanian Banat, invasive.*

INTRODUCTION

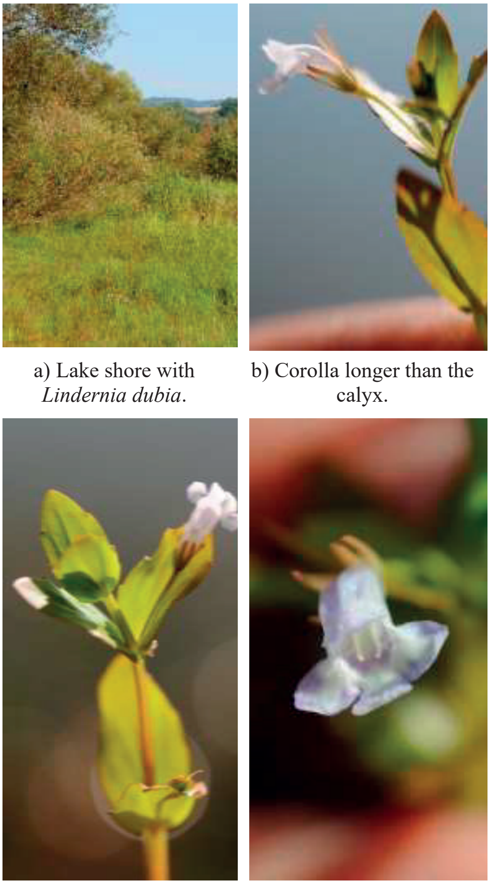
The genus *Lindernia* Allioni encompases about 70 species distributed throughout warm tropical and warm-temperate regions of New and Old World (Flora of China, 1998); recently, based on cpDNA sequences, many *Lindernia* species have been transfered to different genera within *Linderniaceae* (Les, 2017).

Formerly included in *Scrophulariaceae*, this genus belongs to *Linderniaceae* Borsch et al., K. Müller, & Eb. Fischer (Stevens, 2017). In Europe, the genus *Lindernia* is represented mainly by two species*: Lindernia procumbens* (Krock.) Philcox, native and *Lindernia dubia* (L.) Pennell, from North America, naturalized (Tutin et al., 1972). Marhold (2017,
The Euro+Med PlantBase) mentions also *Lindernia anagallidea* (Michx.) Pennel (*Lindernia dubia* var. *anagallidea* (Michx.) Cooperr.) from Italy, a species native from North America (Cook, 1985).

The most significant morphological differences between *Lindernia procumbens* (Krock.) Philcox and *Lindernia dubia* (L.) Pennell are documented by Molnár et al. (2000): length of leaves, length of pedicels, ratio of pedicels/leaf length. These two species may occur simultaneously in a habitat and may interbreed (Yoshino et al., 2006, in Schmotzer, 2015). *Lindernia dubia* (L.)
Pennell is characterized by: serrated leaves, floral pedicels shorter than the bracts, corolla longer than the calyx, two fertile stamens (Figure 1). *Lindernia procumbens* (Krock.) Philcox (Natura 2000 species code: 1725) is included in the Annex IV of the Council Directive 92/43/EEC among the species of community interest in need of strict protection.
Lindernia dubia (L.) Pennell is an invasive, accidentaly introduced in France in 1850, by ships on the Loire river banks (Fournier, 1940, Simons & Jansen, 2018).

According to Marhold (2017), the species is to be found in: Bulgaria, France, Germany, Switzerland, Italy, Portugal, Slovenia, while DAISIE Database mentions *Lindernia dubia* (L.) Pennell present in: Belgium, Bulgaria, Czech Republic, France, Germany, Greece, Hungary, Italy, Macedonia, Portugal, Romania, Spain.

The Euro+Med PlantBase presents the following countries as a distribution for genus *Lindernia*: Armenia, Austria, Bulgaria, Czech Republic, Croatia, Egypt, France, Germany, Greece, Switzerland, Spain, Hungary, Italy, Portugal, Poland, Central European Russia, South European Russia, Romania, Slovakia, Slovenia, Serbia (including Kosovo and Vojvodina), Asiatic Turkey, Ukraine and for the species *Lindernia dubia* (L.) Pennell, the following: Bulgaria, France, Germany, Spain, Italy, Portugal, Slovenia. The European areal of the species included also Poland (first mention: 2003, Drobnik & Buchalik, 2004).



a) Lake shore with *Lindernia dubia*.

b) Corolla longer than the calyx.

c) Pedicel shorter than leaf length.

d) The two fertile stamens.

Figure 1. *Lindernia dubia* (L.) Pennell - overwiew of the habitat (a) and morphological aspects (b, c, d).

In Romania, *Lindernia dubia* (L.) Pennell have been identified for the first time in the Danube Delta by Ciocârlan & Costea, 1994, and is now one of the most abundant invasive species in the Danube Delta Nature Reserve (Doroftei & Anastasiu, 2014, Sîrbu et al., 2011, Anastasiu et al., 2014). *Lindernia* species are among the few annual species whose germination doesn't diminish in floodable conditions; *Lindernia dubia* (L.) Pennell germinates very well in the riparian areas of the rivers Loire and Allier (France), but if the water layer persists the plants do not grow (Abernethy & Willby, 1999). *Lindernia dubia*'s capacity to germinate both in floodable conditions and in non-flooded soil is also highlighted by Neff et al. (2009), Šumberová et al., 2012 and http://beta.floranorthamerica.org/Lindernia_dubia. In seasonally flooded habitats the ability to preempt the growth of flooding-sensitive annuals by germinating before water levels have fully receded will bestow a considerable competitive advantage and may account for the present abundance of *Lindernia dubia* (L.) Pennell in the riparian zone of sections of the Loire and Allier (Abernethy & Willby, 1999).

In the Czech Republic, Šumberová et al. (2005) discussing the two species, they appear in the marginal vegetation; *Lindernia dubia* (L.) Pennell regenerates earlier from the stock of seeds, while *Lindernia procumbens* (Krock.) Philcox appears at the end of June when there are high temperatures necessary for germination.

In this country, the species is also study by Horáková et al. (2005), Kaplan et al. (2016). In 1995, Seliškar et al. revised the *Lindernia* genus and first mentioned the *Lindernia dubia* (L.) Pennell species in the flora of Slovenia. In Slovakia, Hrivnák et al. (2016) discussing *Lindernia dubia* (L.) Pennell in its Central European context. In Bulgaria, both species grow in humid sandy places, in ponds and rice paddies. *Lindernia procumbens* (Krock.) Philcox is cited in 1889, *Lindernia dubia* (L.) Pennell appearing much later in 1984. Stojchev & Cheshmedziev (2005) investigate the anatomy of the two species and complete their chorology in Bulgaria with new data. The anatomical characteristics remain in close parameters, the observed differences refer only to a greater density of the epidermal cells and the stomata from the *Lindernia procumbens* (Krock.) Philcox leaf and the higher number of cavities and medullary rays from the *Lindernia dubia* (L.) Pennell stem.

Pignatti (2005) considers as main ecological differences between the two species of *Lindernia* the fact that *Lindernia procumbens* (Krock.) Philcox has slightly higher requirements for light, slightly lower for temperature, and slightly lower for humidity,

compared to *Lindernia dubia* (L.) Pennell. Ellenberg et al. (1992) present the two species as having the same light requirements (high) and *Lindernia procumbens* (Krock.) Philcox slightly more thermophilic.
Julve (2020a; 2020b) presents *Lindernia dubia* (L.) Pennell as a more thermophilic and more trophic soils. Both species are intolerant to salinity. Sanda et al. (2003), consider that there are no ecological differences between the populations of the two species in Romania, while Sarbu et al. (2013) say that *Lindernia dubia* (L.) Pennell needs less light and slightly higher temperature.

Figure 2. Geographical position of Surduc Lake.

During some researches on the flora and vegetation around Surduc Lake (Timiș County, W Romania, Figure 2), during 2005-2019 we followed the presence of invasive species populations, given that this area is subject to a chaotic tourist development (Neacșu et al., 2017). *Lindernia dubia* (L.) Pennell is among the species we found here.

MATERIALS AND METHODS

We conducted field research in July-September, during 2005-2019. The identification of the species was made according to the current determinants in Romania (Ciocârlan, 2009, Sârbu & Oprea, 2011), and according to Molnar et al. (2000) and Flora of China, 1998 (online edition). In the areas with populations of *Lindernia dubia* (L.) Pennell we also noticed the presence of other species of cormophytes. We also made observations on anthropogenic pressures. Nomenclature is according to Euro+Med PlantBase. Voucher specimens are stored in the Herbarium of Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania" from Timisoara.

RESULTS AND DISCUSSIONS

We found populations of *Lindernia dubia* (L.) Pennell in six areas of the shores of Surduc Lake (Figure 3). Given the characteristics of the habitat (accumulation lake on the edge of a small river, with a narrow valley), two ways in which *Lindernia dubia* (L.) Pennell got here seem more likely: together with the fish brought here for breeding or by seeds in the mud encrusted in birds' feathers and legs (natural zoochory).
Both anthropic and natural *Lindernia dubia* (L.) Pennell dispersion is considered by various authors (e.g. Thiébault, 2007, Schmotzer, 2015). Simons & Jansen (2018) present as a way of introduction the zoochory.

Figure 3. Zones with *Lindernia dubia* (L.) Pennell populations on the Lake Surduc shore.

We noticed that the morphology of *Lindernia dubia* (L.) Pennell specimens depends on the characteristics of the microhabitat:
a) along the shore with clay soil we met abundant populations, with vigorous specimens, ample, ascending or slightly procumbent stems, which take root at the nodes. When the soil is dry, it blooms. In autumn, the stems turn reddish. In these microhabitats are also found: *Eleocharis palustris* (L.) R. Br., *Eleocharis acicularis* (L.) Roemer et Schultes, *Lythrum portula* (L.) D. A. Webb, *Echinochloa crus-gali* (L.) Beauv., *Gypsophilla muralis* L., *Gnaphalium uliginosum* L., *Polygonum aviculare* L., *Polygonum lapathifolium* L., *Xanthium orientale* subsp. *italicum* (Moretti) Greuter;
b) on the waterfront, in the sandy areas, *Lindernia dubia* (L.) Pennell grows together

with: *Polygonum aviculare* L., *Trifolium repens* L., *Potentilla reptans* L, *Plantago media* L., *Bidens tripartita* L., *Alisma plantago-aquatica* L., *Echinochloa crus-galli* (L.) Beauv. These forms are vigorous, with ascending stems.

c) in the agricultural crops near the shore we found isolated specimens, small in size, with weak rooting.

In the whole studied area, the shores of the lake are occupied by populations of the following species: *Alisma plantago-aquatica* L., *Bidens tripartita* L., *Calystegia sepium* (L.) R.Br., *Carex riparia* Curtis, *Cyperus fuscus* L., *Echinochloa crus-galli* (L.) Beauv., *Eleocharis acicularis* (L.) Roemer et Schultes, *Eleocharis palustris* (L.) R. Br., *Gnaphalium uliginosum* L., *Gypsophila muralis* L., *Impatiens noli-tangere* L., *Juncus bufonius* L., *Juncus effusus* L., *Juncus tenuis* Willd., *Leersia oryzoides* (L.) Sw., *Leontodon autumnalis* L., *Lotus corniculatus* L., *Lysimachia vulgaris* L., *Lythrum hyssopifolia* L., *Lythrum salicaria* L., *Mentha pulegium* L., *Oenanthe aquatica* (L.) Poir., *Plantago media* L., *Polygonum aviculare* L., *Polygonum hydropiper* L., *Polygonum lapathifolium* L., *Polygonum persicaria* L., *Pulicaria vulgaris* Gaertn., *Ranunculus repens* L., *Rorippa amphibia* L. (Besser), *Rorippa sylvestris* L. (Besser), *Salix alba* L., *Salix cinerea* L., *Stachys palustris* L., *Trifolium pratense* L., *Trifolium repens* L., *Xanthium orientale* subsp. *italicum* (Moretti) Greuter.

In our research (Neacşu, 2008), we encountered on the shores of Lake Surduc and *Lindernia procumbens* (Krocker) Philcox and we included these phytocenoses in the vegetal association *Eleocharidetum acicularis* W. Koch 1926 em. Oberd. 1957, which we did not find later. Near the lake, *Lindernia procumbens* (Krock.) Philcox is also reported by Karácsonyi & Negrean (2012). From a phytosociological point of view, *Lindernia procumbens* (Krock.) Philcox is presented by Sârbu et al. (2013) as a characteristic species for *Isoëto-Nanojuncetea*, while *Lindernia dubia* (L.) Pennell is considered characteristic for *Nanocyperion*.

Mucina et al. (2016) presents the unit *Cypero-Lindernion dubiae* Müller-Stoll et Pietsch in T. Müller 1963 as synonym for *Eleocharition soloniensis* Philippi 1968, described as pioneer ephemeral rush-vegetation in temporarily flooded mesotrophic habitats of Central and Western Europe. *Lindernia dubia* (L.) Pennell is considered in Mucina et al. (2016) classification as a diagnostic species for the class *Oryzetea sativae* Miyawaki 1960 (www.synbioosys.alterra.nl/evc/).

CONCLUSIONS

In the paper, we signal a new place in Romania for *Lindernia dubia* (L.) Pennell: the shores of Surduc Lake. This habitat is represented by depression areas, flooded in spring, whose existence is conditioned by the water levels. When these areas are covered in water the species takes refuge on the nearby arable lands.

In the habitat near Surduc Lake, *Lindernia dubia* (L.) Pennell grows alongside other cormophyte species, most frequently being accompanied by: *Echinochloa crus-galli* (L.) Beauv., *Eleocharis acicularis* (L.) Roemer et Schultes, *Gypsophilla muralis* L., *Polygonum lapathifolium* L., *Alisma plantago-aquatica* L., *Bidens tripartita* L.

Due to the area occupied by the species on the lake shores and large population fluctuations from one year to another, as well as the status of the lake (protected and tourist area), we do not recommend drastic measures to manage the species, especially since there is a probability of reappears *Lindernia procumbens* (Krock.) Philcox. However, it is necessary to monitor the area and the lakes and ponds in the area, where *Lindernia dubia* (L.) Pennell could be spread by birds.

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ROMANIAN CONSUMERS BEHAVIOR TOWARDS DOMESTIC FOOD WASTE

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Abstract

It has been reported that worldwide, food waste has become a problem of great importance due to population growth and the daily need for food. Thus, in this present paper, the consumer's attitude from Cluj County, Romania, regarding food waste and ways to prevent, was analysed. An on-line survey on a sample of 114 respondents was conducted in order to achieve the objectives of the research. Descriptive statistics were used to analyse the collected data. The results reveals that main category of wasted food is represented by cooked food, due to the fact that the respondents are cooking more than they need. Buying local products and using a shopping list are two of the main actions that could be taken in order to reduce the food loss and waste. At the other end respondents agree that educational campaign in order to educate and inform people about reducing food wasting are important.

***Key words**: consumer, food, food waste, waste reduction.*

INTRODUCTION

Food waste and food losses are an interesting topic in almost the last decade. Food waste is defined as the drop in quantity and quality of food while being transferred from the producer to the consumer due to the fact that the food hasn't been consumed, has expired or deteriorated as a consequence of consumer's behaviour, stock handling or negligence (Aktas et al., 2018). Food waste is strongly associated with the environment protection and the supplies usage in an efficient way, having a serious impact on the environmental degradation, climate change (for example biodiversity loss, fertile soil loss and the increase of greenhouse effect gas emissions and global temperature) and people's health (Franzo et al., 2018; Lindgren et al., 2018). Poore et al., 2018 claims that food systems constitute 26% out of the total gas emissions with greenhouse effect). As a consequence, the proper handling of food waste from the sustainability point of view has been a major problem for many countries and many organizations, even ONU considers it a major priority (European Parliament. Report on How to Avoid Food Wastage: Strategies for a More Efficient Food Chain in the EU 2011).

According to FAO, 32% of the food produced for human consumption is wasted or lost, approximately 1.3 billions of tones annually (Liu et al., 2016) whereas there is a number of countries which have to face food insecurity and malnutrition. The World Food Program (WFP, 2015) estimates that globally about 805 billion people have no sufficient food and their health and lifestyle are affected. The most important element of food security (there are four: availability, access, usage and stability) (Schmidhuber et al., 2007), is for sure food availability. This element refers to the food supply at the market level and reflects the economic development of a country very well (Patel D.R. et al., 2016). As a consequence there are ongoing debates all over the world regarding ways of reducing food waste for a more sustainable society (Abdel, 2018).

Most research in the field of food waste were focused in the area of final sellers (large store chains) and consumers. Store chains encounter a problem with excessive product stocks, and consumers encounter a problem with food consumption. Due to the fact that consumers contribute significantly to food waste, other studies have investigated the consumer behaviour regarding food waste from different perspectives, such as factors affecting food choices (De Boer et al., 2007), the effect of social influence upon food waste (Comber & Thieme, 2013), or food shopping practices and their relationship with food waste (Farr-Wharton et al., 2014). Therefore, wasted food cannot be defined only by a single behaviour, but rather by a combination of multiple behaviours that can increase or decrease the probability of being wasted.

Researches revealed that financial constraints are stronger, than those related to environment protection regarding consumers' actions in order to reduce food waste (Graham-Rowe et al., 2014; Stancu et al., 2016). This is stronger in the case of younger people, than older whom are more concern about social aspects and environmental consequences of food waste (Tucker & Farelly, 2015). Using a shopping list, checking inventories before shopping are strategies used to reduce food waste and lose (Jorrisen et al., 2015; Far-Wharton et al., 2014). There are many studies that are analysing the consumers' behaviour towards food waste generation (Schanes et al., 2018). The household routines such as planning, shopping, cooking, eating, management of leftovers play an important role in domestic food waste (Evans, 2012). The influence of the socio-demographic characteristics on food waste generation was also deeply analyzed. There are studies that revealed that is a direct link between the size of the household and the quantity of food waste (Jorissen et al., 2015; Silvennoinen et al, 2015). At the same time in the families with children the trend is to produce more food waste (Parizeau et al., 2015; Visschers et al., 2016). Cecere et al. (2014) concluded that older people are wasting more, than younger, while other researches underline that older people tend to waste less due to better knowledge regarding the impacts of food waste (Quested el al., 2013).

According to Public Health National Institute (insp.gov.ro), In Romania 10% of the purchased food is being thrown away, which represents 350g per day or 129 kg per year for each inhabitant, given that 4.5 million people in Romania are facing difficulties when it comes to day-to-day food acquisition. The same source mentions that the people who have high income waste the most, and the bigger the family, the more food is being disposed of. The largest waste of food is recorded in urban areas: while rural communities use traditional methods of recovery of food waste in the household, in urban areas over 95% of food waste ends up in landfills, making it impossible to be recovered neither as food nor as non-food (http://foodwaste.ro/wpcontent/uploads/2018/10/ FoodWasteRO-Anexa21).

MATERIALS AND METHODS

The aim of the research was to analysed to consumers attitude regarding the food waste and food combat. The survey method and the structured questionnaire tool were used to collect the data from the respondents. The sample consisted of food consumers from Cluj County, Romania. A convenience sample method was applied in order to achieve the objective of the research. A questionnaire of 17 questions was applied on social media groups of consumers during March-July 2020. A total number of 114 questionnaires were validated and furthermore was analysed using descriptive statistics indicators and chi-square test. Data was analysed by using SPSS, Statistics for Windows, Version 23.0. The questionnaire consists of three main parts: type, frequency and place of purchasing food products; type, frequency and reasons of food waste, and perception of the respondents of food waste on the environment; and the third part was represented by the socio-demographic data.

From the total number of respondents (Table 1) 78.9% were female, while 21.1% were male. This could be explained by the fact traditional the women are those whom are responsible are enjoying more household shopping (Ramprabha, 2017) and are responsible for cooking (Hamasalih et al, 2019). It was observed as well that 45.5% of the respondents are less than 40 years old, and 43.9% of the cases they declared that in the household are also children. More than 45% of the respondents are employee, with an average monthly income ranged from 2000 to 4000 RON, in 40.4% of the cases.

Table 1. Socio-demographic characteristics

Characteristic	Category	Percent %
Age	Less than 40 years old More than 40 years old	45.5% 55.5%
Children in the household (under 18 years old)	Yes No	43.9% 56.1%
Level of education	Less than university degree University degree or more	68.4 31.6
Socio-professional status	Student Employee Freelance Farmer Entrepreneur Unemployed Other	3.5% 47.4% 1.8% 28.1% 5.3% 1.8% 12.3%
Gender of respondents	Female Male	78.9% 21.1%
Average monthly household income (RON)	< 650 650-1000 1001-2000 2001-4000 > 4000	5.5% 8.8% 15.8% 40.4% 29.8%

RESULTS AND DISCUSSIONS

The main aspects that were considered in the present research were grouped into three categories of results.

1. Opinions regarding categories of purchased food, frequency and place of purchase and the degree of using the shopping list

The exact causes of food waste vary around the world and depend greatly on the specific conditions and local situation in a particular country (Nică, 2017). These food losses can be influenced by crop production choices and marketing patterns and distribution channels, as well as by consumers' purchasing and food use practices. So, at the beginning of the research we wanted to reveal the categories of purchased products. The obtained results following the application of the research questionnaire, reveal that the majority of respondents (82.5%) buy bread and bakery products, followed by those who purchase fruits and vegetables (73.7%), while other basic products (oil or sugar) are purchased at a rate of 12.3% (Table 2). Given that fact that on 15 December 2020, FAO launched the International Year of Fruit and Vegetables 2021 with a call for improving healthy food production and reducing food loss and waste (www.un.org), we consider the results obtained in the present research are worrying. Wasting this category of food could lead to higher prices, but also to a lack of fruits and vegetables.

Table 2. Categories of purchased food

Type of products	%
Fruit and vegetables	73.7
Meat and meat products	52.6
Bread and bakery products	82.5
Milk and dairy products	43.9
Sweets	24.6
Others (oil, sugar)	12.3

Analyzing the purchase frequency (table 3) it was observed that 61.4% of respondents buy bread and bakery products daily, followed by those who buy fruits and vegetables (21.1%) and milk and dairy products (14%). All these products are purchased weekly.

The possibility to purchase food products in Cluj County is varied. In this purpose, another objective of the research (Figure 1) aims to locate the most frequented commercial space from which the county's residents usually buy food.

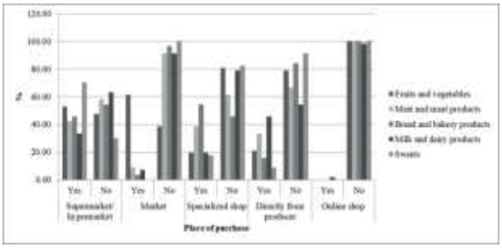

Figure 1. Place of purchase of products

The food products were divided into six groups, for which five places for purchasing were allocated. Thus, it can be observed that fruits/vegetables are purchased by consumers in agro-food markets (35%), while in the case of meat and meat products (33%), bread and bakery products (31%) respectively milk and dairy products (38%) consumers have chosen to purchase them from the supermarket. The explanation of this phenomenon is given by the period in which this research was conducted

(May-June 2020), namely, the pandemic caused default in Cluj County, which has limited the consumers in frequenting specialised stores, by the SARS Cov 2 virus in Romania, and by respectively, the direct purchase from the manufacture (Table 3).

Table 3. Frequency of food purchases

Categories	Daily	Weekly	Monthly	A few times per year	I do not buy	I do not know
Fruits and vegetables	21.1%	64.9%	8.8%	5.3%	0%	0%
Meat and meat products	12.3%	42.1%	22.8%	10.5%	7%	5.3%
Bread and bakery products	61.4%	28.1%	0%	5.3%	3.5%	1.8%
Milk and dairy products	14.0%	59.6%	5.3%	3.5%	5.3%	12.3%
Sweets	10.5%	40.4%	24.6%	8.8%	5.3%	10.5%

In the context of the current crisis in the field of environmental pollution and food inequity which is affecting the entire population of our country, in this context the use of the shopping list could be a cornerstone in reconfiguring consumer's opinion about food waste. Furthermore it was evaluated the degree in which the shopping list is used during the act of acquisition, and as a result, the survey data showed that 36.8% of respondents are not using it frequently, while 31.6% use it frequently or sometimes (Table 4).

Table 4. The degree of use of the shopping list

Use of shopping list	%
Yes	31.6
No	36.8
Sometimes	31.6

2. Opinions regarding the categories and frequency of food waste

Being asked which are the main types of food that the respondents are thrown away, it was observed that 22.5% of the respondents totally agree that cook food (mean=2.42) ends up to garbage, followed by bread and bakery products (12.3% of respondents totally agree, with and average score of 2.36) (Table 5).

Table 5. Food categories that are most often wasted

Statements	Appreciation scale (%)					NR	Mean	SD
	1	2	3	4	5			
Cooked food	43.9	17.5	14	1.8	22.8	0	2.42	1.603
Bread and bakery products	43.9	17.5	7.0	17.5	12.3	1.3	2.36	1.507
Milk and dairy products	56.1	12.3	15.8	7.0	7.0	1.8	1.95	1.299
Meat and products	50.9	24.6	10.5	3.5	10.5	0	1.98	1.316
Fruits	45.6	22.8	17.5	5.3	8.8	0	2.09	1.286
Vegetables	40.4	35.1	8.8	8.8	5.3	1.8	2.02	1.168

Previous studies revealed that people are cooking a larger quantity that they need (Graham-Rowe et al., 2014; Silvennoinen et. al, 2015). At the other end the less thrown away food products are dairy (56.1% totally disagree with an average score of 1.95) and meat products (50.9% totally disagree, with an average score of 1.98).

The acute lack of food for a large part of the country's population is known as food crisis. Consequently, it's every day waste (32.1%) (Table 6) as being shown by the research results, leads us to the idea that the residents of Cluj County could suffer in the future from lack of food, could spend more for their purchase and not in the least, the environment will suffer from the need to increase cultivated areas and the usage of inputs in agriculture.

As it can be observed there are significant difference (χ^2=18.038, df=4, p=0.001) between families with children and families without children regarding the frequency of throwing out the food. More 45% of the families in which are children are throwing out food daily, while in the families without children this could be observed only in 21.9% of the cases (Table 6). It was observed that there is a significant difference between the education level and the frequency of throwing food (χ^2=16.432, df=4, p=0.002**). Less educated people are throwing out food often than more educated ones. In 42.1% of the cases the respondents with maxim high school degree declared that they are daily throwing food, while this was noticed only in 11.1% of the cases of respondents with more than university degree. The results are different that those recorded by Cecere et al. (2014) and Neff at al. (2015). Regarding the average monthly house hold income it was noticed that in general with lower amount of income tend to produce more food waste, being a significant difference between the income and frequency of throwing food (χ^2=41.225, df=16, p=0.001***). This is contrary to the results of other researchers which show a positive correlation between income and food waste (Stancu et. al, 2016) or no between income and food waste correlation (Visscherts et al., 2016). This could be explain by the fact that by general the respondents with higher income are also persons more educated (table 6).

At the same time (table 6) younger respondents tends to throw out food more often than older respondents. Around 39% of the respondents less than 40% declared that they are daily throwing food, while only 29.4% of the respondents older than 40 years stated that they throwing food out daily. Chi-square test reveal that there are significant differences between the two groups of respondents (χ^2=12.104, df=4, p=0.017). Previous studies underlined the fact that household with children are producing more waste than other types of households (Tucker & Farrelly, 2016). This could be explained by the fact that for families with children is difficult to predict if the children will be eat home at all and their food preferences (Cappelini & Parsons, 2012; Ganglbauer et al., 2015). There was no significant difference between male and female and the frequency of throwing food (χ^2=3.507, df=4, p=0.477).

Table 6. Correlation analysis between socio-demographic characteristics and frequency of throwing out food

Variables	Frequency of throwing out food				
	Daily	Each two days	Twice a week	Others (once a week/give to animals)	I do not know
Total	32.1	8.9	32.1	21.4	5.4
Type of household					
With children	45.8	12.5	33.3	4.2	4.2
Without children	21.9	6.3	31.3	34.3	6.3
χ^2=18.038, df=4, p=0.001**					
Age					
Less than 40 years old	38.9	16.7	16.7	16.7	11.1
More than 40 years old	29.4	5.9	41.2	20.6	2.9
χ^2=12.104, df=4, p=0.017*					
Education					
Less than university degree	42.1	7.9	26.3	15.8	7.9
More than university degree	11.1	11.1	44.4	33.3	0
χ^2=16.432,df=4, p=0.002**					
Average monthly house hold income (RON)					
< 650	100.0	0	0	0	0
650 – 1000	60.0	0	40.0	0	0
1001 – 2000	55.6	11.1	22.2	0	11.1
2001- 4000	22.7	13.6	36.4	22.7	4.5
> 4000	11.8	5.9	35.5	41.2	5.9
χ^2=41.225,df=16, p=0.001***					
Gender					
Male	34.1	9.1	34.1	18.2	4.5
Female	25.0	8.3	25.0	33.3	8.3
χ^2=3.507,df=4, p=0.477					

3. Opinions regarding the causes of food waste, ways and benefits on the environment

Since the most important goals of the present generation when purchasing food, are related to the sanogenetic virtues of the products, another aspect of the paper was to identify the reasons why food is thrown away and the ways to prevent food waste. The study consisted of 5 statements, which were evaluated on a scale from 1 to 5, where 1 means "total disagreement" and 5 means "total agreement". The results confirmed what was observed by other researches as well that by general people do not estimate correctly the amount of need food and they end up to cook more than they need (Ghram-Rowe et al., 2014). As it can be observed just 10.5% (Table 7) of the respondents claimed that the reason of throwing is related to overproviding food.

Table 7. Causes of food waste

Main reasons for throwing out food	%
Improper estimation at purchase	10.5
Fast degradation	40.4
Preparing large quantities for a meal	66.7
The food is very cheap and it doesn't matter how much you buy	0
Others	7.0

This aspect was highlighted by Parizeau et al. (2015) as well, while Evans (2012) claimed that by general people follow of routine of buying more than they need.

Analysing the actions that could be taken in order to reduce the food wasting it was observed that 68.4% (table 8) of the respondents totally agree that is necessary to buy an accurate quantity of food (mean 4.32±1.208), followed by donation of food (mean 4.05±1.446), while 43.9% of the respondents strongly agree that a shopping list should be used in order to prevent food waste. Jorissen et al. (2015) found out the using a shopping list reduces by 20% the amount of food through away by capita. Buying local products represents for 43.9% of the respondents a tool for reducing food loss and waste (3.76±1.464) (Table 8). Buying local products has a positive effect on reducing food loss and waste. Setti el al. (2016) found out that in case of local food buyers the frequency of wasting vegetables tends to be lower. Food waste was noticed to be higher in the case when people exclusively purchase in supermarkets, and tends to decrease when people are buying food from local and small shops (Jorrisen et al., 2015).

Table 8. Actions of preventing food waste

Statements	Appreciation scale					NR	Mean	SD
	1	2	3	4	5			
Proper assessment of food needs	7	1.8	12.3	8.8	68.4	1.8	4.32	1.208
Shopping according to a list	8.8	12.3	17.5	12.3	43.9	5.3	3.74	1.403
Food donation	14	0	12.3	10.5	59.6	3.5	4.05	1.446
Awareness campaigns on food waste provided by the authorities	14	12.3	3.5	17.5	47.4	5.3	3.76	1.541
Purchasing mainly local products	12.3	5.3	17.5	10.5	43.9	10.5	3.76	1.464

Analysing consumers' perception regarding the environmental impact of the food waste, it was observed that 42.7% believes that surfaces covered with waste would reduce, 33.7% believes that it would lead to rational use of the resource, while 23.6% believes that the emissions of the greenhouse gas will decrease (Table 9).

Table 9. Environmental benefits by reducing food waste

Items	%
The reduction of land areas occupied by waste	42.7
The rational use of resources	33.7
Reducing greenhouse gas emissions	23.6

CONCLUSIONS

Studying the consumer's attitude towards food waste reveals an original contribution of the paper, which enriches the literature, by examining how consumers of different food products can become an important factor in avoiding food waste. At the same time, the article can bring an important contribution to the specialized literature by providing a model for analysing the problem of food waste, which can be applied to any region.

Previous researches proved that there are several factors that are influencing consumers' behavior towards domestic food waste. The results showed that the participants in the study are aware about the methods of reducing the food waste and food waste and its effects on the environment; however this is not reflected in their behavior regarding planning, shopping, cooking meals.

In the case of larger household with children and younger respondents it was noticed that the frequency of throwing out food is higher, most often cooked food is thrown out. In this context in could be concluded that is a need of educational campaigns against food waste and sustainable cooking. Therefore, women should be targeted for providing information on food waste and ways to avoid it.

The number of dairy cows has continuously decreased during the period 1990-2010, with a negative impact upon milk production.

Milk yield is the only positive aspect, because it has increased reaching 3,980 kg per cow in the year 2010.

As a consequence of the reduced number of cows but an increased milk yield, milk production has continuously increased, except the year 1995 when it recorded the lowest level.

The North Eastern region is traditionally suitable for cow rearing, due to its pastures and meadows, the important number of cow livestock and possibilities to produce ecological milk.

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GMO IDENTIFICATION IN FOOD AND FEED FOR 2019 AND 2020 (PANDEMIC YEAR) - A COMPARATIVE STUDY

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Abstract

In this article, we will present a comparative study by the incidence of genetically modified soy and corn, in food and feed, in Romania, during 2019 and 2020. The laboratory analyzes performed on their determination are compared. These data refer to the samples from different food and feed matrices. This paper includes the data provided by the molecular biology laboratories from Braila, Bucharest, Calarasi, Iasi, Satu-Mare, Salaj, Suceava, and Tulcea and the data provided by the County Veterinary Sanitary and Food Safety Directorates. The paper presents the data for each county that has been processed for the indicators: the number of samples of food or feed processed annually and analyzed to identify genetically modified soy and corn. During this period, were analyzed in 2019 between 0 samples by Tulcea laboratory and 158 samples by Salaj laboratory, in 2020 (known as the Covid year) were analyzed fewer samples. The matrix samples were cereals, flour, mixed feed, textured, soy isolate, lecithin, starch, drinks, sauces, pastries, pasta, and popcorn. For the analyzed samples, genetic transformation processes were not detected. In conclusion, for the period 2019-2020 on the Romanian market were marketed safe food and feed, non-genetically modified products, and their traceability was following the European and national legislation.

Key words: *GMO, determination, soy, corn, 2019, 2020.*

INTRODUCTION

GMO means an organism that possesses foreign genes obtained using modern biotechnology. Generally, methods for obtaining transgenic plants are based on the use of the *Agrobacterium* system. (i.e. genetic engineering) (Cornea, 2010).

Biotechnology has revolutionized crop improvement by producing genetically modified crops with increased availability and the use of important traits (Icoz and Stotzky, 2008).

Researchers are constantly contributing through innovations in biotechnology, creating new GMOs beneficial to farmers around the world. Improving genetically modified crops that are obtained through genetic engineering methods is particularly important, because the issues of environmental protection, maximizing crops to combat or mitigate malnutrition around the world, the needs of a growing population are evident worldwide.

This comparative study presents an analysis of the number of samples by food or feed examined for the identification and incidence of genetically modified events into products obtained from soy and corn or containing soy and corn. The study includes data provided for the period 2019 – 2020, by the official national laboratories.

Only genetically modified MON 810 corn is allowed to be cultivated in Romania by national and European legislation.

The evidence of genetically modified corn crops is public on the website of the Ministry of Agriculture and Rural Development, accessing the link https://www.madr.ro/organism-modified-genetic.html.

The Romanian cultivators can establish crops with genetically modified corn MON 810 Bt but recently have chosen not to establish such cultures.

This type of corn is resistant to lepidopteran insects, identification code MON-ØØ81Ø-6, according to the European Commission Regulation no. 65/2004 establishing a system of elaboration and allocation of unique identifiers for genetically modified organisms.

After 2015 year, the corn line MON 810 Bt was not cultivated in Romania. In Secuieni commune, Neamt county were cultivated 2.5 ha (www.madr.ro, January 2021).

Data were collected and were statistically processed and interpreted from laboratories that can analyze the identification and quantification of genetically modified events in plants. These laboratories are Veterinary Sanitary and Food Safety Laboratory (LSVSA) by Romanian counties: Brăila, Călăraşi, Iaşi, Satu Mare, Sălaj, Suceava, Tulcea, and the Institute of Diagnostic and Animal Health (IDSA) from Bucharest.

Genetically modified food and feed are currently limited almost exclusively to agricultural plants.

The indicators used for the characterization of the Genetically Modified Organisms and the possible events necessary for this comparison were the total number of samples during the whole period of study, annually and in each county laboratory of the food and feed samples processed for the determination of genetically modified organisms, species of vegetal material or food and feed product obtained from soy and corn.

Generally, the methods to obtain transgenic plants are based on the use of the *Agrobacterium* system (Cornea, 2010; Kado, 2015; Parmar et al., 2017).

Countries that have developed specific legislation for foods and feed products containing, consisting of, or produced from genetically modified organisms, focus mainly on the analysis of risks to consumers' health, possible environmental problems, as well as the practical control (possibilities for detection, testing, and labeling of these foods and feed products).

The methods used for the efficient transfer of genes of interest to plant organisms have progressed a lot, from indirect methods such as bacterial-mediated transformation to methods of direct DNA shooting into target tissues.

The European Union has been developed laboratory methods to control plants or products containing genetically modified plants, most of which are based on DNA testing by PCR or protein testing by enzyme-linked immunosorbent assay.

The detection international standards are also used in the Romanian county laboratory's activity and this is:

• ISO 21569 – quality standard,
• ISO 21570 – quantitative standard,
• ISO 21571 – extraction of nucleic acid standard.

The methods used in Romanian county laboratories for detecting GMOs in food and feed are:

• PCR identification of p35S and tNOS genetic elements from soy or corn
(the RENAR accredited method);
• Real-Time PCR quantification of soy-specific genetically modified line GTS 40-3-2, (the RENAR accredited method).

Methods are based mainly on molecular techniques (PCR, qPCR) (Levy et al., 2014) and could be applied to various plant species (corn, soybean, rice, tomato, papaya, carnation, cotton) for specific transformation event or sequences (Bonfini et al., 2012).

MATERIALS AND METHODS

The research methodology used in this study has the following main aspects:

- Bibliographic study of the national literature;
- Collecting the concrete information within the research area;
- Ranking, processing, and presentation of results in synthetic form;
- Analysis and interpretation of results and formulation of conclusions.

RESULTS AND DISCUSSIONS

The European and national markets allow the marketing and use of genetically modified plants for the production and processing of food and feed for animals (especially soybeans and corn) and in general for the food industry. These foods and feeds are used in various forms, such as cereals, bran, flour, soy isolate, textured. The purpose of this paper is to make public the data on food and feed samples examined for GMO detection.

For a genetically modified plant or part of a plant to be used in the production of food or feed, there must be mandatory conditions such as: it must be authorized for commercialization on the European market by the European Food Safety Authority and not exceed the maximum limit established conventionally at the legislative level, represented by a

percentage of 0,9%, which also requires the appropriate labeling.
It is also mandatory to indicate the presence of genetic modification events in plants, explicitly stating on the label of a food or feed products = "contains GMOs".
These products can be used for the production or processing of finished food or feed and can therefore be marketed on the intra-Community market, therefore they can be found throughout the food chain, "from the fork to the farm".
Traceability of genetic modification events of plants used in the food or feed industry is an essential indicator for human and animal health.
Therefore, the expression "contains GMOs", the content was expressed as a percentage of modification and the specific code of the modified plant species are useful information for the traceability of genetically modified food or feed.
The National Veterinary and Food Safety Authority from Romania is a control authority in the domain of genetically modified food and feed, ensures that only genetically modified foods and feed authorized at the European level are placed on the market and labeled according to the specialized European and national legislation.
There are 8 specialized laboratories for molecular biology and GMOs in Romania, which perform the analysis of food and feed samples taken under the official national control program and the analysis of samples received from economic operators from the food and feed industry in the process of self-control of own unit.
In (Table 1) is the number of food and feed samples examined in 2019 and 2020 (927 samples) the distribution of samples analyzed in the national laboratories to determine genetically modified organisms.
We note that the difference between 2019 and 2020 is 57 more samples in 2019.
In 2019 were examined in total 492 samples and in 2020 the number of samples was 435, with an average of 463.5 samples. The difference comes from the LSVSA Braila, Calarasi, Tulcea, and IDSA laboratories (Figure 1).
Within LSVSA Braila, half of the number of samples from 2019 was analyzed in the 2020 pandemic year. Within the LSVSA Iasi, Salaj, and Satu Mare, the number of analyzed samples for the two years was maintained. Within LSVSA Suceava and Tulcea, the number of analyzed samples for the pandemic year 2020 was increased, compared to 2019. For LSVSA Tulcea is a resumption of this activity in 2020.

Table 1. Comparative annually samples analyzed for GMOs detection during 2019-2020 and their average

	2019	2020	Total	Average
Braila	97	52	**149**	**74.5**
Calarasi	48	37	**85**	**42.5**
Iasi	63	64	**127**	**63.5**
Satu Mare	10	9	**19**	**9.5**
Salaj	158	157	**315**	**157.5**
Suceava	11	18	**29**	**14.5**
Tulcea	0	16	**16**	**8**
IDSA	105	82	**187**	**93.5**
Total	**492**	**435**	**927**	**463.5**

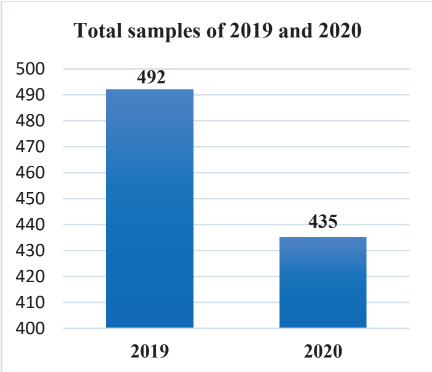

Figure 1. 2019 and 2020 annually total samples/analysis laboratory for GMO identification

The distribution of the total number of samples analyzed during the period 2019-2020 on each laboratory (Figure 2) shows that the laboratories within LSVSA Salaj (Transylvania area), IDSA, and Braila (south Moldova area) examined a lot more samples, indicating increased economic activity in the western area and the center area of Romania. Within LSVSA Salaj, 315 samples were taken for GMO analysis, which is the maximum cumulative for the two years of study.

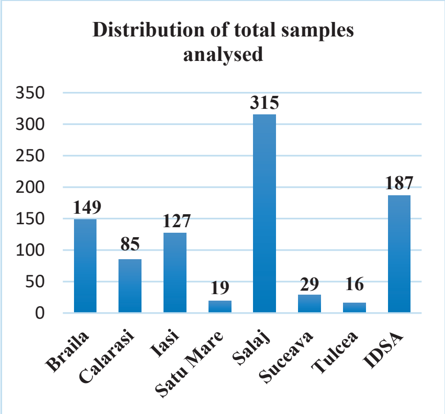

Figure 2. The distribution of total samples/analysis laboratory for GMO identification (2019-2020)

Within LSVSA Braila, Iasi and IDSA we noticed that the number of samples is approximately half, compared to the data provided by LSVSA Salaj for the same period. A much smaller number of samples were taken at LSVSA Satu Mare (19 samples), Suceava (29 samples), and Tulcea (16 samples).

The comparative annually distribution for 2019 and 2020 respectively for each laboratory of the number of samples analyzed for the identification of GMOs (Figure 3), shows a maximum of 158 samples examined in 2019 by LSVSA Salaj laboratory and 0 samples in 2019 on LSVSA Tulcea.

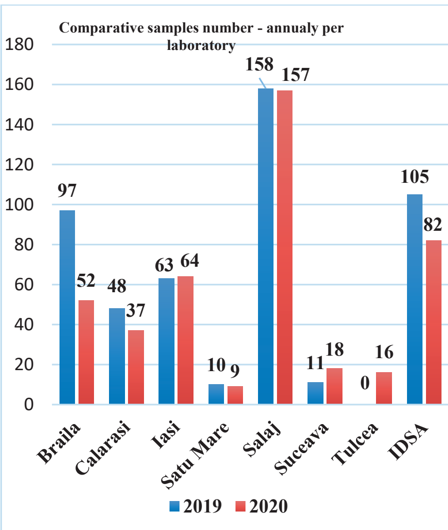

Figure 3. Comparative annually distribution by each laboratory of samples analyzed for GMO identification

Within LSVSA Braila, twice as many samples were processed in 2019 (97 samples), compared to 2020 (52 samples).

A number with 10 fewer samples is observed for LSVSA Calarasi in the pandemic year 2020 (37 samples), compared to 2019 (48 samples).

About a quarter fewer samples were processed at IDSA in the pandemic year 2020 (82 samples), compared to 2019 (105 samples).

The variability of the data regarding the number of examined samples expressed by the measure of their scattering, represented by the standard deviation is S = 50.58784, so about half of the data are located on either side of the average (Table 1).

The measure of the degree of statistical correlation between the data of Table 1. is represented in Figure 4. by the linear correlation coefficient. This indicator has values between -1 and 1. For this data the calculated value is r = 0.06928 for 2020 and r = 0.1 for 2019. Because the value of r is a positive number, the correlation is a direct one, and because the value of r has a small value, the correlation is very weak between 2019 and 2020 (Figure 4).

In the laboratories of molecular biology from Romania is used the PCR method to identify genetically modified soy the GTS 40-3-2 (Roundup Ready) line and the p35S promoter and the tNOS terminator for identify genetically modified corn.

The total number of 541 samples of food and feed analyzed by all laboratories to identify the modified soy line (Table 2) is much higher than the number 391 samples examined for modified corn (Table 3).

For genetically modified soybeans, we noticed that for the eastern part of Romania, a number of 133 samples were examined by the molecular biology and GMO laboratories within the county LSVSA, for the western area a number of 252 samples, and for the southern area a number of 156 samples (the southern area also includes samples examined within IDSA, which is the national reference laboratory for determining genetically modified organisms), according to the data provided by the county LSVSA laboratories and which are included in Table 2.

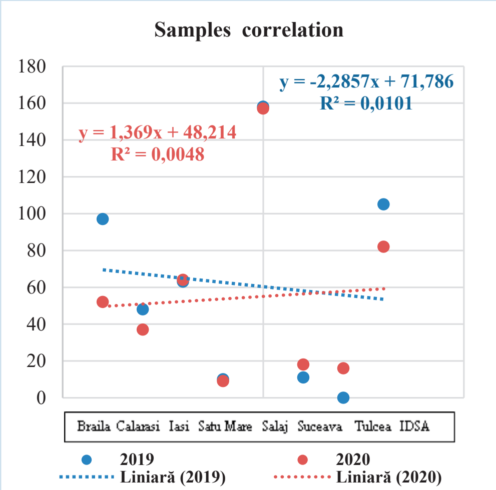

Figure 4. Correlation of samples analyzed for GMO detection, for 2019 and 2020

Table 2. Total samples number of food or feed processed for identification of modified DNA soy line during 2019 - 2020

	Species	Total
Braila	soy	63
Calarasi		85
Iasi		44
Satu Mare		19
Salaj		233
Suceava		26
Tulcea		16
IDSA		55
Total		**541**

During the study period, LSVSA Salaj examined for identification of genetically modified soy most food and feed samples, a total of 233 samples.

For genetically modified corn, we noticed that for the eastern part of Romania, a number of 177 samples were processed within LSVSA Braila, Iasi, and Suceava.

For the western area within LSVSA Salaj we observed a number of 82 samples and for the southern area within IDSA a number of 132 samples (Table 3) Data on the analysis of food and feed for genetically modified corn were received from 5 molecular biology and GMO laboratories among the 8 county laboratories, data that were included in this study.

Table 3. Total samples number of food or feed processed for identification of modified corn

	Species	Total
Braila	corn	86
Iasi		88
Salaj		82
Suceava		3
IDSA		132
Total		**391**

The graphical representation of the distribution on laboratories of the samples examined during this study shows that all 8 laboratories examined samples for the identification of genetically modified soy (Figure 5) and 4 laboratories for the identification of genetically modified corn (Figure 6).

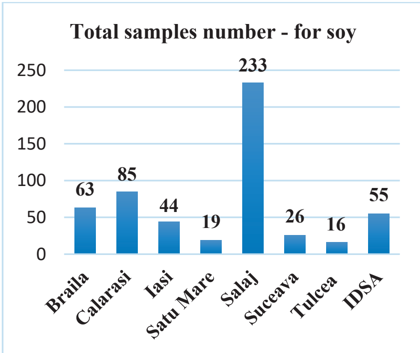

Figure 5. The total samples number analyzed for the identification of the specific modified DNA of line GTS 40-3-2 (Roundup Ready) during 2019-2020

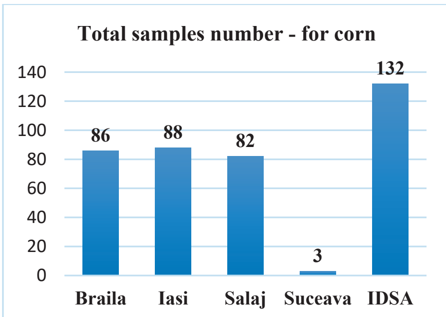

Figure 6. The total samples number analyzed for P-35S and T-NOS identification from corn during 2019-2020

In comparison, for the years 2019 and 2020 (Table 4.) is approximately equal to the annually number of food or feed samples for which it was examined to identify the genetically modified soybean line, by the PCR detection method to find the p35S and tNOS sequences.

Table 4. Annually samples number of food or feed by soy analyzed in 2019 – 2020 and their average

	Species	2019	2020	Average
Braila	soy	40	23	**31.5**
Calarasi		48	37	**42.5**
Iasi		22	22	**22**
Satu Mare		10	9	**9.5**
Salaj		116	117	**116.5**
Suceava		8	18	**13**
Tulcea		0	16	**8**
IDSA		32	23	**27.5**
Total		**276**	**265**	**270.5**

In 2019 (276 samples), they were analyzed with only 10 more samples than in 2020 (265 samples), with an average of 270.5 samples.

In the LSVSA Tulcea, we noticed that no samples were analyzed for the identification of genetically modified soybeans in 2019, but in 2020 the analyzes for this species were resumed in food and feed products and we observed a number of 16 samples examined in this molecular biology and GMOs laboratory.

All 8 county laboratories for the determination of genetically modified organisms examined samples of food and feed to analyze the genetically modified soybean line.

We observe from the following graph (Figure 7) that we have a maximum of 117 samples examined in 2020 at LSVSA Salaj and a minimum of 8 samples examined in 2019 at LSVSA Suceava.

For genetically modified soybean, in the LSVSA Braila, Calarasi, Satu Mare, and IDSA a smaller number of samples were analyzed in the pandemic year 2020 (Figure 7).

In the LSVSA Iasi and Salaj were analyzed an approximately equal number of samples for the two years of study. In the LSVSA Suceava and Tulcea, the number of analyzed samples was increasing in 2020, compared to 2019. For LSVSA Suceava there is a doubling of the number of samples, and for LSVSA Tulcea we can say that the laboratory resumed the determination of GMOs (16 samples) after what the year 2019 meant the lack of this activity (Figure 7).

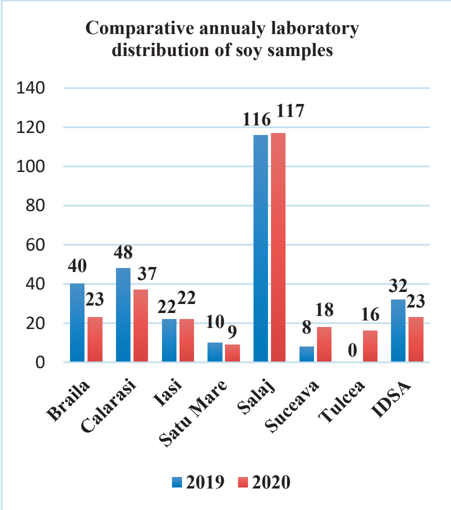

Figure 7. Comparative laboratory distribution of the samples number of food or feed containing soy

The number of samples examined to identify the genetically modified corn (Table 5) presents a maximum of 73 samples in 2019 at the IDSA laboratory.

In 2019, 221 food and feed samples were taken for the determination of genetically modified corn, compared to 170 samples in 2020, with about a quarter fewer samples observed for the pandemic year 2020 and an average of 195.5 samples (Table 5).

The variability of the data on the number of food or feed samples examined to identify the genetically modified soybean line is the standard deviation S = 34.64144, so about one-third part of the data is located on both sides of the average (Table 4).

The rank of statistical correlation for the data from Table 4 is represented by the linear correlation coefficient.

For this data, the calculated value is r = 0.18357 for 2019. The linear correlation coefficient r = 0.00006 for 2020 and the correlation is the direct correlation.

Because the value of r for 2019 is a positive number, the correlation is a direct correlation, and because the value of r has a small value, the correlation is very weak (Figure 8).

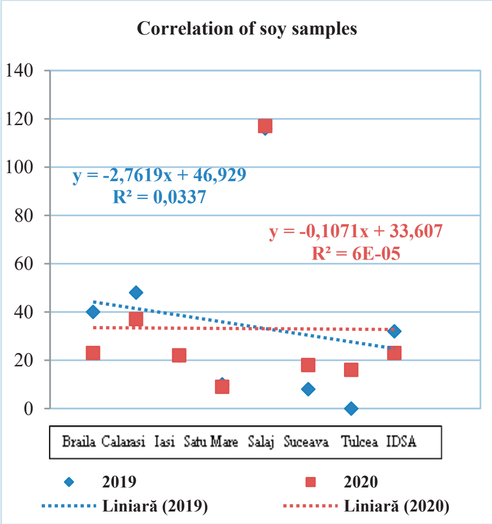

Figure 8. Correlation of samples of food or feed by soy analyzed for GMO detection, for 2019 and 2020

Table 5. Annually samples of food or feed by corn analyzed in 2019 – 2020 and their average

	Species	2019	2020	Average
Braila	corn	57	29	**43**
Iasi		46	42	**44**
Salaj		42	40	**41**
Suceava		3	0	**1.5**
IDSA		73	59	**66**
Total		**221**	**170**	**195.5**

Within LSVSA Suceava (Moldova area), no samples were analyzed for genetically modified corn in 2020.

Within LSVSA Braila, approximately half of food and feed samples were processed in 2020 (29 samples), compared to 2019 (57 samples).

In the LSVSA Iasi were analyzed an approximately equal number for the two years of study, 46 samples in 2019 and 42 samples in 2020.

The same for LSVSA Salaj was analyzed an approximately equal number for the two years of study, 42 samples in 2019 and 40 samples in 2020.

The laboratory within IDSA examined a smaller number with 14 samples in 2020, compared to the previous study year.

The following graphical representation also shows that in 2019 and 2020, the laboratory within IDSA had a capacity and received for analyzing much more samples than the other laboratories (Figure 9).

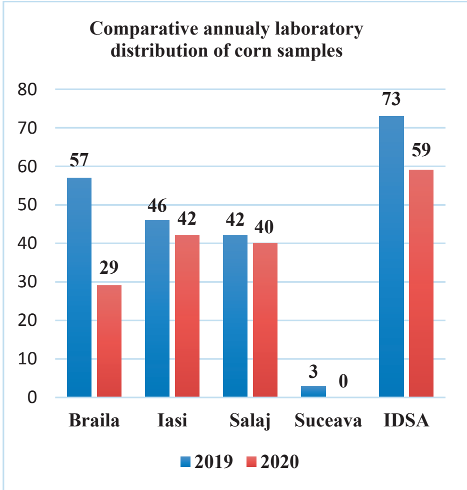

Figure 9. Comparative laboratory distribution of the samples number of food or feed containing corn

The variability of the data on the number of food or feed samples examined to identify the genetically modified corn is the standard deviation S = 23.24961, so about one-fifth part of the data is located on both sides of the average (Table 5).

The statistical correlation coefficient for the data contained in Table 5. is represented graphically by Figure 10.

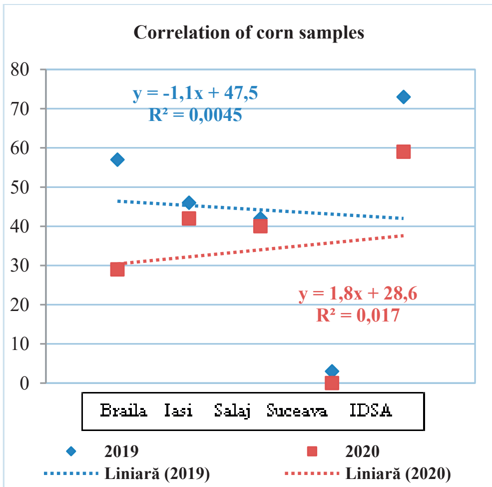

Figure 10. Correlation of samples of food or feed by soy analyzed for GMO detection, for 2019 and 2020

For this data the calculated the correlation coefficient value is r = 0.13038 for 2020 and r = 0.06708 for 2019.
Because the value of r for 2019 and 2020 is a positive number, the correlation is a direct correlation.
Because the value of r has a small value, the correlation is very weak between the two years of the study (Figure 10).
During the study period 2019 – 2020, there were 0 positive results for GMO determination for genetically modified soy and corn.
Were examined samples from food or feed products and the samples were collected from the marketing, processing, production, warehousing units.
Sampling is carried out according to specific procedures regarding the size of the lots, the packaging or bulk products, the diversity of the products and the observance of national and European hygiene norms, and prevention of direct or indirect contamination of these food or feed samples.
These results in fact without positive samples for the determination of GMOs are by the data provided by the 8 laboratories of molecular biology and the determination of genetically modified organisms in Romania accredited for performing these analyzes.
The purpose of this paper is to make public the data on food and feed samples examined for GMO detection within the molecular biology and GMOs laboratories in Romania during 2019 - 2020 and to be a starting point for future specialized studies.
The European Commission considers that legislation needs to be amended to strike a stable balance between maintaining the European Union licensing system based on scientific health and environmental risk assessment and the need to give Member States the freedom to address specific national or local issues raised by cultivation Genetically Modified Plants.
This approach is expected to respond to the demands of several Member States and to receive public support while maintaining the European Union authorization system for Genetically Modified Organisms, as well as the free movement and import of food, feed, and modified genetic seeds.

CONCLUSIONS

During 2019-2020, eight molecular biology laboratories processed 927 samples of food and feed products of plant origin to identify specific genetic modification sequences of soy or corn genetically modified 492 samples in 2019 and 435 samples in 2020.
The number of samples processed for identifying genetically modified soybeans (541 samples) is higher than the number of samples processed to identify genetically modified corn (391 samples).
The total number of samples analyzed during 2019 – 2020 show that the laboratories within LSVSA Salaj (Transylvania area), IDSA, and Braila (south Moldova area) examined a lot more samples.
For the analysis of GMOs, LSVSA Salaj examined the largest number of samples for each of the two years of study. In comparison, LSVSA Braila, Iasi, and IDSA the number of samples is approximately half.
For the identification of genetically modified soybeans: the LSVSA Salaj laboratory (116 samples in 2019 and 117 samples in 2020) processed the largest number of samples in comparison with all the other laboratories, whereas the LSVSA Iasi and Satu Mare examined an approximately equal number in each of the study years.
Within the LSVSA Braila, Calarasi, Satu Mare, and IDSA a smaller number of samples were analyzed in the pandemic year 2020.
In 2019, 221 food and feed samples were taken for the determination of genetically modified corn, compared to 170 samples in 2020. The samples processed to identify genetically modified corn have been processed mostly by the IDSA laboratory (73 samples in 2019 and 59 samples in 2020).
Within LSVSA Braila, approximately half of samples were processed in 2020, compared to 2019, and in the LSVSA Iasi and Salaj were analyzed an approximately equal number of samples for the two years of study.
During 2019 – 2020, the study period there were no positive results for genetically modified soy and corn determination, according to the data provided by the laboratories of molecular biology and determination of genetically modified organisms from Romania.

From a statistical point of view, several statistical indicators were calculated, such as: arithmetic average, variability by the standard deviation (S), and correlation by correlation coefficient (r).

The average was calculated for:

- the total samples examined (463.5 samples);
- the total sample examined by each laboratory;
- the total samples examined for the determination of the genetically modified soy (270.5 samples);
- samples examined for the determination of genetically modified soy by each laboratory;
- the total samples examined for the determination of genetically modified corn (195.5 samples);
- sample examined for the determination of genetically modified corn by each laboratory.

The standard deviation was calculated for:

- the total samples examined for the two years of study (S = 50.58784);
- samples examined for the determination of genetically modified soy (S = 34.64144);
- samples examined for the determination of genetically modified corn (S = 23.24961).

The correlation coefficient was calculated for:

- samples examined in 2019 and 2020 (r = 0.06928 for 2020 and r = 0.1 for 2019);
- samples examined for the determination of genetically modified soybeans (r = 0.00006 for 2020 and r = 0.18357 for 2019);
- samples examined for the determination of genetically modified maize (r = 0.13038 for 2020 and r = 0.06708 for 2019).

This is a direct correlation because r is a positive number and is very weak correlations because is a number between 0 and 0.2.

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THE INFLUENCE OF DIFFERENT SUCROSE CONCENTRATIONS AND GENOTYPES OVER PLANTLETS GROWING PARAMETERS

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Abstract

The objective of this study was to determine the effect of sucrose concentrations (2 and 3%) added to nutritive culture medium over growing parameters: plantlets length, number of leaves/plantlets, root length, fresh plantlet weight, fresh root weight. The experience is bifactorial (2 x 3), on 3 repetitions, with next factors: experimental factor A - the sucrose concentration on nutrient medium with two graduations (2, as control and 3%) and experimental factor B: variety, with 3 graduations (Marvis, considered as control, Castrum and Ervant). The use of 3% sucrose concentration has a positive influence in vitro, for leaves number, fresh plantlet weight and fresh root weight. Castrum variety has a significant positive and a distinctly significant positive differences (1.69 cm and 3.44 cm) for the length of the plantlets, respectively the number of leaves/plantlets. Also, Ervant variety had a good in vitro behaviour with a distinctly significant positive (2.50 cm) and significant positive differences (2.11 leaves and 0.052 g) for three of the analysed parameters (plantlet length, number of leaves/plantlet and fresh plantlet weight).

Key words: *potato, genotypes, plantlets, in vitro, sucrose concentrations.*

INTRODUCTION

By cultures of plant tissues and cells, respect-tively by cultures of explants, it is understood their growth *in vitro*, for different purposes, on artificial culture media having complex chemical compositions (Cachiţă-Cosma D. and Sand C., 2000). Recently, plant tissue culture technology has become very popular and has a visible impact on the production of virus-free seed potatoes (Sadek et al., 2017). Micropropagation becomes the alternative to conventional propagation of potatoes and includes two main steps: multiplication and acclimatization of the *in vitro* obtained plants to growing under field conditions (Salem and Hassanein, 2017). Tissue culture has been applied to improve production of potato by germplasm conservation, pathogen free potato plants and micropropagation (Khalafalla, 2001). *In* vitro propagation of potato using single nodal cutting is the best method of rapid multiplication brates with maximum genetic stability (Chandra and Naik, 1993). The quality potato seed is one of the major constraints in potato production, especially in developing countries where the cost of seed potatoes alone accounts for about 40-60% of the total production cost (Shekhawat et al., 1997). The nutritional composition varied depending on the type of cells, tissues, organs, protoplasts and the plant species used (Badr, 2011). Plant cells, tissue or organ is requiring carbon source (George, 1993; Gopal, 2004). The concentration of sucrose is one of the factors controlling the induction and growth of *in vitro* shoots (Gibson, 2000; Gurel and Gulsen, 1998, cited by Ndagijimana et al., 2014). The optimum sucrose level for shoot development may vary among species and genotypes (Nowak et al., 2004, cited by Ndagijimana et al., 2014). According to the study approached by Durnikin et colab. (2019), The addition of sucrose in the concentration of 3-5% contributed to the formation of more internodes. Good development of plants from cuttings is the main criterion for evaluation of micropropagation efficiency. The main factors influencing the parameters of growth and development of microplants are variety features and composition of the nutrient medium (Ryabtseva et al., 2017, cited by Durnikin et colab., 2019). Factors of nutrient environment is one of the important parameters that should be optimized to obtain successful plant regeneration (Durnikin et al., 2019). Plants produce sucrose through photosynthesis and can transport this molecule through the vascular system (Taiz and Zeiger, 2006). Carbohydrate is supplemented as a carbon source to maintain carbon supply as well as to maintain osmotic potential of cells. Sucrose is widely used in plant tissue culture due to its most favourable effect on growth and relatively low cost (Sumaryono et al., 2012). Sucrose in concentrations between 2-4% is usually added into culture medium and is a major component (George and Sherrington, 1993/1996). Sucrose also supports the maintenance of osmotic potential and the conservation of water in cells (Gago et al., 2014). Sucrose in culture media is usually hydrolysed totally, or partially, into the component monosaccharides glucose and fructose (George, 1993).

MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of National Institute of Research and Development for Potato and Sugar Beet Brasov during the period from December 2020-January 2021. This study is part of the research activity from ADER 5.1.2. project *Research on the production of minitubers under specific isolation conditions*. The main objective of the laboratory activity is to obtain a biological material with a high value, healthy, free of diseases and viruses. The obtaining methodology for this material, corresponding from phytosanitary point of view, is based on the *in vitro* multiplication technology of the potato, starting from the meristem culture. *In vitro* technology uses as first material the potato tubers sprout (Figure 1), which reached the desired length of 2-3cm are detached and disinfected (Figure 2) for meristematic sampling (Figure 3). In order to obtain a virus-free material, in addition to the meristem culture, chemotherapy was applied with 4 antiviral agents: acyclovir, ribavirin, 5-bromouracil and 2-thiouracil (all of them in 2 concentrations: 15 and 30 mg/l). After 6-8 months, depending by genotypes, from meristematic explants plantlets are developed.

The phytosanitary quality of the plantlets is determined by the ELISA test. All plantlets on medium with 30 mg/l ribavirin were virus free.

Plantlets were subjected to fragmentation in order to obtain minicuttings (Figure 4).

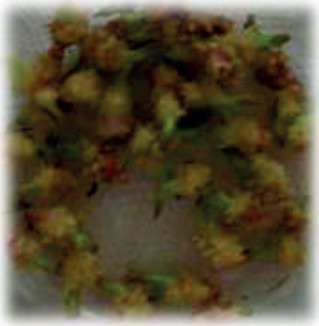

Figure 1. Potato sprouts

Figure 2. Sterilisation of potato sprouts

Figure 3. Meristematic prelevation

Figure 4. Minicuttings

Each minicutting contained a single node with the corresponding leaf. The minicuttings were placed in a culture medium in an upright position, respecting their polarity. The nutrient medium Murashige-Skoog (1962) (MS) was agarized and alpha naphthylacetic acid (NAA) was added as a growth medium regulator at a concentration of 0.5 mg/l. After inoculation, the inoculated tubes were placed in the growth chamber at 22°C with a photoperiod of 16 hours of light and 8 hours of darkness. Murashige-Skoog (1962) was supplemented with 20 g/l sucrose and 30 g/l sucrose respectively and 0.9% phyto-agar. The goal is to improve *in vitro* plantlets development and to determine the beneficial sucrose concentration for plantlets. The pH was reduced to 5.6-5.8. The medium was sterilized in an autoclave for 15'.

Marvis, Castrum and Ervant, Romanian genotypes created at National Institute of Research and Development for Potato and Sugar Beet Brasov were analysed to determine the regeneration of minicuttings produced under micropropagation conditions and plantlets development. At four weeks after inoculation of minicuttings, measurements were made for: plantlets height, number of leaves, root length, weight of the fresh plantlets and the weight of the fresh root. The research consisted on a bifactorial experience (2 x 3), on 3 repetitions, comprising the following factors: experimental factor A - the sucrose concentration on nutrient medium with two graduations: a1-MS control medium, to which 2% sucrose was added; a2- MS medium, to which 3% sucrose was added; experimental factor B: variety, with 3 graduations: b1- Marvis, considered as control; b2- Castrum; b3- Ervant. The obtained results were processed by the analysis of variance (Săulescu N.A & Săulescu N.N., 1967).

RESULTS AND DISCUSSIONS

The statistical interpretation of the number of leaves/plantlets shows that the difference for 3% sucrose concentration reported to control (concentration of 2%) is significantly positive (1.85 leaves). Regarding the fresh weight of the plantlets (g) and the fresh root (g), the differences for the concentration of 3% are distinctly significant positive (0.05 g/0.07 g), compared to the control (Table 1).

In the case of variety influence (Table 2), it can be seen that the differences for plantlets length (cm) and number of leaves/plantlets are big compared to the Marvis variety control. Thus, the Castrum variety registers significant positive and distinctly significant positive differences (1.69 cm and 3.44 cm) for the length of the plantlets, respectively the number of leaves/plantlets.

The Ervant variety shows distinctly significant positive (2.50 cm) and significant positive (2.11 leaves and 0.052 g) differences for three of the analysed parameters (plantlet length, number of leaves/plantlet and fresh plantlet weight). Regarding the root length, the Ervant variety has an opposite behaviour, presenting a significant negative difference (-2.89 cm, with a value of 3.58 cm.

Research on the combined influence of sucrose concentration and genotype on plantlet length (Table 3) shows that at a concentration of 2% sucrose, the Castrum variety obtains a high value (8.28 cm) and a significant positive difference (2.61 cm).

The concentration of 3% sucrose determined for the Ervant variety a distinctly significant positive difference (3.11 cm), with a value of plantlet length by 10.39 cm. Thus, this concentration (3%) determined for the Ervant variety the development of a bigger plantlet. As greater plant height (Table 3) and number of leaves (Table 4) are higher, the multiplication coefficient is bigger. The concentration of 3% (compared to 2%) had a beneficial effect for the Ervant variety, standing out with a significant positive difference (2.83 cm).

From the examination of the differences obtained between the 2 sucrose concentrations, a larger value for plantlet length it is ascertained (7.28 cm) for the Marvis variety, by applying 3% sucrose in culture medium, but the difference relative to 2% sucrose, although it is positive, is insignificant (1.61 cm).

Table 1. Influence of sucrose concentration in the nutrient medium on the elements studied: plantlet length (cm), leaves number/ plantlet, root length (cm), fresh plantlet weight (g), fresh root weight (g)

Sucrose conc. (%) (a)	Plantlets length (cm)	Dif. (cm)	Sign.	Leaves no.	Dif.	Sign.	Root length (cm)	Dif. (cm)	Sign.	Fresh plantlet weight (g)	Dif. (g)	Sign.	Fresh root weight (g)	Dif. (g)	Sign.
2 (Ct)	7.17	-	-	9.48	-	-	5.56	-	-	0.11	-	-	0.05	-	
3	8.57	1.41	ns	11.33	1.85	*	6.04	0.48	ns	0.16	0.05	**	0.12	0.07	**

LSD 5%=2.07 cm; LSD 1%=4.78 cm; LSD 0.1%=15.21 cm.
LSD 5%=1.84 leaves; LSD 1%=4.24 leaves; LSD 0.1%=13.50 leaves.
LSD 5%=1.90 cm; LSD 1%=4.39 cm; LSD 0.1%=13.98 cm.
LSD 5%=0.02 g; LSD 1%=0.04 g; LSD 0.1%=0.13 g.
LSD 5%=0.01 g; LSD 1%=0.03 g; LSD 0.1%=0.09 g.

Table 2. The influence of the varieties on studied the elements

Varieties(b)	Plantlets length (cm)	Dif. (cm)	Sign.	Leaves no.	Dif.	Sign.	Root length (cm)	Dif. (cm)	Sign.	Fresh plantlet weight (g)	Dif. (g)	Sign.	Fresh root weight (g)	Dif. (g)	Sign
Marvis (Mt)	6.47	-	-	8.56	-	-	6.47	-	-	0.103	-	-	0.08	-	-
Castrum	8.17	1.69	*	12.00	3.44	**	7.33	0.86	ns	0.142	0.039	ns	0.10	0.02	ns
Ervant	8.97	2.50	**	10.67	2.11	*	3.58	-2.89	ooo	0.154	0.052	*	0.08	0.00	ns

LSD 5%=1.40 cm; LSD 1%=2.04 cm; LSD 0.1%=3.06 cm.
LSD 5%=2.02 leaves; LSD 1%=2.93 leaves; LSD 0.1%=4.40 leaves.
LSD 5%=0.91 cm; LSD 1%=1.33 cm; LSD 0.1%=1.99 cm.
LSD 5%=0.045 g; LSD 1%=0.066 g; LSD 0.1%=0.099 g.
LSD 5%=0.05 g; LSD 1%=0.07 g; LSD 0.1%=0.10 g.

Table 3. Combined influence of sucrose concentration and variety on plantlet length (cm)

Conc. (%) (a)/ Variety (b)	2% (a_1)	Dif. (cm)	Sign	3% (a_2)	Dif. (cm)	Sign	a_2b_1-a_1b_1 (cm)	Sign
Marvis (Ct)	5.67	-	-	7.28	-	-	1.61	ns
Castrum	8.28	2.61	*	8.06	0.78	ns	-0.22	ns
Ervant	7.56	1.89	Ns	10.39	3.11	**	2.83	*

LSD 5% =1.98 cm; LSD 1% = 2.88 cm; LSD 0.1% = 4.33 cm.
LSD 5% =2.51 cm; LSD 1% = 4.79 cm; LSD 0.1% = 12.11 cm.

The combined influence of sucrose concentration and variety on the number of leaves/plantlet (Table 4) confirms the superiority of the Castrum variety, with a value of 14.00 leaves and a distinctly significant positive difference 5.44 leaves, followed by the Ervant variety, with a value of 11.44 leaves (with a significant positive difference of 2.89 leaves) for the concentration of 3% sucrose. Analysing the two concentrations of sucrose, the superiority in the production of the number of leaves of the concentration of 3% is highlighted, for the Castrum variety, with a significant positive difference (4 leaves).

Table 4. The combined influence of sucrose concentration and variety on the number of leaves

Conc. (%) (a)/ Variety (b)	2% (a_1)	Dif. (g)	Sign.	3% (a_2)	Dif. (g)	Sign.	a_2b_1-a_1b_1 (g)	Sign.
Marvis (Ct)	0.08	-	-	0.12	-	-	0.04	ns
Castrum	0.13	0.05	ns	0.15	0.03	ns	0.02	ns
Ervant	0.11	0.03	ns	0.20	0.08	*	0.09	*

LSD 5% =2.85; LSD 5% =2.86;
LSD 1% = 4.15; LSD 1% = 7.86;
LSD 0.1% = 6.22. LSD 0.1% = 22.42

When examining the combined influence of sucrose concentration and variety on root length (Table 5) it is observed that the difference obtained compared to the control (Marvis variety) is significantly positive for the Castrum variety (1.83 g), with a value of 7.44 cm. Ervant variety had an opposite behaviour, with a value of root length by 3.61 cm and a distinctly significant negative difference (-2.00 cm). Regarding the concentration of sucrose in the culture medium, the experience with 3% sucrose determined the obtaining for the Marvis variety of a root with a positive but insignificant difference (1.72 cm).

Table 5. Combined influence of sucrose concentration and variety on root length (cm)

Conc. (%) (a)/ Variety (b)	2% (a_1)	Dif. (cm)	Sign.	3% (a_2)	Dif. (cm)	Sign.	a_2b_1-a_1b_1 (cm)	Sign.
Marvis (Ct)	5.61	-	-	7.33	-	-	1.72	ns
Castrum	7.44	1.83	*	7.22	-0.11	ns	-0.22	ns
Ervant	3.61	-2.00	oo	3.56	-3.78	ooo	-0.06	ns

LSD 5% =1.29 cm; LSD 5% =2.08 cm;
LSD 1% = 1.88 cm; LSD 1% = 3.01 cm;
LSD 0.1% = 2.82 cm. LSD 0.1% = 6.76 cm.

If we examine the fresh plantlet weight of the three genotypes for the two sucrose variants (Table 6), it is observed that by using the concentration of 3%, the Ervant variety achieves a vigorous plantlet, with an average plantlet weight of 0.20 g and a significant positive difference (0.08 g). By comparing the concentration of 3% to 2%, the first one determines for the same variety (Ervant) a significant positive difference (0.09 g).

Table 6. Combined influence of sucrose concentration and variety on plantlet weight (g)

Conc. (%) (a)/ Variety (b)	2% (a_1)	Dif.	Sign.	3% (a_2)	Dif.	Sign.	a_2b_1-a_1b_1	Sign.
Marvis (Ct)	8.56	-	-	8.56	-	-	0	ns
Castrum	10.00	1.44	ns	14.00	5.44	**	4.00	*
Ervant	9.89	1.33	ns	11.44	2.89	*	1.56	ns

LSD 5% =0.06 g; LSD 5% =0.05 g;
LSD 1% = 0.09 g; LSD 1% = 0.21 g;
LSD 0.1% = 0.14 g. LSD 0.1% = 0.65 g.

The statistical interpretation on the fresh root of the plantlets shows that the combined influence of the sucrose concentration and genotype resulted in insignificant differences for the Castrum and Ervant genotypes, at a concentration of 2% and with low values (0.08 and 0.02 g).

The concentration of 3% in the culture medium, determined for Ervant variety obtaining a root with a higher average weight value (0.14). The difference registered between the 2 sucrose concentrations detaches 3% sucrose for Ervant variety, with a significant positive difference (0.11 g) (Table 7).

Table 7. Combined influence of sucrose concentration and variety on root weight (g)

Conc. (%) (a)/ Variety (b)	2% (a_1)	Dif. (g)	Sign.	3% (a_2)	Dif. (g)	Sign.	a_2b_1-a_1b_1 (g)	Sign.
Marvis (Ct)	0.05	-	-	0.10	-	-	0.05	ns
Castrum	0.08	0.02	ns	0.12	0.01	ns	0.04	ns
Ervant	0.02	-0.03	ns	0.14	0.03	ns	0.11	*

LSD 5% = 0.07 g; LSD 1% = 0.10 g; LSD 0.1% = 0.15 g.

LSD 5% = 0.06 g; LSD 1% = 0.23 g; LSD 0.1% = 0.72 g.

CONCLUSIONS

In the experimentation of the two sucrose concentrations for the analysed genotypes: Marvis, Castrum and Ervant, they behaved differently for all parameters.

The use of 3% sucrose concentration has a positive influence *in vitro*, for leaves number, fresh plantlet weight and fresh root weight.

The reaction of the varieties analysed shows the superiority of Castrum variety which had a significant positive and a distinctly significant positive differences (1.69 cm and 3.44 cm) for the length of the plantlets, respectively the number of leaves/plantlets. Also, Ervant variety had a good *in vitro* behaviour with a distinctly significant positive (2.50 cm) and significant positive differences (2.11 leaves and 0.052 g) for three of the analysed parameters (plantlet length, number of leaves/plantlet and fresh plantlet weight).

The plantlet height was between 10.39 cm and 7.28 cm for Ervant and Marvis varieties for 3% sucrose. Also, for the same concentration, Castrum variety had a superior number of leaves/plantlet (14.00), followed by Ervant variety (11.44).

Root length oscillated between 7.44 cm and 3.61 cm (for Castrum and Ervant varieties), but for 2% sucrose concentration. For the second concentration no significant differences were obtained (for the two varieties, compared to control) for root length.

The plantlet weight (g) was 0.20 g for Ervant variety and 0.12 g for Marvis variety, when it was used 3% sucrose.

At the analysis of the variety's behaviour at the concentration of 3%, compared to that of 2% it was found Ervant superiority with a significant positive difference (0.11 g).

Following this research, we recommend using in the culture medium of sucrose in a concentration of 3%.

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CURRICULUM DESIGN OF "SUSTAINABLE FOOD PRODUCTION SYSTEMS" MASTER PROGRAMME IN WESTERN BALKANS

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Abstract

The economic sectors involved in the search for a new balance in era of globalization and labour market flexibility are crying out for human resources capable of performing job activities to required standards in a variety of contexts and conditions. In this context, food sector and the education system are facing a lot of issues that are related not only with the strengthening of policies but also with the modernization of food engineering and food management practice. This paper presents the curriculum design of Master programme of Sustainable Food Production Systems according to the specific training needs of target groups, the vision and the strategic goals of the national educational policies regarding food sector for six universities from Western Balkans: Agricultural University of Tirana and European University of Tirana (Albania); University "Haxhi Zeka" of Peje and Universum College (Kosovo); University of Bihac and University of Sarajevo (Bosnia and Herzegovina). University of Agronomic Sciences and Veterinary Medicine of Bucharest was responsible to design this Curriculum, as partner of Erasmus plus project "MSc in sustainable Food Production Systems".

***Key words**: curriculum, master program, sustainable food production, Western Balkans.*

INTRODUCTION

A sustainable food system is a food system that delivers food security and nutrition for all in such a way that the economic, social and environmental bases to generate food security and nutrition for future generations are not compromised (FAO, 2018). Sustainable food is food that is healthy for the consumers and produced in ecologically and socially responsible and economically fair way (Byloo, 2011). Also, sustainable food production systems offer opportunities for economic benefits, creation of jobs, enhanced food safety and security. According to European Commission (EU), on the Common Agriculture Policies, there are many different views as respect to what constitutes a "sustainable" food system, and what falls within the scope of the sustainability" term (EU, Sustainable Development Strategy, 2016). For food, a sustainable system might be seen as encompassing a range of issues such as security of the supply of food, health, safety, affordability, quality, a strong food industry in terms of jobs and growth and, at the same time, environmental sustainability, in terms of issues such as climate change, biodiversity, water and soil quality (EU, Sustainable Development Strategy, 2016).

According to the Institute of Food Technologists (IFT), a global organization with members in more than 90 countries, dedicated to advancing the science of food and its application across the global food system, "the food sciences draws from many disciplines, including biology, chemical engineering, and biochemistry to better understand food processes and improve food products for the general public" (Food Sciences Institute, 2020).

The economic sectors involved in the search for a new balance in this era of globalization and labour market flexibility are crying out for human resources capable of performing job activities to required standards in a variety of contexts and conditions. In different EU countries the competency certification of training systems is in an advanced stage of development, the training on offer already incorporates a competency-based approach, whereas for other countries this objective has yet to be achieved. The absence of a system of competency standards appears to be the brake that is holding back its adoption, although the

need to modernize training has been clearly expressed in recent policies, legal instruments and educational reforms ("Steps" project proposal, 2018).
On the other hand, Western Balkans countries (in this case, Albania, Bosnia and Herzegovina, Kosovo) are all facing similar challenges regarding agricultural and food production and rural development (D1.1 report of "Steps" project). The latter are mostly related to the modernization of food engineering and food management practices. Organic agriculture production, post-harvest processes, environmental footprints, supply chain management, industrial ecology have to be addressed by educational programmes on the road to social, economic growth and integration with the EU (D2.1 report of "Steps" project). Such challenges are not limited within national borders; they are related to regional and global issues and call for cooperative actions. The Western Balkans region has clear aspirations to improve its economic competitiveness and integrate further into Europe. A highly skilled population is critical to achieving these goals, which makes creating and maintaining high quality and equitable education systems a vital part of regional development efforts (OECD, 2020).
In this context, the motivation behind the Erasmus plus project "Steps – Master Sciences of Sustainable Food Systems" is to build the capacity of partner countries from Western Balkans, to improve the quality of the education offered, and provide an education that is more aligned to the needs of the labour market and society. Capacity building of these countries will also offer the opportunity to engage in research, innovate, collaborate with EU and international partners in joint programmes and activities and face the challenges of the modern world. This programme offers advanced knowledge to graduates who work or aim to work in private companies and national bodies or start new businesses in particular, in rural, agricultural areas and in this way, can contribute to the transition to sustainable food production systems. The consortium includes partners that have diverse backgrounds and expertise's, so that they deal successfully with the complexities of project: Agricultural University of Tirana (coordinator) and European University of Tirana (Albania); University of Sarajevo, University of Bihac and Ministry of Education, Sciences, Culture and Sport of Una-Sana Canton (Bosnia and Herzegovina); University "Haxhi Zeka" of Peje and Universum College (Kosovo); Czech University of Life Sciences (Czech Republic); University of Agronomic Sciences and Veterinary Medicine of Bucharest (Romania); Agricultural University of Athens and Research Innovation and Development Organisation (Greece).

MATERIALS AND METHODS

In order to design the Curriculum, in the first year of the "Steps" project (2019), the stakeholders of MSc in Sustainable Food Systems: private sector companies SMEs and industry and SMEs, in terms of engineers and managers, rural society, farmers, students, national organizations, policy makers, national priorities, teaching staff, etc., from Higher Educations Institutions (HEIs) partners from Albania, Bosnia and Herzegovina and Kosovo, were investigated about topics of training needs. Also, other elements were analysed and taken into account for developing of specialised Curriculum, such as: the scientific background and the experience of the universities; the vision and the strategic goals of the national educational policies, other best practice in MSc in Food Sciences in the word.
The "Steps" Curriculum was developed by each HEIs partners through complex work teams (see name in Curriculum tables), which included both teaching staff and staff with experience in the certification of study programs, also, teams of EU project partners. University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania, due to its previous experience in creating educational curricula was coordinator of Working Package which had as subject the "Steps" structure and course design (WP2). MSc "Steps" Curriculum includes: the Core (mandatory, compulsory) Courses (subjects, disciplines) and Elective (optional); the number of hours reserved per week for a subject (and how many are intended for lectures, seminar, laboratory or projects); the type of assessment (evaluation, verification during the course); the number of related European Credit Transfer and Accumulation System (ECTS) per semester.

RESULTS AND DISCUSSIONS

The Curriculum is a set of courses that are considered basic and essential for future class work and graduation. Students may receive a grade and academic credit after completion of the course.

The Curriculum is the document that includes all the disciplines that must be passed in order to obtain a university qualification, divided by years of studies.

The list of disciplines included in the Curriculum, as well as their content, reflected in the analytical programmes, correspond to the legal profile and respond to the current training requirements of lawyers, with fundamental knowledge and the ability to adapt to the requirements of the practical activity,

The "Steps" MSc programme was designed according to the Bologna convention.

According to the target groups, the needs analysis, the scientific background, the expertise of the partners, and laboratories that were set up during the project (Figure 1), the Core Courses and Elective Courses will be organized in two working groups: *Food engineering, quality and safety* and *Food production systems management*.

Workloads were measured in ECTS credits and have defined 60 ECTS as a fulltime year of studies. The number of credits differs from country to country and is specified when the master's program is accredited. ECTS credits assigned to courses, in accordance with the estimated workload in terms of formal lectures, laboratory activities, projects and reports to be delivered by students, individual or team-based activities.

MSc Steps programme targets graduates of agriculture, food science and engineering, management, economics, business, engineers and managers already working in private companies or national organizations.

Attendees will have the opportunity to acquire knowledge of sustainability as related to the engineering and socio-economic aspects of food production systems. New teaching methodologies will focus on equip them with soft skills, including problem solving, team work, decision making.

Curriculum Design in Albania. For Agricultural University of Tirana (AUT) and European University of Tirana (EUT), the master program will be organizing by 6 ECTS credits per course, 30 ECTS per semester and 120 ECTS of two study years. The two universities offer a joint master's program in Albania. AUT will contribute with courses in the category of Food Engineering Quality and Safety and Master thesis and EUT will contribute with courses in the category of Food Production Systems Management and Master thesis. The Curriculum has the 6 common Core Courses, 7 Elective Courses for 1st year, 8 Elective Courses for 2sd year, with a total of 21 courses (Table 1).

Curriculum Design in Bosnia and Hertegovina. In University of Sarajevo (UNSA), the Master program was organised into four semesters and account for a total of 120 ECTS credits (Table 2). The Curriculum has a total of 26 courses, from which 6 Core Courses, 10 Elective Courses for 1st year and 10 elective courses for 2^{sd} year. University of Bihac (UNBI) organise only one year of study, with 60 ECTS, and 6 Core Courses and 10 Elective Courses (Table 3). Jointly on the national level based on a bilateral agreement between UNSA and UNBI meaning: exchange all teaching staff during the first semester of the Master study, and joint mentoring and co-mentoring on student Master's thesis and membership in the Master's thesis defence commissions.

Curriculum Design in Kosovo. University of Peja decide to have 30 ECTS credits per semester, divide by 5 ECTS per course, with a total of 120 ECTS. Also, it has 6 Core Courses, 7 Elective Courses in 2^{sd} semester, and 10 Elective Courses for 3rd semester (Table 4). Universum College will have the same structure in first year of study and in second year will have 7.5 ECTS per each course (Table 5). The two universities agree to do joint Master program to following principles: University of Peje should be home University and will prepare Self Evaluation Report; advertisement and enrolment conditions will be decided by home University; first semester with obligatory courses will be organised at University of Peje; second and third semesters and Master thesis will be organised in both universities based on student's interests.

Table 1. MSc - Sustainable Food Production Systems in Albania
Course Curriculum for Agricultural University of Tirana and European University of Tirana
(Renata Kongoli, Luziana Hoxha, Enkeleda Berberi, Anila Kopali, Myqerem Tafaj, Klotilda Marku, Alketa Shehaj, Anisa Peculi, Spase Shumka, Mariola Kodra, Erjon Mamoci (AUT); Kebjana Haka, Blerjana Bino, Elena Kokhti, Ermira Qosja, Arlinda Ymeraj, Klementin Mile, Kreshnik Bello, Irina Canco, Besarta Vladi, Selami Xhepa (EUT))

No.	Course title	Formative category	1st Semester (15 weeks)						2nd Semester (15 weeks)						
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab	P.	ECTS	ET	Total per sem.
	I. CORE COURSES														
1	Fundamentals of sustainable agri food systems	Core; FEQS	30	6	8	16	6	Written and oral							6
2	Agricultural and food industry waste management	Core; FEQS	30	12	0	18	6	W+O							6
3	Advanced food science and technology	Core; FEQS	30	6	8	16	6	W+O							6
4	Governance, policy and legislation in the agri-food sector	Core; MFPS	30	30			6	W+O							6
5	Food Ethics	Core; MFPS	30	30			6	W+O							6
6	Research methodologies and tools	Core; MFPS							30	6	6	18	6	W+O	6
	Total core courses: ECTS/semester						30								30
	II. ELECTIVE COURSES														
2	Quality System Development, Management and Shelf Life Assessment of Food	Elective; FEQS							30	12	0	18	6	W+O	6
3	Quality and Sustainability of Animal-source Food Production	Elective; FEQS							30	8	10	12	6	W+O	6
4	Traceability systems of food products	Elective; FEQS							30	10	10	10	6	W+O	6
5	Innovative product development	Elective; FEQS							30	6	12	12	6	W+O	6
6	Innovative practices of harvesting and post harvesting	Elective; FEQS							30	2	10	18	6	W+O	6
7	Ecological sustainability for Fish Management and Conservation	Elective; FEQS							30	6	8	16	6	W+O	6
8	Environmental Chemistry towards Food Processing	Elective; FEQS							30	12	8	10	6	W+O	6
Total elective courses: ECTS/semester													30		30
Total year courses: ECTS/year							30						30		60
			3rd Semester (15 weeks)						4th Semester (15 weeks)						
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab.	P.	ECTS	ET	
	III. ELECTIVE COURSES														
9	Management of Sustainable Food Supply Chain	Elective; MFPS	30	30			6	W+O							6
10	Marketing of Sustainable Agri-Food Products	Elective; MFPS	30	30			6	W+O							6
11	Innovation and Entrepreneurship for Sustainable Food Production Systems	Elective; MFPS	30	30			6	W+O							6
12	Sustainable Food Value Chain Management	Elective; MFPS	30	30			6	W+O							6
13	Consumer science and sustainable consumption	Elective; MFPS	30	30			6	W+O							6
14	Data Analysis and Decision-making	Elective; MFPS	30	30			6	W+O							6
15	Total Quality Management in the Agri-Food Sector	Elective; MFPS	30	30			6	W+O							6
16	Business economics and international trade in the agri-food sector	Elective; MFPS	30	30			6	W+O							6
Total elective courses: ECTS/semester							30								30
	Professional Practice	Compulsory							0			60	6	W+O	6
	MASTER THESIS	Compulsory							0		240		24	W+O	24
Total compulsory professional practice and master thesis: ECTS/semester													30		30
Total year courses: ECTS/year							30						30		60

Lect. – Lectures; S – Seminars; Lab.- Laboratory; P – Projects (hours/week); ECTS – credits/semester; ET – Evaluation type
FEQS – Food Engineering, Quality & Safety; MFPS – Management of Food Production Systems

Table 2. MSc - Sustainable Food Production Systems in Bosnia and Hertegovina
Course Curriculum for University of Sarayevo
(Sabahudin Bajramović, Milenko Blesić, Zlatan Sarić, Dragana Ognjenović, Nermina Spaho, Asima Akagić, Emir Džomba, Emir Bećirović)

No.	Course title	Formative category	1st Semester (15 weeks)						2nd Semester (15 weeks)						Total per sem.
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab	P.	ECTS	ET	
	I. CORE COURSES														
1	Fundamentals of sustainable agri food systems	Core; FEQS	30	-	15	-	5	W							5
2	Agricultural and food industry waste management	Core; FEQS	30	15	-	-	5	W							5
3	Advanced food science and technology	Core; FEQS	15	-	30	-	5	W							5
4	Governance, policy and legislation in the agri-food sector	Core; MFPS	30	15	-	-	5	W + O							5
5	Food Ethics	Core; MFPS	30	15	-	-	5	W							5
6	Research methodologies and tools	Core; MFPS	15	15	-	15	5	W+ O							5
Total core courses: ECTS/semester							30								30
	II. ELECTIVE COURSES														
7	Sustainable land management	Elective; MFPS							30	-	15	-	5	W + O	5
8	Waste and recycling technologies in agriculture	Elective; FEQS							30	-	-	15	5	W + O	5
9	Nutritionism	Elective; FEQS							30	15	-	-	5	W	5
10	Rural development	Elective; MFPS							30	15	-	-	5	W	5
11	Harvesting and post-harvesting technologies for agricultural products	Elective; FEQS							30	15	-	-	5	W	5
12	Low input agriculture	Elective; FEQS							30	-	15	-	5	W	5
13	Consumer science and sustainable consumption	Elective; MFPS							30	15	-	-	5	W	5
14	Total quality management in the agri-food sector	Elective; MFPS							15	-	15	15	5	W + Practical	5
15	Agri-food economics	Elective; MFPS							30	-	-	15	5	W	5
16	Business economics and international trade in the agri-food sector	Elective; MFPS							30	-	-	15	5	W	5
Total elective courses: ECTS/semester													30		30
Total year courses: ECTS/year							30						30		60
			3rd Semester (15 weeks)						4th Semester (15 weeks)						
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab.	P.	ECTS	ET	
	III. ELECTIVE COURSES														
17	Sustainable technology of dairy products	Elective; FEQS	30	-	15	-	5	W + O							5
18	Sustainable technology of fruit and vegetable processing products	Elective; FEQS	30	-	15	-	5	W + O							5
19	Sustainable technology of meat products	Elective; FEQS	30	-	15	-	5	W + O							5
20	Sustainable technology of wine, beer and spirits	Elective; FEQS	30	-	15	-	5	W + O							5
21	Sustainable technology of bakery products	Elective; FEQS	30	-	15	-	5	W + O							5
22	Packaging technology	Elective; FEQS	15	-	15	15	5	W + O							5
23	Innovation and entrepreneurship for sustainable food production systems	Elective; MFPS	30	15	-	-	5	W							5
24	Marketing of sustainable agri-food products	Elective; MFPS	30	15	-	-	5	W							5
25	Project cycle management	Elective; MFPS	30	-	-	15	5	W							5
26	Sustainable food value chain management	Elective; MFPS	30	-	-	15	5	W							5
Total elective courses: ECTS/semester							30								30
	MASTER THESIS	Compulsory							0		240		30	O	30
Total compulsory master thesis: ECTS/semester													30		30
Total year courses: ECTS/year							30						30		60

Lect. – Lectures; S – Seminars; Lab.- Laboratory; P – Projects (hours/week); ECTS – credits/semester; ET – Evaluation type
FEQS – Food Engineering, Quality & Safety; MFPS – Management of Food Production Systems

Table 3. MSc - Sustainable Food Production Systems in Bosnia and Hertegovina
Course Curriculum for University of Bihac
(teaching staff: Emir Mujić, Refik Šahinović, Suzana Jahić, Halid Makić, Jasmine Ibrahimpašić, Husein Vilić, Vildana Jogić Aida Džaferović, Melisa Oraščanin; students: Adnan Kovačević Amina Selimanović, (UNBI); Adnan Kreso (Ministry of Education, Science and Sport USK))

No.	Course title	Formative category	1st Semester (15 weeks)						2nd Semester (15 weeks)						
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab	P.	ECTS	ET	Total per sem.
I. CORE COURSES															
1	Fundamentals of sustainable agri food systems	Core; FEQS	45	15	-	15	5	W +O							5
2	Agricultural and food industry waste management	Core; FEQS	30	15	15	-	5	W +O							5
3	Advanced food science and technology	Core; FEQS	30	15	15	-	5	W +O							5
4	Governance, policy and legislation in the agri-food sector	Core; MFPS	15	15	-	15	5	W +O							5
5	Food Ethics	Core; MFPS	30	15	-	15	5	W +O							5
6	Research methodologies and tools	Core; MFPS	30	15	-	15	5	W +O							5
Total core courses: ECTS/semester							30								30
II. ELECTIVE COURSES															
7	Sustainable Land Management	Elective; MFPS							30	15	15	-	5	W +O	5
8	Harvesting and Post-Harvesting Technologies for Agricultural Products	Elective; FEQS							30	15	15	-	5	W +O	5
9	Low Input Agriculture	Elective; FEQS							30	15	-	15	5	W +O	5
10	Total Quality Management in the Agri-Food Sector	Elective; MFPS							30	15	-	15	5	W +O	5
11	Sustainable Technology of Dairy Products	Elective; EQS							30	15	15	-	5	W +O	5
12	Sustainable Technology of Meat Products	Elective; EQS							30	15	15	-	5	W +O	5
13	Sustainable Animal Production	Elective; FEQS							30	15	-	15	5	W +O	5
14	Sustainable Plant Production	Elective; FEQS							30	15	-	15	5	W +O	5
15	Animal Food Technology Science	Elective; FEQS							30	15	15	15	5	W +O	5
16	Marketing of Sustainable Agri-Food Products	Elective; MFPS							30	15	-	15	5	W +O	5
Total elective courses: ECTS/semester													15		15
	MASTER THESIS	Compulsory							0				15	O	15
Total compulsory master thesis: ECTS/semester													15		15
Total year courses: ECTS/year							30						30		60
Lect. – Lectures; S – Seminars; Lab.- Laboratory; P – Projects (hours/week); ECTS – credits/semester; ET – Evaluation type FEQS – Food Engineering, Quality & Safety; MFPS – Management of Food Production Systems															

Figure 1. Aspects of laboratories that were set up during the "Steps" project
(WP5, Development of infrastructures, 2020)

Table 4. MSc - Sustainable Food Production Systems in Kosovo
Course Curriculum for University of Peja
(Nexhdet Shala, Agym Rysha, Florin Peci, Ibish Mazreku, Afrim Selimaj, Fadil Millaku, Sabiha Shala, Ibrahim Hoxha, Defrime Berisha, Arsim Elshani, Naser Bajraktari, Astrit Bilalli, Bakir Kelmendi, Nazmi Hasanaj)

No.	Course title	Formative category	1st Semester (15 weeks)						2nd Semester (15 weeks)						Total per sem.
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab	P.	ECTS	ET	
I. CORE COURSES															
1	Fundamentals of sustainable agri food systems	Core; FEQS	30		30		5	W+ O							**5**
2	Agricultural and food industry waste management	Core; FEQS	30		30		5	W+ O							**5**
3	Advanced food science and technology	Core; FEQS	30		30		5	W+ O							**5**
4	Governance, policy and legislation in the agri-food sector	Core; MFPS	30	15	15		5	W+ O							**5**
5	Food Ethics	Core; MFPS	30	15	15		5	W+ O							**5**
6	Research methodologies and tools	Core; MFPS	30	15	15		5	W+ O							**5**
	Total core courses: ECTS/semester						30								30
	II. ELECTIVE COURSES														
7	Quality System Development, Management and Shelf Life Assessment of Food	Elective; FEQS							30		30		5	W+ O	**5**
8	Quality and Sustainability of Plant-source Food Production	Elective; FEQS							30		30		5	W+ O	**5**
9	Traceability systems of food products	Elective; FEQS							30		30		5	W+ O	**5**
10	Nutrition	Elective; FEQS							30		30		5	W+ O	**5**
11	Innovative practices of harvesting and post harvesting	Elective; FEQS							30		30		5	W+ O	**5**
12	Sustainable Ecology for Fish Management and Conservation	Elective; FEQS							30		30		5	W+ O	**5**
13	Environmental Chemistry towards Food Processing	Elective; FEQS							30		30		5	W+ O	**5**
	Total elective courses: ECTS/semester												30		30
	Total year courses: ECTS/year						30						30		60
			3rd Semester (15 weeks)						4th Semester (15 weeks)						
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab.	P.	ECTS	ET	
	III. ELECTIVE COURSES														
14	Sustainable technology of dairy products	Elective; FEQS	30		30		5	W + O							5
15	Sustainable technology of fruit and vegetable processing products	Elective; FEQS	30		30		5	W + O							5
16	Sustainable Use of the plant protection products	Elective; FEQS	30		30		5	W + O							5
17	Sustainable technology of wine, beer and spirits	Elective; FEQS	30		30		5	W + O							5
18	Sustainable technology of bakery products	Elective; FEQS	30		30		5	W + O							5
19	Consumer science and sustainable consumption	Elective; FEQS	30		30		5	W + O							5
20	Innovation and entrepreneurship for sustainable food production systems	Elective; MFPS	30		30		5	W							5
21	Marketing of sustainable agri-food products	Elective; MFPS	30		30		5	W							5
22	Total quality management in the agri-food sector	Elective; MFPS	30		30		5	W							5
23	Sustainable food value chain management	Elective; MFPS	30		30		5	W							5
Total elective courses: ECTS/semester							30								30
	MASTER THESIS	Compulsory							0				30	O	30
	Total compulsory master thesis: ECTS/semester												30		30
	Total year courses: ECTS/year						30						30		60

Lect. – Lectures; S – Seminars; Lab.- Laboratory; P – Projects (hours/week); ECTS – credits/semester; ET – Evaluation type
FEQS – Food Engineering, Quality & Safety; MFPS – Management of Food Production Systems

Table 5. MSc - Sustainable Food Production Systems in Kosovo
Course Curriculum for Universum College
(Uran Rraci, Elejtin Berisha, Luan Vardari, Gezim Turkeshi, Muhamet Hajdari)

No.	Course title	Formative category	1st Semester (15 weeks)						2nd Semester (15 weeks)						Total per sem.
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab	P.	ECTS	ET	
	I. CORE COURSES														
1	Fundamentals of sustainable agri food systems	Core; FEQS	30		30		5	W+ O							**5**
2	Agricultural and food industry waste management	Core; FEQS	30		30		5	W+ O							**5**
3	Advanced food science and technology	Core; FEQS	30		30		5	W+ O							**5**
4	Governance, policy and legislation in the agri-food sector	Core; MFPS	30	15	15		5	W+ O							**5**
5	Food Ethics	Core; MFPS	30	15	15		5	W+ O							**5**
6	Research methodologies and tools	Core; MFPS	30	15	15		5	W+ O							**5**
	Total core courses: ECTS/semester						30								30
	II. ELECTIVE COURSES														
7	Quality System Development, Management and Shelf Life Assessment of Food	Elective; FEQS							30		30		5	W+ O	**5**
8	Quality and Sustainability of Plant-source Food Production	Elective; FEQS							30		30		5	W+ O	**5**
9	Traceability systems of food products	Elective; FEQS							30		30		5	W+ O	**5**
10	Nutrition	Elective; FEQS							30		30		5	W+ O	**5**
11	Innovative practices of harvesting and post harvesting	Elective; FEQS							30		30		5	W+ O	**5**
12	Sustainable Ecology for Fish Management and Conservation	Elective; FEQS							30		30		5	W+ O	**5**
13	Environmental Chemistry towards Food Processing	Elective; FEQS							30		30		5	W+ O	**5**
	Total elective courses: ECTS/semester												30		30
	Total year courses: ECTS/year						30						30		60
			3rd Semester (15 weeks)						4th Semester (15 weeks)						
			Lect.	S.	Lab.	P.	ECTS	ET	Lect.	S.	Lab.	P.	ECTS	ET	
	III. ELECTIVE COURSES														
14	Management of Sustainable Food Supply Chain	Elective; FEQS	30	5	13	25	7.5	W+O							7.5
15	Marketing of Sustainable Agri-Food Products	Elective; FEQS	26	8	13	30	7.5	W+O							7.5
16	Innovation and Entrepreneurship for Sustainable Food Production Systems	Elective; FEQS	30	5	13	25	7.5	W+O							7.5
17	Sustainable Food Value Chain Management	Elective; FEQS	30	8	8	30	7.5	W+O							7.5
18	Consumer science and sustainable consumption	Elective; FEQS	26	13	5	30	7.5	W+O							7.5
19	Data Analysis and Decision-making	Elective; FEQS	30		13	30	7.5	W+O							7.5
20	Total Quality Management in the Agri-Food Sector	Elective; MFPS	30	13	13	15	7.5	W+O							7.5
Total elective courses: ECTS/semester							30								30
	MASTER THESIS	Compulsory							39	20	50	45	30	W+ O	30
Total compulsory master thesis: ECTS/semester													30		30
Total year courses: ECTS/year							30						30		60

Lect. – Lectures; S – Seminars; Lab.- Laboratory; P – Projects (hours/week); ECTS – credits/semester; ET – Evaluation type
FEQS – Food Engineering, Quality & Safety; MFPS – Management of Food Production Systems

The courses efficiency and relevance are presented based on high-level learning outcomes. The design provided guidelines for the development of the content of the courses, the educational methodologies and material, the utilisation of Information and communications technology (ICT) tools, the combination of traditional teaching with student-centred or blended learning approaches etc.
Scientific staff of the Agricultural University of Tirana, University of Peje, University of Bihac, University of Agronomic Sciences and Veterinary Medicine of Bucharest and Research Innovation and Development – ReadLab design

the courses related to food engineering, quality and safety.

Scientific staff of the European University of Tirana, Universum College, University of Sarajevo, Czech University of Life Sciences, and Agricultural University of Athens design the courses related to Food production systems management. Ministry of Education, Science, Culture and Sport of Una-Sana Canton provided guidelines in order to ensure that the courses of the MSc programme are designed in accordance with the requirements of the educational systems of the partner countries.

The level of education offered by Western Balkans Higher Education Institutions has to be improved, in order to support the implementtation of national policies and priorities related to agriculture restructure, rural development, food safety and security and sustainable food production systems (D1.1 report of "Steps" project).

By taking advantage of the technological growth, the existing agriculture-related study programmes, do not need to increase but rather be combined with Curriculum based on Science, Technology, Engineering, and Mathematics (STEM) oriented subjects ("Steps" project proposal).

Universities themselves need to enhance their networks with the labor market and society, improve the quality of the education offered, help graduates to be engaged with the world of work according to their skills and increase their perception and role considering social and economic growth (EU Commission, 2006). Update of educational programs, modernization of teaching methodologies, development of infrastructures and professional development of scientific staff are critical, if the educational systems of Western Balkans countries are expected to develop the human capital that will be able to tackle the challenges of modern food production systems .

The goal is to develop capacities and infrastructures and improve the level of education offered, by delivering a new joint MSc programme, which, compared to existing courses and learning programmes, will offer a holistic approach of sustainability aspects of food production systems.

The Core Courses of Curriculum for all HEIs institutions are: Fundamentals of Food Production Systems, Food Industry Waste Management, Advanced Food Science and Technology, Food Legislation, Food Ethics, and Research Methodologies and Tools.

Elective Courses will have subjects: food engineering, including food quality monitoring techniques and safety and management, including supply chain, economics and environmental management of food production systems, sustainable of vegetal and animal production, environment protection issues, agroecology, organic agriculture, etc.

Each Course Description will include:

▪ course unit title;
▪ type of course (compulsory or optional);
▪ semester of delivery;
▪ number of ECTS credits;
▪ course description and link with the problems and needs that it intends to address;
▪ scientific topics, methods and approaches that will be analysed in relation to the specific problems and needs;
▪ high-level learning outcomes;
▪ course contents and proposed sections;
▪ teaching methods and learning activities proposed, including laboratory experiments and software simulations;
▪ proposed evaluation methods and grading criteria.

The working groups are comprised by scientific staff participating also in seminars/lectures during the workshops and open seminars in HEIs countries and it's are involved in the development of research labs and the experiments and simulations after the installation of the modern equipment in partner countries HEIs.

Institutions participating in the "Steps" project should assure themselves of the competence of their teachers. They should apply fair and transparent processes for the recruitment and development of the staff (D2.2 report of "Steps" project). The teacher's role is essential in creating a high quality student experience and enabling the acquisition of knowledge, competences and skills. Also, scientific staff of HEIs partners will be involved in the development of educational material, research labs, experiments simulations and accompanying material for the STEPS platform.

CONCLUSIONS

The MSc Steps program aims to analyse and to put into service the agri-food production chains, while it is considered to have a considerable impact on economic, social and environmental points.

The mission of the STEPS MSc program is justified by elements of relevance and opportunity in relation to the objectives of education and scientific research, as well as with the national qualification list and, respectively, with the requirements of the labor market.

All documents (Course Curriculum, List of Courses – Core and Elective, Number of Hours per activity, Number of ECTS per course, Names of Teachers, List of Topics for lectures and List of topics for practical applications) were in direct connection with the documents for the accreditation elaborated at National level by HEIs universities.

Descriptions of the MSc programme courses will be provided along with the key scientific topics addressed.

Courses efficiency and relevance will be presented based on high-level learning outcomes.

The design of courses will also provide guidelines for the development of the content of the courses, the educational methodologies and material, the utilisation of ICT tools, the combination of traditional teaching with student-centred or blended learning approaches etc.

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DEVELOPMENT, PRODUCTIVITY AND QUALITY OF NAKED OAT GRAIN AFTER TREATMENT WITH BIOFERTILIZER IN THE CONDITIONS OF ORGANIC AGRICULTURE

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Abstract

The importance of oats as a healthy food for people is growing, and this draws attention to the production of environmentally friendly products, using environmentally friendly approaches and tools in agriculture. The study was conducted at the Demonstration Center for Organic Agriculture at the AU, Bulgaria during the 2013-2015 period. The aim of the study is the influence of biofertilizer Amalgerol on germination, development, productivity and some quality indicators of the grain in naked oats. Applied by seed treatment and vegetation on plants, increases the percentage of sprouted and harvested plants by 10% at a dose of 5 l/ha and by 13.4% at a dose of 10 l/ha. Amalgerol increases the weight of the grain from one plant in the adverse weather conditions of 2014 by 0.25 g to 0.30 g at different fertilization doses, as well as the weight of 1000 grains by 3.70 to 4.19% compared to the control. The effect on grain yield was positive by 17.26% at a dose of 5 l/ha and by 35.69% at a dose of 10 l/ha above the control. Amalgerol (10 l/ha) increases the hectolitre weight of the grain. There is more crude protein and crude fat in the grain in the unfertilized variant, and starch in the variants treated with Amalgerol (5 l/ha) - 51.18% and (10 l/ha) - 49.43%.

Key words: *naked oats, organic agriculture, organic fertilizer, grain yield.*

INTRODUCTION

Oats (*Avena sativa* L.) are becoming an increasingly attractive crop for Bulgarian agriculture. Its production is still insufficient to meet the needs of the market. Grain production is a major sector covering nearly 60% of the country's arable land and provides about 30% of the commodity production of the crop sector. The general vulnerability of production is determined by the strength of the negative impacts of climate change, globalization and regional integration, as well as by its adaptive capabilities (Alexiev A., 2008). In Bulgaria, oats are grown mainly for grain, as fodder for young animals and breeding animals. In countries with a tradition of oat production, special interest is in the multifaceted use of grain - for feed and human food (Cudderford, 1995). The good absorption of nutrients and vitamins in oatmeal make it particularly suitable for children's and dietary nutrition (Welch, 1995). In Bulgaria, oats are grown at about 50.000 ha and in recent years with the development of private agriculture there has been an increase in areas. Compliance with the conditions in the agroecosystem and agro-technological requirements of the culture is a basic prerequisite for the realization of high productive opportunities. The main points in the cultivation of oats are the observance of the term for sowing, the plant protection measures and the terms of harvesting. They are also leading for its successful cultivation in organic farming. The aim is to create varieties of oats suitable for the specific climatic and agro-ecological conditions of our country. As a result, new varieties of oats with high productive potential and ecological plasticity are created. Drought is the most important factor limiting grain production. The studies of a number of researchers are focused on studying the adaptation of new crop varieties to water stress in the conditions of increasingly prevailing extremely high air temperatures in the environment: 40-44°C (Slavov N. et al., 2000). In France, studies have focused on the length of growth periods, cold resistance, disease and their productivity in different agro-ecological regions of the country. As a result of a study by on sowing dates, it was found that plant deaths during the winter range from 48% to 50%. At the lowest crop density of 200 g.s./m², the losses were greatest. The optimal sowing period according to the author is 20.IX. to 1.X., and the optimal density is 400 g.s/m². According the Chisinau region, the most suitable time for sowing is the middle of month IX. Research shows that the optimal sowing rates are different, depending on the different agro-ecological areas (Georgieva T. et al., 1994). According to organic and mineral fertilization significantly increases the yield of oats. In the study of other authors (Turkin A., 1987; Savova T., 2001) it is stated that the utilization rate of nitrogen varies sharply by year depending on rainfall and soil moisture, and in dry years it decreases, and in wet increases significantly to 60-90%. The research of show that nitrogen uptake with primary fertilization plays an important role in growth and development in the early stages of organogenesis. A similar effect from nitrogen nutrition and of precrop (Delibaltova V. et al., 2010) was reported in other cereals in another studyes (Neshev N. et al., 2018). In the Czech Republic, a study was conducted on the influence of climatic factors on oat yield (Tichy F. et al., 1992), as a result of which the closest correlation between grain yield was reported with the number of grains in the panicle. The yield is negatively affected by the high air temperatures in the period from germination to harvest. High temperatures for 5 days after the panicle emergence reduce the number of grains in the panicle (Tichy F. et al., 1992). In other experiments, the dependences of yield and agri-environmental factors are analyzed. The size of the yield is more influenced by the sowing period than the sowing rate. The sowing rate and sowing date affect the weight of the grain in the panicle. Absolute and hectolitre weight change significantly depending on the meteorological conditions in the individual years and to a lesser extent on the date of sowing and the sowing rate. Many of the studies with oats are focused on the biological manifestations and economic qualities of the grain intended for fodder (Georgieva T. et al., 1990; Georgieva, T. et al., 1994). The potential of wintering naked oats in Bulgaria has also been assessed, but it has low winter hardiness (Antonova N., 2005). The first spring naked oat variety Mina was registered in Bulgaria (Antonova N. et al., 1995), and in 2010 the first naked wintering oat variety was registered in the selection.

A connection has been established between early maturity and drought resistance - varieties with low and medium drought resistance avoid water stress by shortening the growing period (Savova T., 2002). Studies on fertilization have shown that oats respond to fertilization especially with nitrogen, which is characterized by strong growth and high yields (Savova T. et al., 2005). In organic farming, oats are suitable for growing due to stable grain yields even on nutrient-poor soils and the valuable nutritional qualities of the produce.

The interest in organic farming, improving the quality and yield of naked oats, obtaining ecologically clean products, provokes the purpose of this study.

MATERIALS AND METHODS

The experiment was conducted at the Demonstration Biological Farm at the Agroecological Center at the Agricultural University - Plovdiv, Bulgaria in the period 2013 and 2015. A two-factor field experiment using the block method in three replications with the size of the reporting plot 10.5 m^2 after the predecessor pepper was set. Amalgerol biofertilizer and Mina spring naked oats were used in the conditions of organic farming. Sowing was done with 600 g.s./m^2 in early March. The factors were studied: A - the influence of the year; B) biofertilizer: B1 - control - without fertilization; B2 - Amalgerol - pre-sowing treatment of the grain in a semi-wet way in a dose of 0.5 l/100 kg grain + two vegetation treatments in doses of 5 l/ha in phenophases: I - in: tillering - stem elongation; II: panicle emergence; B3 - Amalgerol - pre-sowing treatment of the grain in a semi-wet way in a dose of 10 l/100 kg grain + two vegetation treatments in doses of 10 l/ha in phenophases: I - in: tillering - stem elongation; II: panicle emergence. The indicators were studied: number of sprouted plants/m^2, duration of phenological phases, overal and productive tillering, elements of the panicle, mass of 1000 grains (g), hectolitre weight, kg/100 l grain, grain yield kg/ha. Biochemical parameters of the grain: content of crude protein (Keldal's method according to BSS 13490 in wheat), starch (by polygraph method as a percentage of

absolute dry matter), crude fat (according to BSS 3412).
Amalgerol - has a certificate and can be used in organic production according to Regulation (EC) 834/2007 and Regulation (EC) 889/2008. Statistical data processing was performed using SPSS V.9.0 for Microsoft Windows (SAS Institute Inc. 1999).

RESULTS AND DISCUSSIONS

The meteorological situation during the study period is relatively favorable for the development of spring oats. In general, the temperature values are above the norm during all months of the vegetation period, but the precipitation is unevenly distributed over months. The shift of sowing outside the optimal agro-technical terms (April) due to heavy rainfall in March 2014, does not reflect well on the development and overall oat yield.

1. Phenological development

During the study period, the development of oats took place in conditions of completely different meteorological years. During the first vegetation year 2013 the sowing was carried out in an optimal agro-technological term (March) and the plants germinate in 14 days in all variants (Table 1).

Table 1. Duration of the interphase periods and vegetation by years in days

Variants	Interphase periods								
	Sowing - Germinations	Germinations - 3-rd leaf	3-rd leaf – tillering	Tillering– Stem elongation	Stem elongation - Panicle emergence	Panicle emergence – milk ripeness	Milk ripeness – Wax ripeness	Wax ripeness – Ful ripeness	Vegetation duration
2013									
Control	14	13	6	21	22	12	14	16	118
Amalgerol 5	14	13	6	21	22	12	14	16	118
Amalgerol 10	14	13	6	21	22	12	14	16	118
2014									
Control	9	5	5	22	20	19	11	13	106
Amalgerol 5	9	5	5	22	20	19	11	13	106
Amalgerol 10	9	5	5	22	20	19	11	13	106
2015									
Control	13	10	5	20	22	11	12	15	108
Amalgerol 5	13	10	5	20	22	11	12	15	108
Amalgerol 10	13	10	5	20	22	11	12	15	108

19 days after sowing the plants enter the tillering phase, and another 21 days pass until the stem elongation phenophase. Panicle emergence occurs after 22 days, when the second vegetation treatment with Amalgerol is applied. Milk and wax ripeness pass in conditions of abundant rainfall and temperature values above the norm for the long term, which favors the normal nutrition of the grain in the panicle. Full ripeness occurs 118 days after sowing. In 2014, sowing was hampered by heavy rainfall in March and April. Germination occurs 9 days after sowing. Phenophase tillering occurs on day 19 and lasts 22 days. The period from panicle emergence to waxy ripeness passes at high temperatures, which adversely affects the pollination and subsequent pouring and feeding of the grain. The duration of these periods is 26 days. Full ripeness occurs 106 days after sowing. The plants provide the necessary number of days for development, but due to the delayed sowing and the shift of the vegetation to the unfavorable warmer months of the year, the interphase periods are greatly shortened, which adversely affects the yield.
In 2015 the meteorological conditions are close to those of 2013. The plants germinate in 13 days and the duration of the vegetation is 108 days.
From our observations on the variants treated with Amalgerol at a dose of 5 l/ha and Amalgerol at a dose of 10 l/ha, no effect on the duration of the interphase periods compared to the untreated control was found.

2. Number of germinated and harvested plants

Ensuring a normal number of germinated plants is the first step in forming a normally, with optimal number of plants per dka, crop (Table 2). During the study period, the plants germinated normally with over 500 pieces per m^2. All variants germinate with between 561 and 570 plants/m^2 which determines the good sowing density. Although there is no statistically proven difference between the fertilization and control variants, the highest percentage of sprouted plants compared to the sowing rate was reported in the variant treated with Amalgerol - 5 l/ha - 95%, which is 1.48% above the untreated control.

Table 2. Number of germinated and number of harvested plants by variants on average for the study period

Variants	Number of germinated plants/m^2	% relative to sowing rate	% relative to contol	Number of harvested plants/m^2	% relative to germinated	% relative to contol
Control	561.66a	93.61	0	378.33b	67.36	0
Amalgerol 5	570.00a	95.00	1.48	422.67ab	74.15	10.08
Amalgerol 10	563.66a	93.94	0.4	430.67a	76.40	13.42

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

A positive effect of seed treatment before sowing with Amalgerol is possible.

During the vegetation, a reduction in the number of plants was observed, harvesting 378 plants/m^2 (67.36%) in the untreated control variant to 431 plants/m^2 (76.4%) of the germinated in the variant treated with Amalgerol – 10 l/ha.

This difference compared to the control is statistically proven and the treatment with Amalgerol - 10 l/ha has been proven to increase the number of harvested plants per ha. There is a percentage increase in the treated variant with Amalgerol 5 l/ha - 74.15% compared to the control (67.36%), but this difference is not proven statistically.

3. Tillering

One of the indicators that strongly influence the yield is the overall and productive tillering of the variety (Savova et al., 2005).

From the data in Table 3 there is a slight increase in the number of overall and productive tillering in both treatment options with Amalgerol, better expressed during the unfavorable for the development of oats 2014.

This fact is explained by the stimulating effect of the applied organic fertilizer Amalgerol.

Table 3. Overall and productive tillering (number of tillers/plant)

Variants	Overall tillering			Average	Productive tillering			Average
	Year				Year			
	2013	2014	2015		2013	2014	2015	
Control	1	1	1	1	1	1	1	1
Amalgerol 5	1.01	1.2	1	1.06	1.1	1	1	1.03
Amalgerol 10	1	1	1.1	1.02	1	1	1.1	1.02

4. Structural elements of the panicle

The structural elements of the yield associated with the panicle directly affect the grain yield (Table 4). In 2013, the formation of a longer panicle length was observed during treatment with Amalgerol, respectively by 3.65 cm for Amalgerol - 5 l/ha to 11 cm for Amalgerol - 10 l/ha, compared to the control. A higher value of the other indicators of the panicle is reported.

As a final result, the weight of the grain in the panicle was increased in the treated variants by 0.19 g for the Amalgerol 10 l/ha variant to 0.44 g for the Amalgerol 5 l/ha variant, compared to the untreated control. In the second year of the study, a longer panicle length was again reported by 2.20 cm for the Amalgerol 5 l/ha variant, up to 10.31 cm for the Amalgerol 10 l/ha variant compared to the control.

Table 4. Elements of the panicle by variants in the study period

Variants	Panicle length (cm)	Number of grains in a panicle	Grain weight in a panicle (g)	Grain weight of one plant (g)
2013				
Control	11.33	16.57	0.33	0.33
Amalgerol 5	14.98	36.20	0.74	0.77
Amalgerol 10	22.33	22.67	0.52	0.52
2014				
Control	10.01	10.02	0.20	0.20
Amalgerol 5	12.21	21.20	0.45	0.45
Amalgerol 10	20.32	22.11	0.50	0.50
2015				
Control	11.12	20.51	0.35	0.35
Amalgerol 5	13.65	35.21	0.61	0.61
Amalgerol 10	19.24	38.67	0.67	0.67
Average for the period				
Control	10.82c	15.7b	0.29b	0.29b
Amalgerol 5	13.61b	30.87a	0.60a	0.61a
Amalgerol 10	20.63a	27.72a	0.56a	0.56a

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

The values of the other indicators are also higher. Same trend was observed in the third year of the study. On average for the study period, treatment with Amalgerol at a dose of 10 l/ha has been shown to increase the length of the panicle compared to other variants - 20.63 cm. The positive effect of treatment with Amalgerol to increase the values of number of grains, grain weight in a panicle and grain weight per plant has been proven.

5. Grain yield, kg/ha

The years of research differ categorically from each other, with more favorable meteorological conditions for the formation of high yields in 2013. The data in table shows that the conditions of the year are a strong factor determining the yield. With a proven highest grain yield is 2013 - 1265.5 kg/ha and 2015 - 1182.5 kg/ha between which there is no proven difference, and with a proven lowest yield is 2014 - 809.8 kg/ha (Table 5).

The independent effect of Amalgerol biofertilizer is also proved statistically, as higher yields were reported when treated with a dose of 10 l/ha - 1252.5 kg/ha.

The yield values in the year vary, as they are proven to be higher when fertilizing with a higher dose in 2013 - 1485.7 kg/ha and 2014 - 911.8 kg/ha compared to other variant.

Table 5. Influence of the year and organic fertilizer on grain yield by years and average for the study period, kg/ha

Influence of year factor on yield	
2013	1265.5a
2014	809.8b
2015	1182.5a
Influence of biofertilizer factor on yield	
Control	942.6b
Amalgerol 5	1105.3ab
Amalgerol 10	1252.5a

Variant	2013		2014		2015		Average for the period, kg/ha	Average % relative to control
	Grain yield, kg/ha	% relative to control	Grain yield, kg/ha	% relative to control	Grain yield, kg/ha	% relative to control		
Control	1066.6 c	100	709.2 b	100	1052.1a	100	942.6b	100
Amalgerol 5	1244.2 b	117	808.5 b	114	1263.2a	120	1105.3ab	117.26
Amalgerol 10	1485.7 a	139	911.8 a	128	1439.8a	142	1279.1a	135.69

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

In 2015, no proven difference between the variants in the study was found, although the two variants of fertilization with Amalgerol achieved higher yields compared to the untreated control. Another study found positive economic efficiency of the application of Amalgerol in wheat in organic farming (Atanasov D. et al., 2020).

On average over the study period, a higher grain yield was reported in the Amalgerol-treated variants compared to the control, by 17% for Amalgerol 5 l/ha and 35% for Amalgerol at a dose of 10 l/ha, respectively. We have a proven difference with increase compared to the control in the variant treated with Amalgerol 10 l/ha - 1279.1 kg/ha. It can be summarized that the treatment with biofertilizer Amalgerol has been proven to increase grain yield in naked oats Mina.

6. Mass of 1000 grains, g

The results in Table 6 show that the conditions of the year have a strong influence on the seed mass index. With proven most favorable conditions for the realization of higher values of the indicator are 2013 (22.01 g) and 2015 (21.95 g) years compared to 2014.

Table 6. Mass per 1000 grains by variants and years

Influence of year factor	
2013	22.01a
2014	20.80b
2015	21.95a

Variant	2013		2014		2015		Average for the period, g
	(g)	Rank , %	(g)	Rank , %	(g)	Rank , %	
Control	22.37 a	100.00	20.27 a	100.00	21.53a	100.00	21.38a
Amalgerol 5	22.00 a	98.34	21.02 a	103.70	22.15a	102.88	21.72a
Amalgerol 10	21.66 a	96.83	21.12 a	104.19	22.17a	102.97	21.64a

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

Within the year the values vary and no proven differences between the options are found. On average for the study period, the values of the indicator are close - from 21.38 g in the unfertilized control to 21.72 g in the variant treated with Amalgerol at a dose of 5 l/ha, and no proven difference was found.

7. Hectolitre weight, kg

The hectoliter weight serves as a guide for the potential yield and quality or the so-called grain flour yield. In 2013, with the largest hectolitre weight of 97.55 kg was the variant treated with Amalgerol at a dose of 10 l/ha, and between the variant Amalgerol 5 l/ha and the control, the difference was 0.11% in favor of Amalgerol. During the second and third years of the study, a higher hectolitre weight was again observed when treated with Amalgerol at a dose of 10 l/ha (Table 7).

From the obtained results on average for the period, it can be said that fertilization with Amalgerol at a dose of 10 l/ha has been proven to increase the hectolitre weight of grain in the variety Mina - 96.78 kg (by 2.29% above the unfertilized control). At the lower treatment dose, the values of the indicator are close to the control.

Table 7. Hectolitre weight of grain, kg

Variant	2013	2014	2015	Average for the period	Rank, %
Control	94.48	94.94	94.42	94.61b	100
Amalgerol 5	94.58	94.30	94.70	94.53b	99.91
Amalgerol 10	97.55	96.44	96.35	96.78a	102.29

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

8. Content of crude protein, starch and fat in the grain. Although with small differences, the highest crude protein content was reported in the control - 15.22%, followed by the variant treated with Amalgerol at a dose of 10 l/ha (14.38%) (Table 8). The grain in the variant treated with Amalgerol at a dose of 5 l/ha has a lower protein content, it has the highest starch content (51.18%), followed by the control. In terms of fat content in the grain, the control variant has the highest value - 7.92%, followed by the variant with a lower fertilization dose, and the lowest is the fat content in the fertilization variant Amalgerol 10 l/ha. Although there are no major differences between the fat content options, low values of this indicator are important for longer grain storage. From the data for the chemical analysis of the grain obtained from organic cultivation of the variety it can be seen that the grain has quality indicators close to those obtained from conventional cultivation of the same variety (Geogieva T. et al., 2011). This confirms the possibility of obtaining environmentally friendly, quality products using Amalgerol in organic farming.

Table 8. Content of crude protein, starch and crude fat by variants

Variant	Absolutely dry matteri, %	Results to absolute dry matter, %		
		Crude protein	Starch	Crude fat
Control	89.90	15.22	48.87	7.92
Amalgerol 5	90.32	13.69	51.18	7.81
Amalgerol 10	90.03	14.38	49.43	7.53

CONCLUSIONS

There are no differences in the duration of the interphase period in the variety Mina after application of biofertilizer Amalgerol in the studied doses. The treatment of the seeds before sowing with Amalgerol leads to an increase in the percentage of sprouted plants, and applied and vegetatively increases the percentage of harvested plants by 10% at a dose of 5 l and by 13.4% at a dose of 10 l/ha. At the higher fertilization dose, the difference was statistically proven compared to the control. Amalgerol has been shown to increase grain weight per plant by 0.32 g at 5 l/ha and 0.27 g at 10 l/ha. The positive effect for increasing the grain yield after application of Amalgerol by an average of 17% at a dose of 5 l/ha and by 35% at a dose of 10 l/ha in the conditions of organic farming has been proven. By increasing the dose of Amalgerol, the mass of 1000 grains increases, but this is not proven. Treatment with a dose of 10 l/ha has been shown to increase the hectoliter weight of the grain - 96.8 kg compared to 94.6 kg for the control. Between the variants with Amalgerol in doses of 5 and 10 l/ha, the differences in the content of crude protein, starch and fat in the grain of the Mina variety are insignificant. The highest amount of crude protein and crude fat is in the control variant, and starch in the variants treated with Amalgerol 5 and 10 l/ha, respectively 51.18% and 49.43%.

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