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SUMMARY

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MISCELLANEOUS

SOIL SCIENCES

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CHANGES IN THE HUMUS STATE OF CHERNOZEMS OF UKRAINE AND MOLDOVA UNDER IRRIGATION

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Abstract

The humus state of irrigated soils in Ukraine is determined mainly by the structure of sown areas, the specific growth of perennial legumes and the level of organic fertilizers application. A deficit-free humus balance is formed in crop rotations with perennial leguminous herbs (more 21-25%) and manure doses of 8-10 t ha^{-1}. When mineralized waters are used for irrigation, a deficit-free humus balance is achieved in crop rotations with alfalfa and organic fertilizers of 12-15 t ha^{-1}. In crop rotations without perennial leguminous grasses, there is a gradual decrease in the humus content from 3-5% in field crop rotations to 11-23% in vegetables. Research conducted in the conditions of the Republic of Moldova has shown that the use of weakly mineralized water, with a neutral reaction, has no negative consequences on the humus content of the soil. The humus state of soils is determined by the ratio of humic acid: fulvic acid. This integrated criterion, in the irrigation conditions of chernozems with Dniester water, also remains unchanged. Chernozem irrigated with alkaline mineralized water causes essential changes in the fractional composition of humus.

***Key words**: humus, crop rotation, irrigation, chernozem, Ukraine, Moldova.*

INTRODUCTION

The issue of humus state transformation in the soils under the irrigation influence was most actively studied at the first stages of the irrigation development. In many cases, a decrease in the content of humus was noted (Ковда, 1981; Крупенников et al., 1985). This was explained by the more activity of microflora on irrigated lands and increased mineralization of organic matter (Агроэкологическая, 1997). Loss of humus as a result of its migration with infiltration waters could also take place. Some studies noted both an increase in the content of humus (Позняк, 1989) and the absence of a change in its content (Приходько, 1988).

It was noted that with the beginning of irrigation, the humus content decreases, and with further irrigation, it increases. In the irrigation conditions of chernozems, the chemical composition of irrigation water and the irrigation regime must also be taken into account. If these factors contribute to the accumulation of calcium, then we can expect a decrease in the mobility of humus as a result of irrigation (Орлов et al., 1980). Long-term irrigation with waters, in which sodium cations predominate, cause the opposite effect, and in an alkaline environment, rapid decomposition of specific humus substances can be expected (…).

However, in the period of the modern development of land melioration, in conditions of changing methods, regimes of irrigation, land removal from irrigation, periodic irrigation at reduced rates, publication are few (Щедрин et al., 2017; Докучаева & Юркова, 2012). The published data are often contradictory, which is understandable, since the processes of organic matter humification and mineralization are closely related to the soil and climatic conditions of the irrigation region, the quality and quantity of irrigation water, the crop rotation system, doses of organic and mineral fertilizers and other measures applied to irrigated black soils (chernozems).

It is not possible to unambiguously assess the direction of the long-term dynamics of the humus content during the irrigation of chernozems on the basis of published data. This question is especially poorly studied for the chernozems of Ukraine and Moldova using for irrigation the water of different quality, in various reclamation conditions and crop rotation.

MATERIALS AND METHODS

The researches in Ukraine were conducted within the Experimental Stations (ES).

ES 1 - typical chernozem, low humiferous, clayey-loamy. Left-bank of Ukraine Forest-Steppe, Kharkov district of Kharkov region. Institute of Vegetable and Melon Growing, long-term experimental field of vegetable - fodder crop rotation. Irrigation water mineralization - 0.7-0.8 g dm^{-3}.

ES 2 - typical chernozem, moderately humiferous, clayey-loamy and light-clayey. Left-bank of Ukraine Forest-Steppe, Chuguevsky district of Kharkov region. National Scientific Center "Institute for Soil Science and Agrochemistry O.N. Sokolovsky", long-term experimental field of grain-fodder crop rotation (the variants with perennial grasses and without them). Irrigation water mineralization - 0.4-0.6 g dm^{-3}.

ES 3 - ordinary chernozem, moderately humiferous, clayey-loamy. Ukraine Northern Steppe, Pervomaisky district of Kharkov region, monitoring site with a long term grain-fodder crop rotation. Mineralization of irrigation water - 1.1-1.2 g dm^{-3}.

ES 4 - ordinary chernozem, low humiferous, deep effervescence, light-clayey. Ukraine Northern Steppe, Slavyansk district of Donetsk region, monitoring site with long-term of grain crop rotation, mineralization of irrigation water - 0.6 g dm^{-3}.

ES 5 - ordinary chernozem, low humiferous, second weakly solonetzated, light-clayey. Ukraine Northern Steppe, Maryinsky district of Donetsk region, monitoring site with a long-term vegetable-fodder crop rotation, mineralization of irrigation water - 2.9 g dm^{-3}.

ES 6 - ordinary chernozem, low humiferous, second weakly solonetzated, light-clayey. Ukraine Northern Steppe, Yasinovatsky district of Donetsk region, monitoring site with a long-term vegetable-fodder crop rotation, mineralization of irrigation water - 2.3 g dm^{-3}.

Groundwater level at all Experimental Station is more than 5 m. The data are given for 0-30 cm soil layer. Field studies were carried out according to the methods of conducting stationary experiments (Доспехов, 1985) and route surveys - method of analog - keys.

The researches in Moldova were carried out in different agropedoclimatically zones on the experimental key polygons with drip irrigation. The main subtypes of chernozems that predominate in the composition of the irrigation fund of the republic were selected as a study objects. As sources of water for irrigation, the Dniester water, accumulation basins (ponds) and artesian water were chosen. These water sources are essentially different from the chemical composition and quality indices. Therefore, they have different effects on the improvement status of irrigated soils, properties, including humus.

The organic carbon content in soils of Ukraine was determinate by Tyurin method (ГОСТ 26213-84), in soils of Moldova - the same method with Nichitin modification (Практикум…, 1989).

RESULTS AND DISCUSSIONS

In the intensive vegetable crop rotation of ES 1, there is a constant, from rotation to rotation, decrease in the humus content in the unirrigated chernozems (Figure 1).

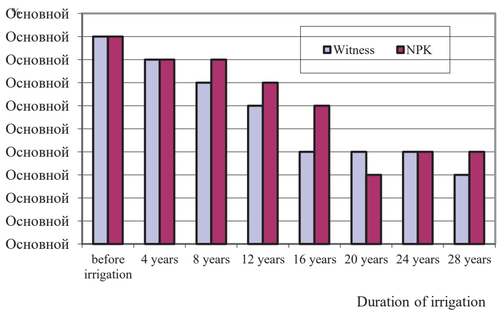

Figure 1. The dynamics of humus content (%) in the chernozems of ES 1 during irrigation

Reconstruction of the vegetable crop rotation of ES 1 after 20 years of irrigation and the introduction of perennial grasses (25%) into it led to a change in the balance of humus and the stopping the fall of its content. The noticeable loss of humus observed in intensive vegetable crop rotation is explained by increased mineralization of organic matter and weakened of humification process.

In the ES 2 - in the field crop rotation with perennial grasses (25-30%) without manure, is observed a tendency in the humus decrease content, the losses were 5-6% of the initial one (Figure 2).

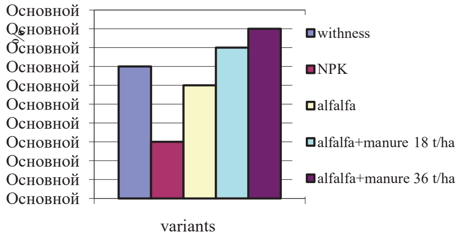

Figure 2. The changes in the humus content in the chernozems of the ES 2 during irrigation

The decrease in the humus content of the weakly humiferous typical chernozems does not occur in crop rotation with perennial grasses and manure. A tendency to increase the humus content in these soils takes place in crop rotation with perennial grasses (alfalfa) and manure in doses of 18 t ha^{-1}.

In a field crop rotation of the ES 3 without perennial grasses using NPK and manure of 9-10 t ha^{-1}, there is a tendency of decrease in the humus content in ordinary chernozem by 2-3% from the initial one (Figure 3). The tendency for an increase in the humus content in these soils is formed in crop rotation with alfalfa, NPK and manure in doses of 8-10 t ha^{-1}.

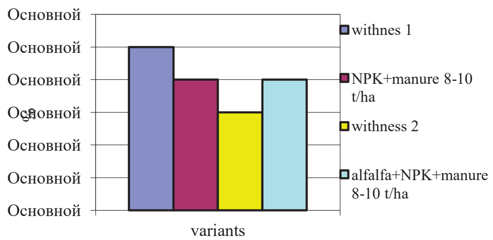

Figure 3. The changes in the humus content in the chernozems of ES 3 during irrigation

In the irrigated soils of ES 4 (Table 1), irrigated with fresh water, there was a slight decrease in the content of humus compared with the non-irrigated analogue (4.3% and 4.6%, respectively), which is associated with the agricultural system, the saturation of crop rotation with row crops, and the introduction of low doses of mineral and organic fertilizers.

Table 1. Humus content in ordinary chernozems of the Northern Steppe of Ukraine

ES No	Humus content, %	
	without irrigation	under irrigation
4	4.6	4.3
5	4.0	4.3
6	5.3	4.4
HCP_{05}	*0.1*	*0.1*

In ES 5 (Table 1), in conditions of equilibrated vegetable and fodder crop rotation, perennial grass cultivation, organic and mineral fertilizers during irrigation with reduced irrigation norms of mineralized waters show a significant increase in the humus content in irrigated soil compared to non-irrigated analogue (4.3% and 4.0%, respectively). In the irrigated soils of ES 6 (Table 1) during irrigation with fresh water, a decrease in the humus content was noted compared with the non-irrigated analogue, which is associated with a low level of agriculture, saturation of crop rotation with row crops, and the introduction of low doses of mineral and organic fertilizers (Щедрина et al., 2017; Докучаева & Юркова, 2012).

In the Republic of Moldova, the chernozems constitute about 74% of the soil's surface suitable for irrigation. Currently only 25-30 thousand ha of vineyards and orchards are irrigated. Changing the natural water regime of these soils through the irrigation one, even in the case of the use of good quality water, leads to degradation of the structure, compacting the surface layer, reducing the lacuna space and permeability for water. Negative effects are recorded in the adsorbent complex, expressed by decalcification and accumulation of sodium and magnesium cations (Крупеников & Подымов, 1978; Подымов, 1976; Орлов, 1980; Рекомендации…, 1991).

The research on the impact of drip irrigation on Moldavian soil regimes and properties has been carried out within systems equipped with sprinkler watering equipment. It was found that studies on the drip irrigation impact on the soil quality are very limited (Ursu, 2012; Andrieș & Filipciuc, 2015). The influence of irrigation on the organic matter content and composition is treated in the opposite way. The multiple researches carried out under different pedoclimatic conditions, including the Republic of Moldova, highlight the decrease of humus content in the irrigation process (Фильков & Попова, 1978; Filipciuc & Andrieș, 2015; Boaghe, 2015).

The impact of drip irrigation on the properties of cambic chernozem using the Dniester river water. The chernozem cambic (leached) was irrigated with Dniester river water - the mineralization: minim - 240 mg l^{-1} and maxim - 490 mg l^{-1}. Published data confirming the reduction of the humic acid content and increase the fulvic acid fraction content under the irrigation influence (Hera & Eliade, 1978; Щербаков & Рудай, 1983; Синкевич, 1989).

Research on the cambic chernozem does not show the decrease in humus content. At the same time, there was a tendency to increase the fraction of humic acids associated with R_2O_3 and to reduce the fraction related to Ca. It is important to emphasize that the solubility of soil organic matter increases considerably in irrigation. After 4 years of irrigation the soil carbon content in the water-soluble substance increased from 7 to 19 mg/100 g soil (Table 2).

Table 2. The humus composition of the cambic chernozem

Depth, cm	C total, %	C, %			$\frac{C_{AH}}{C_{AF}}$	C fraction of AH, %		C	
		extracted with $Na_4P_2O_7$+NaOH	AH	AF		unassociated and associated with R_2O_3	associ-ated with Ca	in the non-hydrolyzed residue, %	in the water-soluble substance, mg/100 g sol
Cambic chernozem - unirrigated									
0-5	1.97	0.96* / 48.7**	0,64 / 32.5	0.32 / 16.2	2.0	8.1	91.9	1.01 / 51.3	8
5-10	1.94	0.91 / 46.9	0.67 / 34.5	0.24 / 12.4	2.7	6.9	93.1	1.03 / 53.1	6
10-20	1.95	0.90 / 46.2	0.66 / 33.8	0.24 / 12.3	2.7	6.2	93.8	1.04 / 53.3	8
20-30	2.00	0.91 / 45.5	0.65 / 32.5	0.26 / 13.0	2.5	6.5	93.5	1.10 / 55.0	7
Cambic chernozem - irrigated 4 years									
0-5	1.97	0.96 / 48.7	0.66 / 33.5	0.30 / 15.2	2.2	9.5	90.5	1.01 / 51.3	16
5-10	1.95	0.97 / 49.7	0.66 / 33.8	0.31 / 15.9	2.1	9.8	90.2	0.99 / 50.8	19
10-20	1.98	0.99 / 50.0	0.67 / 33.8	0.32 / 16.2	2.1	10.0	90.0	0.99 / 50.0	21
20-30	1.97	0.99 / 50.2	0.67 / 34.0	0.32 / 16.2	2.1	9.4	90.6	0.98 / 49.7	21
*% from the soil mass; **% from the C total; AH - humic acid; AF - fulvic acid									

The impact of drip irrigation with water from local sources (ponds) on the properties of typical chernozem. Researches on typical chernozem confirm the evolution of negative changes in humus composition. The irrigation changes the humification process and the composition of the organic matter in the soils (Boaghe, 2015).

The water used for irrigation of the typical chernozem has a total soluble salt content of 605-676 mg l^{-1} and is characterized by weak and moderately alkaline reaction (pH = 8.05-8.40). The data from Table 3, shows that drip irrigation with water from local sources has a pronounced influence on the fraction of humic acids unassociated and coupled with iron and aluminum oxides, but also on the fraction of these calcium-related acids.

Thus, in the typical chernozem under irrigation regime the carbon content of free and bound with R_2O_3 - humic acids increased by 7.8% in the 0-30 cm layer and in the same proportion decreased the fraction coupled with Ca.

The impact of drip irrigation with groundwater on the properties of the ordinary chernozem. Research on ordinary chernozem irrigated with groundwater shows that humus content does not show appreciable changes (Table 4).

In both cases the total carbon has the average value of 2.09% in the 0-30 cm layer. In the soil under irrigation, the decrease in the amount of carbon extracted with the solution of sodium pyrophosphate mixed with sodium hydroxide is noted. The fraction of humic acids in irrigated soil is decreasing, which has changed the main evaluation indicator of the humic state of C_{AH}: C_{AF}. In the unirrigated soil this ratio has values between 2.0 and 2.2; therefore, the humus type is the humatic one. In the soil under irrigation, the ratio between humic and fulvic acids decreases to 1.7-1.9, the type of humus being appreciated as humato-fulvic (Table 4).

Table 3. The humus composition of the typical chernozem

Depth, cm	C total, %	*C, %			C_{AH}/C_{AF}	C fraction of AH, %		C_{RN}, %
		in the extract	AH	AF		unassociated and associated with R_2O_3	associated with Ca	
Typical chernozem - non-irrigated								
0-5	1.71	50.3	32.7	17.6	1.9	7.1	92.3	49.7
5-10	1.68	45.2	31.5	13.7	2.3	6.8	93.2	54.8
10-20	1.62	45.0	30.9	14.1	2.2	7.6	92.4	55.0
20-30	1.57	44.6	29.9	14.6	2.0	7.6	92.4	55.4
Typical chernozem - irrigated 7 years								
0-5	1.54	46.8	30.5	16.2	1.9	21.1	78.9	53.2
5-10	1.55	49.0	33,5	15.5	2.2	14.2	85.8	51.0
10-20	1.59	47.8	33,0	14.5	2.3	13.2	86.6	52.2
20-30	1.60	48.8	35,0	13.8	2.5	12.0	88.0	51.2
*% din C total; C_{RN} - carbon of the non-hydrolyzed residue								

Table 4. The humus composition of the ordinary chernozem

Depth, cm	C total, %	C, %			CAH/C_{AF}	C fraction of AH, %		C in the non-hydrolyzed residue, %	Gh, %
		in the extract	AH	AF		unassociated and associated with R_2O_3	associated with Ca		
Ordinary chernozem - non-irrigated									
0-5	2.10	0.96* 45.7**	0.64 30.5	0.32 15.2	2.0	6.2	93.4	1.14 54.3	30
5-10	2.12	0.94 44.3	0.65 30.7	0.29 13.6	2.2	7.4	92.3	1.18 55.7	31
10-20	2.06	0.95 46.1	0.64 31.1	0.31 15.2	2.1	4.7	95.3	1.11 53.9	31
20-30	2.08	0.91 43.6	0.61 29.3	0.30 14.4	2.0	3.3	96.7	1.17 56.2	29
Ordinary chernozem - irrigated - 3 years									
0-5	2.11	0.85 40.3	0.54 25.6	0.31 14.7	1.7	16.7	83.3	1.26 59.7	26
5-10	2.06	0.87 42.2	0.57 27.7	0.30 14.5	1.9	17.5	82.5	1.19 57.8	28
10-20	2.06	0.84 40.8	0.54 26.2	0.30 14.6	1.8	14.8	85.2	1.22 59.2	26
20-30	2.12	0.86 40.5	0.55 35.0	0.31 14.6	1.8	12.7	87.3	1.26 59.5	26
*% from the soil mass; **% from the C total; Gh - humification degree									

Significant changes record the fractions of free humic acid and associated with R_2O_3, but also its fraction related to Ca. On the whole 0-30 cm thickness of irrigated soil, the carbon content of humic acid coupled with R_2O_3 is on average 9.9% higher compared to the non-irrigated soil. It is noteworthy that the content of human acids coupled with Ca on average decreased from 94.5% in the non-irrigated soil to 84.6% in the irrigated one.

CONCLUSIONS

It was established that the humus state of irrigated chernozems of Ukraine is determined mainly by the structure of sown areas and the presence of perennial grasses in crop rotation, the application level of organic and mineral fertilizers, and, to a lesser extent, the quality of irrigation water, since reduced irrigation rates are used in Ukraine in modern conditions. To ensure a positive balance of organic matter, it is necessary to perform a high agricultural background, introduce organic and mineral fertilizers, and perennial and sidereal crops.

The water of the Dniester river corresponds to the quality requirements for irrigation of Moldovan soils, but it has an alkaline potential and causes the decalcification in the surface soil layer. Drip irrigation results in decreased the hydrostability of structure and secondary compaction of chernozems.

The application for soil irrigation the water from the accumulation lakes with poor quality indices has the following consequences: modification of the soluble salts composition with predominance of the toxic compounds of sodium and magnesium; changing the current reaction in the direction of alkalization; secondary salinization of the soil, including magnesium salinization; increasing the content of fine hydropeptized clay and increasing the dispersion factor; increasing the degree of compaction and worsening the potential ventilation conditions of soils; destructuring and reducing the hydrostability of the structure; increasing the degree of swelling and penetration resistance; decreasing the water permeability of the soils (Filipciuc & Boaghe, 2017; Filipciuc et al., 2016).

The groundwater is characterized by moderate to strong alkaline reaction and unfavorable chemical composition. Soluble salts include carbonate and sodium bicarbonate. The use of groundwater for irrigation of Moldavian soils has the effect of salinization in the first year of irrigation. Intensely degrades the physical, chemical, hydro- and physico-mechanical properties of the soils. Irrigation with groundwater has a negative impact on the organic matter composition of chernozems. In the irrigation regime the organic matter composition of the chernozems records unfavorable changes related to the reduction of the huminic acids content and the increase of the fraction of acids associated with R_2O_3.

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CLIMATE CHANGE IN THE REPUBLIC OF MOLDOVA - CAUSES AND THEIR INDUCED CONSEQUENCES

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Abstract

This study presented in the article directly correlates with a series of sustainable objectives regarding the causes of climate change in the Republic of Moldova and their consequences. For more than 100 years, specialists have known the meaning of the phrase "greenhouse effect". This process contributes to maintaining a warm atmosphere, preventing the Earth from cooling and becoming hostile to life (the average global temperature is + 15°C versus - 18°C in the absence of this natural greenhouse effect). Of course, a drastic way in the influence of climate change is effected by burning forests - made to obtain arable land, roads, housing or shopping centers - produces over 25% of global carbon dioxide emissions. The conclusion that can be drawn very easily is that the current heating is not caused by an intensification of the solar activity, but it is in close correlation with the emission of carbon dioxide. In the Republic of Moldova, the inventory of greenhouse gas emissions was carried out during the years 1990-2002, the reference year recommended by the UN being 1990. During this research period, the main place of the total emissions belongs to it CO_2. There is a considerable decrease in greenhouse gas emissions. The cause of the decrease is the closure of emission sources: factories, factories, etc. Comparative assessments with other neighboring territories, using data from the Center for Epidemiological Research of Natural Hazards (Belgium, Luviana University) allow us to ascertain that the risk of catastrophic flooding is 1-2 times in 10 years, which is less than in some regions of Romania, where they have the frequency of 2-3 times in 10 years. Systematic observations on climate indices in the Republic of Moldova, initiated since 1986, have shown gradual warming as a consequence of global warming. Climate change that has occurred over the years in the Republic of Moldova seems to have significant impacts on biodiversity, starting with the level of individual law and up to the level of ecosystem or biometrics.

Key words: *consequences of climate change, ecosystems, greenhouse gas emissions, vulnerability agriculture*.

INTRODUCTION

Climate is the multiannual regime of the weather or all the possible successive changes of the atmospheric processes that characterize the regime of the weather of a certain region.
In any specific locality, the weather can change very quickly from day to day and from year to year, even when the climate does not change. These changes involve variations in temperature, precipitation, wind and cloudiness.
Unlike time, the climate is generally affected by slow changes, for example, changes in the surface of the oceans, the land, and the inclination of the Earth's axis and the intensity of solar radiation. (Cazac et al., 2005).
In the second half of the century. XX, ideas about climate change are becoming more frequent, and in the 80^s, scientific evidence appeared regarding the risk of global climate change (Daradur et al., 2007).
For these reasons, a detailed assessment of the effects of climate change on economic, social and environmental aspects is needed now.
The list of human activities that lead to climate change is: electricity and heating: 30.6% - burning fossil fuels to produce electricity and heat is the biggest cause of climate change, according to the World Resources Institute (WRI). Emissions account for almost one-third of total greenhouse gas emissions. Transport: 14.8%. Production and construction: 13.3%. Agriculture: 11.1%. Burning of other fuels: 8.2% of this category includes wood and burned fuels in residential and commercial buildings, but also those used for agriculture; industrial processes: 5.8% - cement and aluminum production are the largest emitters of greenhouse gases; deforestation and land use change: 5.7% - forests prevent carbon emissions, and deforestation dramatically influences climate change; accidental

emissions: 5.3% - emissions associated with energy production are a factor of climate change. Waste: 3.1% - garbage dumps produce methane gas, which contributes to air pollution. The last ten years in the Republic of Moldova (1996-2005) compared to the multiannual period are characterized by a somewhat higher temperature regime. The average annual air temperature during this period averaged 8.7-10.4°C in the territory, which exceeds the norm by 0.5-1.0°C. It is worth mentioning that during these ten years the average annual air temperature reached its highest values during the 100-year period of instrumental observations - 9.7 and -11.5°C (2000). Its lowest values were reported in 1996 and 1997 and were 7.1-9.3°C. The lowest air temperatures (-21 and -30°C) were reported in December 1996, and the highest (37-40°C) - in July-August 2000 and July 2002, isolated exceeding historical maximums (IPCC, 2007; UNFCCC, 2007).

MATERIALS AND METHODS

The research method consisted in the bibliographic analysis of the chemical changes and the statistical analysis of the data.

Climate change refers to statistically significant changes in the average state of the climatic parameters or their variability, observed over time, either due to changes occurring within the climate system or to the interactions between its components, or because of the action of external factors natural or result from human activity. These changes involve variations in temperature, precipitation, wind and cloudiness.

An analysis of the climatic elements of the last century can serve as evidence for the argument of the existence of climate change. Thus, during the last century, at global level, the average terrestrial temperatures increased by 1°C. The rate of global warming has increased from 0.1°C per decade over the past 100 years - to 0.2°C per decade over the past few decades. Compared with the pre-industrial period (average for 1850-1899) the average temperature (on land and in the ocean) over the last century is 0.8°C higher.

In Europe there is a warming higher than the global average 2007 data show that average annual temperatures in Europe were 1.2°C higher than in the pre-industrial era in land areas, and 1°C higher in the combined space of the sea and land. Eight of the 12 years from 1996-2007 were among the hottest years after 1850.

Annual rainfall (Figure 1) in the 20th century showed a 10-40% increase in Northern Europe and a decrease (up to 20%) in some parts of Southern Europe. Average rainfall increased in most of Western and Northern Europe (by 20-40%); and in southern Europe and partly central Europe the winters were drier (Nedealcov, 1998; Taranu et al., 2009).

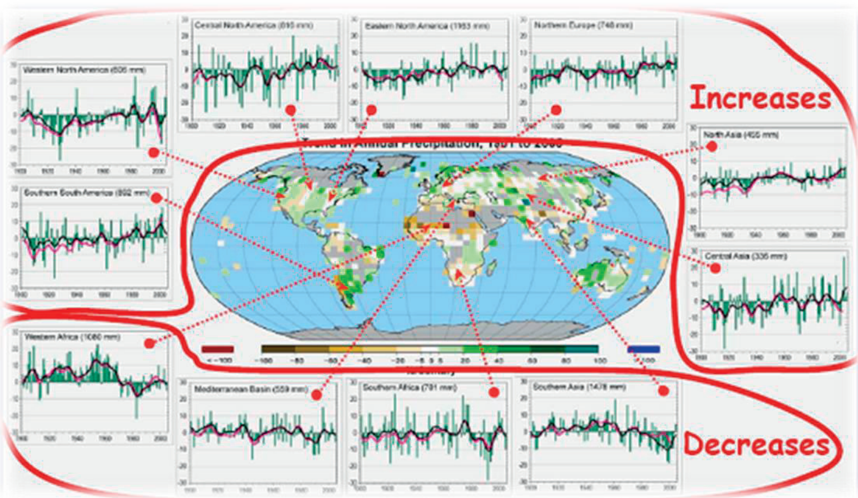

Figure 1. Changing the annual amount of global rainfall in the period 1900-2005

The global lifting of the air temperature was materialized by a series of changes that were observed and measured as: the increase of the global sea level by 0.1-0.2 m in the 20th century, the decrease by about 10% of the snow covered areas, compared to 1960, the retreat of the alpine glaciers from the nonpolar regions during the twentieth century. The warmest 10 years recorded were after 1991. Global evidence from the last decade shows that many natural systems are affected by regional climate change, many of which are also relevant to the Republic of Moldova (Mihăilescu et al., 2006; Nedealcov, 1998).

In the Republic of Moldova - part of Europe - there are also changes in climate. Under its current conditions, the average annual air temperature ranges from 8.0ºC in the north (Briceni) to 10.0ºC (Cahul, Comrat) in the south. The character of spatial distribution of air temperature is determined by the geographical situation and the extent of the southernmost territory. The analysis of the data, carried out by the specialists of the State Hydrometeorological Service, for a period of more than a century (1886-2004) shows us that since the end of the 19th century and until now the average annual temperature in Moldova has increased by 0.78ºC (www.old.meteo.md).

On the territory of the Republic of Moldova the average annual amount of precipitation in the period 1996-2005 constituted on the territory 500-655 mm, which is close to the norm, calculated for the entire observation period. In some years, the amount of rainfall that has fallen has fluctuated to a considerable extent - from 300 mm (2003) to 890 mm (1998).

The most common natural hazards of hydrometeorological origin on the territory of the Republic of Moldova, according to the statistical data of the Center for Epidemiological Research of Natural Hazards (Belgium, Luviana University), are floods, droughts, strong winds, extreme temperatures. In the period 1992-2003, 90% of the total natural hazards in the Republic of Moldova were extreme hydrometeorological phenomena, the most numerous being the floods (50%), during which 92% of the total human victims, strong winds were recorded (25.5%) and droughts (12.5%).

According to the evaluations of the United Nations Development Program, 280 thousand people suffer from catastrophic drought on average. For comparison, the average numbers for floods and earthquakes are 193 thousand and 19 thousand inhabitants respectively.

RESULTS AND DISCUSSIONS

The heating of the climate system can be seen in several ways. We assist in changing the temperature of the atmosphere, the surface of the oceans (the warming of the oceans descends to hundreds of meters depth) and in increasing their level. Rising temperatures in the troposphere and falling in the stratosphere are most likely caused by greenhouse gases and ozone depletion, respectively.

For more than 100 years, specialists have known the meaning of the phrase "greenhouse effect". Solar radiation passes through the Earth's atmosphere and reaches its surface. Instead of reflecting on the surface of the Earth and returning to space, the radiation stays in the atmosphere and produces a heating of it. The process contributes to maintaining a warm atmosphere, preventing the Earth from cooling down and becoming a hostile environment (the average global temperature is +15°C compared to -18°C in the absence of this natural greenhouse effect).

The current trends of modification of the natural risk factors in the Republic of Moldova, their frequency allows us to assume that the risk of manifestation of hydrometeorological hazards will continue to increase. Since the 1950^{s} (www.old.meteo.md), the number of destructive phenomena caused by the weather has increased 5.5 times globally, while the number of other extreme natural phenomena has increased only 2.4 times (Figure 2).

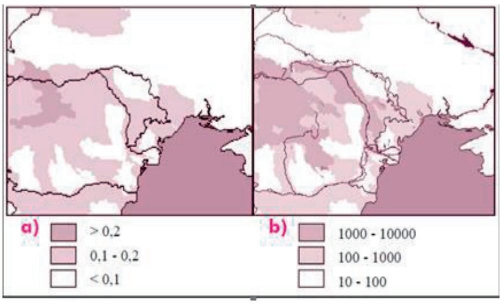

Figure 2. a - Distribution of the flood risk index (no/year); b - number of victims (no/year)

Comparative assessments with other neighboring territories, using data from the Center for Epidemiological Research of Natural Hazards (Belgium, Luviana University) allow us to find out that the risk of catastrophic flooding is 1-2 times in 10 years, which is less than in some regions of Romania, where they have a frequency of 2-3 times in 10 years.
There are obvious effects on aquatic systems, which are manifested by higher water leaks and early spring maximum flow in many rivers fed by snow, as well as surface water heating, which affects their thermal structure and water quality.
It is important to mention some consequences such as (Figure 3): reducing the water supply in the groundwater and lowering the water level in the wells, sometimes even drying them, which causes insufficient drinking water; increased mineralization of groundwater, concentration of inorganic pollutants in water, etc.

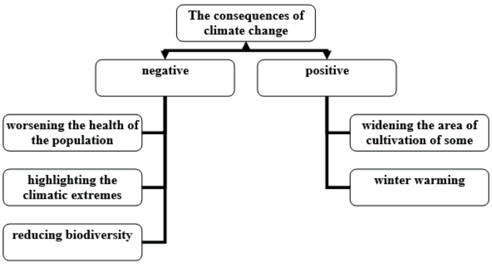

Figure 3. The consequences of climate change in the Republic of Moldova

Climate change of this size appears to have significant impacts on biodiversity, starting with the level of individual law and up to the level of ecosystem or biometrics. It is assumed that the climate in Moldova will change from semi-arid to arid, especially in the south and east of the country. Rising temperatures will force many living organisms to migrate to cooler areas in the northern part of the country, while new organisms will arrive here. Such movements can involve many species, including plants and trees (Mihăilescu et al., 2006; Дарадур, 2001).
Climate change is quite chaotic, with unexpected effects and difficult to anticipate. In Central and Eastern Europe, the scenarios show a decrease in precipitation, especially in the summer season. The water deficit will affect cereal and grazing crops, with the orientation towards tolerant agricultural crops at high temperatures and hydrological stress being recommended.
However, some species of flora and fauna may have low elasticity when changing temperature and precipitation, because climate systems move faster than they can accept.
Some species will seek to settle at higher altitudes, in the Central part of the country (the forest area Codru), others will move further to the northern part of Moldova. Steppe plants, in general, adjust well to high temperatures, although, due to excessive grazing, the population of some species may shrink and disappear. Fragmentation of the landscape and human activity are all likely to increase the vulnerability to changing climatic conditions of steppe species with limited dispersal capacity, such as snowflakes (Andrieş, 1999; Banaru, 2001).
Droughts reduce the presence of water on the floodplains and intensify soil salinization processes. These developments can have a significant impact on grassland species by extending the surfaces with halophyte plants, well adapted to salt and drought conditions, and by reducing the areas occupied by flood tolerant plants such as. In general, forest eco-systems are more drought resistant; however, some oak species in the central and southern parts of the country may dry out in the event of a massive insect invasion. Floods and droughts or other extreme climatic phenomena (storms, hail, etc.) have an influence on human well-being, as they cause losses in the ecosystems on which human prosperity depends.
Agroecosystems and the livestock sector will suffer greatly from climate change. What is the action of changing the temperature of the environment on animals? Higher organisms have a constant body temperature, characte-ristic of the given species. For example, raising the temperature of the environment is accompanied by the need for the body to make extra efforts to maintain the constant temperature; breathing becomes more frequent, disturbances of the work of the cardiovascular system, often of the digestive system, as well as in the reproduction of animals. This set of deviations, of course, leads to a decrease in the level of animal productivity (Ursu, 2000).

The vulnerability of agriculture as a whole is based on two main components. First, it is determined by a permanent tendency to limit the spectrum of the species used. Currently, to satisfy 90% of the food requirements, the human uses only 0.5% of the total number of edible plant species. This condition has led to the so-called "genetic vulnerability", because for each climatic zone, the number of cultivated plant species is always decreasing, and the varieties and hybrids used are of a higher degree of kinship. Secondly, the vulnerability of agriculture is determined by the essential and very rapid changes, especially lately, of the environment (Mihăilescu et al., 2006; www.statistica.gov.md.)

In order to minimize the impact of climate change on the various systems and sectors of activity, a system of adaptation measures has been developed. The most important of these measures provide the elaboration and extension of forests, the adaptation of the management of natural resources to the principles of sustainable development of the basic sectors of the economy.

In particular, for the natural ecosystems the adaptation measures are: the extension of the protected areas for the most vulnerable ecosystems to climate change; creation or restoration of interconnected areas (creation of the ecological network) of fragmented and dispersed ecosystems; organizing the monitoring, in order to assess the stability of species and ecosystems according to climate change; directing their evolution vector towards increasing the resistance to these changes; elaboration and implementation of forest restoration and expansion programs; restoration of wetlands.

According to the above-mentioned conception, the environmental protection requirements will be integrated in the economic reform and in the sectoral policies.

In this way, the properties related to waste management, the reduction and capture of harmful emissions, the increase of energy efficiency, the use of renewable energy sources and the broadening of the spectrum of research on climate change would be taken into consideration. All these, in conjunction with other components of environmental policy, will determine a sustainable socio-economic development.

CONCLUSIONS

Soils of the Republic of Moldova are severely degraded and highly vulnerable to climatic conditions. They no longer have the capacity to cope with the drought, even the short one, and at national level, over the years; there is a decrease in agricultural productivity.

In the Republic of Moldova, research shows that in the coming years, climate warming will have a negative impact on autumn wheat. Similarly, maize and sunflower, which represent the main crops in the national agricultural sector, will suffer. However, such research must be carried out continuously, because the frequency of disasters differs, their geography changes and the degree of exposure.

For the natural ecosystems, the degree of vulnerability can also be conditioned by risk factors, such as the reduced surfaces, the degraded state, the fragmentary distribution, the insufficiency of the soil moisture, the unevenness of the distribution of precipitation.

In the conditions of climate change for the problems related to the adaptation of ecosystems to the new climate conditions, a particular current importance is the elaboration of the regional climate forecast.

The changes that take place, both at the climate level and at the level of the ecosystems, endanger the health of millions or even billions of people all over the world and are at this moment the biggest challenge of the 21st century. These changes globally threaten even the fundamentals of human health: access to adequate food resources, clean air, safe water and safe housing. For the Republic of Moldova, the priority areas in this regard are ecosystems, the agricultural sector and human health.

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AMPLIFICATION OF SOIL COMPACTION BETWEEN RIDGES AFTER USING PLASTIC MULCH ON ENTIRE SURFACE IN PLASTIC TUNNELS FROM NORTH-EAST OF ROMANIA

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Abstract

Our studies performed on the plastic tunnels from North-East part of Romania included a field work phase (soil and biological material sampling, soil profiles description, penetration resistance determination, digital images capture), laboratory phase (chemical and physical soil analysis). Based on field and laboratory data were estimated packing density and bulk density restricting rooting. The obtained results have shown that black plastic films on the entire surface of soil favors soil compaction, in the areas located between plants rows (i), the ploughpan conducted to the waterlogging on the bottom part of ploughed layer (ii), root system poorly developed favored some physiological disorders such as Blossom end rot on tomatoes fruits (iii). Soil determination in field (penetration resistance, morphological soil indicators) and laboratory (water distribution, bulk density, size particles distribution) and quality of fruits reveled negative influence of plastic mulch. Strong soil compaction between ridges allows us to recommend avoiding plastic mulch over the entire soil surface.

Key words*: plastic mulch, ridges, penetration resistance, ploughpan.*

INTRODUCTION

In Romania, growing vegetables in plastic tunnels has greatly expanded. High plastic tunnels are simple, tall, plastic-covered structures used for early vegetables production. It is also recognized that the intensive agricultural system has a great impact on soils properties. The application of a suitable agricultural management system, according to the soil characteristics can have positive effects on the prolonging service life of the greenhouses soil.
Interest concerning to processes of physical greenhouses soil degradation developed nowadays, mainly due to increasing of technological inputs which has the effect amplification of soil degradation processes such as hardsetting, crusting, cementation, compaction etc.
Starting with 1960 plastic mulching is currently used in horticulture especially for warm-season crops such as tomatoes, cucumbers, melons, peppers, eggplants (Incalcaterra et al., 2004).
In numerous publications are mentioned some advantages of plastic mulching. The main benefits of using plastic mulch include: increasing soil temperature (i), decreasing of water loss by direct evaporation (ii), increased yield (iii), reduced nutrient leaching (iv) and improved nutrient uptake (v), getting early production (vi), ensure full control of weeds (vii), ensure maintenance of optimal water content in soils from zone with high rainfall (Streck, 1995; Henry, 2010).
Nowadays, it is using a wide range of films covering both greenhouses and soil surface as mulch. Among the main varieties of films frequently used for mulching we mention: transparent polyethylene film; black film; transparent biodegradable Mater-BiTM film; black biodegradable Mater-BiTM film, red plastic mulch, green or brown Infra-Red Transmitting (IRT) mulches, white or co-extruded white-on-black mulch, yellow and silver plastic mulch, shiny aluminum plastic mulches, blue plastic, olive plastic etc. (Incalcaterra et al., 2004; Nair et al., 2013).
Soils with clay content higher than 33% belong to the third class of capabilities for greenhouses (Florea et al., 1987) and have high susceptibility to degradation by compaction.
Frequently, greenhouses or high plastic tunnels have been set up on soils with a compact layer, such as plough pan. A plough pan layer

(pressure pan, tillage pan) is a subsurface horizon having a high bulk density and a lower total porosity than vicinity soil layers (above or below layer). The plough pan is formed as a result of pressure applied by normal tillage operations, such as plows, discs or other tillage implements.

Degradation of soil by compaction processes had evidenced in soil with coarse and medium texture, especially as cumulative effects of drip irrigation and if it was used plastic mulch (Filipov et al., 2013, 2018).

Knowing the influence using plastic mulch on entire surface between ridges is important for making decision on the sustainable exploitation of the soil resources. Some soils profiles from poly-tunnels have been studied in order to point the changes of soil properties after 5 years of vegetables grown in organic system.

MATERIALS AND METHODS

The study site was located at poly-tunnels Roznov from Moldavian Subcarpatians (Romanian: Subcarpaţii Moldovei) Cracau-Bistrita Depression-geographic area spanning in the NE of Romania, situated to the east of the Eastern Carpathian Mountains. The studied area included soils from 4 plastic tunnels.

The penetration resistance of the soils was determined using a digital penetrologger (Eijkelkamp Equipment, Model 0615-01 Eijkelkamp, Giesbeek, The Netherlands) which had a cone angle of 30° and a base area of 1 cm^2. It was carefully inserted into the soil profiles in 1 cm increments from the surface to a depth of 80 cm. Ten parallel records were made in each plot and averaged for analysis.

In order to establish the effect of black foil, used to cover the entire surface of the soil in the plastic tunnels, on the state of compactness of the soil we made 4 cross section in the middle area of the plastic tunnels.

This section comprised two ridges grown with tomato and cucumber plants and a rill between ridges. After morphological description of soil from ridges and rill, soil samples were taken from soil layers with a thickness of 10 cm to a depth interval of 0-50 cm.

The collected soil samples were analyzed in the lab, in three replicates independent each of layer. In the lab conduct size particles analyses: pH, bulk density, content of water-according to the current methodology (Dumitru et al., 2009; Lăcătușu et al., 2017). The period of study was 2017-2019.

Following the processing and analysis of the data obtained in the field and laboratory, several reclamation measures have been recommended.

RESULTS AND DISCUSSIONS

The studied soils have been diagnosed as Hipohortic Phaeozems. The clay content of these soils range from 32.4 to 38.3%.

Following studies of the soil profiles, the presence of the plough pan (Figure 1) was noticed, which we consider to be a residual effect of the agricultural technologies practiced before the establishment of the high plastic tunnels and the growing of vegetables in an ecological agricultural system. The soil is strong compacted in the plough pan layer and in the upper part of the soil (Figure 1). High compactness state of plough pan favors water stagnation, especially on the bottom part of ploughed horizon, even if it is drip irrigation practice

The strong compaction of the soil is also highlighted by the high values (3.8-5.8 MPa) of the penetration resistance recorded in the plough pan (Figure 2).

Although penetration resistance values are lower than 2.5 MPa soil is compacted, as evidenced by pedomorphological parameters in the field and by the bulk density values higher than 1.4 g/cm^3 (Figure 3).

The high values of water content in the layer 0-10 cm currently determined decreasing of penetration resistance value. Soil profile on the top of ridge with tomato plants has a smaller content of water on the depth interval 0-20 cm. We mention that the soil sampling was done after 4 weeks of the last irrigation.

The data in Figure 4 highlights the negative effect of the plastic foil on the soil by increasing the water content (resulting from the condensation of evaporated water from the irrigated soil surface) and strong compaction due to repeated movements for various works.

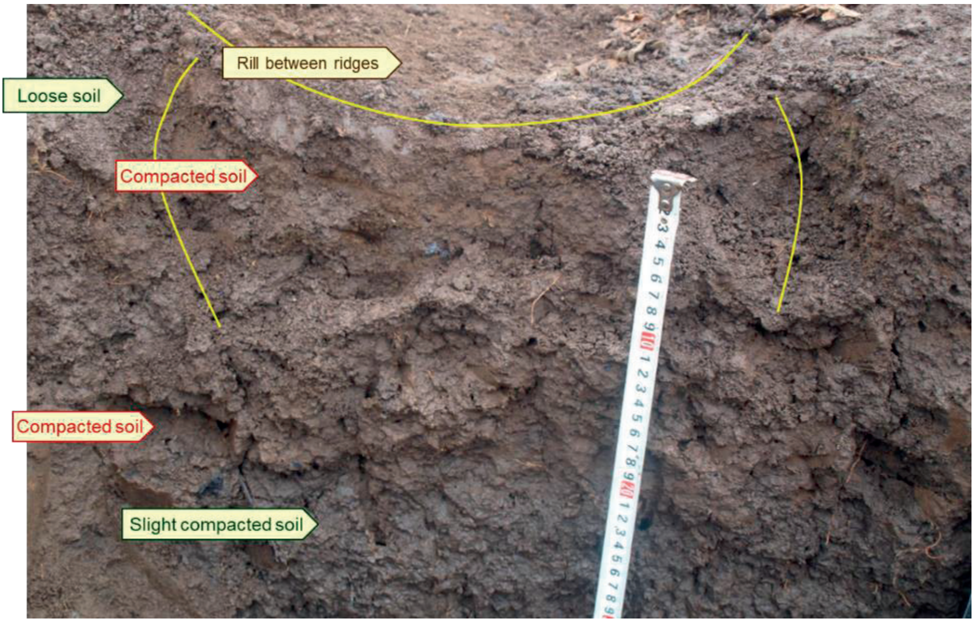

Figure 1. The state of compaction of the soil within the cross section between the two ridges

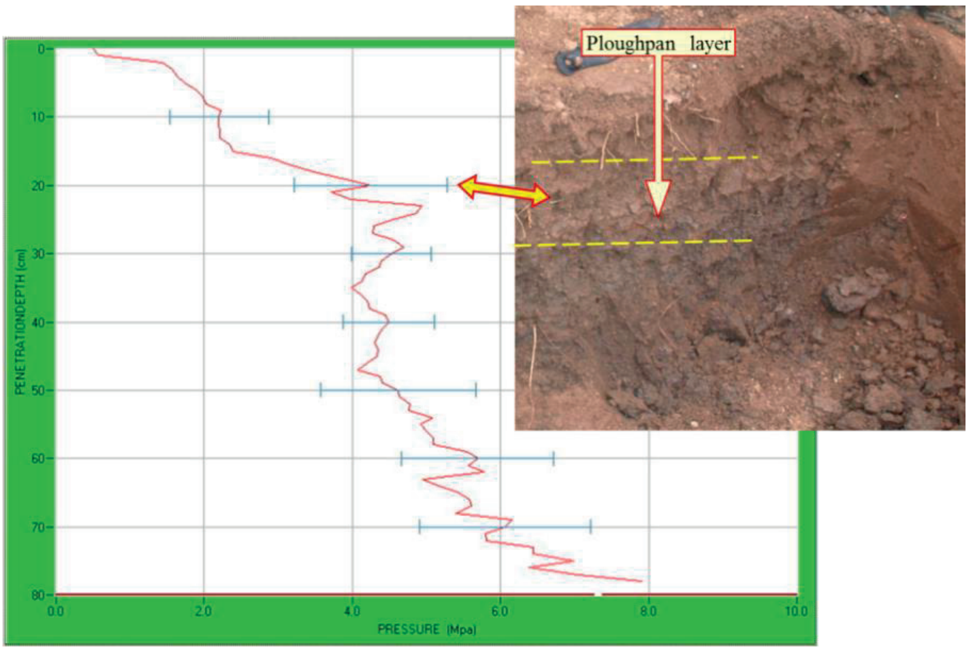

Figure 2. Strong compaction of the ploughpan layer on the rill between rows plants highlighted by high values of penetration resistance (3.8-5.8 MPa)

Depth (cm)	BD g/cm³	Average values BD (g/cm³)	Wg %	Average values Wg (%)
0-10	1,43	1,4	27.602	24.706
	1,43		22.799	
	1,34		23.717	
10-20	1,54	1,49	21.769	20.584
	1,40		19.555	
	1,52		20.430	
20-30	1,51	1,49	19.491	19.958
	1,49		20.301	
	1,48		20.082	
30-40	1.39	1,42	25.680	25.653
	1.40		25.285	
	1.43		25.994	
40-50	1.41	1,42	27.151	26.845
	1.44		26.522	
	1.42		26.861	

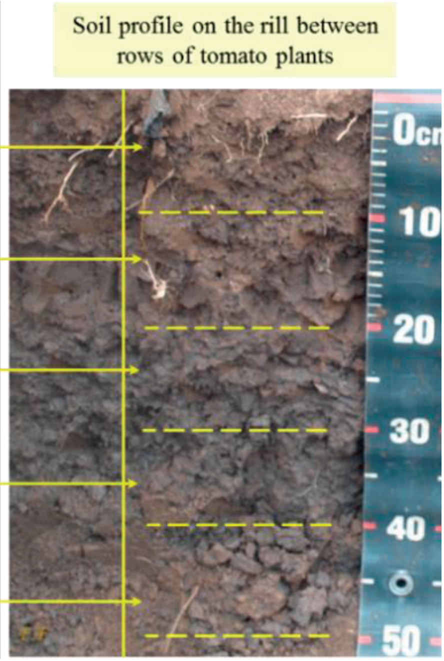

Figure 3. Soil profile on the rill between ridges with rows of tomato plants. Values of Bulk Density (BD) and gravimetric content of water (Wg) registered on the depth interval of 0-50 cm

Depth (cm)	BD g/cm³	Average values Wg (%) BD (g/cm³)	Wg %	Average values Wg (%)
0-10	1.42	1.40	15.982	16.412
	1.38		16.627	
	1.39		15.719	
10-20	1.21	1.16	18.425	16.667
	1.12		15.787	
	1.16		15.470	
20-30	1.49	1,51	19.623	19.624
	1.55		19.624	
	1.50		19.825	
30-40	1.51	1.51	21.815	23.290
	1.53		24.028	
	1.49		23.635	
40-50	1.40	1.40	23.796	23.812
	1.42		23.820	
	1.38		23.572	

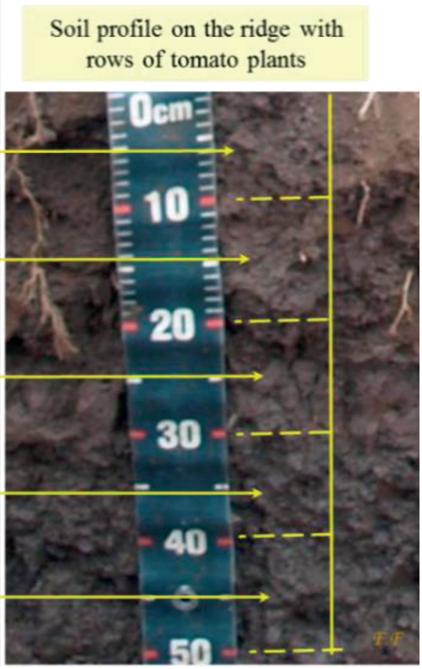

Figure 4. Soil profile on the top of ridge with tomato plants. Values of Bulk Density (BD) and gravimetric content of water (Wg) registered on the depth interval of 0-50 cm (V2)

CONCLUSIONS

The obtained results have shown that black plastic films on the entire surface of soil favours soil compaction between plants rows.

The ploughpan conducted to the waterlogging on the bottom part of ploughed layer and poorly developing of root system.

High content of water favoured some physiological disorders such as Blossom end rot on tomatoes fruits. Strong soil compaction between ridges allows us to recommend avoiding plastic mulch over the entire soil surface.

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SEED GERMINATION AND EARLY SEEDLING DEVELOPMENT OF MAIZE (*Zea mays* L.) UNDER THE STRESS OF DIFFERENT HEAVY METAL CONCENTRATIONS

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Abstract

Since the lifecycle of plants begins with the germination phase, evaluating this process under different environmental factors may reveal some parameters with great importance for the further development of plants. Also, it can represent a base for other studies. The purpose of this paper was the evaluation of germination and seedling growth of maize under different levels of heavy metal contaminations. The following heavy metals were used as mixtures: Cd, Cu, Ni, Pb, Co and Zn. Five mixtures with different concentrations of Cd (3 to 20 ppm), Cu (20 to 200 ppm), Ni (25 to 200 ppm), Pb (30 to 150 ppm), Co (20 to 100 ppm) and Zn (100 to 700 ppm) were used as variants, and distilled water as control variant. Seed germination percentage was determined in accordance to ISTA (International Seed Testing Association). A series of physiological indicators associated with germination and seedling growth were calculated. The germination was observed for ten days, until no changes occurred for three consecutive days. Several effects were observed at the end of the experiment regarding the seed germination and especially to seedling development. Although there were some differences on germination between control variant and high contaminated variants, the growth of seedlings showed a high variation as the concentration of heavy metals increased.

Key words: *Zea mays L., heavy metals, germination, seedling*.

INTRODUCTION

As the first stage of plant life, the germination plays a substantial role on plant development. This process is easily influenced by environmental factors, which most often have negative effects on germination parameters, reducing growth, development and production of plants.

Some of these factors have been studied in many works, each of them influencing germination parameters differently. Important changes were noted due to humidity stress (Shaban, 2013; Khodarahmpour, 2012), temperature (Farooqi & Lee, 2016; Wang et al., 2018; Bano et al., 2015), salt stress (Mirosavljević et al., 2013; Sozharajan & Natarajan, 2014; Turk & Eser, 2016) and heavy metals (Bashmakov et al., 2005; Sethy & Ghosh, 2013; Oladele et al., 2018). Maize (*Zea mays* L.) are known to be a good accumulator of heavy metals and lot of experiments was conducted to study the effects of these contaminants on growth and yield of maize (Aladesanmi et al., 2019; Ahmed & Hanan, 2015).

The capacity of plants to accumulate heavy metals and other contaminants can vary with the type of plant species, the nature of metal, but also other environmental factors or soil properties (Oladejo et al., 2017). The existence of heavy metals in soil can lead to their accumulation in different parts of plant with major consequences to health. The excessive amounts can determine physiological and biochemical toxic effects (Shahid et al., 2015). On a study in 2005, Bashmakov et al., observed that on high concentrations of heavy metals, the growth of shoots and roots was inhibited, followed by death of the majority of seedlings. Heavy metals can also determine other physiological responses like necrosis, browning of roots or chlorosis, but the effects may differ in function of environmental condition, plant type or metal amount (Pourrut et al., 2013).

The majority of metals are barely soluble and mobile in soils, with very low phytoavailability. Almost all heavy metals occur in soils as residual forms, due to strong binding with different ligands (Uzu et al., 2009).

Even the plants can easily accumulate metals that are dissolved as ionic compounds in the soil solution, also chelated or complexed metals can be absorbed due the complex structure of soils and complex absorption mechanisms of plants (Fairbrother et al., 2007). This would be the main different between laboratory tests and "in situ" growth of plants. Yet, the influence of increasing doses of heavy metals on the behavior of seeds and seedlings can be observed in laboratory, with very good remarks. This work aimed to observe the behavior of seeds and seedlings of maize (*Zea mays* L.) in the presence of increasing doses of soluble heavy metals (Zn, Cu, Ni, Co, Pb and Cd).

MATERIALS AND METHODS

In order to characterize the effects of heavy metals on seed germination and seedling growth, six soluble salts of heavy metals were used ($Cd(NO_3)_2 \cdot 4H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Pb(CH_3COO)_2 \cdot 3H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Zn(CH_3COO)_2 \cdot 2H_2O$), representing those heavy metals (Cd, Cu, Ni, Pb, Co and Zn). The salts were mixed together in five concentrations, representing five variants of treatments. A control variant represented by distilled water was used also, resulting a total of six variants. The concentrations for the five variants were chosen according the Romanian regulation (Order 756/1997), aiming to cover the whole interval from reference value to values over intervention threshold for sensitive use of soils, as described in the order mentioned above. The variants are showed in the Table 1.

Table 1. Variants of concentrations used in experiment

Metal	Variants (ppm)					
	M	C1	C2	C3	C4	C5
Ni	0	25	75	100	150	200
Cu	0	20	60	100	150	200
Cd	0	3	5	10	15	20
Zn	0	100	300	450	600	700
Pb	0	30	50	75	100	150
Co	0	20	30	50	75	100

The biological material was represented by maize seeds (DKC 3969 hybrid). The experiment was conducted using 20 centimetres Petri dishes, with two filter paper disks which have been soaked in heavy metal solutions. Each variant had four replicates (two per vessel), and each replicate used 25 seeds (Figure 1), resulting a total of 600 seeds. The germination took place at room temperature (22°C during daytime and 19°C during night), at natural light, excepting first 24 hours when the vessels were kept in dark conditions. The seeds were considered to have germinated after radicle emerged 2 mm through tegument. Germination test was ended when no seeds have germinated for 3 days consecutively. The germination period ended after 8 days, the seeds being monitorized for 10 days.

Figure 1. The arrangement of seeds in Petri dishes (two replicates: 2 x 25 seeds per vessel)

A series of physiological indicators were determined, as presented on the Table 2.

Table 2. List of determined physiological indicators

Indicator	Unit	Formula	Source
Final germination percentage	FGP (%)	(total number of germinated seeds / total number of planted seeds) x100	Ranal & Santana, 2006, according to ISTA, 1999
Length of radicle, shoot and total length of seedling	Lr, Ls, T	Metric measurements	-
Maximum germination value ("peak value")	PV	FGP / the number of days required to reach the maximum germination value	Czabator, 1962
Seedling growth inhibition	SGI (%)	(Seedling length of control variant - Seedling length of tested variant) / length of control variant x100	Ali et al., 2015
Seedling vigor	PVI	PVI = seedling length x FGP	Pati & Chowdhury, 2015

RESULTS AND DISCUSSIONS

After eight days, the germination ended resulting the daily evolution presented in Figure 2. The last three days are identical, meaning that germination is over.

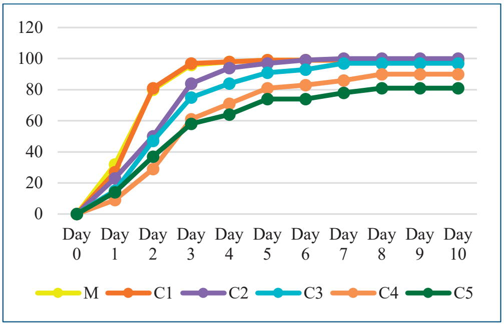

Figure 2. Germination dynamics by days

There were counted the seeds whose radicle reached a length of at least 1 mm. It can be observed that the contamination with heavy metals delayed the germination in concordance with the level of toxicity. The way how the germination influenced the others indicators is shown on the following calculations.

Final germination percentage (FGP) represents the total number of germinated seeds reported to total number of planted seeds (Figure 3).

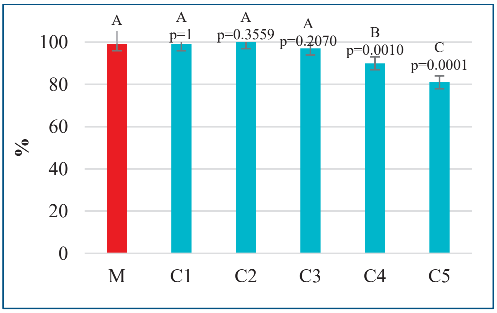

Figure 3. Final germination percentage (FGP) (means±SE)

Although the maximum FGP was obtained for C2 treatment, the difference between this variant and the control (M) in not significant (p-value > 0.05), according to ANOVA unifactorial test (α = 0.05). Actually, there are no significant difference between control M and C1, C2 and C3 variants. In the case of C4 variant, there is a significant difference beside control M, and moreover, between M and C5 variant is highly significant difference.

Seedling length was obtained by adding the length of radicle with the length of shoot (Figure 4).

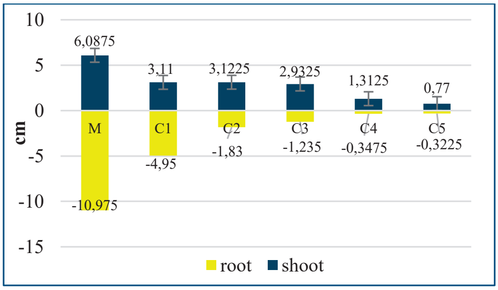

Figure 4. Seedling length (radicle + shoot) (means ± SE)

Although the FGP decreases from 99% to 81% with the increase of concentrations, the effect of heavy metals is not so relevant on germination as it is observed on seedling length (Figures 4 and 5).

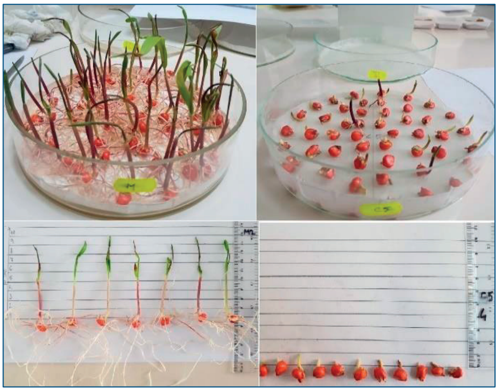

Figure 5. Differences between M and C5 seedling length

Running ANOVA test unifactorial test (α = 0.05), it is shown that there are highly significant differences between control variant and all treatments, from C1 to C5 (Table 3).

Table 3. ANOVA P-value comparing control variant with the rest of variants regarding seedling length

Variant	M	C1	C2	C3	C4	C5
P-value	A	D 4.95E-05	D 4.38E-06	D 3.73E-06	D 1.13E-06	D 7.28E-07

As can be seen, the length of seedlings dropped significantly from an average of 17.06 cm (M) to an average of 1.09 cm(C5). The root length followed the same trend dropping from average of 10.97 cm (M) to an average of 0.32 cm (C5).

Maximum germination value (PV) represents the mean daily germination of the most vigorous component of the seed lot. It has a high relevance in seed germination interpretations (Czabator, 1962). It is determined by reporting FGP to the number of days required to reach the maximum germination value.

For all tested variants, the germination started on Day 1 and ended differently, the latest germination being observed on C5 variant. The highest PV was reported for control variant, followed at slight difference by C1 variant. Running ANOVA unifactorial test (α=0.05), it was noted that there is no significant difference between M, C1 and C2 variants. For C3 and C4 variants, the values of PV differ significantly in in relation to M. Also, between C5 and M the ANOVA test showed a highly significant difference (Figure 6).

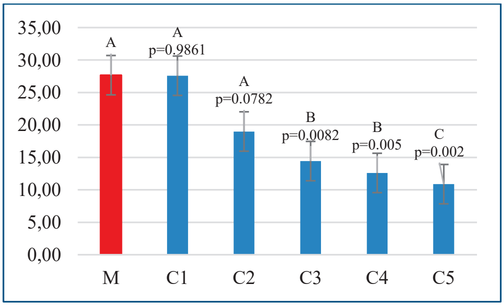

Figure 6. Maximum germination value (PV) (means±SE)

Seedling growth inhibition (SGI) indicator show how much a seedling growth is inhibits by a toxic factor. It is determined by the difference between control variant and tested variant length, reported to control length. Actually, the control variant has zero inhibition and as the length of a seedling decreases, the SGI indicator will increase.

As the control variant was not inhibited at all by heavy metal treatment (SGI = 0), the Figure 7 show the evolution of this indicator as for the rest of the variants.

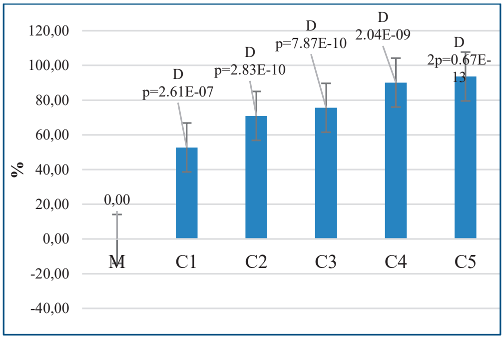

Figure 7. Seedling growth inhibition (SGI) (means±SE)

Given that for control variant the SGI is null, from statistical point of view (ANOVA unifactorial test α=0.05), between control and all other variants there are highly significant difference. As well, it can be observed that the seedling growth inhibition follows the trend of contamination level (Figures 7 and 8).

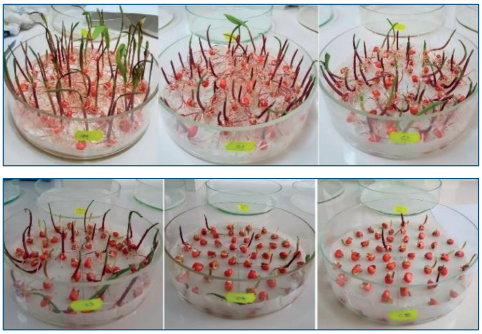

Figure 8. The seedling growth inhibition at the end of the experiment, from control variant (M) to highest used concentration (C5)

Seedling vigour (PVI) indicator is derived from final germination percent and seedling growth parameters (i.e. length) and is calculated as seedling length multiplied by FGP (Figure 9).

The ANOVA test unifactorial test (α = 0.05) reported that all variants differ significantly from control variant. This shows that seedling vigour is substantially reduced by any contamination with heavy metals.

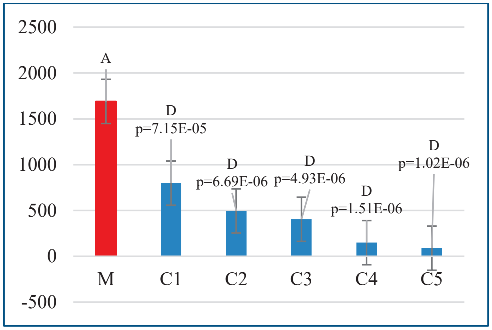

Figure 9. Seedling vigour (PVI) (means ± SE)

CONCLUSIONS

The results showed that the germination percent did not decrease significantly for the lower level of contamination, but increasing the concentration of heavy metals close to intervention threshold for sensitive soils the germination percent dropped significantly.
Regarding the seedling development, the high concentration of heavy metals inhibited the growth, especially the radicle evolution which was almost stopped. The radicle length was reduced by 17 times on highest heavy metal treatment. Although the shoot continued to grow slowly, it may be due to seed nutrient reserve which did not end in the supervised period.
The maximum germination value showed that the higher the contamination was, the harder the seed germinated. The highest level of contamination increased the germination time almost twice compared to non-contaminated seeds.
Considering the seedling growth inhibition, it can be noted that the first level of contamination reduced the length of seedling more than half of the control variant and the highest level of contamination, the length of seedling was reduced over 93%.
The same way, the seedling vigour is reduced by half at the first contamination and at the highest contamination is reduced about 20 times than the control variant.
Overall, even the germination indicators of some variants did not suffer significant modifications related to control variant, the indicators concerning the seedling growth are totally different beside the control variant, heavy metal contaminations affecting much more the early development than the germination.

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MONITORING OF THE THERMAL AND HYDRIC REGIME OF THE SOILS FROM THE SOMEȘAN PLATEAU

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Abstract

The purpose of monitoring the thermal and hydric regime of the soils from the Someșan Plateau is to set up the evolution tendency of pedoclimate parameters and to establish the agrotechnical measures in order to adapt agricultural technologies to climate changes. Romania is among the areas with the lowest capacity to adapt to existing climate changes and to those which are going to come and the Someșan Plateau is among the most affected areas due to the reduced degree of afforestation and the lack of forest curtains. Monitoring and variability of climate elements was achieved during 2013-2018, through a network of 10 HOBO microstations which stored soil temperature data electronically (at 10, 30, 50 cm deep) and air (at 1 m height), soil moisture (at 10 cm depth) and rain gauges. The thermal regime of the soils from the Someșan Plateau is mesic, with values of the annual average temperature of the soil 50 cm deep ranging between 8-15°C, and the differences between the average of summer temperatures and the average of winter temperatures are higher than 6°C at 50 cm depth (the difference ranging between 10.3-15.6°C). The annual values of the soil humidity ranged between 0.153-0.344 m^3/m^3. The annual average of the air temperature during 2013-2018 ranged between 9.21-11.03°C. The annual average of rainfall ranged between 476.3-879.4 mm/an.

***Key words**: thermal and hydric regime of soils, the Someșan Plateau.*

INTRODUCTION

The Earth's climate is generated by factors like: insolation, excentricity of the Earth's orbit, the movement of earthly precession, obliqueness compared to the Sun, terrestrial albedo, anthropic factors, humidity etc., cannot remain constant as long as there are changes, evolution at the level of the earthly bark (Eastwood et al., 2006; Casas-Prat, 2012; Murante et al., 2020). Climate changes are currently a political issue worldwide (Ramirez-Villegas et al., 2012; Cattaneo et al., 2019).

Scientific evidence and explanations of climate changes have accumulated over decades, being integrated into strategies and adaptation policies (Fuhrer, 2003; Hemadi et al., 2011; Lereboullet et al., 2013; Clar and Steurer, 2019).

Changes in the rainfall regime at a European level show a higher temporal and spatial variability compared to temperatures, increasing in the areas from the north and nort-western Europe, but decreasing in the south of Europe. The majority of projections of climate models show a continuation of increased rainfall in the north of Europe and their decrease in the south of Europe (EEA, 2019).

The effects of climate changes in agriculture reflect in the expansion to the north of areas favorable for certain agricultural crops during blooming and harvesting of crops earlier than the usual ones, in the growth of irrigation need for the crops from the South and South-West of Europe, in the decrease of productivity in the case of certain crops due to heat waves and drought periods, mainly in central and southern Europe, instead there is an increase in productivity for crops from northern Europe, where the vegetation period of crops has been extended.

In terms of the soil humidity, there are no clear clues as to the tendencies of keeping water in

the soil due to the lack of systematic and harmonized data. The projections suggest a reduction of the soil humidity in the greatest part of Europe, significant reductions in the mediterranian region and its growth in the North-East of Europe (Calanca et al., 2006; Sheffield et al., 2012).
The regional distribution of the impact of climate changes on agricultural production can vary a lot (Donatelli et al., 2012). Thus, the biggest drops in crops are forecast in the southern part of Europe, of approximately 25%, up to 2080 based on a temperature increase by 5.4°C (Ciscar et al., 2011). Under these conditions, Bindi and Olesen (2011) also estimate an increase of the risk of failure, especially in the case of summer crops which are not irrigated.
Romania is among the areas with the lowest capacity to adapt to the existing climate changes and to those which are going to come and the Someşan Plateau is among the most affected areas due to the reduced degree of afforestation and the lack of forest curtains. Right now and in perspective, a series of strategies and plans to fight climate changes are being proposed, but in order to put them into practice, a strict monitoring of the thermal and hydric regime of the area is needed in order to identify and implement the measures to adapt to the effects of climate changes.
The afforestation percentage of the current geographical space of Romania has slowly decreased from around 80%, which was in the recent past to 55-60% at the beginning of the XIXth century and to 23%, which is now. Therefore, a considerable damage of the environment has been produced, including an incredible narrowing of biodiversity at all levels (Baciu, 2006). The Someșan Plateau is a subdivision of the Transilvanian Basin together with the Transylvanian Plain and the Târnavelor Plateau. The Someșan Plateau is aproximately a quadrilateral, as a form, with a surface of approximately 510000 hectares, having around 100 km on the South-West : North-East direction, respectively 60 km on the North-West : South-East direction. In this region, high erosion is not only the result of combined processes of two neotectonic movements (the uplift of the hill compartment and local subsidence), but also the friability of geological formations. The Someșan Plateau is a “hilly complex” of plateaux and basins, with many corridors on the side and it is very fluctuant both in terms of relief and as lithological structure. The lithology from the Someşan Plateau is a dominant pedogenic factor, influencing the type of relief, the diversity and territorial distribution of soils. Its influence reflects in the texture, depth, fertility and humidity regime of soils, being at the same time the main cause of slope processes. The zonal soils are: phaeozems, preluvisols and luvisols, to which intrazonal lithomorph soils are added: rendzina, pseudorendzina, hydromorph, halomorph, less spread. Less developed soils like lithosols, regosols, alluvial soils and river deposits are also met. The analysis of crop structure from the Someșan Plateau shows a higher share and considers main agricultural plants: autumn wheat, triticale, maize, potato and clover.
The annual average temperature has values ranging between 6.5-9ºC, the lower value is very rarely reached in higher areas, and the higher value of 8.5-9ºC is rarely met, only during very hot years. The annual average rainfall has values ranging between 600-800 mm, rarely dropping or exceeding this limit.
The purpose of the paper is to monitor the thermal and hydric regime of the soils from the Someşan Plateau in order to set up the evolution tendency of the pedoclimate parameters and to set up the agrotechnical measures to adapt agricultural technologies to climate changes.

MATERIALS AND METHODS

Monitoring and variability of climate element from Someșan Plateau was achieved during 2013-2018, through a network of 10 HOBO-MAN-H21-002 (On-set Computer Corp., Bourne, MA, USA) stations, which store soil temperature data electronically (at 10, 30, 50 cm deep) and air (at 1 m height), soil moisture (at 10 cm depth) and rain gauges. HOBO Smart Temp (S-TMB-M002) temperature sensors and Decagon EC-5 (S-SMC-M005) moisture sensors were connected to HOBO Micro Stations. Additionally, tipping bucket rain gauges (RG3-M) were deployed to measure rainfall. Data was downloaded from the Micro

Stations every four months via laptop computer using HOBOware Pro Software Version 3.7.2. Soil types, land slope and exposition, altitude and geographic coordinates of the locations in which stations were set are shown in Table 1 (Duda, 2018).

Table 1. Configuration of stations in the Someșan Plateau

No. station	Location (County)	Altitude (m)	Soil type	Exposition	Slope, %
1	Cristorel (Cluj)	404	Preluvosol	N	8-10
2	Borșa (Cluj)	332	Faeoziom	S	2-3
3	Lelești (Bistrița-Năsăud)	606	Regosol	V	25-26
4	Șomcutu Mic (Cluj)	271	Aluviosol	S	2-3
5	Căprioara (Cluj)	416	Preluvosol	S	4-5
6	Almașu (Sălaj)	323	Aluviosol	S	8-10
7	Racâș (Sălaj)	253	Preluvosol	S-E	2-3
8	Șimișna (Sălaj)	256	Preluvosol	N-E	7-9
9	Ileanda (Sălaj)	225	Aluviosol	S	2-3
10	Bunești (Cluj)	209	Preluvosol	N	6-8

When calculating the average, minimum and maximum values of daily, monthly and annual temperatures from the soil and air, as well as the average, minimum and maximum values of humidity from the soil and rainfall, the initial data recorded by the HOBOware Pro Software Version 3.7.2 Program mentioned earlier shall be used, which is exported in Microsoft Excel table worksheets. Subsequently, the data is analyzed, it is graphically represented and statistically interpreted by using both functions specific for Microsoft Excel and specific statistic methods of interpreting data. Regression is used as the best interpolation method in order to spatialize climate information, taking into account the nature of the variable (continuous spatial character) and the time scale (monthly, annual and multiannual average values).

In order to estimate the evolution tendency of climate parameters we used the regression analysis which supposes quantifying relations from one (simple regression) or several (multiple regression) independent variables and a dependent variable (of reply), quantifying cause relations being made by linear equations (linear regression). The estimation of evolution tendencies was applied in the evolution analysis of climate parameters during 2013-2018, with regards to the soil temperature at the surface and 10 and 30 cm deep in the soil, the air temperature 1 m from the soil surface, the evolution of rainfall and soil humidity. The values of the correlation coefficients in relation to the tendencies of linear evolution of the calculated parameters were graphically represented for each of the parameters previously mentioned. The correlation analysis was used in order to measure the intensity among the variables by offering information regarding the existance, the sense, the form and the intensity of the connection among the variables analyzed.

For a synthetic image of the space-time variability of the variation of a meteorologic parameter, we used standard deviation (standard deviation), frequently used in climatology, an index with the same unit measure as that of the values from the data string used. The evolution of quantitative elements were shown by the graphical representation of standard deviations, for example, of the annual amount of rainfall compared to the multiannual average, which indicates the value of positive or negative deviations from each year compared to the multiannual average, also called normal, considered absolute zero arbitrary. For the calculation of rainfall, the HOBOware program allowed to record the events (daily counting of the number of tippings of the cup to collect rainfall), which were exported in their turn in Excel calculation sheets, where the monthly and annual amount of rainfall was calculated by multiplying the number of events by 0.2 (mm), a value which corresponds to each balance of the cup mechanism the rain gauge is equipped with.

RESULTS AND DISCUSSIONS

The thermal regime of the soil depends on a complex of factors, first on the intensity of solar radiation and its periodic variations in

time, to which physical properties of the soil, composition, structure, texture, degree of humidity or dryness of the soil, specific heat and thermal conductivity, orientation and slope inclination as well as nature and degree of covering soil surface with vegetation are added.
The soil surface receives a certain amount of energy which is converted into thermal energy, which subsequently propagates and/or it is taken over, then, by the soil layers through conduction.
The thermal regime of the soil influences in its turn the growth of plants, the biological activity and movement of water inside the soil. In evaluating the thermal regime of the soil, the multiannual average of temperature is the value mostly used.
The analysis of the thermal regime of the soils from the Someșan Plateau during 2013-2018 by indirect determination according to SRTS, 2012 (adding 2°C to the annual average temperature of the soil 50 cm deep from the surface) is not confirmed as, in the case of most stations from the Someșan Plateau, the temperature values 50 cm in the soil are higher than those of the air.
Figure 1 shows the multiannual average temperatures (2013-2018) of the temperatures in the soil, at 10 cm, 30 cm and 50 cm in the case of the 10 stations from the Someșan Plateau:

- the multiannual average of the soil temperature 10 cm deep ranged between 10.59°C at Cristorel station and 12.04°C at Căprioara station;
- the multiannual average of the soil temperature 30 cm deep ranged between: 10.62°C at Cristorel station and 11.98°C at Căprioara station;
- the multiannual average of the soil temperature 50 cm deep ranged between: 11.03°C at Almașu station and 11.91°C at Căprioara station.

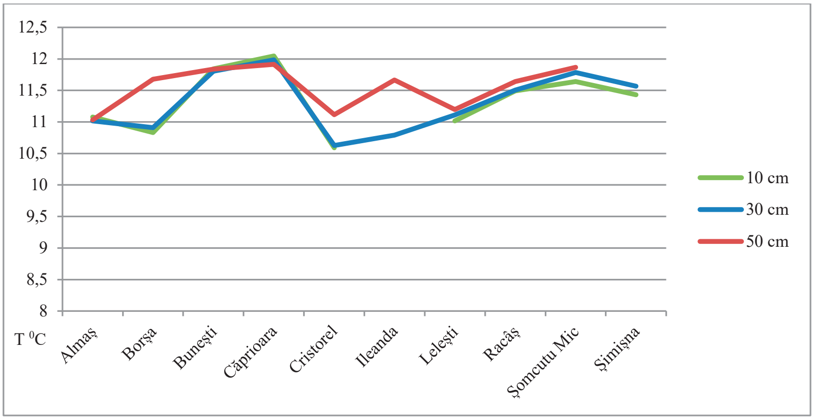

Figure 1. Multiannual average (2013-2018) of soil temperature in the Someșan Plateau

From the data recorded during 2013-2018, it results that the thermal regime of the soils from the Someșan Plateau is ***mesic***, with values of the annual average temperature of the soil, 50 cm deep, ranging between 8-15°C, and the differences between the average of summer temperatures and the average of winter temperatures are higher than 6°C at 50 cm deep. The annual average of the soil temperature has values ranging between 10.3°C and 15.6°C. In terms of the differences between the average of the summer and winter temperatures, 50 cm deep into the soil, they range between 12.07°C and 19.91°C. The differences between the annual average of the summer and winter temperatures, 50 cm deep into the soil, range between 12.07°C at Borșa, in 2015 and 19.19°C at Cristorel in 2017. The lowest differences of temperature were recorded at Borșa (12.07°C) and Almașu (12.6°C) stations in 2015, and the highest differences of temperature were recorded at Cristorel in 2017 (19.19°C) and Șomcutu Mic in 2017 (18.91°C).
The soil humidity is one of the derived meteorological elements of atmosphere, with a

determining role, together with other factors, in the ongoing under the best conditions of the vegetation cycle of plants. The determination of the soil humidity was made with soil humidity sensors located 10 cm deep into the soil. The annual values of the soil humidity are presented in Figure 2, from which one can notice that the lowest annual values of the soil humidity are recorded at Cristorel station in 2016 of 0.153 m^3/m^3, in 2013 of 0.1762 m^3/m^3 and in 2018 of 0.176 m^3/m^3; followed by Căprioara station of 0.185 m^3/m^3 in 2017. The highest values were obtained in 2013 at Leleşti station, of 0.344 m^3/m^3, followed by those at Şimişna station in 2018, of 0.327 m^3/m^3 and Ileanda in 2017, of 0.322 m^3/m^3.

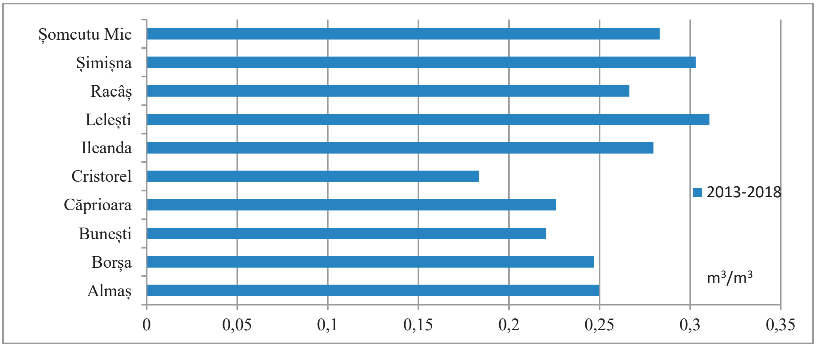

Figure 2. Multiannual averages of soil moisture in the Someșan Plateau

The air temperature was recorded at the stations which were equipped with rain gauge, 1 m high from the soil surface. The annual average of the air temperature during 2013-2018 ranges between 9.21°C at Cristorel station in 2017 and 11.03°C at Şomcutu Mic station in 2015. The lowest values of the annual average of air temperature during 2013-2018 were recorded at Cristorel and Şomcutu Mic stations and the highest values were recorded at Buneşti and Almaşu stations (Figure 3).

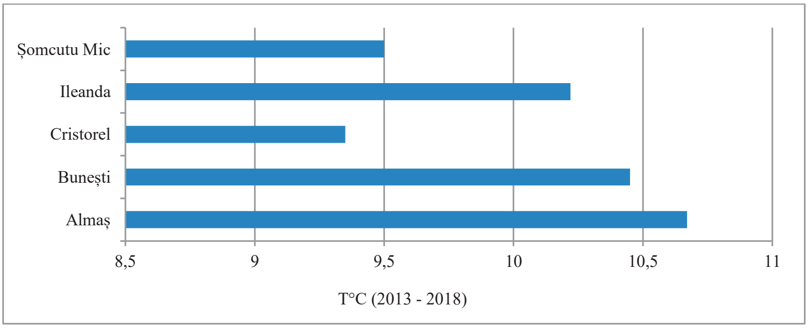

Figure 3. Multiannual averages of air temperature during 2013-2018

From the data recorded at the stations equipped with rain gauges, the highest amount of rainfall from the Someşan Plateau during 2013-2018 was recorded at Ileanda station in 2016 (879.4 mm/year), followed by the ones from Buneşti station in 2016 (708.8 mm/year) (Figure 4).

In order to statistically analyze the general direction in time of the soil temperature, *the monthly rolling average for 12 months* was used of temperatures 10, 30 and 50 cm deep during 2013-2018. Then, *the linear tendency* was applied to the values of the rolling average based on the size of R^2 determination index and the correlation coefficients, the first 12 months of data was excluded from the graphs in order to allow the rolling average to accumulate all 12 months for which calculations are made and thus to become relevant for 1 year every month when it is calculated. Figure 5 shows the values of the correlation coefficients associated to the trend evolution of the annual average temperatures calculated by the average of temperatures at 10, 30 and 50 cm. The values range between 0.69 at Almaşu station and 0.19 at Buneşti station.

The average of the rolling temperatures is presented in Figure 6. Temperature values between 11.5 and 13.5°C can be noticed, the only exception is at Ileanda station, where on an average, 14.85°C was calculated.

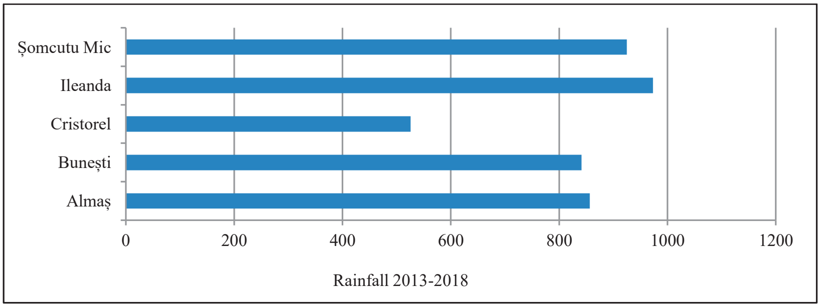

Figure 4. Multiannual rainfall the Someşan Plateau

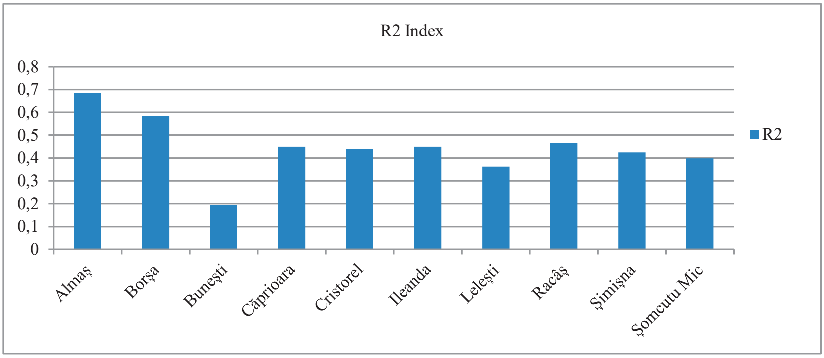

Figure 5. Values of correlation coefficient associated with trend evolution of annual average temperature averaged at 10, 30 and 50 cm

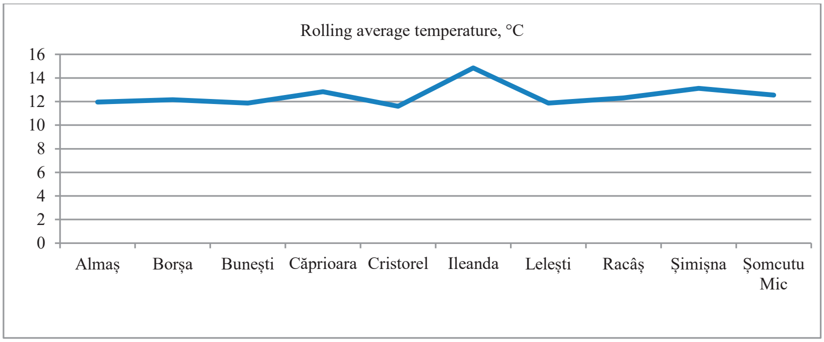

Figure 6. Temperature rolling average in the Someşan Plateau, 2013-2018

The values of the correlation coefficients associated to the evolution tendencies of the annual average temperatures calculated through the soil humidity are shown in Figure 7.

The values range between 0.6922 at Bunești station and 0.0097 at Șomcutu Mic station. The evolution tendencies of humidity are described in Figure 8.

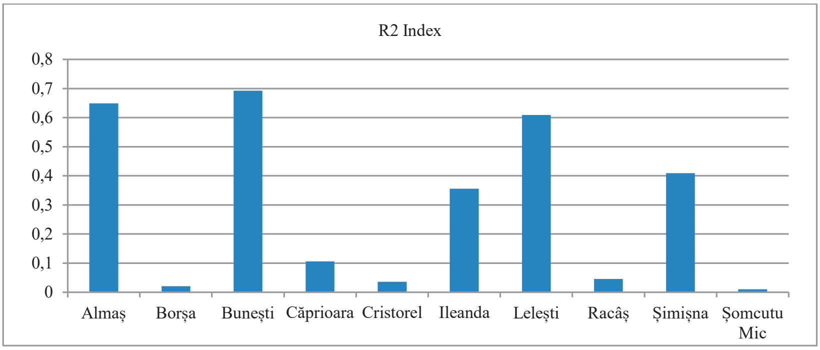

Figure 7. Values of the correlation coefficients between temperature and humidity, Someșan Plateau, 2013-2018

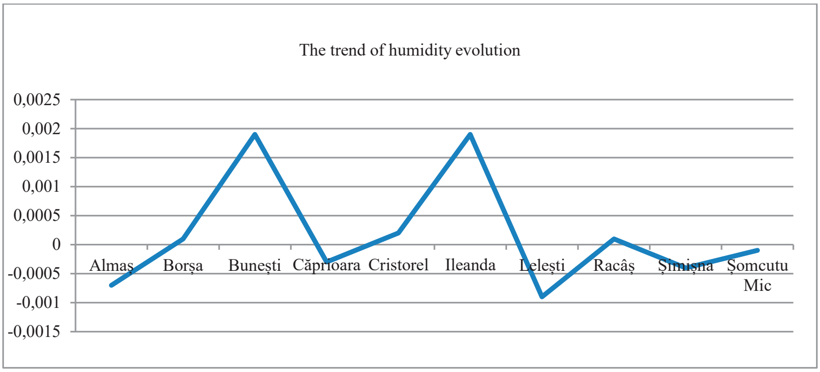

Figure 8. The trend of humidity evolution in Someșan Plateau, 2013-2018

At Bunești and Ileanda stations, tendencies of easy growth (0.002) of humidity were recorded, and at the other stations decreases or an almost constant evolution resulted. At Lelești, the highest decreasing tendency was recorded (-0.0009). The highest multiannual values of the rolling average (Figure 9) were calculated at Lelești and Șimișna stations (of over 0.3 m^3/m^3), and the stations with the lowest averages were Bunești and Cristorel - the only ones with values under 0.2 m^3/m^3 (0.173 and, respectively, 0.178 m^3/m^3).

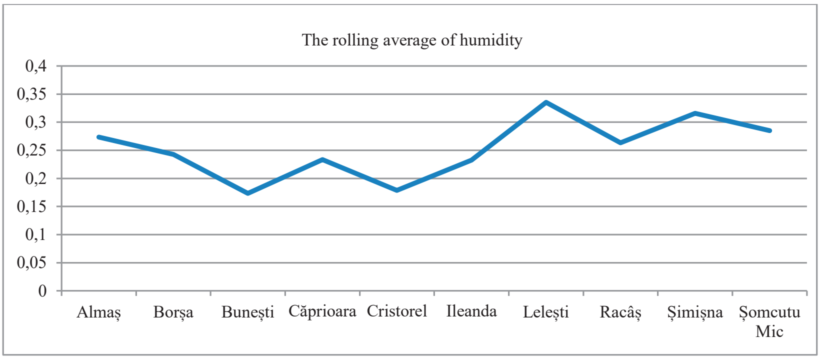

Figure 9. The rolling average of humidity in the Someșan Plateau, 2013-2018

For the stations equipped with rain gauge, the correlation index between the amount of rainfall and the soil humidity was calculated, too and its results are shown in Figure 10. Thus, a very high R^2 correlation index (0.8507) between the annual rainfall recorded at the stations with rain gauge and the records offered by the humidity sensors in the soil is confirmed.

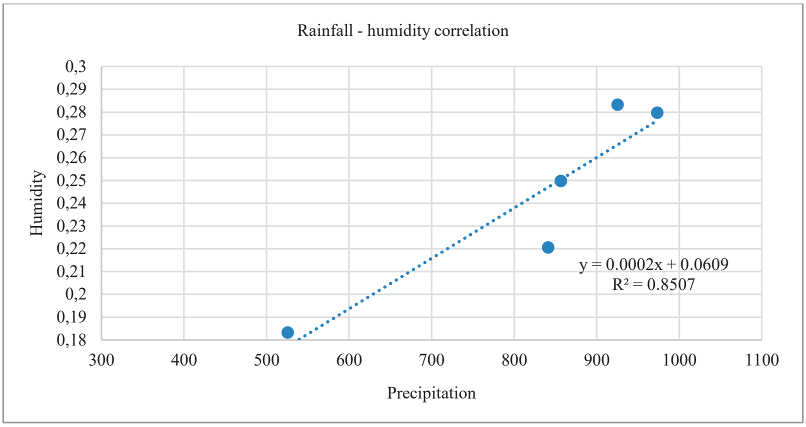

Figure 10. The correlation index between rainfall and soil moisture, Someșan Plateau, 2013-2018

CONCLUSIONS

The thermal regime of the soils from the Someșan Plateau resulted from the data analysis of soil temperature during 2013-2018, indicates a mesic-type regime, with values of the annual average temperature of soil at 50 cm deep ranging between 8 and 15°C, and the differences between the average of summer temperatures and winter temperatures are higher than 6°C at 50 cm into the soil. The differences between the annual average of summer temperatures and winter temperatures at 50 cm deep range between 12.07°C at Borșa, in 2015, and 19.19°C at Cristorel, in 2017. The lowest differences of temperature were recorded at Borșa (12.07°C) and Almașu stations (12.6°C), in 2015, and the highest differences of temperature at Cristorel, in 2017 (19.19°C) and Șomcutu Mic, in 2017 (18.91°C).
The evolution of temperatures at 10, 30 and 50 cm into the soil during 2013-2018, but also of the average of the 3 temperatures indicates evident decreasing trends of temperature in most of the stations. The coefficients of linear correlation among the data strings analyzed indicate a dropping synchronous evolution of the annual average temperature in the soil, with more evident tendencies at Almașu, Borșa and Cristorel (-3%), the only station which recorded an increase in temperature was Ileanda, by 4%. The R2 coefficients of linear correlation for the rolling average, calculated every month, of the average of temperatures range between 0.35 and 0.8 for all stations.
Following the analysis of evolution trends of annual average temperatures, based on the data recorded during 2013-2018 we can notice that the latter recorded decreases, even more important than those for the soil temperature. Drops of up to 10-15% of the rolling average of temperatures were recorded, the only station with a relatively constant evolution is Șomcutu Mic, with an unimportant decrease of -0.5%. The values of correlation coefficients associated with the evolution trends of annual average air temperature are lower than soil temperatures. An R2 index of 0.62 was calculated at Almașu and approximately 0.3 at the other stations.
From the data analysis regarding the amount of rainfall recorded, one can observe their linear falling trend, the highest values of the annual average are recorded in 2016, an average value of 616.9 mm, this year is considered a year with rainfall close to the normal of the area. The lowest amount of rainfall was recorded in 2017 (476.3 mm), a dry year from the point of view of rainfall. The value of the correlation

coefficient associated with the falling evolution trend of rainfall R2 is smaller, which expresses the unpredictable character of rainfall from the area.

Following the comparative analyses between the monthly average amount of rainfall during 2013-2018 and the monthly average amount of rainfall from the data series for 30 years and respectively 100 years, one can notice evident decreases of the average amount of rainfall, especially in critical periods, with high demands for water, mainly during the blooming and filling the seed/grain periods.

The evolution of soil humidity during 2013-2018, recorded at 10 cm deep indicates its general stagnation trend. The values of R2 correlation coefficients associated to the linear trends of soil humidity range between 0.35 at Ileanda and 0.69 at Bunești.

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COMPARATIVE ANALYSIS ON A BALANCED FERTILIZATION FOR QUALITY OF SOME ARABLE SOILS FROM SOUTH OF ROMANIA

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Abstract

One of the requirements of quality management of soils in general, and of arable soils in particular, is knowledge of the dynamics of physical and chemical characteristics especially of those which are the most sensitive under human activities. The main objectives of the present paper were to measure the physical, chemical and hydrological properties of soil collected from three agricultural soils, and to carry out the fertilization plan accomplished by using modern agricultural practices and technologies for a balanced fertilization in order to preserve soil fertility and environment. Soil samples were collected from three locations around South of Romania. The selected soils for this study are located in the Calarasi county, namely: profile P1 in Sarulesti location - Typical Chernozem (Calcic Chernozem), profile P2 in Tamadau Mare location - Cambic Chernozem (Haplic Chernozem), and Arges county respectively: profile P3 in Costesti location - Vertic-Stagnic Preluvosol (Vertic-Stagnic Luvisol). The investigated soils are medium to fine textured, starting from medium clayey-loam, clayey-silty loam to loamy clay. High contents in clay result in higher values for bulk density and penetration resistance. On the contrary, the saturated hydraulic conductivity (K_{sat}) of such fine textured soils records low values, the most significant decrease being encountered in the soil profile P3. The highest values of K_{sat} were recorded in the topsoil layer of the soil profile P2 where the total porosity was also the highest. The deeper layers of the soil profiles (> 80 cm depth) have lower values for S index. That means the soil physical quality as quantified by S is better in the topsoil horizons than in the subsoil horizons. The soil reaction values highlighted a lightly acid soil in P3, a lightly acid to lightly alkaline soil reaction in P2, and a lightly alkaline soil reaction in P1. The total nitrogen content of all three soils varied from very low-low to moderate values. The available phosphorus content ranged from very low-low in P3, low-high in P2, up to moderate-high in P1. As for the available potassium content, all the studied soils were moderately supplied. The fertilization plans were accomplished based on agrochemical studies. For each of the three sites cartograms were done for soil reaction, phosphorus and potassium. Also synthetic cartograms with the average values of soil reaction (pH), humus content, available phosphorus (P_{AL}), available potassium (K_{AL}), and nitrogen index (IN) for the established fertilization parcels from each location were realized. The fertilization plans contained economic and technical optimum doses - DOE and DOT (which ensure a certain level of crop yield at which the maximum benefit is achieved) for a four years crop rotation in case of P1 and P2 soils and five years crop rotation in case of P3 soil.

***Key words**: soil quality, balanced fertilization, arable soils, optimum economic dose.*

INTRODUCTION

One of the requirements of quality management of soils in general, and of arable soils in particular, is knowledge of the dynamics of physical and chemical characteristics especially of those which are the most sensitive under human activities.

It was widely recognized and accepted that soil structure represents the most important physical characteristic for all aspects of soil use and management due to its great impact on other soil physical properties.

Soil structure can change as a result of the use of various agricultural practices and can, in turn, influence both the productivity arising from agricultural practices and the impact of these practices on the environment. In order to have soil with a high physical quality it is necessary to have two main structural features: firstly, the soil must be stable. That means, the clay particles must flocculate and not disperse.

Flocculation is enhanced by calcium ions adsorbed on the clay surfaces and by maintaining the electrolyte concentrations above certain critical values. Also high levels of organic matter content are essential for the stability of micro-aggregates. Secondly, the soil must have a wide distribution of pore sizes and a high total porosity that will make the soil able to absorb, store and release water for plant use in response to transpirational demand. Such soils will have also a good aeration status and will be more easily penetrable by plant roots.

The interactions between soil chemical, physical and microbiological properties define a specific soil's "quality" and influence how effectively the soil carries out ecosystem functions such as: a) retain and release nutrients and other chemicals, b) distribute rainfall at the soil surface into runoff and infiltration, c) hold and release soil water to plants, streams and groundwater, d) withstand wind and water erosion, and e) buffer against the concentration of potentially toxic materials (Larson and Pierce, 1991; Karlen et al., 1997). Moreover, soil quality is an inherent property of a soil, a result of the factors of soil formation (i.e. climate, vegetation, parent material, time and topography). Therefore, from a productivity point of view, each soil has an innate capacity to function, and some soils will be inherently more productive than others.

An important measure from the Code of Good Agricultural Practices (2015), which transposes the European Nitrates Directive in Romania, refers to the standards on maximum quantities of nitrogen fertilizers which may be applied on agricultural lands in order to prevent or reduce water pollution with nitrates. For this, fertilizer plans at farm level are recommended to be carried out. A fertilization plan is based on a soil agrochemical study. Within the agrochemical study, the maximum nitrogen doses which might be applied in soil are calculated. For calculation of the maximum nitrogen doses, soil nitrogen content, soil physical and chemical properties as well as the expected crop yields are taken into account. If the maximum calculated nitrogen (mineral and organic) dose is lower than 170 kg nitrogen/ha/year, the maximum nitrogen dose from animal manure which might be applied on agricultural land should not exceed this value.

In order to obtain high yields and to increase the soil fertility, a proper fertilizer dose should be applied for increasing soil nutrients content as well as the soil fertility without losing nitrates by surface runoff or by leaching and avoiding water bodies pollution.

The fertilization plan is accomplished for a period of 4-6 years for crops within a certain rotation at farm level and contains nutrients economic optimum doses (which ensure a certain level of crop yield at which the maximum benefit is achieved) and technical doses (which take into account the ecological potential and the amount of nutrients needed to maintain/increase the soil fertility and to achieve high crop yields without a certain benefit and possible losses) (Lacatusu, 2016). All the fertilization doses are established in kg/ha.

The main objectives of the present paper were to measure the physical, chemical and hydrological properties of soil collected from three agricultural soils, and to carry out the fertilization plan accomplished by using modern agricultural practices and technologies for a balanced fertilization in order to preserve soil fertility and environment.

MATERIALS AND METHODS

Sample collection and preparation

Soil samples were collected from three locations around South of Romania. The selected soils for this study are located in the Calarasi county, namely: profile P1 in Sarulesti location, profile P2 in Tamadau Mare location, and Arges county respectively: profile P3 in Costesti location. According to the Romanian soil classification - SRTS 2012 (Florea and Munteanu, 2012) and FAO-WRB (1998), the soil types used in this paper are: P1-Typical Chernozem (Calcic Chernozem), P2- Cambic Chernozem (Haplic Chernozem), P3-Vertic-Stagnic Preluvosol (Vertic-Stagnic Luvisol).

All the investigated areas were under the arable land use. The soils were characterized according to the instructions for accomplishing the agrochemical studies by using standardized measurement methods.

Fertilization plan execution

The fertilization plan was carried out going three stages, namely: field stage, laboratory stage and desk stage. The field stage included activities such as: obtaining information about the farm specific conditions (physical blocks, crop location on physical blocks, previous agrochemical treatments, soil types). Two soil samples were collected from one physical block within the Sarulesti sampling point, 11 soil samples were collected from four physical blocks within Tămădău Mare sampling point

and 10 soil samples were collected from one physical block within Costești sampling point.
The laboratory stage included the measurement of soil indicators used for nitrogen doses calculation, such as: soil reaction (pH), organic carbon (Corg), available phosphorus (P_{AL}), available potassium (K_{AL}). The nitrogen index (IN) was calculated. During the desk stage cartograms related to soil reaction (pH) and availability of phosphorus (P_{AL}) and potassium (K_{AL}) were accomplished.

RESULTS AND DISCUSSIONS

Comparative analysis of soil quality

The soils from this study were characterized in terms of hydro-physical and chemical properties. In Table 1 the hydro-physical characterization of the soils is presented. It can be observed that the investigated soils are medium to fine textured, starting from medium clayey-loam (P1), clayey-silty loam (P2) to loamy clay (P3). High contents in clay result in higher values for bulk density (BD) and penetration resistance (RP). On the contrary, the saturated hydraulic conductivity (K_{sat}) of such fine textured soils records low values, the most significant decrease being encountered in the soil profile P3.
Soil penetration resistance and bulk density are often used in studies concerning soil degradation by compaction. In this investigation, in case of soil profiles P2 and P3, the values of penetration resistance and bulk density increased down the soil profile, the subsoil layers showing a greater mechanical strength than the topsoil layers. Plots of penetration resistance and bulk density as functions of soil depth are presented in Figure 1. The compacted layer is observed just below the ploughing depth, values of both bulk density and penetration resistance increasing sharply at this depth.
Penetration resistance is used to simulate the mechanical impedance encountered by growing roots (Whitebread et al., 2000). Several authors (e.g. Ferreras et al., 2000) suggested that the level of mechanical strength, as recorded by penetrometer, can severely restrict root growth, particularly in the plough pan. A value of penetration resistance of 2-2.5 MPa is quoted in the literature as a critical value above which root growth is reduced significantly (Busscher et al., 1986).
Degradation of soils due to compaction is a worldwide problem, and the problems caused by this were intensively studied and reported in many articles (e.g. Defossez and Richard, 2002). Lipiec and Nosalewicz (2004) showed that a characteristic response of a root system to increasing soil compaction level is a decreased root length, retarded root penetration and shallower rooting depth. The authors in their work showed that irrespective of soil type and site the soil compaction resulted in greater concentration of roots in upper soil (0-10 cm) and reduced root growth in deeper soil, mostly due to excessive mechanical impedance such as hard pan.
Soil structure represents one of the major attributes of soil quality (Dexter, 2004a). It affects the soil pore system and through it the water movement processes in soil. The highest values of saturated hydraulic conductivity (K_{sat}) were recorded in the topsoil layer of the soil profile P2 where the total porosity (PT) was also the highest (Figure 2).

Table 1. Physical characterization of the soil profiles

Location	Profile No.	Depth	Particle size distribution			Soil texture class	BD	RP	K_{sat}	PT	$W_{pF\,0}$
			sand	silt	clay						
		(cm)	(% g/g)				(g/cm^3)	(MPa)	(mm/h)	(% v/v)	
Sarulesti	P1	0-18	37.2	27.7	35.1	TT	1.45	4.4	3.95	45.9	45.2
		18-28	40.0	27.6	32.4	LL	1.44	3.9	7.24	46.2	45.5
		28-45	37.7	29.1	33.2	TT	1.37	3.1	4.44	48.9	45.3
		45-65	37.6	29.8	32.6	TT	1.27	2.4	18.23	52.7	45.6
		65-92	35.2	32.5	32.3	LP	1.28	2.6	13.49	52.1	46.4
		92-138	40.1	30.0	29.9	LL	1.46	3.4	2.14	45.7	44.7
Tamadau Mare	P2	0-16	37.1	28.2	34.7	TT	1.18	2.5	16.93	56.0	47.8
		16-28	34.8	28.0	37.2	TT	1.45	4.0	5.70	45.9	41.5
		28-45	34.8	26.5	38.7	TT	1.40	4.7	1.21	47.6	41.3
		45-63	37.6	24.0	38.4	TT	1.43	4.8	1.04	46.8	42.1
		63-90	28.8	31.6	39.6	TT	1.40	4.3	2.07	47.9	41.7
		90-130	33.8	32.5	33.7	TP	1.48	4.0	3.46	44.9	41.1
		130-150	37.9	29.9	32.2	LL	1.36	3.0	5.87	49.3	42.4
Costesti	P3	0-20	29.6	29.2	41.2	TT	1.61	2.5	0.95	39.2	48.7
		20-39	33.2	24.7	42.1	TT	1.46	5.5	14.20	44.9	41.1
		39-57	21.1	23.5	55.4	AL	1.48	5.3	2.40	44.2	47.4
		57-74	20.9	22.8	56.3	AL	1.47	6.2	1.18	44.5	49.7
		74-96	19.9	24.5	55.6	AL	1.50	6.2	1.18	43.4	49.2
		96-138	23.1	23.1	53.8	AL	1.51	6.9	0.80	43.0	44.7

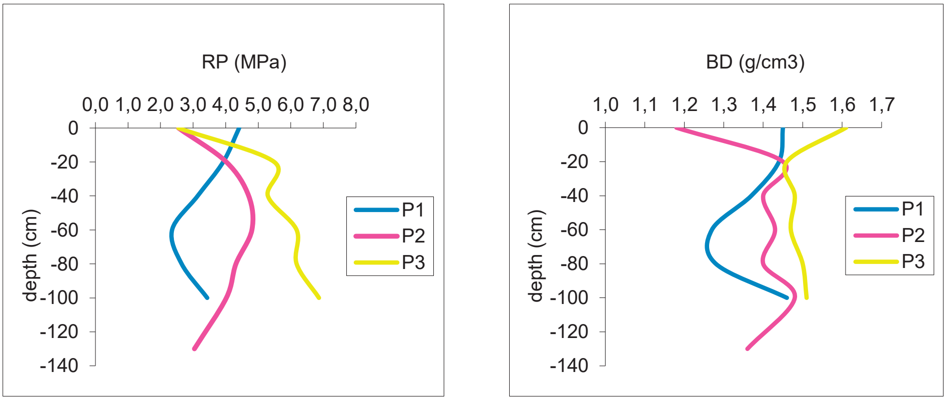


Figure 1. Plots of penetration resistance and bulk density as functions of soil depth

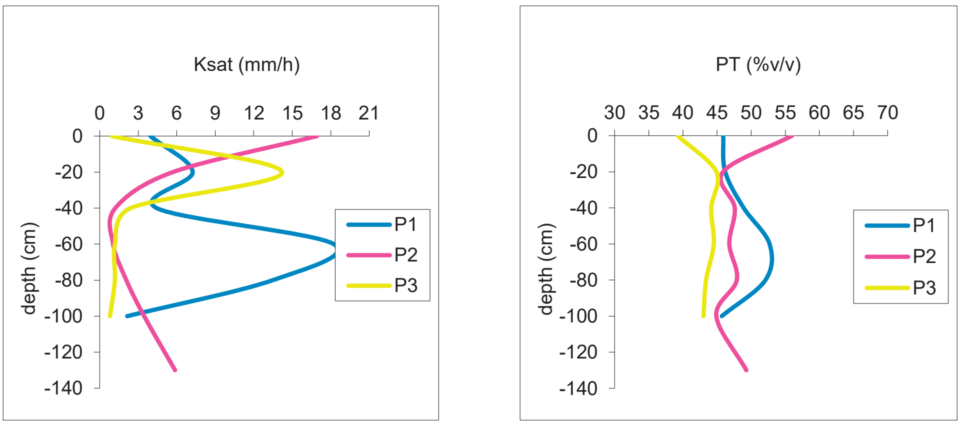


Figure 2. Plots of saturated hydraulic conductivity and total porosity as functions of soil depth

Pagliai et al. (2004) stated the significant role played by soil porosity in evaluation of the impact of management practices on the quality of soil structure. They found that adopting alternative tillage systems, such as ripper subsoiling, on a cambisol the macro-porosity was generally higher and more-homogeneously distributed through the profile when compared with a conventional tillage system, and the resulting soil structure had a better quality, as confirmed by the higher hydraulic conductivity measured in the soil tilled by ripper subsoiling.

As a general trend, the topsoil layers had greater values of water content at saturation (W_{pF0}) when compared with subsoil layers, except for the profile P1 where the values of water content at saturation were constant within the soil profile (Table 1). This may be attributed to a decrease of organic matter content and an increase of bulk density values within the soil profiles.

Figure 3 shows the soil water retention curves of the three investigated soils. The soil water retention curves were obtained after using the Arya-Paris model for estimating the van Genuchten parameters (Arya and Paris, 1981).

The differences in shape of water retention curves between topsoil and subsoil layers may be as a result of either externally-applied mechanical stress by agricultural machinery which can lead to compaction of the layer below ploughing depth, or internally-applied mechanical stress due to drying of the soil which causes shrinkage due to the effective stresses generated by the pore water suction and the surface tension in the water menisci. It is known that both increasing bulk density and soil drying reduces the volume of the soil pores (Vizitiu et al., 2010).

The fitted van Genuchten (van Genuchten, 1980) parameters were then used to calculate S index using Eq. (1) from below.

$$S = -n\left(\theta_{sat} - \theta_{res}\right)\cdot\left[1 + \frac{1}{m}\right]^{-(1+m)} \qquad (1)$$

where: n, m - adjustable shape factors;
θ_{sat} - water content at saturation (kg kg^{-1});
θ_{res} - residual water content (kg kg^{-1}).

The van Genuchten parameters were used to calculate the values of S index by using the equation 1.

The resulting values of S are presented in Figure 4 and show that these soils have different pore size distributions along the soil profile which is mainly due to differences in their micro-structure. According to Dexter's theory (2004a,b,c) the values of S index were in the range of the S values defining a poor (S = 0.028-0.034) and good (S = 0.036-0.088) soil physical quality, with the mention that based on the S index values, all the soil profiles fall into the class of good soil physical quality.

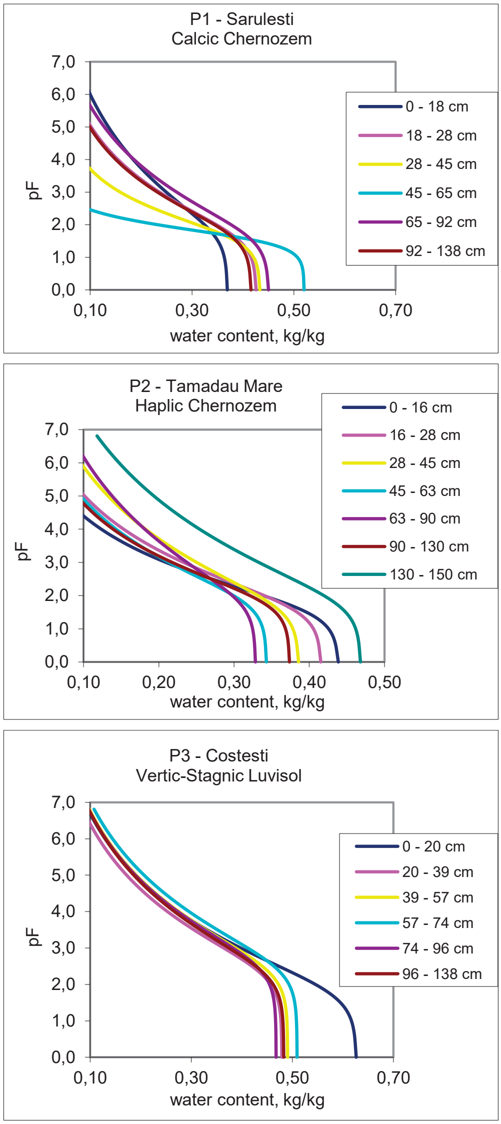


Figure 3. The soil water retention curves of the soils

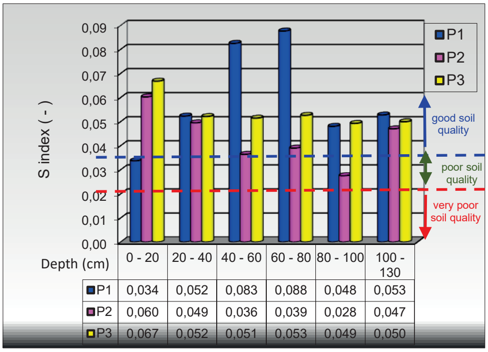


Figure 4. Values of the S index for the studied soils

From the Figure 4 it can be seen that the deeper layers of the soil profiles (> 80 cm depth) have lower values for *S* index. That means the soil physical quality as quantified by *S* is better in the topsoil horizons than in the subsoil horizons, except for the profile P1 where the values of *S* index in the top-soil layer is the lowest, and then showed an increase up to 80 cm depth and then decreased again with depth. This can be due to both higher contents in organic matter of the top-soils and lower values of bulk density.

The soil fertility and availability of the nutrients are strongly affected by the soil properties. Because of these, it is very important to evaluate periodically the soil fertility and to correlate the applied fertilization doses with the plant needs.

The chemical characterization of the studied soils is presented in Table 2. The soil reaction values, in case of P3 soil profile, varied between 5.5 and 6.7, which highlighted a lightly acid soil. The soil profile P2 had soil pH values ranging between 6.7 and 8.2, which means that this soil has a lightly acid to lightly alkaline soil reaction. At last, the soil profile P1 had values of pH between 7.5 and 8.5, meaning that this soil had a lightly alkaline soil reaction.

Organic matter content of the studied soils varied between very low and low contents (0.6-3.3% and 0.9-2.7% respectively) in case of fine textured soils (P2 and P3 respectively) up to low – moderate contents (1.2-3.9 %) in case of medium textured soil profile P1.

The total nitrogen content of all three soils varied from very low - low to moderate values (0.090-0.225%). The available phosphorus content ranged from very low - low (2-12 ppm) in case of soil profile P3, low - high (17-56 ppm) in case of soil profile P2, up to moderate - high (22-52 ppm) in case of soil profile P1. As for the available potassium content, all the studied soils were moderated supplied (100-200 ppm).

Table 2. Chemical characterization of the soil profiles

Location	Depth	pH	Hu-mus	N_{total}	P_{AL}	K_{AL}
	(cm)	(-)	(%)	(%)	(ppm)	
Sarulesti	0-18	7.5	3.9	0.225	52	180
	18-28	8.1	3.6	0.179	28	159
	28-45	8.4	2.6	0.158	22	179
	45-65	8.5	2.3	0.157	22	180
	65-92	8.5	2.3	-	-	-
	92-138	8.5	1.2	-	-	-
Tamadau Mare	0-16	6.7	3.3	0.186	56	170
	16-28	6.7	3.3	0.183	51	167
	28-45	6.7	3.0	0.144	23	177
	45-63	7.1	2.5	0.118	17	200
	63-90	7.3	2.4	-	-	-
	90-130	7.3	1.2	-	-	-
	130-150	8.2	0.6	-	-	-
Costesti	0-20	5.5	2.7	0.196	12	100
	20-39	5.8	2.1	0.153	2	109
	39-57	5.8	1.3	0.090	2	131
	57-74	6.0	1.0	-	2	171
	74-96	6.2	0.9	-	4	138
	96-138	6.7	1.0	-	3	123

The nutrients uptake by absorption in plants from the soil, by leaching or by other processes related to the natural dynamics of the soils, result in a decrease of the contents of mobile forms of nutrients and the gradual decline of soil production capacity. Therefore, both nutrients consumption by crops and decreased nutrient availability through natural processes (adsorption, fixation, immobilization in humic substances, etc.) is necessary to be compensated by applying of mineral and organic fertilizers (Borlan et al., 1994).

Fertilization plan - a tool for managing and controlling the fertilizers use

To maximize their productive potential, the cultivated plants need appropriate amounts of water, light, carbon dioxide and mineral nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and a number of trace elements). Soil is the main source of mineral nutrients and water for plants. Its capacity to provide the nutrients needed by plants varies depending on its fertility level.

The plant nutrients availability and soil fertility are strongly influenced by soil properties.

Consequently, is very important to periodically evaluate the soil fertility and to correlate the applied fertilization doses with the plant requirements. Moreover, in the Action Plan for water protection against nitrates pollution from agricultural sources it is mentioned that for the farms which practice irrigated agriculture and for that's where the planned crop yield requires higher amounts of nitrogen than those given by the maximum standards set out in the Code of Good Agricultural Practices (GAP) for water protection against nitrate pollution from agricultural sources (Dumitru et al., 2015), it is mandatory to accomplish the fertilization plan based on agrochemical study.

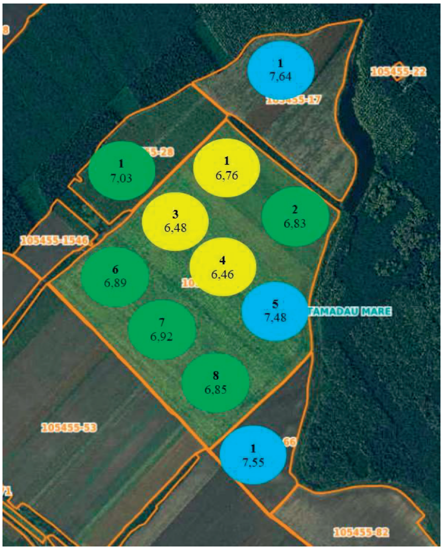
Cartograms of soil reaction, available phosphorus and available potassium were accomplished. In Figure 5 the cartograms of soil reaction and fertilization parcels are presented as an example for all the three studied areas. Each soil sample was located by numbers and agrochemical values on cartograms. It can be seen that the two soils from Calarasi county (P1 - Sarulesti and P2 - Tamadau Mare) had similar values as those found when soil smaples were collected from profiles. The pH values varied between 6.13 (P1) and 7.64 (P2). Also the soil from Arges county (P3 - Costesti) maintained its lightly acid reaction, with values ranging between 5.03-5.87.

The available phosphorus content ranged from low (8-15 ppm) in case of soil profile P1, low - high (26-91 ppm) in case of soil profile P2, up to low - moderate (15-33 ppm) in case of soil profile P1. As for the available potassium content, the studied soils from Calarasi county (P1, P2) were good - very good suppled (143-267 ppm), while the soil from Arges county (P3) was moderately supplied (87-132 ppm).

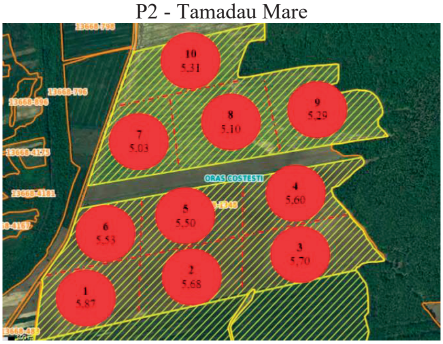
The nutrients uptake by absorption in plants from the soil, by leaching or by other processes related to the natural dynamics of the soils, result in a decrease of the contents of mobile forms of nutrients and the gradual decline of soil production capacity. Therefore, is necessary to compensate by applying of mineral and organic fertilizers, both nutrients consumption by crops and decreased nutrient availability through natural processes (adsorption, fixation, immobilization in humic substances, etc.) (Borlan et al., 1994).

Within the investigated physical blocks, one fertilization parcel (P 1-2) was established in case of P1 - Sarulesti, four fertilization parcels were established in case of Tamadau Mare - P2 and one fertilization parcel was established in case of P3 - Costesti by fitting the agrochemical subparcels with agrochemical values included in the same variation range.

P1 - Sarulesti



P2 - Tamadau Mare



P3 - Costesti

Figure 5. Cartograms of the soil reaction

In Figure 6, as an example, the average values of soil reaction (pH), humus content, available phosphorus (P_{AL}), available potassium (K_{AL}), and nitrogen index (IN) for the established fertilization parcels in case of P2 - Tamadau Mare are presented.

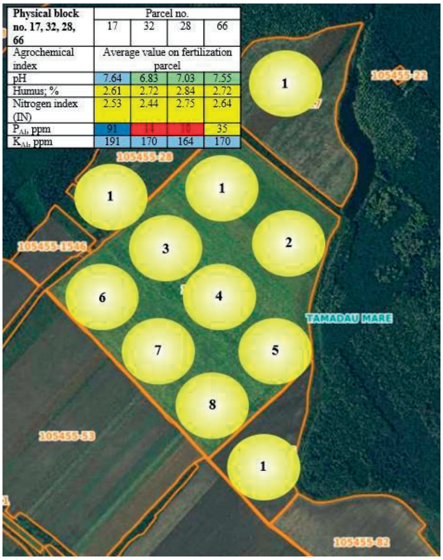


Figure 6. Fertilization parcels established within the physical blocks in case of P2 - Tamadau Mare

The soil pH values within the studied physical block varied between 6.83 and 7.64, which highlighted a lightly alkaline soil (Figure 6). The lightly alkaline reaction of soil indicates a high degree of bases saturation of the soil, which indicates a good soil fertility.

Soil nitrogen content was evaluated by using the nitrogen index (IN) and it was moderate (the IN values were at the lower limit of the variation range) (Figure 6). The average value of nitrogen index (IN) was classified as medium nitrogen content (2.61-2.84). This indicates a good fertility level of the soil in those specific parcels of the farm.

The soil available phosphorus content of P2 was classified as low - moderate - good, with average values between 10 and 91 mg/kg. The available potassium content of P2 was classified as good, with values ranging between 164 - 191 mg/kg. On such soils with a good fertility, the fertilizers doses containing phosphorus and potassium should be applied according to the plant needs, soil phosphorus and potassium contents, expected yield.

Table 3. Expected yields in the studied areas

Crop	Expected yields of the farmer for which optimum economic doses were established		
	P1	P2	P3
Wheat	7.0	7.0	6.5
Maize	9.0	9.0	9.0
Sunflower	4.0	4.0	4.0
Rape	4.0	4.0	4.0
Soybean			3.0

The expected yields at the farm level are presented in Table 3. Based on these expected yields, fertilization plans for the established fertilization parcels were accomplished. These contain economic and technical optimum doses - DOE and DOT (which ensure a certain level of crop yield at which the maximum benefit is achieved) for a four years crop rotation (winter wheat - maize - sunflower - rape) in case of P1 and P2 soils and five years crop rotation (winter wheat - maize - sunflower - rape - soybean) in case of P3 soil.

In Tables 4 a, b, c the fertilization plans are presented for the physical blocks of soils from Sarulesti (P1), Tamadau Mare (P2) and Costesti (P3).

Table 4 a. Fertilization plan in Sarulesti location (P1)

Crop	Physical block no.	**44**	
	Fertilization plan		
	Parcel no.	P 1-2	
	Doses of amendments, organic fertilizers		
	$CaCO_3$; t/ha		
	Partially decomposed animal manure; t/ha		
	Doses of nitrogen, phosphorus and potassium on parcels within the physical block		
	Dose type (kg/ha active subst.)	**DOE**	DOT
Wheat	Nitrogen (N)	**117**	198
	Phosphorus (P_2O_5)	**73**	146
	Potassium (K_2O)	**0**	77
Maize	Nitrogen (N)	**131**	232
	Phosphorus (P_2O_5)	**61**	132
	Potassium (K_2O)	**0**	93
Sun-flower	Nitrogen (N)	**70**	146
	Phosphorus (P_2O_5)	**70**	140
	Potassium (K_2O)	**0**	65
Rape	Nitrogen (N)	**82**	141
	Phosphorus (P_2O_5)	**83**	137
	Potassium (K_2O)	**0**	59

Table 4 b. Fertilization plan in Costesti location (P3)

Crop	Physical block no.	**1348**	
	Fertilization plan		
	Parcel no.	2 a+b	
	Doses of amendments, organic fertilizers		
	$CaCO_3$; t/ha		
	Partially decomposed animal manure; t/ha		
	Doses of nitrogen, phosphorus and potassium on parcels within the physical block		
	Dose type (kg/ha active subst.)	**DOE**	DOT
Wheat	Nitrogen (N)	**126**	214
	Phosphorus (P_2O_5)	**40**	118
	Potassium (K_2O)	**13**	83
Maize	Nitrogen (N)	**150**	261
	Phosphorus (P_2O_5)	**41**	120
	Potassium (K_2O)	**40**	126
Sun-flower	Nitrogen (N)	**86**	165
	Phosphorus (P_2O_5)	**66**	135
	Potassium (K_2O)	**31**	94
Rape	Nitrogen (N)	**96**	159
	Phosphorus (P_2O_5)	**54**	112
	Potassium (K_2O)	**26**	82
Soy-bean	Nitrogen (N)	**10**	72
	Phosphorus (P_2O_5)	**0**	55
	Potassium (K_2O)	**0**	64

For the calculation of the optimum economic doses (DOE) and technical economic doses (DOT) different aspects were taken into account: prices of mineral fertilizers, nutrients crop requirements, soil nutrients content and economic aspects. DOE and DOT are also calculated for achieving at farm level the expected yields.

It is necessary a proper management and fertilizers use at the level of each agricultural or agro-zootechnical holding, both for economic reasons and environmental protection requirements.

Particular emphasis, especially in areas highly vulnerable to water pollution with nitrates from agricultural sources, should be placed on the management of organic and mineral fertilizers with nitrogen, given the particularly complex behavior of this nutrient in soil and the easiness with which it can be lost as nitrates form together with infiltration waters and surface runoff (Code of Good Agricultural Practices, 2015).

Therefore, the fertilization plan is a useful tool both for establishing the organic fertilizers doses (produced within the farm or procured outside the farm) and mineral doses and for making economic decisions related to the availability of any excess organic fertilizers produced within the farm, and for the selection of proper times to purchase the necessary quantitative and qualitative mineral or organic fertilizers (if the farm does not have enough own reserves).

Table 4 c. Fertilization plan in Tamadau Mare location (P2)

Crop	Physical block no.	**17**		**32**		**28**		**66**	
	Fertilization plan								
	Parcel no.	P1		P2		P3		P4	
	Doses of amendments, organic fertilizers								
	$CaCO_3$; t/ha								
	Partially decomposed animal manure; t/ha								
	Doses of nitrogen, phosphorus and potassium on parcels within the physical block								
	Dose type (kg/ha active subst.)	**DOE**	DOT	**DOE**	DOT	**DOE**	DOT	**DOE**	DOT
Wheat	Nitrogen (N)	**119**	199	**121**	204	**117**	201	**118**	202
	Phosphorus (P_2O_5)	**0**	75	**67**	142	**81**	156	**22**	97
	Potassium (K_2O)	**0**	74	**0**	75	**0**	74	**0**	75
Maize	Nitrogen (N)	**134**	235	**136**	244	**131**	240	**132**	240
	Phosphorus (P_2O_5)	**0**	74	**56**	132	**65**	142	**24**	100
	Potassium (K_2O)	**0**	70	**0**	88	**0**	87	**0**	88
Sunflower	Nitrogen (N)	**72**	141	**73**	152	**70**	148	**70**	150
	Phosphorus (P_2O_5)	**0**	73	**84**	153	**70**	140	**41**	110
	Potassium (K_2O)	**0**	57	**2**	65	**4**	67	**2**	65
Rape	Nitrogen (N)	**84**	144	**85**	147	**81**	143	**83**	144
	Phosphorus (P_2O_5)	**79**	56	**77**	133	**80**	130	**31**	87
	Potassium (K_2O)	**0**	50	**0**	58	**0**	57	**0**	58

CONCLUSIONS

The soils from this study were characterized in terms of hydro-physical and chemical properties.

The investigated soils are medium to fine textured, starting from medium clayey-loam (P1 - Sarulesti), clayey-silty loam (P2 - Tamadau Mare) to loamy clay (P3 - Costesti). High contents in clay result in higher values for bulk density and penetration resistance. On the contrary, the saturated hydraulic conductivity of such fine textured soils records low values, the most significant decrease being encountered in the soil profile P3.

The highest values of saturated hydraulic conductivity were recorded in the topsoil layer of the soil profile P2 (Tamadau Mare) where the total porosity (PT) was also the highest.

The topsoil layers had greater values of water content at saturation when compared with subsoil layers, except for the profile P1 where the values of water content at saturation were constant within the soil profile. This may be attributed to a decrease of organic matter content and an increase of bulk density values within the soil profiles.

The soil water retention curves were obtained after using the Arya-Paris model for estimating the van Genuchten parameters. The differences in shape of water retention curves between topsoil and subsoil layers may be as a result of either externally-applied mechanical stresss by agricultural machinery which can lead to compaction of the layer below ploughing depth, or internally-applied mechanical stress due to drying of the soil which causes shrinkage due to the effective stresses generated by the pore water suction and the surface tension in the water menisci.

The deeper layers of the soil profiles (> 80 cm depth) have lower values for *S* index. That means the soil physical quality as quantified by *S* is better in the topsoil horizons than in the subsoil horizons, except for the profile P1 where the values of *S* index in the topsoil layer is the lowest, and then showed an increase up to 80 cm depth and then decreased again with depth. This can be due to both higher contents in organic matter of the top-soils and lower values of bulk density.

The soil reaction values, in case of P3 soil profile highlighted a lightly acid soil. The soil reaction values highlighted a lightly acid soil in P3. The soil profile P2 had soil pH values that led to a lightly acid to lightly alkaline soil reaction. At last, the soil profile P1 had values of pH that led to a lightly alkaline soil reaction.

The total nitrogen content of all three soils varied from very low-low to moderate values. The available phosphorus content ranged from very low-low in case of soil profile P3 to low-high in case of soil profile P2 and up to moderate-high in case of soil profile P1. As for the available potassium content, all the studied soils were moderately supplied.

The fertilization plans were accomplished based on agrochemical studies. For each of the three sites cartograms were done for soil reaction, phosphorus and potassium. Also synthetic cartograms with the average values of soil reaction (pH), humus content, available phosphorus (P_{AL}), available potassium (K_{AL}), and nitrogen index (IN) for the established fertilization parcels from each location were realized.

Based on the expected yields, fertilization plans for the established fertilization parcels were accomplished. These contained economic and technical optimum doses - DOE and DOT (which ensure a certain level of crop yield at which the maximum benefit is achieved) for a four years crop rotation in case of P1 and P2 soils and five years crop rotation in case of P3 soil.

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STUDY ON THE ECONOMIC EFFICIENCY OF SLOPE CONSOLIDATION BY DIFFERENT METHODS

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Abstract

Slope instability and erosion of the soil by water and wind are major environmental hazards. Although they are result of natural geomorphological processes, they are both affected by and have consequences for human activity often incurring economic and social damage. The main objective of this study is to determine the costs of slope consolidation by using different methods. There were studied four techniques for slope consolidation: vegetation, gabions, nets biodegradable jute expanded clay and vegetation, respectively geotextiles. The economic efficiency was determined on an area of 100 m^2 for each case under study. Based on the obtained results, the variant represented by nets biodegradable jute expanded clay and vegetation had a total cost of 4735.12 lei, the cost per square meter of consolidated area being of 47.35 lei. This variant offers a pleasant aspect of the slopes and does not involve very high costs, compared to the variant represented by the arrangement of slopes with gabions.

Key words: *slope, economic efficiency, cost, consolidation techniques, vegetation.*

INTRODUCTION

Slope instability and erosion of the soil by water and wind are major environmental hazards. Although they are result of natural geomorphological processes, they are both affected by and have consequences for human activity often incurring economic and social damage (Morgan and Rickson, 1995). Slope failures are the movement of soil, and they occur on both artificial and natural slopes. Potential causes for slope instability range from deep-scared failures (such as with landslides) to surface erosion (such as when steep slopes cause water to travel in concentrated flows, eroding a series of gullies). There are many types of slopes failures, including rockfalls, rockslides, debris avalanches, debris flows and slumps, earth flows (Fay et al., 2012).

Human-induced modifications that may adversely affect external loads to slopes include grading of the existing slope or adjacent slope, construction adjacent to the slope, construction damage caused by blasting, and vibration of passing vehicles (Turner and Schuster, 1996). Slope regrading can create an over steep toe, or base of the slope, or an accumulation of material at the crest, which can lead to erosion. The shape of the slope can be a defining factor in its stability.

Natural slopes are generally concave, which is the most stable type of slope and experiences the least erosion (Schor and Gray, 2007). Many man-made slopes are linear and research has found that in many cases a linear slope will erode until it becomes concave (Fay et al., 2012). Mechanical stabilization techniques utilize non vegetative or nonliving components such as rock, gabion baskets, concrete, geosynthetics, and steel pins to reinforce slopes. These techniques can provide stability to both cut and fill slopes. Structures are generally capable of resisting much higher lateral earth pressures and shear stresses than vegetation. Mechanical stabilization techniques include retaining walls, mechanically stabilized earth, geosynthetically reinforced soil and other in-situ reinforcement techniques. For anchoring shallow soils, use of *in situ* earth reinforcements and recycled plastic pins has been reported in slope stabilization (Fay et al., 2012).

MATERIALS AND METHODS

The consolidation of slopes is very important in our country as well as worldwide. Nowadays there are numerous different slope stabilization methods but unfortunately many of them are unesthetic and very expensive. The study area is represented by slopes located in Cluj-Napoca.

In order to be able to calculate the economic efficiency, both classical and modern techniques were considered. The calculations were performed on an area of 100 m^2 for each studied variant: using vegetation, gabions, net biodegradable jute + expanded clay + vegetation, geotextiles, geocells and grass.

To consolidate the degraded slopes with vegetation we used the following species *Hedera helix*, *Campsis radicans*, *Cotoneaster horizontalis*, *Euonymus fortunei* and *Chaenomeles japonica.* 150 plants were used, 30 pieces of each species. The expenses for a plant varies between 19 and 35 RON. The planting of the species was done manually in pits of 15 x 15 x 20 cm and 30 x 30 x 35 cm. In order to carry out this work, two workers were needed, who dug, planted and watered the plants.The plants were planted with the root ball in ground. To consolidate the degraded slopes with gabions (Figure 1), in order to carry out this work, an excavator was needed to dig the foundation where the gabions will be located. The following materials were used: galvanized wire mesh with 2 mm wire diameter and 40 x 40 mm eye dimensions - 891 m^2; steel bar with 16 mm diameter - 1188 m; galvanized wire with 2 mm wire diameter - 600 m, broken stone with 44 mm diameter - 223.08 m^3. In order to carry out this work, three workers were needed, who worked 120 hours to complete the task.

Figure 1. Gabions

To consolidate the degraded slopes with neds biodegradable jute, expanded clay and vegetation (Figure 2), was needed nets biodegradable jute having the technical data: 290 g/ml 101 cm x 1 ml. 110 m^2 of biodegradable jute were needed, 100 liters of expanded clay and 100 pieces of stakes to support the nets biodegradable jute on the ground.

Also, the folowing ten species of ornamental shrubs both through the leaves and flowers were used: *Juniperus horizontalis, Forsytia suspensa, Lonicera pileata, Syringa vulgaris, Viburnum opulus, Buxus sempervirum, Weigela florida, Kerria japonica, Cedru feeling Blue, Betula pendula*. In order to carry out this work, two workers were needed, who worked 36 hours to complete this work.

The plants were planted with the root ball in ground. The planting of the species was done manually in pits of 15 x 15 x 20 cm and 30 x 30 x 35 cm.

Figure 2. Neds biodegradable jute, expanded clay and vegetation

To consolidate the degraded slopes with geotextile (Figure 3), were used 110 m^2 of geotextile and 150 pieces of stakes to support the geotextile on the ground. A worker worked 12 hours to complete this work.

Figure 3. Geotextile

To consolidate the degraded slopes with geocells and grass, 100 m^2 of geocells and 5 kg seeds of grass were needed. In order to carry out this work, two workers were needed, for 16 hours.

RESULTS AND DISCUSSIONS

In Table 1 was calculated the economic efficiency for the consolidation of the degraded slope, using vegetation. The total cost for the purchase of the 150 plants was 3420 RON and the total cost of this variant of the study was 4593 RON.

In Table 2 was calculated the economic efficiency for the consolidation of the degraded slope using gabions. Expenses for excavation are 1700 RON.

The material needed for the arrangement slope cost 20159.75 RON and labor cost 3760 RON. So the cost of arranging a slope area of one m^2 is 256.19 RON.

This method of arranging the slopes is the most expensive of the ones presented in this study.

Table 1. The cost of arranging a degraded slope using vegetation (RON)

Costs	Materials (RON)	Manual labor (RON)	Equipment (RON)	Total (RON)
Employee				
Gross salary	-	661	-	-
Direct costs	3420	661	-	4081
Contributions to social insurance (25%)	-	165	-	-
Social Health Insurance (10%)	-	66	-	-
Personal deduction	-	510	-	-
Income tax	-	0	-	-
Net salary	-	430	-	-
Employer				
Insurance Contribution for Work 2.25%	-	15	-	-
The difference in CAS, CASS to their value for minimum salary				
Social insurance (CAS) 53.71%	-	355	-	-
Social Health Insurance CASS 21.48%	-	142	-	
Total taxes				
The employee pays the state	-	231	-	-
The employer pays the state	-	514	-	-
Total taxes collected by the state	-	743	-	-
Full salary	-	1173	-	-
To pay a net salary of 430 RON, the employer spends 1173 RON				
TOTAL COSTS	**3420**	**1173**	**-**	**4593**

Table 2. The cost of arranging a degraded slope using gabions

Costs	Materials (RON)	Manual labor (RON)	Equipment (RON)	Total (RON)
Employee				
Gross salary	-	3677	-	-
Direct costs	20159.75	3677	1700	25536.75
Contributions to social insurance (25%)	-	919	-	-
Social Health Insurance (10%)	-	368	-	-
Personal deduction	-	0	-	-
Income tax	-	239	-	-
Net salary	-	2151	-	-
Employer				
Insurance Contribution for Work 2.25%	-	83	-	-
The difference in CAS, CASS to their value for minimum salary				
Social insurance (CAS) 15.75%	-	0	-	-
Social Health Insurance CASS 6.27%	-	0	-	-
Total taxes				
The employee pays the state	-	1526	-	-
The employer pays the state	-	83	-	-
Total taxes collected by the state	-	1609	-	-
Full salary	-	3760	-	-
To pay a net salary of 2151 RON the employer spends 3760 RON				
TOTAL COSTS	**20159.75**	**3760**	**1700**	**25619.75**

In Table 3 was calculated the economic efficiency for the consolidation of the degraded slope using nets biodegradable jute, expanded clay and ten species of ornamental shrubs both through the leaves and flowers. Total expenses for the consolidation of 100 m^2 of slope were: the net biodegradabile jute cost 1309 RON, the expanded clay cost 163 RON, vegetation cost 1684.12 RON and the pieces of stakes to support the net cost 250 RON, and the labor was 1329 RON.

Table 3. The cost of arranging a degraded slope using nets biodegradable jute, expanded clay and vegetation

Costs	Materials (RON)	Manual labor (RON)	Equipment (RON)	Total (RON)
Employee				
Gross salary	-	893	-	-
Direct costs	3406.12	893	0	4299.12
Contributions to social insurance (25%)	-	223	-	-
Social Health Insurance (10%)	-	89	-	-
Personal deduction	-	510	-	-
Income tax	-	7	-	-
Net salary	-	574	-	-
Employer				
Insurance Contribution for Work 2.25%	-	20	-	-
The difference in CAS, CASS to their value for minimum salary				
Social insurance (CAS) 33.26	-	297	-	-
Social Health Insurance CASS 13.33%	-	119	-	-
Total Taxes				
The employee pays the state	-	319	-	-
The employer pays the state	-	436	-	-
Total taxes collected by the state	-	755	-	-
Full salary	-	1329	-	-
To pay a net salary of 574 RON, the employer spends 1329 RON				
TOTAL COSTS	**3406.12**	**1329**	**0**	**4735.12**

In Table 4 was calculated the economic efficiency for the consolidation of the degraded slope using geotextiles. The necessary for the material amonunted to 1154.9 RON, as follows 7799 RON cost 100 m^2 of geotextile, 375 RON cost 150 pieces of stakes to support the geotextiles and the labor was 950 RON. So the total cost of consolidation of 100 m^2 of slope was 2104.9 RON and the cost for 1 m^2 of consolidation with this material is 21.04 RON.

Table 4. The cost of arranging a degraded slope using geotextiles

Costs	Materials (RON)	Manual labor (RON)	Equipment (RON)	Total (RON)
Employee				
Gross salary	-	331	-	-
Direct costs	1154.9	331	-	1490.9
Contributions to social insurance (25%)	-	83	-	-
Social Health Insurance (10%)	-	33	-	-
Personal deduction	-	510	-	-
Income tax (10%)	-	0	-	-
Net salary	-	215	-	-
Employer				
Insurance Contribution for Work 2.25%	-	7	-	-
The difference in CAS, CASS to their value for minimum salary				
Social insurance (CAS) 132.02%	-	437	-	-
Social Health Insurance CASS 6.27%	-	175	-	-
Total taxes				
The employee pays the state	-	116	-	-
The employer pays the state	-	619	-	-
Total taxes collected by the state	-	735	-	-
Full salary	-	950	-	-
To pay a net salary of 215 RON, the employer spends 950 RON				
TOTAL COSTS	**1154.9**	**950**	**-**	**2104.9**

In Table 5 was calculated the economic efficiency for the consolidation of the degraded slope using geocells and grass. The expenses for the material are 2207 RON and the labor cost 1025 RON for the 16 working hours. The cost for consolidation of one m^2 slope is 32.32 RON.

Table 5. The cost of arranging a degraded slope using geocells and grass

Costs	Materials (RON)	Manual labor (RON)	Equipment (RON)	Total (RON)
Employee				
Gross salary	-	441	-	-
Direct costs	2207	441	-	2648
Contributions to social insurance (25%)	-	110	-	-
Social Health Insurance (10%)	-	44	-	-
Personal deduction	-	510	-	-
Income tax (10%)	-	0	-	-
Net salary	-	287	-	-
Employer				
Insurance Contribution for Work 2.25%	-	10	-	-
The difference in CAS, CASS to their value for minimum salary				
Social insurance (CAS) 92.97 %	-	410	-	-
Social Health Insurance CASS 6.27%	-	164	-	-
Total taxes				
The employee pays the state	-	154	-	-
The employer pays the state	-	584	-	-
Total taxes collected by the state	-	738	-	-
Full salary	-	1025	-	-
To pay a net salary of 287 RON, the employer spends 1025 RON				
TOTAL COSTS	**2207**	**1025**	**-**	**3232**

CONCLUSIONS

Among the presented arranging techniques for the degraded slopes, the less costly technique is the one that use geotextiles in the amount of 2104.9 RON.

The most expensive alternative arrangement for the degraded slopes was the gabions variant, amounting to the sum of 25619.75 RON.

Based on the obtained results, the variant represented by nets biodegradable jute expanded clay and vegetation had a total cost of 4735.12 RON. This variant offers a pleasant aspect of the slopes and does not involve very high costs, compared to the variant represented by the arrangement of slopes with gabions.

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NEW RESULTS OF THE WINTER PEAS BREEDING PROGRAM AT NARDI FUNDULEA

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Abstract

Breeding winter cultivars requires the combination of freezing tolerance as well as high seed productivity and quality.Winter peas have some advantages over spring peas like: better establishment and more efficient use of humidity during the winter season, which makes it less vulnerable to drought over the spring, frequently in Romania in the last years; earlier harvest; has a longer vegetation period and get higher productivity and more stable yield than spring peas type. In this paper we present data obtained from the first F4 and F5 lines of winter peas obtained in the NARDI-Fundulea program with the germplasm of winter peas from USA, France and Austria. A number of 170 lines, selected from winter/winter and winter/spring crosses pea genotypes, have been tested in 2019 in the field of NARDI Fundulea.The conclusion of this preliminary study is that will be possible to realize the genetic progress in breeding in winter peas, to select the new varieties with good enough winter hardiness and being with high yield, different earliness or plant height.

Key words: *winter peas, winter hardiness, earliness, yield.*

INTRODUCTION

Field pea *(Pisum sativum* L.) is an important annual legume grown and consumed extensively both human and animal feed (Mustafa Tan et al., 2012). The use of plant proteins as functional ingredients in the food and feed industry is increasing and special attention has been paid to the use of peas because they are already accepted as a part of the human and animal diet throughout the world (Rodino et al., 2009). It is economically very important for many agricultural systems, but the relative importance of abiotic stresses affecting its production is poorly understood. Field pea has inadequate tolerance at the reproductive stage to winter stresses, particularly frost. (Shaista Shafiq, 2012).

Winter peas (*Pisum sativum*) is a cool-season annual legume that has long been considered a high-preference forage for whitetails. Due to its nitrogen-fixing ability, winter pea has numerous agricultural uses, and it is very easy to establish in fall food plots.

Winter peas are broadly adapted to dryland production in all regions where winter wheat is grown, and the improved cold hardiness of winter peas rivals that of winter wheat.

Winter hardiness in peas is a complex combination of phenotypes that the plants express in response to environmental cues.

Winter hardiness in peas is a combination of acclimation, tolerance, and avoidance. As the autumn temperatures fall, days shorten, and spectral quality of light changes, the plants become acclimated to cold temperatures. Some of the physiological characteristics that have been associated with the process of cold acclimation in peas are the accumulation of sugars in leaves, stems, and roots (Bourion et al., 2003) and an increase of the RuBisCO activity (Dumont et al., 2009). When peas that are cold acclimated do experience freezing temperatures, there is less electrolyte leakage than in non-acclimated peas, and associated QTL have been identified (Dumont et al., 2009).

Winter peas (*Pisum sativum* L.) are an alternative. Planted in late summer to early autumn, winter peas confer all of the advantages of spring-planted peas. With symbiotic Rhizobia bacteria, they fix atmospheric N, making them a low-energy and low-greenhouse-gas-emissions crop; they interrupt weed, disease, and insect cycles; have a relative low water use; and can flourish under current crop management practices with existing farm equipment. In addition, their greater yields compared with spring peas make them economically viable, and late-summer planting shifts fieldwork to avoid the vagaries, narrow planting window, and variable conditions that constrain spring-planted peas.(Rebecca McGee, 2017)

MATERIALS AND METHODS

In 2019, the first 94 F4 peas lines were tested in preliminary comparative cultures (46 F4 lines) and comparative cultures (48 F4 lines) with 25 variants in three replications, on a plot of four m^2 harvested area.

The 76 F5 peas lines were tested in preliminary micro-cultures, each of them with 25 variants, one replication, on a plot of four m^2 harvested area.

The 2019 winter was slight enough, with a short period with negative temperatures of -16°C (the begining of January), with a snow layer, which has protected the crop. There are no damages registered due to frost. The early spring was normal, fact that led to restart the vegetation under optimum conditions.

The level of resistance to winter hardiness was estimated in the field, early in the spring, in a scale 1 to 9, where score 1 is very resistance and 9 very susceptible. Plant height was measure in cm, total length of plant from the ground till the top to the end of flowering time. The earliness was appreciated like number of days from 1st January till the end of flowering time and yield as kg/ha.

The statistic analyses of data have been evaluated by correlations and linear regressions between study traits.

RESULTS AND DISCUSSIONS

Yield performances lines of F4 and F5 generation, tested in advance trials and respectively in preliminary trials in 2019, (Figure 1) shows that the coefficients of correlation are significant high (r = 0.26***) so the coefficient of heredity is in sense large and shows the genetic transfer between generations.

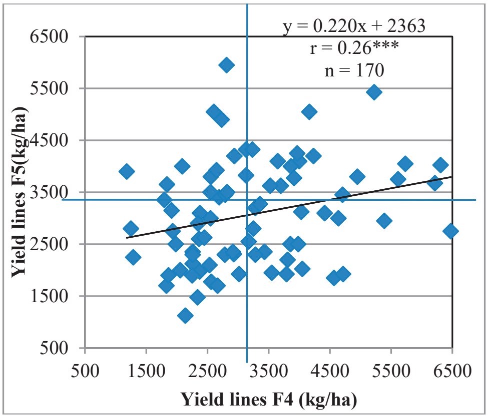

Figure 1. Correlation between yield F4 and of F5 data of 170 lines of the winter peas

Having in view that winter hardiness in winter peas is a very important trait, there was necessary to highlight in what way this trait could be recombined with other important agronomical characteristics, like earliness to flowering, plant height, grain yield as well as the relationship among other traits as plant height/earliness or yield/earliness.

In the Table 1 are presented such correlations using the data collected from 48 F4 lines and 46 F4 lines tested in comparative cultures and respectively in preliminary cultures in 2019.

Table 1. Correlation coefficients among different traits in F4 winter pea lines

The generation these genotypes	Correlation between different characters	The correlation coefficient
48 F4 lines tested in comparative cultures in 2019	Winter hardiness/yield	0.18ns
	Winter hardiness/earliness	0.06ns
	Winter hardiness/plant height	-0.14ns
	Plant height/earliness	0.38***
	Yield/earliness	-0.38***
46 F4 lines tested in preliminary comparative cultures in 2019	Winter hardiness/yield	**-0.28***
	Winter hardiness/earliness	-0.08ns
	Winter hardiness/plant height	-0.24ns
	Plant height/earliness	**0.57*****
	Yield/earliness	-0.57***

The correlation between winter hardiness and yield in F4 lines (Table1) was significantly negative (r = 0.18 and r = -0.28*), what means that in winter peas is absolutely necessary to cultivate genotypes with good level of winter hardiness, to realize high and stable yields.

Also relationship between plant height and earliness was very distinct significantly (r = 0.38*** and r = 0.57***), strong enough in some case, what mains that it quite easily to be recombine such characteristics.

The correlation, between plant height and winter hardiness, was negative significantly (r = -0.14 and r = -0.24), but so that suggests possibility to select the genotypes which recombine both traits.

The relationship between yield and earliness, in F4 winter pea lines was very distinct signify-cantly negative, (-0.38*** and -0.57***), however demonstrate the existence of lines with high yield and with earliness. The correlation between yield and winter hardiness of F5 lines had (Figure 2) was very distinct significantly among those traits (r = 0.25***). So that, the distribution of the lines demonstrated existence the lines with the same level of winter hardiness and with high level of yield.

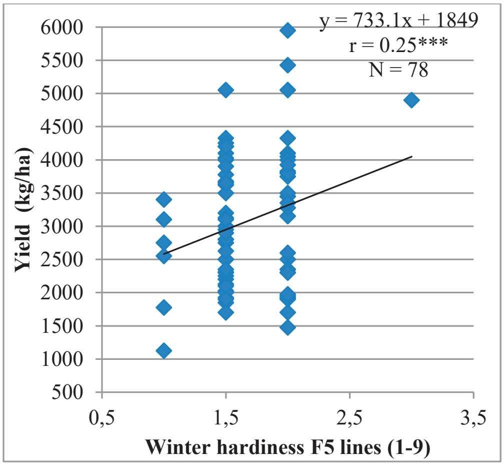

Figure 2. Correlation between winter hardiness and yield of F5 lines winter peas

Also it is very important to select the perspective lines with good yield potential but in the same time to recombine an acceptable earliness for Romanian climate conditions.

The distribution of the 76 of F5 lines for precocity (Figure 3), divides the breeding material in two groups of maturity: late forms and early forms.

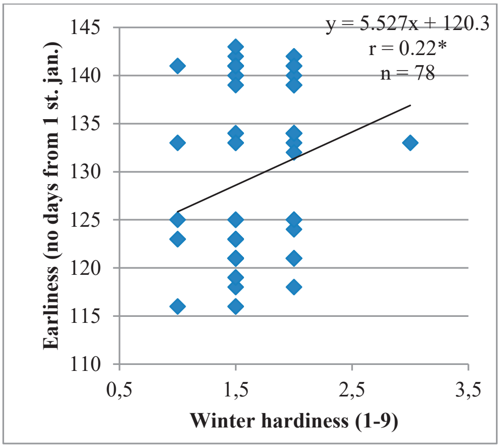

Figure 3. Correlation between winter hardiness and earliness of F5 lines winter peas

The data obtained till now form the study of relationship between winter hardiness and plant height indicated the possibility of recombination of both traits of interest (plant height and winter hardiness) (Figure 4) suggesting that, in functions of the end use the production, for forage need to be a tall variety, for high biomass production or mid tall variety for grain type.

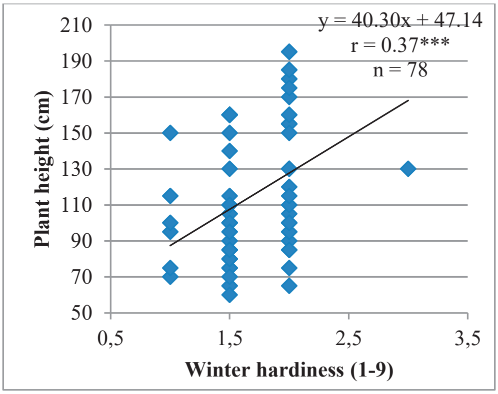

Figure 4. Correlation between winter hardiness and plant height of F5 lines winter peas

The date obtained between correlation plant height and earliness shows separation of the material în two category, with plant height 50-

110 cm and with earliness and with plant height 150-200 cm and tardive (Figure 5).

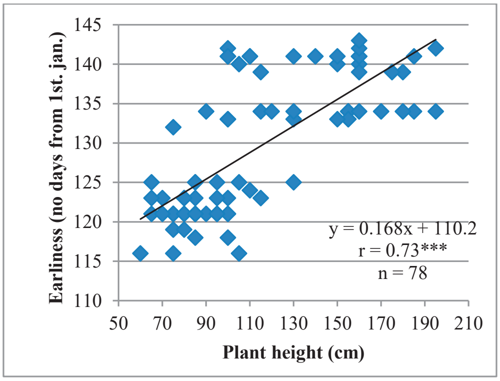

Figure 5. Correlation between plant height and earliness of F5 lines winter peas

The relationship between yield and earliness is insignificant negative (r = -0.15), however demonstrat the existance of lines with high yield and with earliness, those are the most important traits which must present a new varieties winter peas (Figure 6).

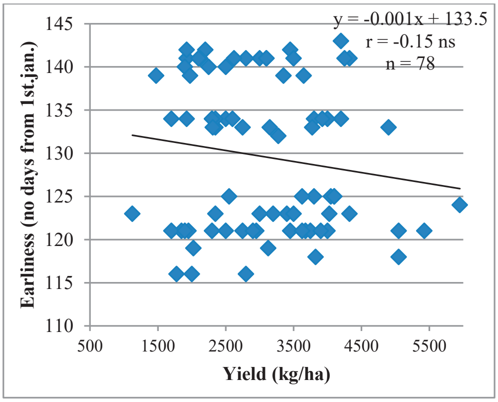

Figure 6. Correlation between yield and earliness of F5 lines winter peas

CONCLUSIONS

The data obtained in these studies, on the advanced lines shown existance the important lines of winter peas which posed high yield, good level of winter hardiness, plant height and earliness.

In this study were remarked winter peas lines F5 with good level of winter hardiness, with high yield (5000-6000 kg/ha), earliness and with the plant height between 130-145 cm, this trait is very important for varieties of winter peas because can utilized both as pure crops and for cereal grain mixtures (high biomass).

In the near future, some of these winter peas lines will be tested for registration of winter peas cultivars, adaptated of the climatic conditions of Romania.

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ACCURATE AND PRACTICAL METHOD TO DETECT PHOTOTROPIC LEAF MOVEMENT OF COTTON: DIGITAL IMAGING

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Abstract

Cotton species have adapted to their originated environment by developing different physiological and morphological features. One of these features is heliotropic leaf movement. Gossypium hirsutum L. (GH) have diurnal leaf movement owing to pulvinus, which is formed by specialized cells on the contrary to Gossypium barbadense L. (GB). There are very few studies in literature about advantages and disadvantages of diaheliotropic leaf movement of cotton. To assess effects of diaheliotropic leaf movement of both cotton genotypes on their adaptation abilities, firstly need to quantify orientation of leaves towards light. Therefore, two different sequential experiments were carried out in this study. First one was angle-based leaf turn experiment that contained two different measurement method for detection of leaf movement which are midrib normal-incident angle (M_I) and midrib-petiole angle (M_P). Second was area-based leaf turn (T_A) experiment. In T_A experiment, digital images of plants from same direction of light source are analysed. Then, area of turning leaf lamina is calculated by pixel counting method using with an image processing software. Results of the study revealed that digital imaging method is easier and faster method for detecting diaheliotropic leaf movement. Furthermore, it is possible to quantify area based diaheliotropic leaf movement as centimetre square in T_A method unlike other two methods. According to our findings, T_A by using digital imaging is featured and accurate method to detect phototropic leaf movement of cotton.

***Key words**: cotton, phototropism, phototropic leaf movement, Gossypium hirsutum L., Gossypium barbadense L., digital imaging.*

INTRODUCTION

Some plants have capabilities that adapt to their environmental conditions in order to survive. This adaptation of plants can be possible by changing some physiological and morphological features. One of these plant's capabilities is leaf movement (Koller, 1990). It is possible to categorize leaf movement as circadian rhythm orientation and light-driven orientation. Some plants can adjust their leaves almost horizontally at day and positioning vertically at night. This type of leaf movement is known as nyctinasty and controlled by circadian rhythm of plants (Ueda and Nakamura, 2007). The other leaf movement is light-driven orientation and known as phototropism (Darwin, 1880). Growth-based phototropism most often observed in almost all plants. However, pulvinus-based phototropism which known as heliotropic leaf movement has developed in some plants by evolution to environment and it is more dynamic form of phototropism (Moore and Hines, 2017). There are two type of heliotropic leaf movements were reported (Darwin, 1880; Yao et al., 2018). One of them is paraheliotropic leaf movement which was developed by plants in order to avoid environmental stresses such as severe light and heat (Inamullah et al., 2006; Bressan, 2004; Kao and Forseth, 1992). The other one is diaheliotropic leaf movement which enables tracking to diurnal sun movement for enhance photosynthetic capacity (Zhang et al., 2009; Yao et al., 2018).

The heliotropic leaf movement commonly occurs in legumes and also in some other plants such as cotton species (Moore and Hines, 2017). *Gossypium hirsutum* L. (GH) have diaheliotropic leaf movement in contrast with *Gossypium barbadense* L. (GB) (Hejnak et al., 2015; Ehleringer and Hammond, 1987). GH leaves have a high sensitivity to different photosynthetic active radiation (PAR) (Yao et al., 2018; O'Carrigan et al., 2014; Arena et al., 2016) and can improve their photosynthetic capacity by the contribution of diaheliotropic leaf movement (Yao et al., 2018). Furthermore, some researchers reported that diaheliotropic leaf movement leads to exposure more incident light to GH leaves, thus these leaves can utilize more light energy (Yao et al., 2018), increase leaf N content (Yao et al., 2015; Werger and Hirose 1991) and leaf mass per area (Witkowski and Lamant, 1991; Niinemets, 1999; Yao et al., 2016) as well as improve leaf longevity (Kitajima et al., 1997). On the other hand, Fukai and Loomis (1976) suggested that diaheliotropic leaf movement of GH leaves lead to enhance total canopy yield in early stage, however canopy productivity could be reducing in late growing stage due to uneven light distribution. Especially in intensive agriculture, outermost leaves may limit penetration of light to inside of canopy in particular period of season due to diaheliotropic leaf movement (Ehleringer and Forseth, 1980; Thanisawanyongkura et al., 1997), thus it could be reducing total canopy photosynthesis (Ehleringer and Hammond, 1987).

Beside all of these discussions, to clarify advantages and disadvantages of diaheliotropic leaf movement of GH in different growing stages has great importance and firstly need to have accurate, practical and quantitative measurement methods. Many studies were conducted in relation to diaheliotropic leaf movement of cotton by using different angle measurement methods such as cosine, azimuth, lamina and midrib angle (Ehleringer and Hammond, 1987; Zhang et al., 2009; Yao et al., 2018; Shell et al., 1974). Ehleringer and Hammond (1987) used cosine method in order to reveal leaf movement differences between GH and GB. Yao et al. (2018) suggested that midrib angle method is the main factor for detecting leaf movement in contrast to lamina and azimuth angle methods (Zhang et al., 2009). In these methods, generally inclinometer, protractor and compass were used on a couple of leaves for angle measurements. When taking account of all plant leaves, these angle measurement methods might have disadvantages especially in terms of practicality. Besides, estimation of area-based leaf turns (T_A) might be more accurate, quantitative and practical method to determine effective leaf movement.

Thus, objectives of this study were (*i*) verifying leaf movement differences between *G. hirsutum* L. and *G. barbadense* L. as revealed in previous studies and (ii) evaluating of angle measurement methods and T_A method in terms of accuracy and practicality by using digital imaging. When considering to diaheliotropic leaf movement as selection criteria, one of these methods is expected to be using in breeding programme for fast selection.

MATERIALS AND METHODS

This study was comprised of two different sequential experiments which have four replications. The experiments were consisted of two contrasting cotton genotypes in terms of heliotropic movement of their leaves: *G. hirsutum* L. (GH) and *G. barbadanse* L. (GB). The plants were grown in pots at fully controlled climate chamber (stable at 30˚C and 50% relative humidity).

Experiment 1th (Angle-based leaf movement)
Angle-based leaf movement experiment was carried out in 2018, in fully controlled climate chamber. Cotton plants were grown under daylight fluorescent lamps until second true leaf fully developed. Then, each plants were transferred to growth cabin which is isolated from light and contain own light source (50W daylight power LED). We have observed in preliminary experiments that GH leaves were showed remarkable leaf movement during first two hours (data not shown). Therefore, the leaves were exposed to one directional light (midrib of first true leaf is vertical to light source) during two hours in the growth cabin. Digital images collected from the leaves as vertical to linear of light source by using with high resolution camera with ten minute intervals during two hours. Then, the images were analysed by an image processing software for different angle measurement methods as described below.

Midrib - Petiole Angle (M_P) Method: M_P refers to angle between midrib and petiole. This measurement was performed by processing images as shown in Figure 1 a. This method could corroborate that leaf movement is pulvinus-based or not.

Midrib normal - Incident Angle (M_I) Method: M_I refers to angle between incident light and midrib normal. It was measured by processing images as shown in Figure 1b. Then, cosine of M_I was calculated to scale leaf movement between 0 to 1 according to Ehleringer and Hammond (1987).

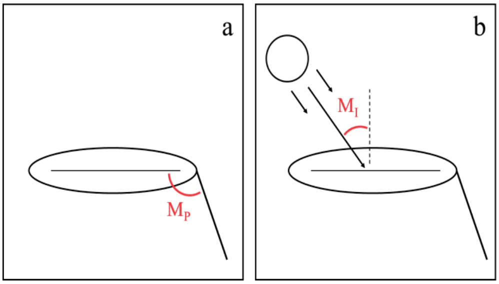

Figure 1. A visual of how measured M_P (a) and M_I (b) in Experiment 1th.
M_P: Midrib - Petiole Angle
M_I: Midrib normal - Incident Angle

Experiment 2nd (Area-based leaf movement)
Area-based leaf movement (T_A) experiment was carried out in 2019 under fully controlled climate chamber conditions. Cotton plants were grown under daylight fluorescent lamps until they had four fully developed leaves. Then, the plants were exposed to light treatment by using lighting equipment which is designed as parallel to ground and consist of four pieces of daylight power LED (50W). This one directional light treatment was implemented during two hours (09:00-11:00) according to the previous experiment during six days. The light treatment wasn't conducted in fully dark ambiance in order to simulate sunrise effect.
Then digital images of plants were collected from 09.00am to 11.00am during the light treatment from the same direction with light source in order to estimation of area-based leaf movement. Adaxial leaf areas were calculated using with an image processing software as centimetre square by using pixel counting method as shown in Figure 2. The leaf area differences between 09:00am and 11:00am indicated leaf movement capability and quantity for each plant.

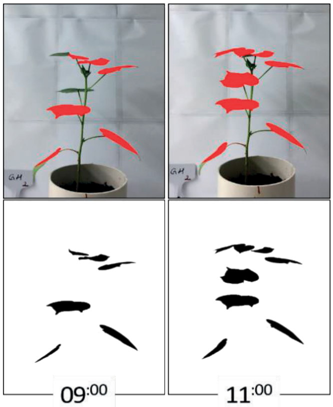

Figure 2. A visual of how measured T_A in Experiment 2nd.
T_A: Area - based leaf movement

RESULTS AND DISCUSSIONS

In order to quantify phototropic movement of plants, all leaves including cotyledons have to be analysed to examine changes in full-plant position. Furthermore, need to non-destructive and zero-touch method while measuring leaf movement for conducting reliable studies. Some digital methods were used to detect heliotropic response in many previous studies (Bawhey et al., 2003; Rakocevic et al., 2010). In our study, we observed that digital imaging method is appropriate method for measuring leaf movement.
Two types of angle measurement (M_I and M_P) were performed during ten minutes interval in the first experiment. Leaf movements of both cotton species were clearly detected at minutes of 0, 50, 80 and 110. M_I of GH had decreasing trend during two hours of light treatment. However, M_I of GB was stable and only decreased in 50 min (Figure 3 a). It is possible to suggest that GB leaves had no diaheliotropic feature, only showed growth-based phototropism in 50 min; however, GH had linear increase in diaheliotropic leaf movement during light treatment. Earlier, this method to measure diaheliotropic leaf movement was examined by Ehleringer and Hammond (1987). Shell et al. (1974) estimating light interception capability of plants.

Joint-like pulvinus is located between leaf lamina and petiole (Moore and Hines, 2017). These specialized cells enable diaheliotropic feature to plants by help of turgor pressure (Moore and Hines, 2017). Therefore, M_P of both GH and GB were measured in order to support previous suggestion that is related to diaheliotropic leaf movement. M_P of GH had decreasing trend similar to M_I of GH, but GB had almost same M_P during two hours (Figure 3 b). It is proving that GH had diaheliotropic leaf movement however, GB only showed growth-based phototropic leaf orientation.

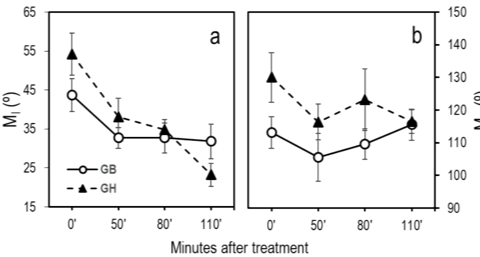

Figure 3. Effects of light treatment on M_I (a) and M_P (b) value of GB and GH during minutes after treatment.
M_P: Midrib - Petiole Angle
M_I: Midrib normal - Incident Angle
GB: *Gossypium barbadense* L.
GH: *Gossypium hirsutum* L.

Ehleringer and Hammond (1987) reported that when cos (M_I) value close to 1, there is a strong diaheliotropic feature in leaves. According to this suggestion, in the previous study, GH had remarkable increase in leaf movement and higher orientation than GB at the end of the light treatment (Figure 4).

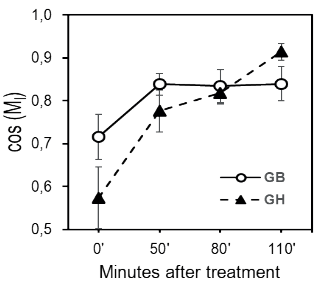

Figure 4. Effects of light treatment on cos (M_I) value of GB and GH during minutes after treatment.
cos (M_I): Cosine of Midrib normal - Incident Angle
GB: *Gossypium barbadense* L.
GH: *Gossypium hirsutum* L.

The second experiment which considered area-based full leaves movement (T_A) was more reliable method in principle since it covered changes in whole plant position. In the present study, GB and GH leaves were tending to turn towards light as shown in Figure 5. GH generally had more orientation to light during six days, but T_A of GB and GH were almost same in 3th, 4th and 5th days. This orientation similarity between GB and GH probably was resulted from growth-based phototropism of GB as mentioned before. This finding is also supported by increasing of differences again between T_A of GH and GB at 6th day. The growth-based phototropism of GB is also explained by growing of cotton plants in lower light conditions on contrary to sunlight.

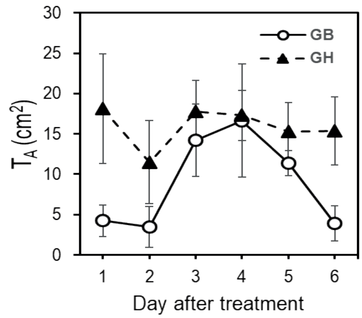

Figure 5. Effects of two hours light treatment on T_A of GB and GH during day after treatment.
T_A: Area-based leaf turn (cm^2)
GB: *Gossypium barbadense* L.
GH: *Gossypium hirsutum* L.

It is possible to estimate and quantify total leaf movement by using T_A method. We found that total T_A of GH is significantly higher than T_A of GB (Figure 6).

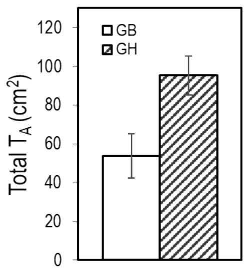

Figure 6. Total T_A of GB and GH through six days of light treatment.
T_A: Area-based leaf turn (cm^2)
GB: *Gossypium barbadense* L.
GH: *Gossypium hirsutum* L.

Although GH has relatively smaller leaf area than GB (Hejnak et al., 2015; Wise et al., 2000), leaf turn of GH were found significantly higher than GB. It is mean that diaheliotropic feature of GH leaves lead to turn more leaves towards light source than growth-based phototropic feature of GB leaves.

CONCLUSIONS

The main objective of study was determining to accurate and practical method for quantifying leaf movement of cotton. All measurement methods of this study were based on digital imaging. It was possible to measuring of phototropic leaf movement via easier and non-destructive way by digital imaging.

Furthermore, estimation of area-based leaf movement (T_A) using with pixel counting method could be suggested as featured method to detecting and quantifying of phototropic leaf movement in terms of accuracy and reliability. According to our results, GH showed strong phototropic leaf movement on the contrary of GB. T_A is expected to use in physiological and breeding studies as accurate and practical method.

However, growth-based phototropic leaf movement of GB were detected in all measurement methods. Therefore, measuring of diaheliotropic leaf movement on same cotton species without lead to growth-based phototropic leaf movement could be more effective to determine accurate method.

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NEW SPICE FORMS OF *Thymus vulgaris* L. ssp. PROMOTED FOR IMPLEMENTATION IN THE REPUBLIC OF MOLDOVA

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Abstract

Thyme (Thymus vulgaris L.) is known and used in various directions since ancient times. Some time it was marginalized due to the impossibility of being cultivated industrially and mechanically harvested (small waist of the plant). Currently, the species returns both in terms of pharmaceutical remedy and good honey-bearing, as well a delicate spice, being absent from the landscape arrangements of parks, gardens and groves. Our research focused on the selection of two forms of Thymus vulgaris (CCT) and Thymus x citriodora (CFL) obtained by clonal selection. Which differ from the initial forms, by the increased productivity of the raw material, the waist of the plant, and production of essential oil. Have been identified the efficient methods of multiplying the seeding material, for various tests to promote and implement new forms.

Key words*: Thymus vulgaris, pharmaceutical remedy, productivity, multiplying, essential oil.*

INTRODUCTION

In the traditional and folk medicine in the countries of the EU and the Middle East, the cultivated thyme has been widely used due to its numerous health benefits, possessing antimicrobial, soothing, antitussive, antiseptic and anthelmintic properties (Chevallier, 1996; Menković et al., 2011; Benítez et al., 2010; Mati, de Boer, 2011; Kavita, 2011; Pieroni et al., 2013; Redžić, 2007; Rexhepi et al., 2013; Kayani et al., 2014; Mustafa et al., 2012).
The spasmolytic effect of flavonoids has been confirmed (Van Den Broucke & Lemli, 1983). Thyme has antimicrobial properties due to some compounds such as terpenoids, essential oil and flavonoids (Behidj-Benyounes et al., 2014; Askun et al., 2009; Horikrrisha et al., 2004). The cultivated species of thyme are used as natural food preservatives, inhibiting microbial growth and extending the shelf-life of food products, and thus they are used to prepare marinades, various canned foods and desserts.
Thymus x *citriodorus* (Pers.) Schreb. is currently used as an ornamental species in landscape architecture.
Some time ago, this plant was introduced as an aromatic species in Turkey (Bagdat et al., 2011) and Iran (Omidbaigi and Rezaei, 2000).
The strong antioxidant activity of thyme is due to phenols - flavonoids (Kulišić et al., 2005; Mihailovic-Stanojevic et al., 2013; Hossain et al., 2013). Hydro-extracts are very powerful antioxidants due to the rosmarinic acid content (Bauermann & Thomann, 2011).
Many varieties have been created, to which different flavours and morphological characters are specific: 'Silver-Edged' - with silver leaf margin, 'Aureus', 'Doone Valley', 'Golden Dwarf', 'Variegatus' - leaves with yellow and white spots.
Since it is a species with numerous medicinal, aromatic and ornamental qualities, it has been used as research object to identify, among various sources, one cultivar with a narrower direction of use (spice - aromatic) and higher resistance to the climatic conditions, which are changing from year to year.

MATERIALS AND METHODS

Three cultivars of *Thymus vulgaris* var. *citriodora* (Pers.) Schreb., which had been purchased at an exhibition with sale of propagating material, and a spontaneous hybrid between *Thymus pulegioides* L. *x T. vulgaris* L. were used as initial material for research.
As a result of the clonal selection, several forms were obtained, and two of them were

chosen because they had higher productivity of raw material and essential oil and the plants were 4-6 cm taller than the original forms.

The new forms are much more cold hardy and thus it is not necessary to protect (cover) them in winter. The two forms are also less vulnerable to the extended periods without rainfall, which have become more frequent in the recent years. The seeds of cultivated thyme are small and they maintain a rather high germination capacity of 65% for three years, under storage conditions without temperature and humidity control.

However, the growth energy, after two years of storage, decreases drastically with each year. The optimization of seed storage conditions makes it possible to extend this term.

Many researchers and growers of the given species propose to sow thyme seeds in early spring (Malankina, 2015), but there are also recommendations to sow them in late autumn or early winter (Rey, 1993).

Because the cultivated thyme is a very polymorphous species, in our opinion, it requires vegetative propagation in order to maintain the obtained characters.

Thus, it was necessary to initiate the vegetative propagation of the new forms with the application of several factors - root stimulators.

For the multiplication of the initial population, which was studied as control, as well as the new lemon-flavoured form, propagation works were initiated.

To identify the most efficient vegetative methods of rooting the forms of *Thymus vulgaris* L. var. *citriodora*, two variants were tested: rooting in filtered water at room temperature; processing of cuttings with Reglal G natural root stimulator solution (1:400 un. and 1:500 un. dilution) - a product obtained at the IGPPP and officially registered as a plant root stimulator.

The mother plants for collecting plant material were prepared (transplanted in pots stored indoors) in February.

The cuttings were taken at the beginning of March and were placed in glass tubes for rooting, filtered water and a natural root stimulator solution at a dilution of 1:400 and 1:500 units served as media.

For rooting, the plant material was freshly collected (cut) with sharp garden shears, the blade of which was treated with ethyl alcohol for purification.

The cuttings were collected in a container with 1% $KMnO_4$ solution. One hundred thirty cuttings of both forms of *Thymus vulgaris* ssp. were subjected to rooting.

Seventy cuttings were soaked in the solution with root stimulator at a dilution of 1:400 and 90 cuttings - at a dilution of 1:500 units, for 24 hours.

The plant material stored for rooting under laboratory conditions, at room temperature of 22-25°C, the humidity of even better results - the rooting rate was by the air being maintained by frequent spraying with filtered hot water (the filter of the "Nobil" brand) with a temperature of 35°C.

The cuttings subjected to the rooting process were kept in tubes placed on a support, being maintained the constant level of the rooting solution for 9 days, after which they were transplanted in a solarium under controlled conditions of humidity and temperature (under agril foil).

At the time of transplantation, at the base of the seedling, several outgrowths appeared - bulbous nodules, of which several actual roots developed in the soil.

The soil in which the cuttings were transplanted for rooting was prepared by adding a specially prepared industrial substrate for plants, peat with neutral acidity, crushed gravel and river sand in the ratio of 3: 3: 3: 1. Further, 20 days until the extraction from soil, the cuttings had been acclimated to the environmental conditions (temperature) and the agril foil was removed. The necessary humidity was provided by spraying the foliage, in the evening or morning, with a minimum amount of water.

RESULTS AND DISCUSSIONS

The cuttings were extracted from soil at the beginning of October, to be tested in the CCC. The obtained planting material was studied to determine the rooting rate and other important characteristics.

Thus, for the form *T. vulgaris* var. *citriodora* - CCT - the control consisted of 370 cuttings placed in filtered water to take roots and then transplanted into a solarium, so, 294 of them took roots and the rooting rate was 79.4%.

The cuttings treated with the natural root stimulator (Reglal G) at the dilution of 1:400 as well as 1:500 had practically the same rooting rate of 48.6 % and 46.7%, respectively (Table 1). As for the form selected of *T. vulgaris* var. *citriodora* - CFL - of 130 cuttings placed for rooting in filtered water, 76.9% took roots, and the rooting rate of those treated with the Reglal G root stimulator at a dilution of 1:400 and 1:500 was 55.7% and 52.2%, respectively. The treatment of cuttings of *Thymus vulgaris* ssp. with the Reglal G root stimulator had no advantages over the rooting in filtered water, which had even better results - the rooting rate was by 25% higher.

Table 1. The rooting rate of cuttings of *Thymus vulgaris* ssp. in various media

Name of the form	Filtered water			Natural root stimulator Reglal G, units					
				1:400 dilution			1:500 dilution		
	Number of cuttings		Rooting rate %	Number of cuttings		Rooting rate %	Number of cuttings		Rooting rate %
	Subjected to rooting	Rooted		Subjected to rooting	Rooted		Subjected to rooting	Rooted	
T. vulgaris var. *citriodora* - CCT - control	370	294	79.4	105	44	48.6	180	42	46.7
T. vulgaris var. *citriodora* - CFL selected form	130	100	76.9	105	39	55.7	180	47	52.2

At harvest, the cuttings were analysed morphologically according to the biometric characteristics - the size of the plants, the length of the roots, the number of the ramifications of the stem, the number of shoots formed on a plant (Table 2). Because the difference between the external morphological characteristics of the treated and untreated cuttings was not essential, the presented data constitute the averages (30 plants) from the measurements of three components in each sample (untreated, treated with Reglal G natural root stimulator solution with a dilution of 1:400 and 1:500).

Table 2. Biomorphological features of rooted cuttings of *Thymus vulgaris* var. *citriodora* ssp.

Name	Values	Height of the cutting, cm	Length of the root, cm	Number of stem ramifications	Number of shoot per cutting
T. vulgaris var. *citriodora* - CCT - control	min	24.0	13.0	20.0	2.1
	max	26.0	17.0	23.0	2.5
	X	24.4	14.0	21.0	2.4
	V%	21.4	28.1	23.0	17.9
	P%	3.8	3.9	3.2	2.3
	DL_{05}	2.3	1.7	2.1	1.0
T. vulgaris var. *citriodora* - CFL - selected form	min	25.0	13.0	19.0	2.0
	max	27.0	16.0	22.0	2.2
	X	25.7	14.7	21.7	2.6
	V	20.4	27.2	22.7	15.6
	P%	3.45	1.3	3.2	2.06
	DL_{05}	2.3	3.8	2.2	1.1

The height of the rooted cuttings was 24.4 cm in the control and 25.7 cm in the selected form. Both the length of the roots and the number of ramifications of the stem had slightly higher values in the selected form than in the control. Each of the cuttings subjected to rooting formed on average 2.4 shoots per plant in the control and up to 2.6 the selected form. The size of the basal ramification (2-3 per plant) was practically equal to that of the mature plants, starting from growing from the base of the cuttings, the smaller apical ones (50% of the length of the basal ones).

Thus, the plant material of *Thymus vulgaris* ssp., necessary to start the testing experience in CCC, was multiplied during a growing season.

The treatment of the cuttings with the Reglal G natural root stimulator did not give any advantages as compared with the untreated cuttings, since the results obtained in all variants were almost identical. The soil preparation works for planting cuttings consisted of fertilization with organic fertilizers as 60-80 kg/ha, applied under the plow, after planting and after the first harvest.

Plow at 28-30 cm, leveling, shredding and superficial loosening of the soil.

The planting norm is 160,000-180,000 threads per/ha, and the distance between nests is 20 cm.

After planting, a mandatory irrigation was applied, which needs to be repeated during the vegetation period.

The care work consists of soil remediation, weed destruction.

Harvesting was done only once because the plantation is young, by cutting the unaligned aerial part, before complete flowering.

During 2018, the planted cuttings developed into mature plants. In 2019, the test plantation was already created, and phenological observations and biometric measurements of the habitus of the plants were carried out.

The year 2019 was very difficult for the initiation of plant growth and development, and adverse weather conditions persisted throughout the growing season.

The lack of rainfall at key moments in plant development and the high temperatures that caused drought did not allow plants to develop normally, and thus productivity indices were very low.

The small stem of the plant, is one of the impediments, making mechanized harvesting impossible on industrial surfaces which prevents the introduction into this culture of this precious species.

During the researches, during several years, the selection on the character of the "plant size" was identified as a priority. The larger the size of the plant in the thyme species, the easier the harvesting, both the manual and the mechanized ones.

The lemon thyme (*Thymus vulgaris* var. *citriodora*) is a species of small plants, but this year, the plants were even smaller than usual - 20-23 cm in the control and 21-23 cm in the new cultivar. The diameter of the bush was equal to average values 19-22 cm from the control and 26-29 cm the new cultivar.

The other morphological indices also did not reach high values.

The number of productive stems at one plant was only 19-22 in the control and 26 in the new cultivar.

The length of the growing season until harvest was 85 days in both forms (Table 3).

The average fresh raw material production was slightly higher in the new cultivar, by 0.99 kg, and it was statistically confirmed.

At the full flowering stage (Figure 1) of plants, the content of essential oil was appreciated and it constituted 0.285%, in the control and 0.32% in the new cultivar, calculated per hectare, it would be equal to 7.24 kg and 10.3 kg/ha, respectively.

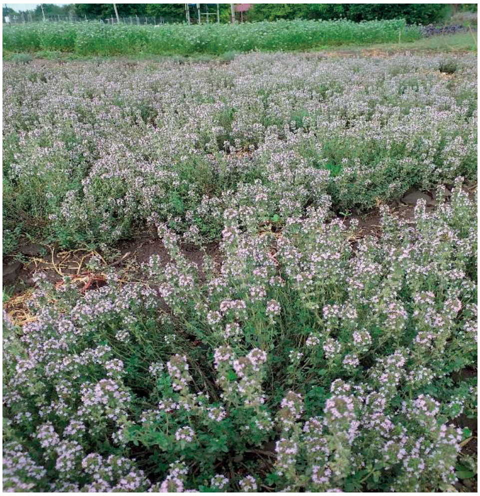

Figure 1. Testing CCT and CFL forms

Plants of both forms tested had abundant flowering, each shoot having flowers all its length. The plants in the plots predestined for seed collection were left until the disappearance of the floral components that constituted 35 days. The harvest for obtaining the seeds, however, was unsuccessful, with no seeds.

The binding of seeds may have been caused by the high temperatures (burning in air) of that period and the long-term lack of precipitation. Testing for seed production will be continued, to elucidate this case.

Table 3. Biomorphological and productivity indices of *Thymus vulgaris* var. *citriodora*, 2019

Name		Height of plants, cm	Diameter of a bush, cm	Number of stems per plant	Length of the growing season, days	Productivity of 10 m² of raw material		
						fresh, kg	dry, kg	essential oil, kg
CCT - control	min	20	19	148	85	2.04	1.06	
	max	23	22	156	-	2.73	1.61	
	x	21	21	153	85	2.54	1.46	0.724
CFL - new form	min	21	23	159	85	3.29	1.62	
	max	23	29	166	-	4.20	1.82	
	x	22	26	161	85	3.72	1.68	1.03

BD_{05} - 0.22 kg - for the production of dry raw material.

Lemon thyme is used as a spice in the well-known mixture of dried herbs "Herbs de Provence". It is also used as a medicinal plant, to produce effective medicinal preparations such as cough suppressants, bronchiolitis - syrups, drops, teas etc. Lemon thyme has high potential as honey plant, making it possible to obtain 160-180 kg/ha of honey. It is also commonly used as an ornamental plant to decorate alpine gardens.

CONCLUSIONS

The "premature" method (February - March) of vegetative propagation by cuttings of *Thymus vulgaris* var. *citriodor*a, transplanted later in soil, is quite efficient, obtaining a rooting rate equal to 77-79% and creating the possibility to reduce the loss of planting material by 25-30%, as compared with other methods of vegetative propagation (division of the mature plant, layering etc.)

The obtained cuttings are vigorous and are able to bloom even in the first year of vegetation.

The new cultivar of lemon thyme *Thymus vulgaris* var. *citriodora* (CFL) has higher productivity indices than the control cultivar (CCT), but it still needs testing to confirm these advantages, especially under adverse conditions of insufficient humidity, and to promote it as an aromatic plant.

The obtained cuttings are vigorous and are able to bloom even in the first year of vegetation.

The new cultivar of lemon thyme *Thymus vulgaris* var. *citriodora* (CLF) has higher productivity indices than the control cultivar (CCT), but it still needs testing to confirm these advantages, especially under adverse conditions of insufficient humidity, and to promote it as an aromatic plant, with unique medicinal properties.

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THE INVESTIGATION OF ENTOMOFAUNA IN THE CROP *Reynoutria sachalinensis* (F. Schmidt) Nakai UNDER THE VEGETATION CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

Giant knotweed, Reynoutria sachalinensis (F. Schmidt) Nakai, is a species native to the Far East, introduced in the Republic of Moldova in the 1980s. The surveys of entomofauna and mites associated with the crop R. sachalinensis were carried out in the experimental sector of the Plant Resources Laboratory of the "Alexandru Ciubotaru" National Botanical Garden (Institute). The research has revealed the structure of the entomofauna on R. sachalinensis plants during the growing season and resulted in the identification of 31 species of insects, in various growth stages, the assessment of the phytoparasitic impact, records and analyses of morphological parameters, taxonomic affiliation, trophic specialization, frequency values and abundance of entomofauna on the entire plant and the biological and ecological significance in the regulation of the ratio of zoophagous/phytophagous insects.

Key words: *biodiversity, giant knotweed, insects, trophic spectrum.*

INTRODUCTION

The species has been introduced in the Republic of Moldova, and a cultivar, named 'Gigant', was officially recognized and registered in the Register of Plant Varieties of the Republic of Moldova in 2012, with the number 2492625. In 2016, it received a Plant Variety Patent, with the number 205/31.05.2016 and the common name "Hrişca-de-Sahalin". The binomial name of giant knotweed, or Sakhalin knotweed, is *Reynoutria sachalinensis* (F. Schmidt) Nakai and it has several synonyms *Polygonum sachalinense* F. Schmidt ex. Maxim., *Fallopia sachalinensis* (F. Schmidt) Ronse Decr., *Pleuropterus sachalinensis* (F. Schmidt) H. Gross., *Tiniaria sachalinensis* (F. Schmidt) Janch. (Yonecura and Ohaschi, 1997; Войлокова, 2007; Fuentes et al., 2011; The plant list). This species, which is native to East Asia, Sakhalin and Kurile Islands, has been studied to determine its potential as medicinal and fodder plant (Schnitzler and Muller, 1998; Mandak et al., 2004; Wittenberg, 2005). It has been studied in several countries in Eastern and Western Europe as a multi-purpose plant. In the Republic of Moldova, it was introduced from the Agricultural Institute of Vladikavkaz, North Ossetia, and until now it has been investigated as a multi-purpose research subject.

Some important aspects of this topic were studied in 2014-2019, focusing on the biological peculiarities of growth and development of this crop, introduced from another eco-geographical zone, with high potential as forage, medicinal, ornamental and energy plant in the Republic of Moldova. At the same time, the environmental conditions in our country are favourable not only for the growth of plants but also for the diverse association of insect complexes and their impact on plants is favoured by the climatic conditions throughout the growing season. An essential risk to which this crop may be subjected and which can affect its harvest is the damage and diseases caused by the species of phytophagous insects. Some of them play a special role in maintaining the biocenotic balance as very efficient pollinators during the long flowering phases. Overall, associations of numerous insects that reproduce on this plant are created by means of adaptation and resistance in the interaction with the given plant, environmental factors and anthropogenic influence (Perju, T., 1995; Lupaşcu, 1998; Busuioc M., 2004;Voloşciuc, 2009).

The main purpose of the study on the entomofauna associated with this crop consists in establishing the ways in which the associations of parasitic insects - entomophages are formed, their role in maintaining the ecological-trophic balance in the system host plant - harmful organism - environmental factors, which can progress depending on species associations, the trophic spectrum and the amount of nutrients, the growth and development phase and the relationships between them (Perju, 1995; Busuioc, 2004).

Based on the actuality of the approached topic, the purpose of the research carried out over four years was to perform the entomological phytosanitary control by estimating the diversity of the associations of insects detected in the plantation of the given species, by establishing the degree of beneficial and parasitic impact, the trophic spectrum and useful contributions as pollinating species on this plant.

MATERIALS AND METHODS

Observations were carried out during the growing season of 2016-2019, with records of the entomofauna and mite diversity on *Reynoutria sachalinensis* plants. The research was conducted in the "Alexandru Ciubotaru" National Botanical Garden (Institute), in the experimental sector of the Plant Resources Laboratory, during several successive growing seasons. Phytosanitary control observations and insect samplings were made using the entomological net, visually, in sequence of the developmental phenophases of plants, periodically, with an interval of 10-15 days, starting in May (the phase of the formation of the stem and leaves) and ending in October (full maturity of plants, ripening of seeds and partial fall of leaves). In total, over 12 observations were made with the help of visual and optical analyzes (portable binoculars and magnifying glasses) on the experimental sector of the laboratory with a surface of 0.25 ha, on perennial plants aged 4-7 years (Figure 1).

At each observation, about 100 plants were investigated at certain intervals on the diagonal of the sector under study, sampling insects by collecting them manually and by mowing the plants, besides, we examined the plants and their organs affected by phytoparasitic insects in various stages of phenological development, their numerical densities and parasitic impact.

A.


B.


Figure 1. A - Plantation of *Reynoutria sachalinensis* (F. Schmidt) Nakai: formation of vegetative organs (April 2016); B - mature plants (July 2016), phytosanitary observations on the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute)

Later, under laboratory conditions, the peculiarities of morphological structure, the taxonomic rank and the diversity of insect species detected in the plantation, found on different organs and in different development phases and trophic specialization spectrum, were established and pictures were taken for documentary purposes. The papers on general entomology of Perju, 1995; Oltean, 2001; Lazări and Busuioc, 2002; Cozari, 2010; Talmaciu, 2014, the guides for determining species written by Плавильщиков, 1994, Bei-Bienco, 1962, and the examination under the microscope MBC-10 and the binocular microscope Leica CME were used to confirm the taxonomic identity of the collected insects and mites.

RESULTS AND DISCUSSIONS

The assessment of the efficiency of using the species giant knotweed can be supplemented with investigations on entomoparasites, conducted under the environmental conditions of the Republic of Moldova, with the purpose of highlighting the presence of entomofauna and mites on plants in different growing seasons, and the phytosanitary control can elucidate the diversity of harmful and useful species, the means of reproduction of such insects and the damage caused to this crop.

The phytosanitary research on the giant knotweed plantation was carried out with a purpose and objectives that illustrate, as a result, the impact of entomofauna on the eco-biological integrity of adaptation of the studied crop to the environmental and biotope conditions, on the experimental sector of the "Alexandru Ciubotaru" National Botanical Garden (Institute). The biodiversity of insects and mites on the investigated plants was found to be of a mixed type, very diverse and varied, being favoured by the new and attractive biomorphological characteristics of the species for the respective fauna, related to process of adaptation of the introduced crop to the environmental factors in the territory of the Republic of Moldova.

The composition of entomofauna in the *R. sachalinensis* plantation was studied by laboratory analyses determining the numerical density, the taxonomic units and variations of the trophic spectrum, with the estimation of the diversity of the species of insects and arachnids, the parasitic impact on the plant organs, according to the abundance and frequency of the detected species, the way and the degree of impact. The mass emergence and the fast expansion of entomofauna on the plants were noticed at the end of May - the middle of July, then, when the pedological drought and high air temperatures were attested (the second half of July-August), a considerable decrease in the numerical density and the diversity of insects on the aerial organs of plants was observed. The mass flowering phase is a significant stimulus for the agglomeration of entomofauna and mites on giant knotweed, which, as a long-day species, blooms at the end of August - the middle of October. During this period, a considerable increase in the diversity of phytophagous insect species and their numerical density, in different developmental stages (adult and larval forms), was observed on the nectar-rich inflorescences and leaves found in the apical area. At the same time, the abundant presence of predatory zoophytophages feeding on colonies of aphids and mites was noticed.

The abundance of the numerical density and the frequency of species by the presence of adult individuals and larvae are more significant in late spring, with the emergence of fragile shoots and the development of leaves, the plants being invaded by phytophagous insects, followed by entomophagous insects, in a 60/40% ratio, in favour of phytophagous species. In autumn (the mass flowering phase - the formation of seeds), entomophages with the trophic specialization: omnivorous predators, including pollenophagous and nectarivorous species, predominate more abundantly in the species diversity.

As a result of taxonomical analyses, the diversity of the entomofauna detected in the plantation varied considerably in the range with two maxima during the entire growing season, and during the spring-summer period, beetle species of the order Coleoptera, bugs of the order Hemiptera and aphids of the order Homoptera predominated. All of them behaved like parasitic species and caused damage, such as: bites, stings, embossing, discolorations and tunnels through newly formed leaves and stems. When the mature plants grow very tall, up to 5 m, and this happens in the second half of summer and in autumn, the species of the orders Diptera, Hymenoptera (especially honeybees) predominate in the plantation. The polyphagous insects of the orders Coleoptera and Lepidoptera are more numerous in the flowering phase with abundant nectar production, as an essential nutritional source, on the large inflorescences of giant knotweed plants (Busuioc, 2004; Lupaşcu, 1998; Плавильщиков, 1994).

Table 1 illustrates the diversity of species and the trophic spectrum of insects and mites found on giant knotweed plants. The complex of insects detected in the dynamics of growth stages and development phases included 31 species, with different trophic specialization and variability of the biological development cycle, adapted to certain organs of the plant, depending on the trophic specialization and the reproductive potential (Table 1, Figure 2). By analyzes and taxonomic determinations, the insect species were identified and it was found that they belong to **8 orders**: 1. Neuroptera, 2. Coleptera, 3. Hemiptera, 4. Mecoptera, 5. Hymenoptera, 6. Diptera, 7. Homoptera, 8. Lepidoptera; **21 families**: 1. *Chrysipidae; 2. Elateridae; 3. Cantaridae; 4. Curculionidae; 5. Coccinellida; 6. Pentatomidae; 7. Coreidae; 8. Codnidae; 9. Panorpida; 10. Apidae; 11. Scoliidae, 12. Formicidae, 13. Vespidae, 14. Sarcophagidae, 15. Califoridae,16. Syrphi, 17. Tachinidae, 18. Aphididae,19. Noctuidae, 20. Pyraustidae, 21. Tenthrediideae;* **28 genera**: 1. *Crysoperla,* 2. *Athous,* 3. *Chantaris,* 4. *Rhagonycha,* 5. *Cratopus,* 6. *Coccinella,* 7. *Adalia,* 8. *Harmonia,* 9. *Graphosoma,* 10. *Coreus,* 11. *Tritomegas,* 12. *Panorpa,* 13.*Apis,* 14. *Scolia,* 15. *Formica,* 16. *Lasius,* 17. *Katamenes,* 18. *Sarcophaga,* 19. *Lucilia,* 20. *Syrphus,* 21. *Eristalis,* 22. *Spherophoria,* 23. *Tachina,* 24. *Aphis,* 25. *Myzodes,* 26. *Agrotis,* 27. *Margaritia,* 28. *Athalia.*

At the same time, there was a stable abundance of tiny phytoparasitic mites on the surface of the leaves and young shoots, throughout the growing season, belonging to **3 orders**, **3 families**, **3 genera** and **3 species**, respectively, with phytoparasitic impact on the leaves and flower primordia, especially in spring and autumn, and species of entomophages feeding on aphids and other harmful insects [10, 11].

Table 1. The diversity and the trophic spectrum of insect and mite species found on *R. sachalinensis* plants

No	Species	Trophic spectrum	No	Species	Trophic spectrum
Phylum: ARTHROPODA, class: INSECTA					
1	*Crysoperla carnea*	Zoophagous	17	*Formica rufa* L.	Omnivorous, predatory
2	*Athous niger* L.	Phytophagous	18	*Lasius niger* L.	Omnivorous, predatory
3	*Chantaris fusca* L.	Zoophagous predatory	19	*Katamenes arbustorum* Penzer	Predatory
4	*Chantaris pellucida* Fabricius	Zoophagous predatory	20	*Athalia rosae* L.	Zoophagous, plant liquids
5	*Rhagonycha fulvu* Scopoli	Zoophagous predatory	21	*Sarcophaga carnaria* L.	Plant liquids
6	*Cratopus* sp.	Phytophagous	22	*Lucilia caesar* L.	Pollen and plant liquids, zoophagous
7	*Coccinella septempunctata* L.	Entomophagous, aphidophagous	23	*Syrphus ribesii* L.	Pollen, plant liquids, zoophagous
8	*Adalia bipunctata* L.	Entomophagous, acariphagous, aphidophagous	24	*Eristalis tenax* L.	Pollen and nectar
9	*Adalia quadrimaculata* L.	Entomophagous, acariphagous, aphidophagous	25	*Spherophoria scripta* L.	Pollen and nectar
10	*Harmonia axyridis* Pallas	Entomophagous, acariphagous, aphidophagous	26	*Tachina fera* L.	Pollen and nectar
11	*Graphosoma lineatum* L.	Phytophagous, nectariphagous	27	*Aphis pomi* De Geer	Occasional plant parasite
12	*Coreus marginatus* L.	Phytophagous, nectariphagous	28	*Aphis fabae* Scopoli	Occasional plant parasite
13	*Tritomegas bicolor* L.	Phytophagous, nectariphagous	29	*Myzodes persicae* Sulz.	Occasional plant parasite
14	*Panorpa communis* L.	Omnivorous, predatory	30	*Agrotis segetum* Denis & Schiffermuller	Polyphagous plant parasite
15	*Apis mellifera* L.	Pollen and nectar	31	*Margaritia sticticalis* L.	Polyphagous plant parasite
16	*Scolia hirta* Schrank	Pollen and nectar			
Phylum: ARTHROPODA, class: ARACHNIDA					
1	*Tetranychus urticae*	Plant parasite	3	*Tarsonemus angulatus*	Mycophagous
2	*Paraseiulus incognitus*	Entomophagous, predatory			

A B C D

Figure 2. Insect species found on inflorescences and leaves of *R. sachalinensis*: A - *Scolia hirta*; B - *Sarcophaga carnaria*; C - *Cantharis pellucida*; D - leaves affected by insects

The graphical representation of Figure 3 highlights the maximum share of the population of insects detected on the researched crop, with the predominance of the species of insects of the order Coleoptera, which constitutes 29% of all the diversity of the species of insects collected from the field and analyzed. The species of the orders Diptera and Hymenoptera constitute 19%, followed by species of the orders Hemiptera and Homoptera with 10% each. The number of butterflies and moths of the order Lepidoptera was lower - 6%, in terms of diversity and numerical density, but individuals of the orders Neuroptera and Mecoptera belonged to a single species (Figure 3).

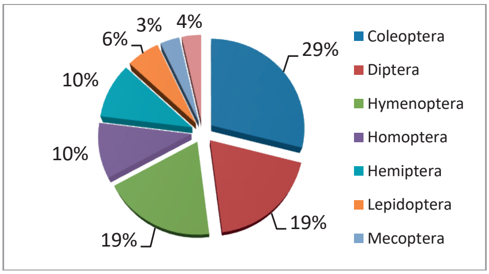

Figure 3. Comparative graphical representation of insect complexes according to the taxonomic abundance by orders

The results of the research on the determination of the taxonomic structure of insect associations in giant knotweed plantations, done by assessing the degree of invasion, by adapting biological strategies to this crop, studying their synergistic and antagonistic relationships and establishing the trophic specialization are estimated comparatively in Table 2.

The distribution through a spectrum related to the zoophagous and phytophagous nutrition differentiation, including the specializations within the groups of insects, the most abundant and frequent species are phytophagous insects that feed on nectar, pollen and other floral exudates, in total, there are 14 such species of detected insects, and this fact denotes the high melliferous potential of the species *R. sachalinensis*.

Table 2. The graphical presentation of the comparative trophic spectrum of insect complexes related to phytophagous and zoophagous specialization

Zoophagous insects		Phytophagous insects	
Trophic type	**№ of species**	**Trophic type**	**№ of species**
Entomophagous	4	Nectar, pollen, plant liquids	14
Omnivorous predators	3	Occasional plant parasites	3
Zoophagous predators	5	Polyphagous	2

The leaves and the flowers are the most attractive to the wild entomofauna, including the entomophagous insects. Parasitic insects with folivorous and anthophagous specialization, represented by a diversity of aphids, ticks and caterpillars of polyphagous parasitic lepidopterans etc. predominate on these parts of the plants. The group with zoophagous specialization is represented mainly by predatory species, followed by entomophages (acariphagous and afidophagous insects) and the omnivorous predators, and it plays a significant role by determining the dynamic balance within the entomofauna of the given crop and diminishing the number of parasitic phytophagous insects. Thus, there is no need to apply chemical phytosanitary treatments because the density of parasitic species of insects is regulated with the help of predatory-omnivorous species.

Species with acariphagous, and predatory-omnivorous zootrophic specialization, such as: *Coccinella septempunctata, Adalia bipunctata, A. quadrimaculata, Harmonia axyridis, Lasius niger*, *Formica rufa*, *Panorpa communis* were found throughout the growing season, they affected the phytophagous species and there was an antagonistic relationship between them, thus, these species play the role of living biological remedies in diminishing and regulating the number and density of phytoparasitic species and the parasitic impact on the polyphagous insect species, such as mites, aphids, beetles, lepidopterans. The diversity of insects of the order Coleoptera with a diverse trophic spectrum was significant; it included hundreds of species of insects, with phytophagous and zoophagous trophic specialization, which regulated, particularly, the presence of parasitic pests such as aphids, mites and polyphagous caterpillars in early stages (Gulii and Pamujac, 1994; Iachimciuc et al., 2012).

CONCLUSIONS

As a result of the taxonomic research and analyses on entomofauna and mites performed in a plantation of *Reynoutria sachalinensis* (F. Schmidt) Nakai, giant knotweed, under the environmental conditions of the Republic of Moldova, a diversity of 31 species of insects associated in complexes with various trophic specialization was found; these species have adapted to the succession of growth/development phenological phases in impact with environmental factors.

The morphobiological characteristics and the trophic specialization of the diversity of insects detected in the giant knotweed plantation were established taking into account their spread on the plants, with the estimation of the frequency and the abundance of the detected insects, the degree of damage caused by them, the trophic spectrum estimated by the presence of 12 zoophagous and 19 phytophagous species, with the predominance of polyphagous-parasitic species, followed by zoophages, entomophages and omnivores-predators with acariphagous and aphidophagous specialization.

Laboratory taxonomic analyzes of insect and mite samples collected from the studied plants were carried out and helped estimating the diversity of insects investigated per year and during the growing season, and, in total, we identified 34 species of insects and mites included in 31 genres, 24 families and 11 orders, with various bio-indications and bioecological importance for giant knotweed during the adaptation time, which were influenced by the environmental conditions of the Republic of Moldova.

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CALCULATION OF THE FERTILISER DOSES APPLIED TO POTATO CROPS BY THE COMPANY SOLFARM SRL, COVASNA COUNTY

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Abstract

The cultivation of potatoes is mainly influenced by the type of fertiliser used, the dose of mineral or organic fertiliser applied based on the soil's supply of nutritional elements and the structure of the soil. The goal of these fertilisation experiments carried out in 2020 was the calculation of optimal and economical doses of NPK fertilisers, based on the soil's supply of nutritional elements, in order to obtain consistent yields of a certain level, using the methodology of an agrochemical study, and to determine the influence of various doses of fertilisers on the yield and on the production efficiency in potato cultivation. To monitor the soil's supply, agrochemical mappings were carried out, used to determine the optimal economical doses required to obtain a yield of 50 t/ha for three potato cultivars: Riviera, Bellarosa ans Arizona. Comparisons were made based on the data obtained, between the doses used by the company Solfarm SRL and the optimal N, P, K fertiliser doses necessary for a yield of 50 t/ha.

Key words: *chemical fertiliser, potato fertilisation, nutritional elements, agrochemical mapping.*

INTRODUCTION

The main purpose of using precision technology in the context of sustainable agriculture is to maintain or increase soil fertility, in addition to ensuring crop efficiency.
The potato is one of the plants that is most recognizant of the soil's natural fertility and of a rational fertilisation, when the other vegetation factors are at an optimal level. The potato has a high specific intake of nutritional elements. Thus, for each 1000 kg tubers and the corresponding surface biomass, it intakes on average 5 kg N, 3 kg P_2O_5, 8 kg K_2O, 3 kg CaO and 1 kg MgO (Ion, 2010).
The doses of chemical fertilisers differ with the purpose of the crop. Following a crop of perennial leguminous plants, the dose of nitrogen is reduced by 20-30 kg/ha, and the dose of phosphate is increased by 20 kg/ha. Following a crop annual leguminous plants, the dose of nitrogen is reduced by 10-20 kg/ha, and the dose of phosphate is increased by 15 kg/ha. Following a crop of technical plants, the dose of nitrogen and phosphate is increased by 10-20 kg/ha (Ion, 2010).
Among the chemical fertilisers containing nitrogen, the ones that are most often recommended are nitrolime and urea in acidic soils, and in neutral soils ammonium nitrate and urea (Miron, 2015).
Nitrogen (N) has a distinguishable effect on the growth of the crop, and is used by the plants throughout the vegetative period.
Nitrogen contributes to the increase of the yield, to the growth of the foliar system, to the increase in the tuber number and size. The potato reaches the highest uptake of nitrogen (150-200 kg N/ha) before bulking, but a smaller quantity continues to be uptaken afterwards. It is an important element for protein synthesis, it stimulates the growth of meristematic tissues, and it encourages stem ramification.
Nitrogen deficiency leads to the growth of light green plants, with small, rigid foliage, with erect leaves, and to a quick (early) maturity.
Nitrogen excess leads to a quick growth of the foliar system during tuber formation. Vines grow very long and fall quickly (Roman et al., 2012).
Phosphate (P_2O_2) is uptaken throughout the entire growth period, especially during foliage growth.
For seed potatoes, the soil must have a good supply of mobile phosphate, while avoiding excess.

Phosphate stimulates root growth and quickens foliage maturity; it stimulates an increase in the number of tubers, but not in their size.
As regards planting material, it provides a vigorous growth of the sprouts and young plants. The optimal phosphate doses encourage quick tuber growth, increased plant resistance against viral infections, increased tuber resistance against shock and storage resistance.
Phosphate deficiency leads to reduced growth, dark green foliage, upward curling of the leaves, lack of lustre and marginal scorching.
Phosphate excess leads to a decrease in the dry matter percentage and can even have a toxic effect, seen in late tuber formation and decreased yields (Burton, 1966).
Potassium (K_2O) stimulates the early growth of the potato, increases stalk vigour and leaf area, without decreasing leaf longevity. Good potassium intake increases plant resistance against pathological and physiological diseases, as well as against draught, amplifies protein synthesis and respiration, helps the plant to grow during dry periods.
The presence of high quantities of potassium in seed tubers encourages early springing. Potassium decreases the blackening of the tuber after cutting and increases the tuber's resistance against breakage, having a positive influence on the normal growth of the tubers.
Potassium deficiency is indicated by a small plant habitus, dark green-bluish colour and the bronzing of the leaves, which turn yellowish as they age, and dry tips. (Burton, 1966).
Potassium excess can negatively affect the yield, especially if the fertiliser contains chloride, to which the potato is particularly sensitive.
The agrochemical mapping paper proposes a synthesis study regarding the pedo-agrochemical conditions, together with recommendations regarding the rational use of agricultural real estate. Agrochemical features allow an in-depth understanding of the land's yield potential. The following can be achieved based on the agrochemical study:

- the rational use of fertilisers;
- the application of preventive measures against soil degradation;
- the increase in natural fertility;
- determining the soils' yield potential.

Soil reaction, expressed as pH, is an indication of the environment where the potato plant grows. Soil reaction can be acid, neutral, or alkaline, with various intermediary levels.
Humus is the main source of nutritional elements in the soil, which, depending on its quantity and quality, determines, to a high extent, soil fertility (Plămădeală, 2008).
The potato plant tolerates a more acidic physiological reaction of the soil, the most adequate pH being 5.5-6.5, but it can be grown without significant damage on soil with pH between 4.5-7.5 (Saulescu, 1971).
With a higher pH there is danger of a stronger presence of the common scab. This is why potato crops are not grown on soil that was treated during the previous year (Axinte et al., 2006).
The nitrogen index (NI) is used to determine the quantity of nitrogen available in the soil. Soil where the NI is lower than 2 is considered to have a low supply of nitrogen, soil where the NI is between 2.1 and 4.0 has a medium supply, and above 4.1 good and very good supply.

MATERIALS AND METHODS

For the production and propagation of potato planting material from higher biological categories, the increase of the propagation index and the optimum efficiency of the yield, are important objectives (Berindei, 2000).
Currently, the company SOLFARM SRL grows seed potatoes on area of 35 ha each year. Starting with the observation that the company, in particular for potatoes, applies larger quantities of chemical fertilisers than needed by the crop for the yield obtained, the purpose of these fertilisation plans for 2020 was to determine the optimal and economical NPK dose, based on the soil's supply of fertilising elements, to obtain consistent yields of a certain level, using the methodology of agrochemical studies in the future.
For trial cultivars, the higher biological category was used when planting, tubers from the 30-55 mm diameter fraction, i.e.:

- Riviera, very early potato cultivar

Riviera

- Bellarosa, extra-early, red, potato cultivar

Bellarosa

- Arizona, medium-early potato cultivar

Arizona

Organic fertiliser was applied in autumn, 30 t/ha per trial lot. Chemical fertiliser was applied on all 3 lots of the company SOLFARM as follows Table 1.

The following are represented in Table 2: soil reaction, the soil's supply of mobile phosphate, the values of the nitrogen index reflecting the soil's supply of available nitrogen, the soil's supply of mobile potassium, the organic matter content (humus).

Table 2. The results of the soil samples

Area (ha)	Prem. Culture (2018-2019)	Recent crop (2019-2020)	pH	NI %	Humus %	Mobile phosphate (ppm)	Mobile potassium (ppm)
2	Rape	Potato	5.92	2.18	2.59	84.63	300
2	Rape	Potato	5.43	2.84	3.68	55.63	260
2	Rape	Potato	5.25	2.41	3.22	55.22	240

The soil samples taken from the 3 lots, the pH varied between 5.25-5.92 indicating a moderate acidity.

As regards the supply of nutritional elements, the soil has a good supply of phosphate (55.22-84.63 ppm) and a very good supply of potassium (240-300 ppm). The values of the nitrogen index were between 2.18-2.84 (medium).

The organic matter content (humus) in the soil falls within the limits of a medium to good supply level (2.59-3.22%).

The recommended quantities of chemical fertiliser were between 372 and 432 kg a.s./ha (Table 3).

Table 1. The influence of fertiliser doses on the yield obtained by SC. SOLFARM SRL

Species Type	Fertilisation 1 with YARAMILA 9-15-25		Fertilisation 2 with YARAMILA 8-11-23		Fertilisation 3 NITROCALCAR		Yield (t/ha)
	a.s. kg/ha	year 2019	a.s. kg/ha	year 2019	a.s. kg/ha	year 2019	
Riviera	430	08.03	430	29.03	350	17.04	40
Bellarosa	430	08.03	430	29.03	350	18.04	55
Arizona	430	08.03	430	29.03	350	19.04	42

Table 3. Fertiliser doses calculated based on an agrochemical study

Species Type	Estimated yield (t/ha)	Fertilisation recommendations for 2020 (a.s./ha)			Total NPK (s.a./ha)	Remarks
		N	P_2O_5	K_2O		Compound +Nitrolime (4 years)
Riviera	50	239.98	86.98	65.44	372	16:16:16
Bellarus	50	226.94	115.41	71.61	414	16:16:16
Arizona	50	234.63	116.68	80.15	432	16:16:16

RESULTS AND DISCUSSIONS

Taking under analysis the influence on the yield of these fertiliser doses, it seems that the highest total yield was obtained for the Bellarosa veriety (55 t/ha), followed by the Arizona variety with a yield of 42 t/ha, and the lowest yield was obtained for the Riviera variety (40 t/ha) (Figure 1).

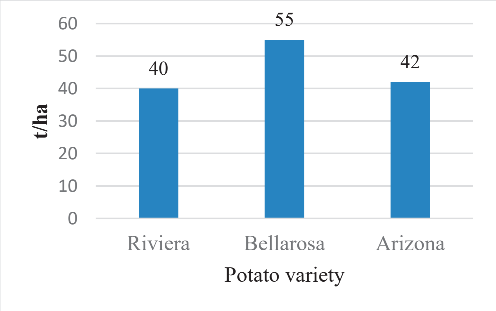

Figure 1. Yield obtained by SC Solfarm SRL in 2019

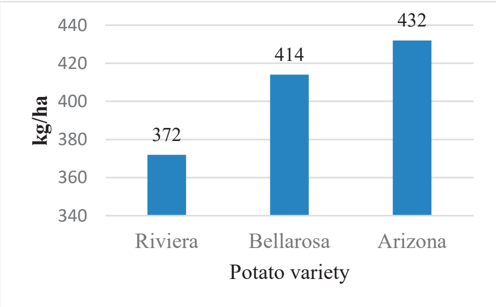

Figure 2. Determining the NPK fertiliser dose for the yield of 50 t/ha

The most efficient yield is obtained based on the recommended system of the NPK dose calculation, based on the data in the agrochemical mapping of the soil for obtaining a potato yield of 50 t/ha (Figure 2).

It can be seen that based on the methodology of the agrochemical study, from the yield perspective, the quantity obtained is almost identical in both cases (Bellarosa variety-yield of 55 t/ha and estimated yield of 50 t/ha), using a fertiliser dose significantly lower, without the risk of reduced yield. There is no business reason either for the fertiliser dose to be too high (Figure 3).

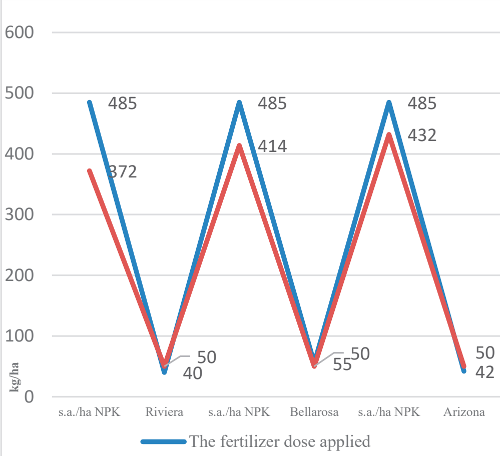

Figure 3. The NPK a.s. quantity used and proposed for 2020

CONCLUSIONS

The potato is a staple crop with a distinct economic and social importance for the agriculture in the Covasna county, first of all as a result of the ecological conditions favourable to this crop and less favourable to others, and secondly as a result of the productivity provided, compared to other crops.

If the soil is supplied with phosphates, it can be seen that good and very good results are obtained for the three lots. This synthesis shows that on most lots there are no special problems regarding phosphate fertilisation.

It must be noted that the soil's supply of mobile potassium on the three lots exceeds the "very good supply" threshold, in which case potassium is applied in very low doses, decreasing the fertiliser cost.

We believe that the results of the agrochemical mapping initiated by SOLFARM SRL aiming to streamline potato fertilisation according to the planned yield will lead to higher and more efficient yields.

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****Cartarea agrochimică a teritoriului* - SC SOLFARM SRL Sfântu Gheorghe, Jud. Covasna.

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COMPARATIVE STUDY OF DIFFERENT COMMON WHEAT (*T. aestivum* L.) CULTIVARS UNDER THE AGROECOLOGICAL CONDITIONS OF SOUTH-CENTRAL BULGARIA

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Abstract

During the period of 2015-2018 in South-Central Bulgaria a field experiment was researched. The cultivars common wheat 'Ekzotik', 'Miryana', 'Avenue', 'Anapurna' and 'Neven', were studied. The experiment was applied in block design with 4 replications and 15 m² plot size, after pre crop sunflower. The growing of plants was performed in compliance with the standard technology. The aim of the present investigation was to carry out a comparative study of the yield and quality of some common wheat cultivars grown in South-Central Region of Bulgaria. The analysis of the results showed that the highest grain yield was obtained from 'Avenue' variety - 7400 kg/ha, followed by 'Anapurna' - 7100 kg/ha and the lowest one - from 'Neven' variety 4067 kg/ha. Among the studied common wheat cultivars, the highest values of thousand kernel (grain) weight and the test weight were reported for 'Miryana' (50.3 g and 81.0 kg, respectively) and the highest wet gluten content was established in 'Anapurna' cultivar (27.0%). The lowest value of the test weight was reported for 'Neven' cultivar (73.3 kg); of the thousand kernel (grain) weight - for 'Anapurna' cultivar (34.3 g) and of the wet gluten content - for 'Avenue' cultivar (22.0%).

Key words*: wheat, cultivars, grain yield, thousand kernel (grain) weight, test weight, wet gluten.*

INTRODUCTION

One of the major ways of increasing the yield and improving the wheat grain quality under the current market conditions is the establishment of new highly productive cultivars and their introduction into practice.

Establishing the proper cultivar structure depending on the concrete agroecological conditions of the region can significantly increase grain yield and quality. That requires a very good understanding of the characteristics of the different cultivars, in order to be able to make the right choice (Dallev and Ivanov, 2015; Dimitrov et al., 2016; Ilieva, 2011; Ivanova et al., 2010; Kirchev and Delibaltova, 2016; Williams et al., 2008).

Studies of a number of authors show that the amount of grain yield is closely related to the cultivar, the use of farming machinery and the soil and climatic conditions of the region (Mut et al., 2017; Stoeva et al., 2006; Studnicki et al., 2018).

Therefore, in order to use the full productive potential of the cultivar, the proper choice of suitable cultivars for each agroecological region is a decisive factor for obtaining high yields. That necessitates systemic studies of the cultivars in the different regions of the country (Aktas et al., 2017; Kaya and Akcura, 2014; Yanchev & Ivanov, 2016).

The aim of the present investigation was to carry out a comparative study of the yield and quality of some common wheat cultivars grown in South Central Region of Bulgaria.

MATERIALS AND METHODS

A field experiment with common wheat was carried out on the experimental field of the village of Carimir (South Central Bulgaria) in the period 2015-2018. The test was performed by means of a block method with four replications; experimental field area - 15 m², after the predecessor sunflower. The following cultivars were tested; 'Ekzotik', 'Miryana', 'Avenue', 'Anapurna' and 'Neven'. All the stages of the established technology for wheat growing were followed.

Soil tillage included single disking (10-12 cm) after harvesting of the previous crop, and double disking after the main fertilization. The area was treated by $N_{120}P_{80}$ and the whole quantity of the phosphorous fertilizer and 1/3 of

the nitrogenous fertilizer were applied before main soil tillage. The remaining amount from the nitrogen norm was applied before the beginning of permanent spring vegetation. Triple super phosphate and ammonia nitrate were used. Sowing was completed within the agrotechnical term optimal for this region at sowing norm 550 germinating seeds/m^2. Control of weeds, diseases and pests was done with suitable pesticides when necessary. Harvesting was done at full maturity. The grain yield is determined with standard grain moisture of 13%.
The indices grain yield (kg ha^{-1}); thousand kernel (grain) weight (g), test weight (kg); wet gluten content (%) and were determined.
For the purpose of determining the quantity dependence between the studied indicators, the experimental data was processed by the method of dispersion and correlation analyses.
The period of the research (2015-2018) is characterized with variety of temperature and rainfall conditions which enables to evaluate the reaction of the studied varieties in accordance with their yields and quality characteristics under different climatic conditions (Figure 1).

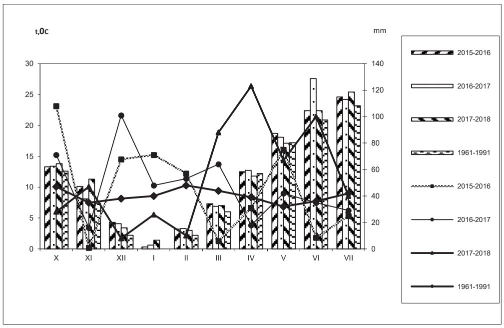

Figure 1. Temperature and rainfall distribution during the period 2015-2018

The results show that the air temperatures were close to or slightly higher than those established for a multiple-year period, with no significant deviations from the crop requirements.
The differences between the three years of the study were established in the amount of rainfall during vegetation. Rainfalls in autumn and during the critical spring period are decisive for the development of the wheat plants. The mean annual precipitation sums during October - March, which formed the autumn-and-winter moisture reserves in soil during the experimental years 2015-2016, 2016-2017 and 2017-2018 were higher with 61, 101 and 7 mm, respectively, than the mean sums of the long - term period. During April-May when plants were at stages booting and heading, the mean annual precipitation sum in 2016 and 2017 was lower than the mean long - term value, while in 2018 this sum was higher with 119 mm. In June and July (during grain filling-maturation) rainfalls in harvest year 2018 was higher with 62 mm, in comparison to the long - term period, while in 2016 and 2017 they were with 44.3 and 14.0 mm, respectively lower.
The most favourable for plant growth and development was the third experimental year (2017-2018), followed by the second (2016-2017), and unfavourable was the first year (2015-2016), of the experiment, having an effect on yield and grain quality of common wheat.

RESULTS AND DISCUSSIONS

Data about the grain yield (Table 1) show that ‘Avenue’ cultivar surpassed the other cultivars included in the experiment, when reporting the harvest by years and in average for the period of study.
In result of the better moisture provision of plants during the vegetation period and in the period of grain formation and ripening, higher yields were obtained in 2017-2018 compared to 2015-2016 and 2016-2017.
Under the conditions of that season they varied from 5000 kg/ha for ‘Neven’ cultivar to 8400 kg/ha for ‘Avenue’. The cultivars ‘Miryana’, ‘Ekzotik’ and ‘Anapurna’ yielded by 2900, 2300 and 200 kg/ha less than ‘Avenue’ and by 500, 1100 and 3200 kg/ha more than ‘Neven’, respectively. All the differences were statistically significant.
The lowest grain yields during the studied period were reported in the first year of the experiment (2015-2016) and it was due to the insufficiency of moisture during the critical stages of growth and development of the wheat plants. In that season the lowest yield was obtained from ‘Neven’ cultivar - 3000 kg/ha, which was by 16.7% less compared to ‘Miryana’, by 83.3% less compared to ‘Ekzotik’, by 190% less compared to ‘Anapurna’ and by 200% less compared to “Avenue’ cultivar.

Table 1. Grain yield - kg/ha

Cultivar	Years of study			Average for the period (kg/ha)
	2015-2016 (kg/ha)	2016-2017 (kg/ha)	2017-2018 (kg/ha)	
Ekzotik	5600 [c]	6500 [c]	6200 [c]	6100
Miryana	3600 [b]	4600 [b]	5600 [b]	4600
Avenue	6100 [e]	7600 [e]	8500 [e]	7400
Anapurna	5800 [d]	7200 [d]	8300 [d]	7100
Neven	3100 [a]	4100 [a]	5000 [a]	4067

*Means within columns followed by different lowercase letters are significantly different ($P < 0.05$) according to the LSD test

In 2016-2017 the yields obtained ranged from 4000 kg/ha to 7500 kg/ha. The differences between the studied cultivars were statistically significant. The grain yield during the second experimental year was by 24.5% higher in average than in 2015-2016 and by 12.5% lower than in 2017-2018.

The highest grain yield, in average for the three years of the study, was reported for ‘Avenue’ cultivar - 7300 kg/ha, surpassing the cultivars ‘Anapurna’, ‘Ekzotik’, ‘Miryana’ and ‘Neven’ by 4.2; 16.7; 55.6 and 75.0%, respectively.

The results of the dispersion analysis about the effect of the factors Cultivar and Year, as well as their interaction, on the indicator grain yield, (Table 2) show a statistically significant effect of the studied factors.

Table 2. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Year**	32980870	2	16490435	386.7212	0.00	3.204317
Cultivar**	111922206	4	27980552	656.1787	0.00	2.578739
Interaction**	5429813.3	8	678726.7	15.91698	0.00	2.152133
Within	1918875	45	42641.67			

*F - test significant at $P < 0.05$; **F - test significant at $P < 0.01$; ns - non significant.

Interactions between the studied factors were also significant for the grain yield.

The thousand kernel (grain) weight is a cultivar specific trait influenced by the agroecological conditions and the growing technology.

The results show that thanks (Table 3) to the favourable climatic conditions during the wheat vegetation period in the third year, the values of that characteristic were significantly higher compared to the other experimental years.

Table 3. Thousand kernel (grain) weight, g

Variety	Years of study			Average for the period (2015-2018)
	2015-2016	2016-2017	2017-2018	
Ekzotik	46.0 [d]	49.0 [c]	48.0 [c]	47.6
Miryana	48.0 [e]	51.0 [c]	52.0 [d]	50.3
Avenue	36.0 [b]	39.0 [b]	43.0 [b]	39.3
Anapurna	31.0 [a]	33.0 [a]	39.0 [a]	34.3
Neven	41.0 [c]	43.0 [b]	44.0 [b]	42.7

*Means within columns followed by different lowercase letters are significantly different ($P < 0.05$) according to the LSD test

In 2017-2018 ‘Miryana’ cultivar produced the largest grains (52.0 g of 1000 grains), followed by ‘Ekzotik’ (48.0 g), while ‘Anapurna’ had the smallest grains (39.0 g). The differences between the cultivars were statistically significant. The thousand kernel (grain) weight of ‘Avenue’ and ‘Neven’ cultivars had similar values (43.0 and 44.0 g, respectively) and the difference was statistically insignificant.

Drought weather combined with high air temperatures at the stage of grain formation and ripening in 2015-2016 had an effect on grain weight.

The lowest weight of thousand kernel (grain) was reported for ‘Anapurna’ cultivar (31.0 g). The cultivars ‘Miryana’ and ‘Ekzotik’ surpassed in weight of thousand kernel (grain) the cultivars ‘Avenue’ and ‘Neven’ by 30.5% and 17.75%, respectively, the differences being significant.

The largest grains in average for the period 2015-2018 were reported for ‘Miryana’ cultivar (50.3 g), followed by the cultivars ‘Ekzotik’ (46.3 g), ‘Neven’ (41.7 g) and ‘Avenue’ (39.3 g). The lowest values of that characteristic were established for ‘Anapurna’ cultivar.

The data of the dispersion analysis about the influence of the factors and their interaction on the thousand kernel (grain), are presented in Table 4. The results show clear statistically significant variations and the interaction between the two factors was statistically insignificant.

Table 4. Analysis of variance ANOVA

Source of Variation	Sum of Square	Df	Mean Square	F	P-value	F crit
Year**	166.2333	2	83.11667	11.24887	0.00	3.204317
Cultivar**	2375.233	4	593.8083	80.36504	0.00	2.578739
Interaction^{ns}	43.76667	8	5.470833	0.740414	0.66	2.152133
Within	332.5	45	7.388889			

*F - test significant at P < 0.05; **F - test significant at P < 0.01; ns - non significant.

Test weight is a commercial indicator showing grain quality and it plays an important role in determining the market price. That characteristic of the studied cultivars in the years of the experiment varied from 69.0 to 82.0 kg (Table 5).

Table 5. Test weight, kg

Variety	Years of study			Average for the period (2015-2018)
	2015-2016	2016-2017	2017-2018	
Ekzotik	77.0 ^{b}	79.0 ^{b}	80.0 ^{b}	78.7
Miryana	80.0 ^{c}	81.0 ^{c}	82.0 ^{c}	81.0
Avenue	78.0 ^{b}	79.0 ^{b}	80.0 ^{b}	79.0
Anapurna	72.0 ^{a}	74.0 ^{a}	79.0 ^{b}	75.0
Neven	69.0 ^{a}	73.0 ^{a}	78.0 ^{a}	73.3

*Means within columns followed by different lowercase letters are significantly different (P < 0.05) according to the LSD test.

The test weight of the cultivars 'Ekzotik', 'Avenue' and 'Miryana' had similar values in all the three years of the study, which is an evidence that for those cultivars, the characteristic is slightly influenced by the climatic conditions in the separate years and it depends more on the cultivar

In contrast to 'Ekzotik', 'Avenue' and 'Miryana', the test weight of the cultivars 'Anapurna' and 'Neven' is significantly influenced by the climatic conditions and less by the cultivar.

The highest test weight of wheat grain, in average for the period of the study, was reported for 'Miryana' cultivar (81.0 kg.), followed by 'Avenue' and 'Ekzotik' (79.0 and 78.7 kg, respectively), and the lowest values were reported for the cultivars 'Anapurna' and 'Neven' (75.0 and 73.3 kg, respectively).

The results of the dispersion analysis about the effect of the factors Cultivar and Year, as well as their interaction, on the indicator test weight, (Table 6) show a statistically significant effect of the studied factors.

Table 6. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Year**	185.4333	2	92.71667	49.81791	0.00	3.204317
Cultivar**	436.2667	4	109.0667	58.60299	0.00	2.578739
Interaction**	68.73333	8	8.591667	4.616418	0.00	2.152133
Within	83.75	45	1.861111			

*F - test significant at P < 0.05; ** F - test significant at P < 0.01; ns - non significant.

The data obtained about the wet gluten content showed that in the studied cultivars, this characteristic was greatly influenced by the production year (Table 7).

In the first year of the experiment the lowest values of that characteristic were reported for the cultivars 'Neven' (22.0%) and 'Miryana' (23.0%), while the highest values of that characteristic for the same two cultivars were established in the third experimental year (30.0 and 28.0%, respectively).

Table 7. Wet gluten, %

Variety	Years of study			Average for the period (2015-2018)
	2015-2016	2016-2017	2017-2018	
Ekzotik	22.0 ^{a}	29.0 ^{b}	26.0 ^{b}	25.6
Miryana	23.0 ^{a}	25.0 ^{a}	28.0 ^{c}	25.3
Avenue	21.0 ^{a}	25.0 ^{a}	20.0 ^{a}	22.0
Anapurna	26.0 ^{b}	30.0 ^{b}	25.0 ^{b}	27.0
Neven	22.0 ^{a}	26.0 ^{a}	30.0 ^{c}	26.0

*Means within columns followed by different lowercase letters are significantly different (P < 0.05) according to the LSD test.

In 2018 a comparatively low wet gluten content was reported for 'Avenue' cultivar (14.0%), i.e. the significant amount of rainfall at the ripening stage led to washing out of the wet gluten, while in 'Neven' cultivar, rains caused an increase of the values of that characteristic (30.0%).
In the second experimental year (2016-2017), the wet gluten content in the cultivars 'Miryana', 'Neven' and 'Avenue' varied from 25.0 to 27.0%, the differences being slight and statistically insignificant, while the cultivars 'Ekzotik' and 'Anapurna' produced grains with 11.5% higher wet gluten content.
The highest wet gluten content, in average for the whole study period 2015-2018, was established in 'Anapurna' cultivar (27.0%) and the lowest - in 'Avenue' cultivar (22.0%).
The dispersion analysis about the effect of the factors Variant and Year, as well as their interaction, on the wet gluten content, shows a significant influence of the factors on the changes of the characteristic and statistically insignificant effect of the interaction between them (Table 8).

Table 8. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Year*	217.2	2	108.6	20.92934	0.00	3.204317
Cultivar*	213.1667	4	53.29167	10.27034	0.00	2.578739
Interaction*	299.1333	8	37.39167	7.206103	0.00	2.152133
Within	233.5	45	5.188889			

*F - test significant at P < 0.05; **F - test significant at P < 0.01; ns - non significant

Table 9. Values of the coefficient of correlation

	Grain yield - kg/ha	Thousand kernel (grain) weight, g	Test weight, kg	Wet gluten, %
Grain yield - kg/ha	1.000			
Thousand kernel (grain) weight, g	-0.314	1.000		
Test weight, kg	0.360	0.614	1.000	
Wet gluten, %	0.029	0.051	0.029	1.000

As a result of the correlation analysis between the grain yield and quality indicators (Table 9), a high positive values of correlation (r > 0.6) were reported for thousand kernel (grain) weight and test weight.
Mean correlation was found between grain yield and test weight (r = 0.360). Weak correlation (r < 0.3) was observed between the wet gluten and all the other indicators. A negative correlation was reported for grain yield and thousand kernel (grain) weight (r = -0.314).

CONCLUSIONS

The highest grain yield, in average for the experimental period 2015-2018, under the climatic conditions of South Central Bulgaria, was obtained from 'Avenue' cultivar (7300 kg/ha), followed by 'Anapurna' (7000 kg/ha), and, the lowest one - from 'Neven' cultivar (4000 kg/ha).
'Avenue' cultivar produced by 300; 1300; 2800 and 3300 kg/ha higher grain yield than the cultivars 'Anapurna', 'Ekzotik', 'Miryana' and 'Neven', respectively.
Among the studied common wheat cultivars, the highest values of the thousand kernel (grain) weight and the test weight were reported for 'Miryana' (50.3 g and 81.0 kg, respectively) and the highest wet gluten content was established in 'Anapurna' cultivar (27.0%).
The lowest value of the test weight was reported for 'Neven' cultivar (73.3 kg); of the thousand kernel (grain) weight - for 'Anapurna' cultivar (34.3 g) and of the wet gluten content – for 'Avenue' cultivar (22.0%).

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THE DYNAMICS OF THE GREEN BIOMASS AND HER RELATIONSHIP WITH THE YIELD, ACCORDING TO HYBRID AND PLANT DENSITY AT AN ASSORTMENT OF SUNFLOWER PLANT, TESTED ON THE CHERNOZEM OF CARACAL

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Abstract

On the ARDS Caracal chernozem, in the last two years, 2018 and 2019, were tested a number of nine sunflower hybrids (Performer, Euromis, Generalis, Terramis, Neoma, Diamantis, Subaro, FD15C27 and FD116M1) sown at three different plant density: 43,000 pl/ha, 57,000 pl/ha and 71,000 pl/ha. The green biomass was determined at 3 different moments: at 44 days, 54 days and 68 days after emergence. The interactions studied showed that the hybrids have a vegetative growth (green biomass) differentiated according to the plant density, hybrid and the moment of determination. As much as the plant density increases, the biomass decreases very significantly. Also, green biomass, regardless of the time of determination, is significantly correlated with production. There are very significant differences between the green biomass determined at the first and the second plant density, at the first and the third plant density, but disappears between the second and the third plant density, at each moments of determination.

***Key words**: sunflower, green biomass, plant density, time of determination, chernozem.*

INTRODUCTION

Helianthus annuus L. (sunflower) is one of the 67 species of Helianthus (from the Greek "helios" - sun; "anthus" - flower). Of these, only two species, *H. annuus* and *H. tuberosum*, are cultivated for food, remaining species being ornamentals species, weeds or wild plants. Originally from the United States and Mexico, the sunflower was introduced in Europe in the 16th century, initially in Spain and later in England and France (Azania et al., 2008).
Sunflower (*Helianthus annuus* L.) is an important oil crop which provides more than 13% of the total amount of oil produced globally (Rauf S., 2008).
The sunflower presents one of the most profitable crops for agriculture, which determines significant growth of the sown areas and increased market demands regarding the created hybrids. The breeding of competitive commercial sunflower hybrids for the European seed market is achievable by diversifying the sunflower germplasm. A major importance in the improvement process is the knowledge of the development phases and the analysis of the transformations that the plants suffers during each phenological phase, determined by a complex of interactions between the genetic and environmental factors, such as temperature, photoperiodicity, rainfall quantity.
The phenological data analysis allowed to highlight the irregularity of the growth and development process and a different speed of interphase growth genotype dependent (vegetative growth - from seedling to button appears; reproductive growth - from button appears to flowering; maturing stage - from flowering to full maturation) (Cucereavîi, 2017).
Detailed research on sunflower biomass was conducted by Tingyue (2013) in order to improve its yield and quality as an alternative to the production and implementation of new energies.
Daugthry et al (1992) showed that the green biomass can be estimated by spectrometry. Wanjura and Hartfield (1985) found that a linear combination between NIR and red ratio was correlated with biomass by the ratio of

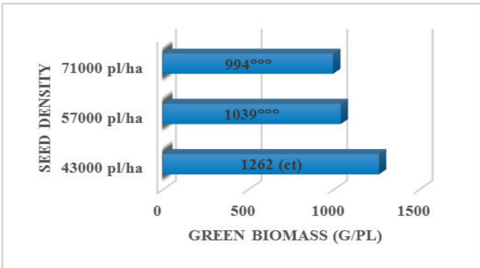
biomass = a + b (NIR/red). At sunflower a = 0.24 and b = 0.69 compared to soybean where a = 0 and b = 1.02.
Serrano et al. (2000) found a strong correlation between biomass and harvested yield.

MATERIALS AND METHODS

On the ARDS Caracal chernozem, in the last two years, 2018 and 2019, were tested a number of nine sunflower hybrids (Performer, Euromis, Generalis, Terramis, Neoma, Diamantis, Subaro, FD15C27 and FD116M1) sown at three different plant density: 43,000 pl/ha, 57,000 pl/ha and 71,000 pl/ha. The green biomass was determined at 3 different moments: at 44 days, 54 days and 68 days after emergence.
The plots were placed in randomized blocks, in 3 repetitions. The length of the plot was 10 m and the width was 2.8 m, the equivalent of 4 rows, two of them, the marginal rows, being eliminated.
The first step in the preparing the land was to make the ploughting at a depth between 25 and 30 cm. In the spring the works continued with the soil disking in 2 passes, process executed using a heavy disc. The last work in preparing the seedbed was performed using a combiner before sowing the sunflower crop.
Fertilization was administered manually in the NPK form, before sowing, and the nitrogen difference was applied in vegetation under the type of ammonium nitrate
In 2018 the sowing was done on 23.04.2018 and the emergence took place on 8.05.2018. In 2019 the sowing was done on 22.04.2019 and the emergence took place on 6.05.2019.
After sowing, the land was pre-emergent herbicidated with Dual Gold 960 EC herbicide (active substance S-metolachlor 960 g/l) in a dose of 1.2 l/ha.
At the stage of 4-6 leaves of the sunflower crop, Select Super 120 EC herbicide (active substance Clethodim 120 g/l) for the annual and perennial monocotyledon weeds was used in a dose of 1.5 l/ha. In the 8-10 leaf phase, a treatment made of 0.6 l/ha Reveller fungicide (active substances Cyproconazole 80 g/l + Picoxystrobin 200 g/l) and 0.15 l/ha Decis Mega 50 EW insecticide (active substance Deltamethrin 50 g/l) was applied to prevent foliar diseases and the attack of pests.
The green biomass was determined at 3 plants from the marginal rows, entirely removed, including roots, at each variant and repetition. The plants were immediately chopped and weighed and then were used to determine the analysis at three different moments in dynamics: at 44, 54 and 68 days after emergence. Between time 1 (T1) and time 2 (T2) the interval was 10 days and between time 2 (T2) and time 3 (T3) the interval was 14 days. The biomass was then reported in g/pl. The harvested production from the surface of 10 square meters has been reported to the number of plants on the same surface, resulting the yield per plant reported in g/pl.

RESULTS AND DISCUSSIONS

The density at sunflower influences the green biomass, it being very significantly lower at 57,000 pl/ha and 71,000 pl/ha plant density compared to 43,000 pl/ha. The influence of density was analysed without taking into account the hybrid and the time of determination (Figure 1).

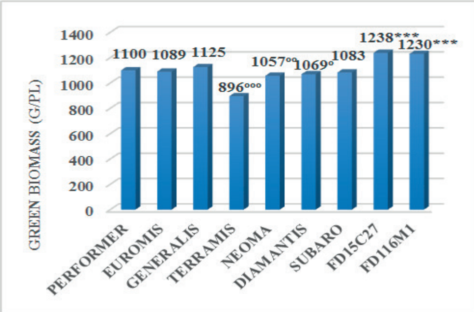


DL 5% = **29 g/pl**; DL 1% = **48 g/pl**; DL 0.1% = **89 g/pl**

Figure 1. Plant density influence on green biomass at sunflower

The green biomass has oscillated to the tested hybrids, irrespective of density and time of determination, between 1,238 g/pl in the Romanian hybrid FD15C27 and 896 g/pl in the hybrid Terramis. Compared to the Performer hybrid, the most wide-spread Romanian hybrid in the country, the FD15C27 and FD116M1 Romanian hybrids are very significantly higher in terms of green biomass. In contrast, the Terramis, Neoma and Diamantis hybrids have a lower vegetative mass with statistical assurance compared to the control hybrid (Figure 2).
Similar results which show that biomass production depends on the hybrid but also on

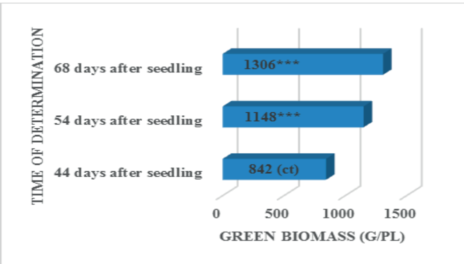
the level of water supply, were obtained by other researchers (Merrien, 1992; Rodriguez et al., 2002).



DL 5% = **26 g/pl**; DL 1% = **35 g/pl**; DL 0.1% = **45 g/pl**

Figure 2. Hybrid influence on green biomass at sunflower

Depending on the time of determination, the results obtained suggest that the green biomass increases very significantly from the first time of the determination (44 days after emergence) to the second time (54 days after emergence) and from the first time of determination (44 days after emergence) to the third time (68 days after emergence). In Caracal conditions it is observed that the biomass grows in the first 10 days from emergence much faster than it grows in the next 14 days (Figure 3).



DL 5% = **45 g/pl**; DL 1% = **60 g/pl**; DL 0.1% = **79 g/pl**

Figure 3. Time of determination influence on green biomass at sunflower

Similar results were presented by Moriondo et al. (2003) who observed that the accumulation of biomass in the sunflower decreases especially in pre-anthesis.

Interaction between plant density, hybrid and the time of determination shows that, at all three density, all hybrids have a very significantly increased biomass at time 2 versus time 1 and at time 3 versus time 1, but with a few exceptions.

If, in a rare crop, this tendency is uniformly maintained, at a density of 57,000 pl/ha and 71,000 pl/ha, the Performer hybrid records a decrease in vegetative mass at the time 2 of the determination in relation to the first one, which suggests that this hybrid does not grow up well under the conditions of a dense crop.

At the density of 57,000 pl/ha, this decrease is even very significantly lower. It is not excluded that this hybrid also has a deficiency because of the applied herbicide which, under high density conditions, acts as a growth inhibitor (Table 1).

At a density of 71,000 pl/ha the hybrid FD15C27 did not showed an increase of green biomass between 44 and 54 days after emergence.

A smaller but still significant increase has recorded the Diamantis hybrid at the density of 57,000 pl/ha between the time 1 and time 2 of determination, the conclusion being that this hybrid has a more pronounced growth over the 54 days after emergence.

The relationship between the 10 days vegetative growth interval (T2-T1) and respectively 14 days (T3-T2) is negative and it is not significant. Only about 5% of growth variability in the first 10 days is associated with vegetative growth in the next 14 days.

Starting from the green biomass value of 182.21 g/pl, an increase in vegetative mass by 1 g/pl in the first 10 days interval is associated with a decrease with 0.0793 g/pl in the following 14 days (Figure 4).

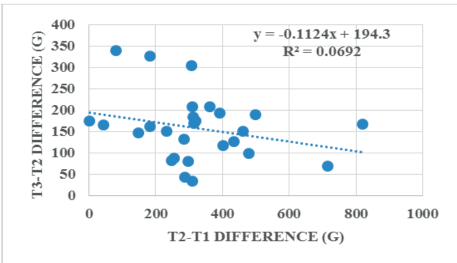


Figure 4. The relationship between the 10 days vegetative growth interval (T2-T1) and respectively 14 days (T3-T2)

However, the hybrids are distinguished from each other also according to their density. The vegetative biomass average between 44 and 54 days after emergence is 305 g/pl and the green biomass average between 54 and 68 days after emergence is 158 g/pl.

Thus, under the cultivation conditions from Caracal, the Generalis hybrid had an above-average increase in both intervals studied at all plant densities. The Diamantis hybrid had an above-average increase only at the 43,000 pl/ha and 71,000 pl/ha plant densities and the Subaro hybrid at 57,000 pl/ha and 71,000 pl/ha plant densities. Growth above-average also registered Euromis hybrid but only at the plant density of 43,000 pl/ha and the FD116M1 hybrid at 71,000 pl/ha plant density.

Table 1. Interaction between plant density, hybrid and the time of determination and its influence on green biomass at sunflower

HYBRIDS	TIME OF DETERMI NATION	43,000 pl/ha			57,000 pl/ha			71,000 pl/ha		
		GREEN BIOMASS (G/PL)	DIF. CT (G)	SIGNIFI CANCE	GREEN BIOMASS (G/PL)	DIF. CT (G)	SIGNIFI CANCE	GREEN BIOMASS (G/PL)	DIF. CT (G)	SIGNIFI CANCE
	44 d.a.s.*	**1,150**	ct		**1,029**	ct		**964**	ct	
PERFORMER	**54 d.a.s.**	**1,405**	255	***	**806**	-223	ooo	**961**	-3	
	68 d.a.s.	**1,492**	342	***	**955**	-74		**1,135**	171	***
	44 d.a.s.*	**768**	ct		**654**	ct		**738**	ct	
EURAMIS	**54 d.a.s.**	**1,078**	310	***	**1,370**	716	***	**1,172**	434	***
	68 d.a.s.	**1,286**	518	***	**1,439**	785	***	**1,298**	560	***
	44 d.a.s.*	**719**	ct		**724**	ct		**792**	ct	
GENERALIS	**54 d.a.s.**	**1,539**	820	***	**1,041**	317	***	**1,112**	320	***
	68 d.a.s.	**1,706**	987	***	**1,210**	486	***	**1,286**	494	***
	44 d.a.s.*	**823**	ct		**701**	ct		**539**	ct	
TERRAMIS	**54 d.a.s.**	**1,121**	298	***	**949**	248	***	**827**	288	***
	68 d.a.s.	**1,201**	378	***	**1,030**	329	***	**869**	330	***
	44 d.a.s.*	**1,102**	ct		**749**	ct		**762**	ct	
NEOMA	**54 d.a.s.**	**1,252**	150	***	**1,035**	286	***	**945**	183	***
	68 d.a.s.	**1,398**	296	***	**1,166**	417	***	**1,106**	344	***
	44 d.a.s.*	**930**	ct		**878**	ct		**614**	ct	
DIAMANTIS	**54 d.a.s.**	**1,292**	362	***	**959**	81	*	**922**	308	***
	68 d.a.s.	**1,499**	569	***	**1,297**	419	***	**1,226**	612	***
	44 d.a.s.*	**934**	ct		**648**	ct		**693**	ct	
SUBARO	**54 d.a.s.**	**1,336**	402	***	**961**	313	***	**1,194**	501	***
	68 d.a.s.	**1,453**	519	***	**1,145**	497	***	**1,383**	690	***
	44 d.a.s.*	**1,049**	ct		**1,098**	ct		**911**	ct	
FD15C27	**54 d.a.s.**	**1,528**	479	***	**1,409**	311	***	**956**	45	
	68 d.a.s.	**1,626**	577	***	**1,443**	345	***	**1,121**	210	***
	44 d.a.s.*	**1,105**	ct		**887**	ct		**781**	ct	
FD116M1	**54 d.a.s.**	**1,567**	462	***	**1,072**	185	***	**1,174**	393	***
	68 d.a.s.	**1,717**	612	***	**1,398**	511	***	**1,367**	586	***

d.a.s.* = days after seedling
DL 5% = 78 g/pl; DL 1% = 103 g/pl; DL 0.1% = 133 g/pl

The second category contains hybrids who had increases below average at both intervals. Thus, the Terramis hybrid highlighted this aspect at all plant densities, while the Neoma and Performer hybrids only at the first and second plant densities.

The hybrids with a significant increase within the 10 days interval and then a decrease within the 14 days interval, depending on the average, were: the Euromis hybrid at the second and third density, the FD15C27 hybrid at the first two densities and the Subaro and FD116M1 hybrids at 43,000 pl/ha.

The last category includes hybrids that had a smaller growth within the 10 days interval and then a more pronounced growth within the next 14 days interval. These hybrids (Neoma, Diamantis, Performer, FD15C27, FD116M)

show this aspect at at one single density, either 57,000 pl/ha or 71,000 pl/ha.
The ratio between the biomass growth within the 14 days interval and the biomass growth within the 10 days interval, showed that the Diamantis and FD116M1 hybrids, at 57,000 pl/ha plant density, and the Performer and FD15C27 hybrids, at 71,000 pl/ha plant density, grow much faster between 54 and 68 days after seedling, the growth being 2 to 4 times higher.
For the rest of the hybrids, at all densities, and even for those hybrids mentioned above, but at other densities than those recorded, the growth is stronger within the 44-54 days interval after emergence.
The correlation between the aquenes production obtained at the sunflower and the green biomass, both expressed in g/pl, is a positive and significant correlation.
As the density increases, the production and the green biomass decrease, as shown in the figure below, taking as landmark the control hybrid Performer (Figure 5).

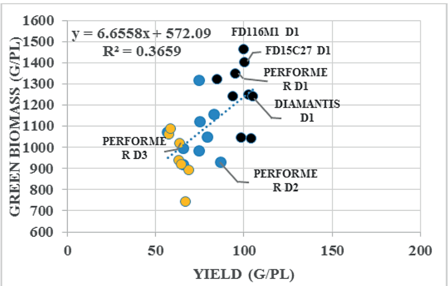


Figure 5. The relationship between production and green biomass at sunflower, without the influence of the time of determination

Published studies have shown that the biomass and the harvest index were strongly correlated with the amount of radiation intercepted during the filling of the grain, which, in turn, were correlated with the duration of the grain filling and the surface of the green leaf at sunflower (Vega and Hall, 2002).

CONCLUSIONS

The density at sunflower influences the green biomass of the sunflower plant, it being very significantly lower at 57,000 pl/ha and 71,000 pl/ha plant density compared to 43,000 pl/ha.

Compared to the Performer hybrid, the most wide-spread Romanian hybrid in the country, the FD15C27 and FD116M1 Romanian hybrids are very significantly higher in terms of green biomass. In contrast, the Terramis, Neoma and Diamantis hybrids have a lower vegetative mass with statistical assurance compared to the control hybrid.
Depending on the time of determination, the results obtained suggest that the green biomass increases very significantly from the first time of the determination (44 days after emergence) to the second time (54 days after emergence) and from the first time of determination (44 days after emergence) to the third time (68 days after emergence).
In Caracal conditions it is observed that the biomass grows in the first 10 days from emergence much faster than it grows in the next 14 days.
Under the cultivation conditions from Caracal, the Generalis hybrid had an above-average increase in both intervals studied at all plant densities. The Diamantis hybrid had an above-average increase only at the 43,000 pl/ha and 71,000 pl/ha plant densities and the Subaro hybrid at 57,000 pl/ha and 71,000 pl/ha plant densities. Growth above-average also registered Euromis hybrid but only at the plant density of 43,000 pl/ha and the FD116M1 hybrid at 71,000 pl/ha plant density.
Hybrids that had increases below average at both intervals were: the Terramis hybrid at all plant densities and the Neoma and Performer hybrids only at the first and second plant densities.
The relationship between the 10 days vegetative growth interval (T2-T1) and respectively 14 days (T3-T2) is negative and it is not significant. Only about 5% of growth variability in the first 10 days is associated with vegetative growth in the next 14 days.

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INFLUENCE OF THE VARIETY AND FERTILIZATION ON THE PRODUCTIVITY OF GRAIN SORGHUM IN MONOCULTURE CONDITIONS

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Abstract

Three grain sorghum varieties have been tested in field trials during the period 2017-2018 with variants of beet and sorghum (monoculture) forerunner crops, and mineral Nitrogen fertilization. The agro-climatic conditions are defining factor for the grain yield and the differences between the varieties in their reaction to fertilization and monoculture growing manifest themselves in the water deficiency conditions of the test period. The Nitrogen fertilization increases the average values of the yield to 113.0% when the applied dose is 20 kg N/da, and to 120.8% when 40 kg/da is the applied Nitrogen dose. The increase of the water deficit decreases the effects of mineralization and the influence of the forerunner crop. The yield of sorghum when grown in monoculture is with 11.6% less than the yield after good forerunner crop as the beet. The Nitrogen fertilization, even with lower doses of Nitrogen overcomes this negative effect. The variety Maxibel manifests the highest adaptability to the stress factors of monoculture growing and reacts with significant increase of the grain yield to fertilization.

Key words: *sorghum, monoculture, fertilization, productivity.*

INTRODUCTION

The optimization of effective crop rotation is a basic factor for the intensive and sustainable agriculture in the conditions of the new economic realities and the tendency for increase of the extremal deviations from the agro-meteorological norms (Vasilev, 1986).
The unique plasticity and drought resistance of sorghum makes actual its place in the agricultural crops structure. A basic factor for the drought resistance is its powerful root system, which absorbs intensively water and nutrition elements even from the deepest soil horizons (Tanchev, 1996).
The breeding of new varieties, the balanced fertilization and the optimization of the modern agro-technics disprove the old standpoint that sorghum is a bad forerunner crop. Thus the potential for ensuring the forage balance is increased in the agriculture in conditions of water deficiency (Hanssmans, 1998; Berenji and Dahlberg, 2004; Kikindonov and Slanev, 2008; Kikindonov et al., 2009).
The intensive market character of the agricultural practice in Bulgaria is concentrated in the narrow parameters of grain production, which brings the necessity of studying the possibilities of monoculture growing of crops. The researches with sorghum started in the 80-s of the last century and are concentrated on the agrochemical sequels from the sorghum as a forerunner crop (Zarkov, 1995; Ran et al., 2009). In the last years the accent is on breeding decisions by selection of forms with increased self-sustainability.
The aim of the study is to assess the genotypic reaction in terms of productivity of sorghum varieties when grown in conditions of monoculture compared to grown in optimal crop rotation with a beet forerunner crop.

MATERIALS AND METHODS

The research was conducted in 2017-2018 on the experimental field of Agricultural Institute - Shumen. The soil is carbonate black soil with weekly alkaline reaction.
In the tests are included the early variety Alise of Euralis Semences, the medium-late variety Maxired and the late variety Maxibel from the list of the Agricultural Institute.
The experimental field is part of a crop rotation wheat/corn/oats/beet/sorghum, and part of the sorghum area is maintained in monoculture. The experiment is conducted according to the

long plots method, in 4 repetitions, with a 3-rows plot of 12 m^2, with 50 cm inter-row space and a sowing rate of 30000 plants per da.

The sowing is at the beginning of May, and the fertilization is made after germination together with a manual hilling. The harvestings in technical maturity stage are made by hand at the end of October. After the threshing of the grain the moisture is measured and the yield is calculated for 13% moisture of grain.

Data are treated by dispersion analysis for calculating the limit values for discernment GD 1% and experimental exactness P%. The control for each variety is the non-fertilized variant after beet forerunner. For determination of the main action of the factors is used dispersion analysis with averaging of values for each variant.

RESULTS AND DISCUSSIONS

The agro-meteorological conditions in years of research are characterized with extreme and continuous drought in the active period of sorghum vegetation during July-August. This opens possibility of manifestation of sorghum as a drought resistant crop and to limit the genotype's influence in the conditions of water deficiency and monoculture. In 2018 is registered a greater moisture retention from the rainfalls in June.

The results for the yield (Table 1) are strongly affected by the extreme drought during the vegetation of sorghum (end of May to September). The yield of the control non-fertilized variants after beet forerunner is 667 kg/da for the late Maxibel, 705 kg/da for the mean-late variety Maxired and 810 kg/da for the early variety Alise. The effect of fertilization is the strongest and statistically proved for the higher fertilizer dose, and for Maxired it reaches 127% of the control. In conditions of monoculture the yields decrease significantly, as the mean-early variety Maxired is the most strongly affected variety – 84% of the control variant. Even the lower dose of 20 kg N/da compensates the negative influence of the forerunner. Maxired is with the strongest reaction to fertilization, no matter the forerunner crop. Comparatively weaker is the reaction of the early Alise, and this affects the yields from the variants grown in monoculture.

Table 1. Influence of the variety, fertilization and the forerunner crop on the yield of grain sorghum, 2017

VARIETY	**Variant**	**Beet forerunner**		**Sorghum forerunner - мonoculture**	
		kg/da	**Rel. %**	**kg/da**	**Rel. %**
MAXIBEL	**Control**	**667**	**100.0**	639	95.8
	+ 20 kg N	703	105.4	685	102.7
	+ 40 kg N	767	114.9	732	109.7
MAXIRED	**Control**	**705**	**100.0**	593	84.1
	+ 20 kg N	769	109.1	718	101.8
	+ 40 kg N	897	127.2	805	114.2
ALISE	**Control**	**810**	**100.0**	718	88.6
	+ 20 kg N	907	111.9	745	91.9
	+ 40 kg N	918	113.3	875	108.0
GD 1%	**97.1 kg/da - 7.8%**				
P %	**3.10%**				

The better moisture retention in 2018 reflects in the comparatively higher grain yields (Table 2). The differences between the control variants with beet forerunner are not proved statistically and vary from 815 to 855 kg/da. The effect of fertilization is high, but with small differences for the fertilization dose. The levels of monoculture influence remain between 82% and 88% of the control.

The late variety Maxibel manifests the strongest reaction to fertilization. The earlier varieties Maxired and Alise form equal to control variants' yield when the higher dose of Nitrogen fertilizer (40 kg) is applied.

On Table 3 are given the results of the dispersion analysis of the averaged data for determining the main action of the studied factors. The agro-meteorological conditions are defining factor for the grain yield. In the water deficiency conditions of the test years the differences between the varieties are manifested depending on their early maturity. In the comparatively damper 2018 the differences significantly increase, and the high productivity of the late Maxibel shows itself.

The fertilization significantly increases the averaged values of yield - mean of 113.1% for the 20 kg dose, and 120.8 - for the higher dose of 40 kg N/da. The differences between the varieties regarding their reaction to fertilization are in narrow frames. More significant, but not proved statistically, is that difference for the Maxibel variety.

The averaged effect of monoculture growing of sorghum is a 11.6% decrease of the yield compared to the variants with a beet forerunner crop.

Table 2. Influence of the variety, fertilization and the forerunner crop on the yield of grain sorghum, 2018

Variety	Variant	Beet forerunner		Sorghum forerunner - мonoculture	
		kg/ da	rel. %	kg/ da	Rel. %
Maxibel	Control	**852**	**100.0**	736	86.4
	+ 20 kg N	1056	123.9	916	107.5
	+ 40 kg N	1080	126.8	948	111.3
Maxired	Control	**815**	**100.0**	724	88.8
	+ 20 kg N	906	111.2	781	95.8
	+ 40 kg N	917	112.5	826	101.3
Alise	Control	**855**	**100.0**	704	82.3
	+ 20 kg N	970	113.5	809	94.6
	+ 40 kg N	1032	120.7	859	100.5
GD 1%	**159 kg/da - 18.4%**				
P %	**7.43 %**				

Table 3. Main action of factors variety, year, fertilization and forerunner on the yield of grain sorghum, 2017-2018

Year		Fertilization with N			Forerunner crop		Mean kg/da
2017 kg/da	2018 kg/da	Control kg/da	+ 20 kg N Rel. %	+ 40 kg N Rel. %	Beet kg/da	Sorghum Rel. %	
Maxibel variety							
699	931	724	116.0	121.8	854	90.9	815
Maxired variety							
747	828	709	111.9	121.4	834	88.8	788
Alse variety							
829	872	772	111.1	119.3	915	85.8	850
Average							
758	877	735	113.1	120.8	868	88.4	818
GD 1% - 56.4 kg/da, 6.94%							
P% - 3.77%							

CONCLUSIONS

The agro-meteorological conditions are defining factor for the grain yield and in water deficiency conditions the differences between the varieties are manifested in their reaction to fertilization and monoculture growing. The averaged values show increase of the yield to 113.0% for the 20 kg of nitrogen and to 120.8% for the 40 kg doses of nitrogen fertilizer application. The increase of the water deficit decreases the effects of fertilization and forerunner crop's influence.

The decrease of yield of monoculture growing is on the average of 11.6% in comparison with the yield after beet forerunner crop. The fertilization with even smaller doses of nitrogen overcomes this negative effect. The variety Maxibel manifests itself with the highest adaptability to the stress factors of monoculture growing, reacting very positively to fertilization.

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CORRELATION DEPENDENCE BETWEEN BIOMETRIC INDICATORS AND PRODUCTIVITY IN THREE COTTON VARIETIES

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Abstract

The purpose of the study is to evaluate, by means of correlation analysis, the correlation between the main biometric indicators for three cotton varieties. The study was conducted in the field of experience of the Department of Crop Production, Faculty of Agriculture at the University of Trakia, Stara Zagora during the period 2018-2019. The field experience is derived by the method of fractional plots. The influence of both factors (fertilization and irrigation) on the development and productivity of the three varieties of cotton was studied. The relationships between the mass of one boll, the number of bolls per plant, the biomass by phase, the total yield per plant and the total yield of cotton per hectare were studied. Correlation dependencies were found, with a high degree of Helius correlation between the structural elements: mass of one boll and bud-formation (r = 0.989), mass of one boll and number of bolls per plant (r = 0.988) under irrigation conditions. Darmi is distinguished by a high degree of correlation between flowering and the number of bolls in a plant (r = 0.996). Colored naturally of Isabell is characterized by a high degree of correlation between positive depending of bud-formation and ripening (r = 0.967) and between the number of bolls in a plant and a total yield (r = 0.958). For irrigation are established correlations between bud-formation and flowering (r = 0.983) and between ripening and number of bolls in one plant (r = 0.979) in Helius. With a high degree of correlation feature dependencies between bud-formation and ripening (r = 0.963), and bud-formation number of bolls in a plant (r = 0.994) in Darmi. Strong positive correlation is drawn between bud-formation and flowering (r = 0.964) and between flowering and ripening (r = 0.956).

Key words: *cotton, irrigation, fertilization, yield, correlations.*

INTRODUCTION

In a market economy, efficient use of water is related to the optimization of mineral nutrition. Dosing of the required amount of mineral fertilizer supplied and distributed evenly over the area, optimizes resources for irrigation water, fertilizers, labor and energy.

The reduction of water resources is forcing researchers to focus on improving the efficiency of water use through introduction of new cotton varieties resistant to drought, or management of irrigation water. In an economic viewpoint, 25.0% saving in irrigation water (T75) resulted in 34.0% reduction in the net income. However, the net income of the T100 treatment is found to be reasonable in areas with no water shortage (Dağdelen et al., 2009).

The application of water-saving technologies has been a priority in the agricultural engineering of crops in recent years. The impact of regulated water scarcity has been the subject of research by many researchers. The cotton response rate was determined by reducing the irrigation rate. Polynomial equations establish the nature of dependencies between vegetative growth, the laying of the generative organs, productivity and irrigation rates (Wanjura et al., 2002; Ibragimov et al., 2007; Wang et al., 2011; Yang et al., 2015).

Study of the technology allowing a regulated water supply at a specific topographical and soil conditions was conducted in Southeast Anatolia (GAP) of Turkey establishes yields of cotton were respectively 4380, 3630 and 3380 kg/ha of a drop, furrow, sprinkler irrigation. Drip irrigation produces 21% more cotton seeds by the method of the furrow and 30% more than the method of spraying. The efficiency of use of water (WUE) proved to be respectively 4.87, 3.87 and 2.36 kg/ha/mm dropping groove and the sprinkler (Cetin and Bilgel, 2002).

Increase of boll number per plant under water stress condition showed that cotton had high ability for adapting water stress conditions. The highest yield was obtained in the I100 treatment. A second degree polynomial relation could adequately describe the cotton seed yield response to the irrigation water amount. The team of scientists found that under I25 (irrigation 25 %), I50 and I75 treatment conditions, evapotranspiration, total cotton seed yield, boll weight, lint percentage, number of sympodial branches and leaf area index decreased while some boll parameters such as boll weights and opened boll numbers increased (Onder et al., 2009).

Cotton grown in different soil-climatic regions manifests itself in different ways their productive potential. Impact on structural elements yields have predecessors, the rate of fertilization, seeding density and variety, the degree of weeding and soil preparation. Each element of agrotechnics is the object of study of a number of researchers (Koleva and Petrova, 2014; Barakova et al., 2018; Barakova et al., 2019)

Balanced diets and optimizing the moisture in the soil affords higher yields. Examining the influence of fertigiraneto with drip irrigation on the efficiency and productivity of cotton Jayakumar et al. (2015) establish a positive influence of a drop fertigirane on the potential of culture and soil fertility. Increasing the efficiency of nitrogen nutrition in drip irrigation was established by Aujla et al. (2005). Agronomic efficiency of nitrogen is increased from 21.65 to 28.59 kg of seed cotton per kg N, applied when applying the same amount of water and N by drip irrigation in comparison to the control pool. The nitrogen is nutrient element influencing the greatest extent on the growth and yield of cotton (Hou et al., 2007; Shah, 2008). The results suggest that once fertilization of the first stage of flowering reduces labor costs without reducing yield and thus can be a practical alternative for fertilization of cotton. Yang et al. (2012) found that fertilization has a higher rate of accumulation of biomass during the rapid accumulation. Single fertilization is a practical alternative to cotton fertilization.

Have been used an interesting approach to manage and comparing data in Bulgaria on some basic food products. It were calculated correlation coefficients and analyzed for all indicators of the products concerned (Dimova, 2018).

The main objective of the development is to evaluate, by means of correlation analysis, the nature of the relationship between certain bio-metric indicators and the productivity in cotton varieties, against the background of different levels of mineral nutrition and moisture supply.

MATERIALS AND METHODS

Establishing ecological plasticity and the impact of mineral nutrition on growth, development, productivity and quality of three varieties of cotton was set field experience in the experimental field of the Department of Plant Agriculture Faculty at Trakia University, Stara Zagora, Bulgaria. During the 2018-2019, three cotton varieties were subject to field research: Helius, Darmi and Isabel. The field study was conducted on soil type typically meadow-cinnamon soil in a fertilizer experiment under irrigation and non irrigation conditions. Based on the method of the small plots in 4 repetitions, with the size of a harvest plot of 15 m^2 (1.80 x 8.34 m), over a two-year period three types of cotton were tested.

The factors of the field experiment are varieties of cotton, the levels of nitrogen fertilization ($N_{0,8,16,24}$), humidity of the soil layer and the influence of climatic elements of the year. A basic fertilization with triple superphosphate norm P_8 in the autumn before sowing of cotton. Nitrogen fertilizer (NH_4NO_3) is imported sowing. Crop density is 12-16 thousand plants per hectare in a depth of seeds 3-5 cm. Irrigation is performed with a drip irrigation system. During the first year they were made two irrigations, and in the second were four irrigations to maintain 75% FC in the soil layer 0–0.50 m. For establishing the timing and duration of phenophase postemergence 40 plants are marked of the embodiment. Beginning of the phase is assumed as 10% of the plants enter the corresponding phase, and when 50% is reported mass occurring. During the growing season, double digging and pesticide treatment were carried out. The first harvest begins in September, after 50-60% of the boxes are burst.

The Bulgarian selection varieties have been studied and they were characterized by medium-sized to large boxes, a high yield of raw cotton and the yield of the fiber. Variety Izabell is representative of a variety of naturally colored fiber with high ecological and economic effect. Inferior to the length of the fiber with the standards, but the extraction of raw cotton and precocity not inferior to standard varieties.

Assessing the impact of mineral fertilization, irrigation, and development of plants in three varieties of cotton is made based on the following biometric indicators: mass of one boll (x_1); bud-formation (x_2); flowering (x_3); ripening (x_4); number of bolls per plant (x_5); total yield (x_6).

Performed a correlation analysis, by means of which it is established and evaluated the relationship between the indicators examined, expressed by the correlation coefficient r. Correlations are the product of mathematical and statistical processing of the output data on Genchev et al. (1975). Are calculated correlation coefficients (r) statistical program SPSS.

RESULTS AND DISCUSSIONS

Since the two years of study in conducted field, the course of major phenophases has been record onset of the phenological observations. Sowing was conducted in optimal time. With regard to the dynamics of the dry biomass of cotton plants under irrigation and irrigation registered higher values under irrigation. Terms of the year, represented mainly by the different security temperatures and rainfall, are affecting plant development.

Values of dry biomass through phenophase budding vary on average depending on soil moisture in all three varieties. Helios variety is characterized by higher biomass values under irrigation and non irrigation conditions. For non-irrigation, biomass values are lower by 4.6% for Darmi and 5.1% for Isabel, compared to Helius.

In terms of irrigation, Darmi yields only 1.2% to Helius, while at Isabel 8.2% for all fertilizer levels is lower for biomass. A tendency for an increase in dry biomass has been reported in the three varieties. Helius under irrigation conditions has an increase rate of 46.1% at N_{24}. Under irrigation conditions, the rate is less than 13.8%. This can be explained by the better natural moisture supply in 2018. During the first year, a higher amount of registered rainfall at the beginning of the growing season provides enough moisture for the normal development of the plants. In Darmi, irrigation provides a growth rate of 28.9%, and without irrigation again the dry biomass is 43.5% higher with the high fertilizer background. Isabell recorded a higher rate of increase in dry biomass under irrigation conditions under the influence of mineral nutrition (35.4%). Under irrigation conditions, the increase in biomass was reported at 14.9%.

The results of flowering phenophase are similar. With the highest dry biomass, Helius stands out with a high fertilizer background (N_{24}) under irrigation and irrigation conditions. Helius exceeds the standards and the Macedonian variety shows a study by Spasov (2010) on productivity and yield of fiber. The results help to define the variety as environmentally friendly. For the other two varieties, irrigation has a greater influence on the formation of plant boss. Darmi by 11.1% is more responsive to irrigation. The naturally colored Isabell variety 9.7% exceeds the biomass formed during irrigation through phenophase flowering.

Cotton varieties have higher values of biomass, under the influence of irrigation and during the ripening phase. In terms of mineral fertilization levels, an increase in dry biomass has been reported for each variety. Under irrigation conditions, Helius produces more biomass, averaging 27.5% over non-irrigation options. From 52.1% to 60.8%, the increase in biomass growth in Darmi. Isabell responds to irrigation, but the growth of biomass during phenophase ripening is narrow in irrigation and non-irrigation range (52.5-55.1%).

Has been reported that with increasing in fertilizer rate, the Helius variety an increase in the generative growth. The results show that the average for irrigation levels under irrigation conditions and without irrigation, the average number of boxes is in the range of 4.56-4.66. The total yield of one plant, first and second harvest averages 20.80 g for non-irrigation variants and 22.38 g for irrigated variants. Optimization of the moisture factor forms a higher yield. The trend observed in the number of boxes at Darmi under the influence of different fertilization levels is similar. Other researchers have come to the same conclusions. According to Shah (2008), nitrogen (N) is the most essential nutrient of a plant, the most important growth limiting factor among all nutrients. Nitrogen is considered to be a major factor in cotton production, according to Bondada and Oosterhuis (2001) and Hou et al. (2007).

Darmi does not show much responsiveness to irrigation. The formed total yield of a single plant in Isabel under irrigation conditions is averages 18.33 g, while under irrigation conditions the variety forms 21.70 g. Under the influence of optimal moisture supply, Isabell forms a total yield of 18.4% per plant. The results show that the higher number of boxes placed, in irrigation conditions, is able to feed and burst in a timely manner.

After correlation analysis, correlation dependencies were found for each cotton variety with a high degree of correlation. The results for the Helius variety show a very high correlation dependence under non-irrigation conditions between the following indicators: mass per boll and bud-formation (r = 0.989); flowering (r = 0.963), number of bolls per plant (r = 0.988).

Table 1. Correlation dependencies of Helius variety under non-irrigation conditions

x	x_1	x_2	x_3	x_4	x_5	x_6
x_1	1.00	0.989*	0.963*	0.909	0.988*	0.691
x_2		1.00	0.965*	0.943	0.959*	0.604
x_3			1.00	0.976*	0.966*	0.495
x_4				1.00	0.891	0.326
x_5					1.00	0.699
x_6						1.00

Also between the indicators bud-formation (x_2) and flowering (x_3); number of bolls per plant (x_5). A positive correlation also was recorded between the indices: flowering (x_3) and ripening (x_4), number of bolls per plant (x_5) (Table 1). The correlation between the indicators is high and positive: mass of one boll (x_1) and bud-formation (x_2), as well as between bud-formation (x_2) and flowering (x_3). The high correlation coefficients reflect the linear relationship between the indicators considered.

For irrigation variants, a high degree of correlation was found between ripening (x_4) and the number of bolls per plant (x_5), with a correlation coefficient r = 0.979 (Table 2). The correlation coefficient between bud-formation and flowering is also high (r = 0.979). This indicates that there are conditions for the buttons that appear to develop and blossom. The lower coefficients between the number of bolls per plant (x_5); total yield (x_6) is an indicator that not all bolls of the laid boxes are guarded, there are dropped boxes and as a result the yield is lower.

Table 2. Correlation dependencies of Helius variety under irrigation conditions

x	x_1	x_2	x_3	x_4	x_5	x_6
x_1	1.00	0.955*	0.939	0.835	0.907	0.269
x_2		1.00	0.983*	0.850	0.870	0.081
x_3			1.00	0.932	0.933	0.219
x_4				1.00	0.979*	0.513
x_5					1.00	0.555
x_6						1.00

When analyzing the indicators of the Darmi variety, recorded under the conditions of natural moisture supply, the correlation between flowering (x_3) is most pronounced; and the number of bolls per plant (x_5) (r = 0.996), and the mass of one boll (x_1) less pronounced between the indicators; and flowering (x_3); ripening (x_4); number of bolls per plant (x_5) (r = 0.955÷0.973) (Table 3). The dependencies establish a strong relationship between the number of bolls filled and the blooms under non-irrigation conditions. The correlation between the number of bolls and the yield is with the coefficient r = 0.884, which is an indicator that the boxes placed under natural moisture supply are able to guarantee a stable yield. Generative growth is weaker, but the organs provided provide good productivity even with unsustainable soil moisture.

Table 3. Correlation dependencies of Darmi variety under non-irrigation conditions

x	x_1	x_2	x_3	x_4	x_5	x_6
x_1	1	0.901	0.963*	0.973*	0.955*	0.769
x_2		1	0.950*	0.970*	0.920	0.946
x_3			1	0.963*	0.996**	0.902
x_4				1	0.938	0.853
x_5					1	0.884
x_6						1

The data in Table 4 shows that a correlation was found with a positive coefficient between the indicators: bud-formation (x_2) and flowering (x_3); ripening (x_4); number of bolls per plant (x_5), with the strongest between bud-formation (x_2) and number of bolls per plant (x_5) (r = 0.994). The analyzes were made on the basis of the results of biometric indicators under irrigation conditions.

Table 4. Correlation dependencies of Darmi variety under irrigation conditions

x	x_1	x_2	x_3	x_4	x_5	x_6
x_1	1	0.850	0.915	0.734	0.833	0.100
x_2		1	0.952*	0.963*	0.994**	0.580
x_3			1	0.935	0.916	0.327
x_4				1	0.937	0.608
x_5					1	0.629
x_6						1

From the attached Tables 5 and 6, a less pronounced correlation between the parameters considered in the naturally colored Isabell variety is established. In it, without irrigation, the dependencies between bud-formation (x_2) and ripening (x_4) (r = 0.967) are derived.

Comparison with the previous varieties considered here with the Isabell variety has a high correlation between the indices: number of bolls per plant (x_5) and total yield (x_6) under non-irrigation conditions. The high number of bolls and the total yield were also characterized by a high ratio (r = 0.958).

When optimizing the irrigation conditions, strongly correlated correlations were found between bud-formation (x_2) and flowering (x_3) with a correlation coefficient (r = 0.964), respectively. The correlation between flowering (x_3) and ripening (x_4) with coefficient r = 0.956 is also highly correlated.

Table 5. Correlation dependencies of Izabell variety under non-irrigation conditions

x	x_1	x_2	x_3	x_4	x_5	x_6
x_1	1	0.769	-0.077	0.904	0.726	0.768
x_2		1	-0.190	0.967*	0.943	0.834
x_3			1	-0.201	0.146	0.365
x_4				1	0.898	0.834
x_5					1	0.958*
x_6						1

Table 6. Correlation dependencies of Izabell variety under irrigation conditions

x	x_1	x_2	x_3	x_4	x_5	x_6
x_1	1	0.670	0.473	0.298	-0.256	-0.159
x_2		1	0.964*	0.855	0.538	-0.022
x_3			1	0.956*	0.730	0.147
x_4				1	0.820	0.410
x_5					1	0.292
x_6						1

As a result of the correlation analysis, correlation dependences between the studied parameters for the three cotton varieties were deduced. The strongest positive correlation is found for the variety Darmi between the number of bolls per plant and bud-formation and flowering. The highest correlation between the indicators is found in the Helius variety under irrigation conditions.

Mathematically unproven are correlations between yield and other parameters considered variety Helius Darmi as well as in a variety Isabell for irrigation regime.

CONCLUSIONS

The results show that when mineral nutrition increases, the vegetative and generative growth increase as well.

Correlation dependencies were found, with a high degree of Helius correlation between the structural elements: mass of one boll and bud-formation (r = 0.989), mass of one boll and number of bolls per plant (r = 0.988) under irrigation conditions. Darmi has a high correlation coefficient between flowering and the number of bolls per plant (r = 0.996). Naturally dyed Isabell is characterized by a high degree of positive relationship between bud-formation and ripening (r = 0.967) and between the number of bolls per plant and total yield (r = 0.958).

Irrigation correlations were found between bud-formation and flowering (r = 0.983) and between ripening and number of bolls per plant (r = 0.979) in Helius. The high correlation coefficient distinguishes between bud-formation and ripening (r = 0.963), bud-formation, and the number of bolls per plant (r = 0.994) in Darmi. A strong positive correlation was found between bud-formation and flowering (r = 0.964) and between flowering and ripening (r = 0.956).

The analysis which has been made, can serve that to predict the productivity of each variety and the advantages of each one of them.

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LIFE CYCLE PARAMETERS OF THE INVASIVE SOUTHERN GREEN STINK BUG (*Nezara viridula*) AT LABORATORY CONDITIONS

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Abstract

The life cycle parameters of Nezara viridula were studied in 2018 under laboratory conditions at the Department of Entomology, Agricultural University of Plovdiv. In the beginning of May field collected adults were placed in Petri dishes for mating and laying eggs (a couple of female and male per dish). A constant temperature of 25 ± 2°C, RH 60-70% and a photoperiod of 16:8 hours L:D were maintained at the laboratory. The larvae, hatching from 15 egg groups, were reared individually in Petri dishes and monitored daily to determine the duration of each stage. The eggs were laid in groups of 27 to 83. The mean duration of the embryonic development was 6.04 ± 0.71 days. The five nymphal stages last as follows: 3.56 ± 1.56 days for the first instar, 9.75 ± 4.99 days for the 2nd instar, 7.16 ± 3.47 days for the 3rd instar, 10.36 ± 2.98 days for the 4th instar, and 13.46 ± 3.39 days for the 5th instar.

Key words*: color morphs, copulation, developmental stages, fecundity, Nezara viridula.*

INTRODUCTION

The geographical origin of the southern green stink bug *Nezara viridula* (Linnaeus) is a matter of debate. The most likely origin is the Mediterranean area and/or the African mainland (Hokkanen, 1986; Jones, 1988). According to DeWitt and Godfrey (1972) Bulgaria falls within the native range, nevertheless the species was mentioned for the first time in 1959 by Strawinski (1959) for the southern part of the country. It was mentioned later by Josifov (1963, 1999) and Josifov & Simov (2004) as part of the heteropteran entomofauna of the country, and only recently there were records in the northern part (Black Sea Coast in 2001, Sofia in 2011) (Simov et al., 2012).

The geographical distribution of the southern green stink bug extends across temperate and tropical areas (Todd, 1989; Waterhouse, 1998). It was reported as a common pest in most European countries like France, Germany, Greece (CABI/EPPO, 1998), Romania (Grozea et al., 2012; 2015), etc. *N. viridula* is classed by Todd (1989), as "... one of the most important pentatomid insect pests in the world ... It is cosmopolitan and highly polyphagous on many important food and fiber crops".

N. viridula causes economic damages to many horticultural and field crops such as tomatoe (*Lycopersicon esculentum*), corn (*Zea mays*), grape-vine (*Vitis vinifera*), pepper (*Capsicum annuum*), raspberry (*Rubus idaeus*) and apples (*Malus domestica*). Feeding causes malformations, discoloration and premature drop of fruits. Feeding on tomato fruits causes reduction of the yield and the fruits become unmarketable. Feeding punctures can also provide access for fungal and bacterial infections (Russin et al., 1988) which can be toxic to vertebrates feeding on maize kernels (Stringer et al., 1983; Payne and Wells, 1984). In USA *Nezara viridula* caused 34-53% fruit drop on pecan (Dutcher and Todd, 1983).

Regarding the pest biology, it is known that is mostly multivoltine with the extent of voltinism related to local differences in climate and the availability of host plants suitable for reproduction (Velasco et al., 1992; Velasco et al., 1995). *N. viridula* overwinter as an adult under bark of trees (Jones & Sullivan, 1981), inside buildings and facilitates. The adults start to emerge in spring, in the beginning of May. They leave their overwintering sites and migrate to different crops to feed and reproduce.

Though *N. viridula* has become a common pest on many agricultural crops in Bulgaria, especially on raspberries and field tomato, since the 90s of the last century (the region of Plovdiv, Harizanova, unpublished data)

(Figures 1 and 2.), it had not been studied so far. Simov et al. (2012) mentioned about some evidence that the species might be a pest in Bulgaria, recording damage (chlorosis) on tomatoes fruits in Gorno Spanchevo Vill., due to many piercings by larvae and adults of the southern green stink bug.
The aim of the present study was to establish lifecycle parameters of the local populations of *N. viridula*.

MATERIALS AND METHODS

The study was conducted in 2018-2019 under controlled laboratory conditions at the premises of the Agricultural University - Plovdiv. A constant temperature of 25 ± 2ºC, RH 60-70% and a photoperiod of 16:8 hours L:D were maintained at the laboratory.

Insect rearing

The laboratory colony of *Nezara viridula* was reared using adults collected in the beginning of May from raspberry plants in a private garden in Stamboliyski, Plovdiv's region. The adults were placed in Petri dishes for mating and laying eggs (a couple of male and female per dish) (Figures 3 and 4). On the bottom of each Petri dish a filter paper for absorbing the moisture was placed. Pods of green bean (*Phaseolus vulgaris*) and pieces from apple (*Malus domestica*) were provided as food. Fan-like folded paper was placed inside the Petri dish where the adults could lay their eggs. The food in the Petri dish was replaced every two days with fresh one.

Developmental Time and Life Table Studies

Egg stage
After eggs were laid the egg group, together with part of the paper on which had been laid, they were transferred to new Petri dishes for hatching and determining the duration of the egg stage
Nymphal stage
Fifteen egg masses were obtained and placed in Petri dishes (one egg mass per dish). The larvae hatched from each egg group were reared individually in Petri dishes and monitored daily to determine the duration of each instar. On hatching first instars have a strong aggregation behavior (Simmons and Yeargan, 1988) and for this reason they were initially left on the egg shells until they molted to the second instar and began to disperse. Each nymph was moved individually to a Petri dish by using a thin brush. On the bottom of the dish a filter paper and a fresh pieces of apple (*Malus domestica*) as a food were placed, together with a folded pieces of paper where the nymphs could hide if disturbed.
Adult stage
At adult emergence, the sex of all individuals was determined by the morphology of their external genitalia. Newly emerged adults were placed in Petri dishes (a couple of male and female per dish) for mating, laying eggs, and to determine longevity and fecundity. The duration of each copulation was measured and recorded in hours. The number of eggs in each newly laid egg group were counted and number recorded for calculating the average fecundity of the females. The adult longevity was calculated by recording the time from emergence to the death. In each Petri dish fresh pieces of apple (*Malus domestica*) as food and folded pieces of paper were placed where the adults can hide for maiting and laying their eggs.
All nymphal instars and adult's development, behavior and survivorship were monitored and recorded at regular basis.
Data were analyzed using Microsoft Excel statistics 2010.

RESULTS AND DISCUSSIONS

Egg stage
The embryonic development lasts 6.04 ± 0.71 days (Table 1), similar to the results reported by Harris and Todd (1980). The eggs are light yellow in color, barrel shaped and with flat tops. They are laid in clusters and firmly glued to each other. Parallel to the embryonic development, changes are observed in the color - eggs become orange three days after laying and a small red triangle starts to appear on the top of each egg. The eggs turn into red color day before hatching (Figures 5 and 6). Young larvae hatch by opening the disk-shape cap. Hatching takes about five minutes for an individual egg, but the entire egg mass is hatching for 1 to 2 hours.

Nymphal stage

As described by Waterhouse (1998), the 1st instar nymphs did not feed and aggregated on the empty egg shells. Newly hatched larvae are light orange with red eyes. Larvae become red in color twelve hours after hatching and dark red twenty four hours after hatching.

Mean development time of the first instar is 3.56 ± 1.56 days (Table 1).

Table 1. Mean developmental time (days ± STDEVA) for *Nezara viridula* (Linnaeus) at constant temperature of 25 ± 2ºC

Duration (days)	Average	STDEVA	Min.	Max.	n
egg stage	6.04	0.71	5	8	419
nymphal stage					
1st instar	3.56	1.56	2	8	286
2nd instar	9.75	4.99	3	29	128
3rd instar	7.16	3.47	3	15	122
4th instar	10.36	2.98	5	19	118
5th instar	13.46	3.39	7	25	113
from egg to adult	41.92	6.87	35	67	52

The mean development time for the second instar was 9.75 ± 4.99 days (Table 1). Third and fourth in stars are different from second in color. The overall green color starts to appear. Third instar nymphs start to disperse and feed mainly on fruits. When disturbed they immediately start to disperse and hide under the leaf surface. The mean development time was 7.16 ± 3.47 days (Table 1).

Fourth instar nymphs live alone. The mean development time of the fourth instar as 10.35 ± 2.98 days. Fifth instar nymphs differ in color among the individuals - part become black in color and the others - green. The percentage of the green against black forms was 60%. The mean development time of the fifth instar was 13.46 ± 3.39 days. A 5th instar nymph molted about 20 to 30 minutes to become an adult.

N. viridula required averagely 41.92 days to develop from first instar to adult. The ratio males: females of the resulting adults was 36: 64.

Males and females start to mate 12.3 ± 4.85 days after becoming adults. Adults mated only in shaded places. This happened under the folded paper inside the Petri dishes. The egg-laying period of a female lasts throughout her lifespan. The mean duration of mating was 32.66 ± 15.26 hours. Females deposited their eggs under the folded paper mainly at night. The eggs were firmly glued to each other. The females laid 1 to 3 egg masses.

Figures 1 and 2. Nymphs and adults of *N. viridula* on raspberry and tomato fruits in Plovdiv's region

Figure 3. Mating in Petri dishes

Figure 4. Eggs laying at laboratory conditions

Figure 5. Freshly laid eggs

Figure 6. Day before hatching

Table 2. Adult longevity, pre-oviposition period and fecundity (days ± standard error) for *Nezara viridula* (Linnaeus) at constant temperature of 25 ± 2ºC

	Female adult longevity (days)	Male adult longevity (days)	Preoviposition period (days)	Fecundity (number)	Eggs per egg group (number)
Mean	47.35	34.81	19.82	89.88	57.27
Standard error	5.02	5.24	1.57	16.61	5.92
Min.	10	11	14	29	27
Max.	88	75	30	201	83
Sample size	26	16	11	11	13

The females lived longer than the males (an average of 47.35 days against 34.81 days) (Table 2). The pre-oviposition period was 19.82 days, the average fecundity – 89.88 eggs per female and the average number of egg per egg group - 57.27.

As described by McPherson (2018) the species has several ‘color types’ (i.e., genetic morphs) of adults but two main morphs exist (i.e., numerically dominant in most regions): var. *smaragdula* F. (G-type, completely green coloration) and var. *torquata* F. (O-type, predominantly green body with anterior yellowish coloration). During the entire study in Plovdiv,s region, the most prevailing was the G-type, *N. viridula* var. *smaragdula* F., comprising 95.4% of all the collected adults.

CONCLUSIONS

Nezara viridula is a pest of many horticultural and field crops in Bulgaria. The populations in the southern part of Bulgaria are predominantly var. *smaragdula* F. (G-type, completely green coloration), and rarely var. *torquata* F.

At constant temperature of 25 ± 2ºC, RH 60-70% and a photoperiod of 16:8 hours L:D one generation may develop for 41,92 days. Female live longer than males and lay an average of 89.88 eggs.

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THE INFLUENCE OF RESIDUAL RED MUD ON WINTER WHEAT PLANTS

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Abstract

Recent researches recommend red mud (red sludge) by using in a possible correction of soils with acid reaction and poor content in nutrients. In addition, it was observed that plants of Poaceae family (called generic herbs) show a certain tolerance to this residue. Wheat plants grown in the second year after applying different doses of red mud have shown significantly positive plants trends. Thus, wheat plants grown at a dose of 60 t/ha compared to those of 10 t/ha red mud, formed ears 1 cm longer, had two (2) additional spikes, and the weight of the ear was larger by 0.4 grams. The number of grains in an ear was greater by seven (7), the weight of the grains in the average ear was 0.3 grams more, while the grains were relatively similar in size. The use of red mud in agricultural field conditions was used for the first time on the white luvic soil at the Pitești- Albora resort.

***Key words**: ears, grains, red mud, variability.*

INTRODUCTION

One of the residual products of alumina extraction is red mud/red sludge. The highly alkaline reaction and its content in different chemical elements (macro and microelements), led in time to a possible preservation through herbs (Xendis et al., 2005). Red sludge is one of the residues obtained in the process of alumina (Al_2O_3) extraction from bauxite. Extraction from the bauxite ore is done with **sodium hydroxide** at high temperature and pressure (Bayer process). This sludge contains **iron oxides**, aluminium oxides, titanium oxides, clay minerals, which together form sodium alumino-silicates. The product as such cannot be used in agricultural fields mainly due to its caustic nature and the content of very fine sand particles that impede the development of the root system of plants. In addition the residue has a high pH (> 10), high electrical conductivity (EC > 30 $dS.m^{-1}$) and a high percentage of exchangeable sodium (> 70%). Therefore, the crude residue is recommended to be separated on one side by sand and the other part by red mud/red sludge (Figure 1).

A treatment with positive effect, namely seawater, has also been suggested. In this case, there is an increase in the concentration available to plants for calcium (Ca) and magnesium (Mg) together with the reduction of pH values.

Figure 1. The PG 102 wheat variety

In contrast, recent research has shown that red sludge resulting from different technological processes can be improved by mixing with different materials used in plant cultivation (Jala & Goyal, 2006). Thus, combinations of different doses of red sludge have been used with manure, compost, domestic sludge and even crop soil (Eastham & Morald, 2006a; Eastham et al., 2006b). From here it was suggested the idea of using improved red mud in agriculture (Gupta et al., 2002; Snars et al., 2004). The high pH values of the red mud could cause a relative improvement of the acidity of the crop soils, while the modest microelement contents could have a beneficial effect on the died of the plants. The first tests from us were carried out in vegetation vessels with soil in which cetain doses of red slurry were introduced. For the vegetation different plants were used, of which also some herbs (plants of the *Poaceae* family). Highlighting the effect of red sludge on culture soil requires longer- term research, usually over 3-4 years. For field conditions an experiment was chosen on the white luvicsoil within the Pitești resort. In addition to the various chemical analyzes of soil and plants, series of morphological determinations on plants have also been carried out. The present material presents results obtained from the winter wheat cultivated in the second year of research.

MATERIALS AND METHODS

In 2018, a stationary experiment was established, consisting of different doses of red mud (factor A with 10, 20, 40 and 60 t/ha) on a constant basis of mineral and organic fertilization (factor B). The duration of the research lasts 3 years (until 2020), in the rotation of the maize- winter wheat- sunflower. Doses of red mud were applied to the maize crop under the plow. The data in this paper refer to the winter wheat of the second year, as a carry over effect of the red mud (Figure 2). The variants had an area of 40 m^2 (5 x 8 m), in three replicates, placed according to the block design method. The winter wheat variety used was *PG 102*. The culture technology was the one recommended by the resort. The chemical analyzes performed complied with the standard methods known in such situations.

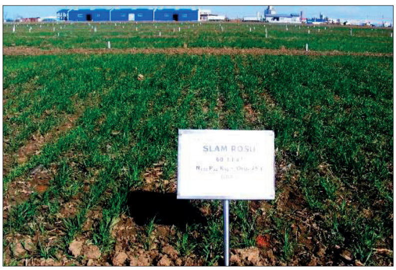

Figure 2. 60 t/ha red mud in wheat crop

In order to observe the influence of exclusive doses of red mud on wheat plants, a number of morphological aspects were analyzed. Among the variants used, we compared wheat plants grown exclusively at the dose of 10 t/ha red mud compared to those obtained exclusively at the dose of 60 t/ha red mud (without fertilizers of any kind). Thus, 100 plants each from three repetitions were chosen, cut and brought to the laboratory. The determinations included: the length of the ear, the weight of the ear, the number of spikelets in the ear, the number of grains in an ear, the weight of grains in the ear, the length and thickness of the grain.

The morphological characters obtained were analyzed by the histograms (or frequency polygons) method. In their expression were used the class intervals established according to the specific string of values obtained. The study carried out highlighted several aspects namely: i) the modal values (with the highest frequencies), ii) the limits of the intervals of variability of the studied characters and iii) the specificity of each character of the wheat variety in the analyzed area. Between the analyzed characters the respective correlations were established, with the help of which they could also observe their tendencies within the studied variety. The Excel program was used to express the values. The significance of the correlation coefficients was obtained by comparing with the r_{max} values (Erna Weber, 1961) for the 5%, 1% and 0.1% levels of the transgression probabilities.

In the statistical calculation of all the obtained values, the analysis of variance (Anova test) was used, namely on the ranges of variation. Statistical parameters were calculated used the formulas: $\bar{a} = \Sigma x/n$, where $\bar{a}$ = media of determinations, and x = values, S^2 (variance) = $1/n\text{-}1.[\Sigma x^2\text{-}(\Sigma x)^2/n]$, S (standard error) = $\sqrt{S^2}$ and S % (variation coefficient,%) = $S/\bar{a}.100$.

RESULTS AND DISCUSSIONS

Characteristics of the contents in chemical elements of soil and red mud. For the purpose of establishing the experiment, chemical analyzes of crop soil and red mud were performed. Thus, the soil was characterized by a moderately acidic reaction, with humus over 2% and with a low supply in macro-elements

(Table 1). The red mud had high pH, very low total nitrogen content (tN), relatively high phosphorus, and potassium- like crop soil. The mobile sulfur was more present in the mud and in lower concentration in the crop soil. The micro-element content of the soil shows relatively low values, both as total forms and as mobile forms (Table 2).

The exception is manganese, which has relatively high values, characteristic of this soil. The red mud contained total forms in micro-elements at higher values, while the mobile forms were very low. Of the chemical elements with a pollutant character, the red mud excelled in nickel and chrome.

Table 1. Contents in macro-elements

Elements	Luvicsoil	Red mud
pH	5.30	10.46
Nt, %	0.142	0.005
P_{AL} mg.kg^{-1}	39	352
K_{AL} mg.kg^{-1}	83	89
S-SO_4 mg.kg^{-1}	23	83
Humus, %	2,41	-

Table 2. Contents in micro-elements

Elements	Luvicsoil		Red mud	
	TF*	MF**	TF	MF
Zn mg.kg^{-1}	51	1.47	33	0.30
Cu mg.kg^{-1}	14	2.80	64	0.85
Mn mg.kg^{-1}	820	50.4	136	0.66
Ni mg.kg^{-1}	25		352	
Pb mg.kg^{-1}	16		5	
Cr mg.kg^{-1}	24		616	
Co mg.kg^{-1}	10		9	

*TF - total forms; **MF - mobile forms

Variability of some morphological characters of wheat plants. By increasing the doses of red mud in the soil of crops, gains of some morphological characters of wheat plants were observed. The data obtained are presented graphically by simultaneous evolutions within each character. Thus, the length of the spike was generally between 2.9 and 8.3 cm. The modal values were in the range 5.1-6 cm (35%) for wheat in the variant with 10 t/ha red mud and between 6.1-7 cm (34) at that of the dose of 60 t/ha red mud (Figure 3). Close limits were at 4.1-5 cm (29%) at wheat from the lower dose of mud and 5.1-6 cm (32%) at the higher dose of red mud. In both cases, the extremes constituted 1-2%. The number of spikelets in a spike ranged from 4 to 17. The modal values ranged from 10-11 spikelets/ spike at 10 t/ha red mud (34%) and 12-13 spikelets/ ear at 60 t/ha red mud (33%) (Figure 4). Close limits were at 8-9 spikelets (25%) at the low dose of mud and at 10-11 spikelets (28%) at the higher dose of red mud. Extreme values were at only 1-4% frequency. The weight of the wheat ear generally ranged between 0.40 g and 3.27 g. In the 10 t/ha mud, the ear had weight values between 0.40 g and 2.9 g, while the ears formed in the 60 t/ha red mud has values between 0.40 g and 3.27 g (Figure 5). The modal values were at 1.0-1.4 g (44%) at the lower dose of mud and the same interval 1.0-1.4 g (33%) at the higher dose of red mud. Close to these maximums were the frequencies of 28% in the range 0.5-0.9 g at the low dose of mud and 31% in the range 1.5-1.9 g at the high dose. And in these case the extreme frequencies constituted 1% of the total. The number of grains in a spike generally ranged from 7 to 51. In the lower dose of red mud the number of grains was between 7 and 40 grains, while in the higher dose they ranged from 8 to 51 grains. Modal values were in range of 11-20 grains (59%) in the low dose and 21-30 grains (36%) in the high dose of red mud (Figure 6). Close values were in the ranges fo 21-30 grains (25%) in the lower dose with 31-40 grains (30%) in the higher dose of red mud. the extreme values of the frequencies of this character constituted 1-2%. The weight of the grains from a spike ranged from 0.23 g to 2.78 g.

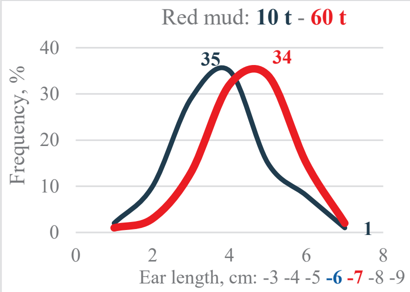

Figure 3. Frequency of ear length

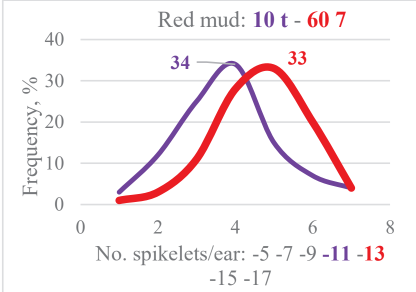

Figure 4. Frequency of no. spikelets/ear

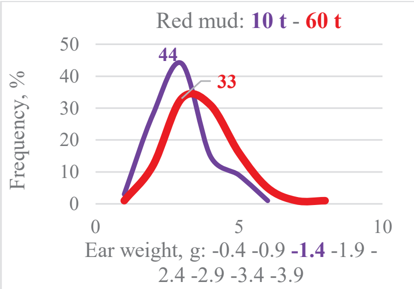

Figure 5. Frequency of ear weight

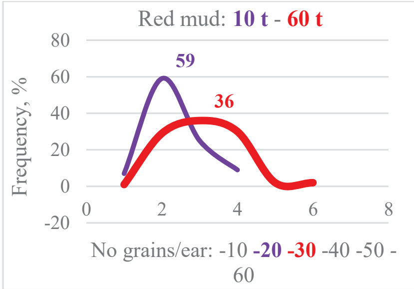

Figure 6. Frequency of no. grains/ear

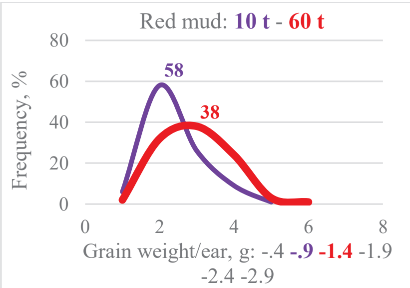

Figure 7. Frequency of grains weight/ear

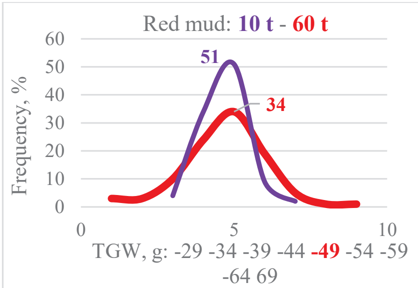

Figure 8. Frequency of a thousand grains weight (TGW)

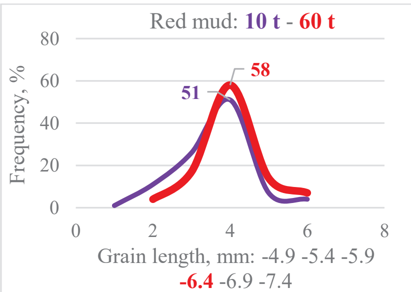

Figure 9. Frequency of grain length

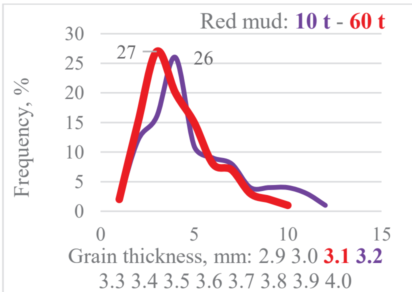

Figure 10. Frequency of grain thickness

The weight of the grains from an ear raised at the lower dose of red mud was between 0.23 and 2.04 g, and under the conditions of the higher mud dose they formed between 0.30 and 2.78 g (Figure 7). The modal values were in the range 0.5-0.9 g (58%) at the lower dose of mud and in the range 1.0-1.4 g (38%). Near values were in the range 0.5-0.9 g (32%) on the low dose of mud and on the 1.0-1.4 g (26%) on the higher dose of red mud. The mass of one thousand grains (MTG/TGW) had absolute values between 26.5 g and 65.5 g (figure 8). The small mud dose was formed with grains between 25 and 69 g, and on the larger mud dose the values were between 35 and 59 g. The modal values were at 45-49 g (34%) at the low dose of mud and in the same range 45-49 g (51%) at the high dose of residue.

The wheat grains formed under the conditions of the two doses of red mud have demonstrated approximately similar dimensions. Thus, the grain length had extreme values between 4.9 and 7.1 mm. The dominant frequencies were in the range 6.0-6.4 mm (51%) on the lower dose of mud and in the same range 6.0-6.4 mm (58%) on the higher dose of red mud (Figure 9). The thickness of wheat grains had extreme values between 2.6 and 4.0 mm, with a slightly greater dispersion in the conditions of wheat grown on the lower dose of red mud. The modal values were at the values of 3.2 mm on the low dose and 3.1 mm on the higher dose of mud (Figure 10).

Statistical analysis of the variability of morphological characters in wheat. The results obtained in the morphological analysis of some wheat characters showed specific aspects. Thus, the length of the spike/ear measured 5.3 cm a the small dose of mud (10 t/ha) and 6.0 cm on the larger dose of red mud (60 t/ha). The variability demonstrated medium to large values (Table 3). The weight of the wheat ear ranged from 1.21 g at the low dose of mud, to 1.57 g at the higher dose of red mud. The variability of this character was particularly high. The number of spikelets in one spike was 10.1 in the low dose and 11.9 in the spikelets raised on the higher dose of red mud. The variability of this character was great to very high. The number of grains in one spike ranged from 19.5 on the low dose of mud and 26.4 on the high ones on the high dose of red mud. The variability was very high in both situations. The grain/ spikelet weight ranged from 0.90 g for the lower dose of mud and 1.21 g for the larger dose of red mud. And for this parameter, the variability was very high in both situations.

Table 3. Statistical indices of winter wheat ears and grains

Indices	Ear length, cm	Ear weight, g	No. spikelets/ ear	No. grains/ ear	Grain weight/ ear, g	TGW, g	Grain length, mm	Grain thickness, mm
10 t/ha red mud								
Media, ā	**5.31**	**1.21**	**10.1**	**19.5**	**0.90**	**45.7**	**6.0**	**3.3**
Variance, s^2	0.381	0.205	6.745	45.06	0.135	47.35	0.194	0.072
Std. error, s	0.617	0.453	2.579	6.713	0.367	6.881	0.440	0.269
Var. coef.,%	**11.6**	**37.5**	**25.7**	**34.4**	**41.0**	**15.**1	**7.4**	**8.2**
60 t/ha red mud								
Media, ā	**6.02**	**1.57**	**11.9**	**26.4**	**1.21**	**45.4**	**6.2**	**3.2**
Variance, s^2	1.131	0.330	5.326	84.82	0.195	30.05	0.160	0.075
Std. error, s	1.064	0.574	2.308	9.210	0.442	5.482	0.400	0.187
Var. coef.,%	**17.7**	**36.5**	**19.4**	**35.0**	**36.6**	**12.1**	**6.5**	**5.8**

The mass of one thousand grains had average of 45.7 g at the wheat raised on the dose of 10 t/ha mud and 45.4 g at the wheat in the high dose of sludge. The grain size, length and thickness, had close relative average values. Thus, the grain length had values of 6.0 mm on small mud dose and 6.2 mm on the larger mud dose. The variability of wheat grain length was reduced in both situations. The thickness of the grain had average values of 3.3 mm for the wheat from the low dose of mud and 3.2 mm for the grains from the higher dose of the red mud. And in this case, the character variables were in the small category.

CONCLUSIONS

The sludge/red mud residue can be used as a product that can improve the chemical regime of the soil. It has been suggested that its agricultural role would be in the form of an amendment. Considering the alkaline reaction and a sensitive content in nutrients, the red mud was tested under the conditions of white luvic soil, an acidic soil with low reserves of plant food. In fact, there was a correction of the acid reaction of the soil together with the contribution of various chemical elements, necessary for the growth and development of the plants. Analyzes of both soil and red mud demonstrated these characteristics.

From the determinations it emerged that the winter wheat plants grew and developed normally, and to prove the effective contribution of the red sludge in the vegetation were made series of determinations on mature wheat plants grown on two doses of mud: 10 t/ha and 60 t/ha, without chemical fertilizers. The obtained data show the gain of the productivity elements from this environment improved with the red mud i.e.: an average ear/spike 0.7 cm longer, a heavier spike 0.36 g, a higher number of spikelets/spike 1.8, and the number of grains from ear was 6.9 higher. The weight of the grains in an ear increased by 0.31 g, and the absolute weight of the grains was similar to 45 g.

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SEED AND OIL PRODUCTIVITY OF SUNFLOWER (*Helianthus annuus* L.) AS AFFECTED BY THE TIMING OF WEED REMOVAL

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Abstract

Sunflower, one of the most important oil crops, is generally considered to be very competitive against weeds. However, this is not valid for all hybrids, weeds and conditions, since in many cases there have been reported yield losses up to 70%. A field study was conducted in western Greece to evaluate growth, grain and oil productivity of sunflower under different weed management treatments (weed free, weed presence for 2, 4 and 8 weeks after sowing). Grain yield of the several treatments resulted to losses up to 64% compared with the weed free plots. Oil concentration was not affected by the presence of weeds, however oil yield was also significantly reduced in the plots with a late weed removal, probably due to the seed yield reduction. The findings of the present study reveal the significant reduction of seed and oil productivity in sunflower due to weed competition and highlight the need of an early weed management.

Key words: *sunflower, seed yield, oil yield, weed competition.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is certainly among the most important oil and bioenergy crops with a high oil content and productivity, high non saturated fatty acids content and absence of cholesterol (De la Vega and Hall, 2002; Papatheohari et al., 2016). It is generally considered to be among the most important oil crops, while it has been already subjected to the improvement by plant breeders.

Biotic and abiotic factors such as weeds and water deficit, respectively, can result in huge yield losses to sunflower and consequently, the selection and cultivation of the most competitive and drought resistant hybrids is crucial (Breccia et al., 2011).

In general, sunflower is considered to be a crop of satisfactory competitive ability against the weeds, mostly due to its large height (Elezovic et al., 2012). However, under the real field conditions, competition with weeds can be high, especially with the earliest growing ones and result in seed yield reduction up to 60-70% (Soares et al., 2019). Competition with weeds begins shortly after emergence, while the low number of registered herbicides makes the situation more difficult. Pannacci et al. (2007) have reported high phytotoxicity of several herbicides to several sunflower varieties, while many herbicide-tolerant varieties (like Clearfield and ExpressSun technologies) have already been developed and globally used (Jocic et al., 2011). However, despite such achievements, there is still a lack of available information regarding the effects of weeds and the timing of their control on sunflower growth and productivity.

The main objective of the present study was to evaluate the growth, grain and oil productivity of a sunflower hybrid with a weed presence for different periods under the conditions of Greece.

MATERIALS AND METHODS

The field experiment was conducted during 2017 in Dokimi area of Agrinio (38º 37’ 13’’ N, 21º 22’ 44’’ E). Soil was a clay loam (CL), the physicochemical characteristics of which (0 to 15 cm depth increment) were clay 341 g/kg, silt 488 g/kg, sand 171 g/kg, pH (1:2 H_2O) 7.4, 1.61 g/kg total nitrogen, 0.054 g/kg available phosphorus (P-Olsen) and 0.98 g/kg available potassium. EC was 2810 μmhos, $CaCO_3$ 270 g/kg and organic matter content 19.4 g/kg (Walkley and Black, 1934). The field was irrigated as needed for the sunflower crop, while this year was of moderate rainfall and temperature (not very low and not very high).

Sunflower was sown on 23 April 2017. Sowing was performed by hand in 75 cm rows with distances within rows of 20 cm. Before sowing, 28 kg N/ha, 15 kg P/ha and 30 kg K/ha were incorporated into the soil. Weed density was moderate, with the dominant weed species being *Chenopodium album*, *Amaranthus retroflexus* and *Echinochloa crus-galli*. These weeds are common for sunflower stands and has been reported by other researchers as well (Tyr and Vavrik, 2015; Jursik et al., 2017). Weed management treatments were: i) weed free, ii) weed presence for 2 weeks, iii) weed presence for 4 weeks and iv) weed presence for 8 weeks after sowing. Weed removal was done by hand-hoeing. The above-mentioned treatments with four replicates were arranged in a completely randomized design with a plot size of 8 m^2.

Several measurements of plant height, leaf area index (LAI) and above-ground plant part biomass were taken from plants in the two center rows of each plot at 40, 60, 80 and 100 days after sowing (DAS). Leaf area was estimated by means of DT-area meter (Delta-T Devices Ltd, Burwell Cambridge, UK), while dry weights were measured after 36 h at 70°C. Harvest was made by hand at 119 DAS and grain yield was measured by harvesting the plants in the two middle rows. Near infrared method was used for oil content measurement. Oil yield was calculated by multiplying seed yields by the concentration of oil in seeds.

Analysis of variance (ANOVA) was conducted for all data and differences between means were separated using Fisher's LSD test at $p < 0.05$. All statistical analyses were conducted using the Statistica 11 software package (StatSoft, Inc. 2300 East 14th Street, Tulsa, OK 74104, USA).

RESULTS AND DISCUSSIONS

The findings of the present study revealed some significant ($p < 0.05$) differences between the several weed management treatments regarding the growth and the yield of sunflower. In particular, weed presence for two weeks had no adverse effects on sunflower's growth, at least for the most cases. However, when removal of the weeds was at 4 or 8 weeks after crop sowing, then plant height, leaf area and dry weight of the plants was significantly lower than the weed-free plots and the plots with a weed presence for two weeks (Table 1).

Table 1. Growth parameters for the several treatments at 60 DAS. Different low case letters in each row denote statistically significant differences between the means ($p < 0.05$)

Growth parameter	Weed free	Weed presence for 2 weeks	Weed presence for 4 weeks	Weed presence for 8 weeks
Plant height (cm)	133 a	125 a	109 b	98 c
LAI	2.6 d	2.1 e	1.7 f	1.4 g
Biomass (g)	128 h	116 h	97 i	78 j

Concerning seed yield, this was significantly reduced as a result of weed competition. Particularly, as shown in Table 2, grain yield of sunflower in the weed-free plots was up to 4.78 tn/ha. However, in the cases of weed removal at 2, 4 and 8 weeks after sowing, yield was reduced by 34, 53 and 64%, respectively. Regarding oil concentration, this was not affected by weed presence and ranged from 37.65 to 39.23%. On the contrary, oil yield was also significantly reduced in the plots with a late weed removal, probably due to the seed yield reduction. Thus, oil yield in the weed free plots was 32, 54 and 74% higher than the plots with a presence of weeds for 2, 4 and 8 weeks after sowing, respectively (Table 2).

Nalewaja et al. (1972) have sown that weed removal at 2, 4, 6 and 8 weeks after crop emergence resulted in yield reductions by 8, 25, 29 and 33% compared to the weed-free plots. Our findings are also in accordance with previous studies highlighting the importance of the first weeks after planting, with a total prevention period of interference ranging from 26 to 43 days (Elezovic et al., 2012). In all cases, yield reductions are related to the weed density, the weed species present (more or less competitive), the cultivated hybrids and varieties and the specific soil and climatic conditions.

It seems that despite the plant height, sunflower needs to emerge and grow at least for the first weeks with the minimum weed presence. Biotic factors like weeds and abiotic stresses are considered major yield limiting factors for sunflower crop and therefore ought to be taken into account. Our findings highlight the importance of early weed management and agree with previous studies showing that the efficacy of several herbicides against many common weeds of sunflower crop (like *C. album*, *E. crus-galli* and *A. retroflexus*) was strongly affected by the growth stages of weeds (Jursik et al., 2017). Therefore, an earlier weed control ensures a higher efficacy of herbicides (in the case of chemical control) and allows a better growth and productivity of sunflower crop due to the absence of weed competition for a longer period.

Harvest measurements also showed that there was not any significant difference between the treatments regarding oil concentration. This is something that could be attributed to the fact that oil content (and even fatty acid profile) of sunflower is known to be mainly affected by the hybrid (Skoric, 1992; Izquierdo et al., 2002).

Table 2. Grain and oil productivity parameters for the several treatments. Different low case letters in each row denote statistically significant differences between the means ($p < 0.05$)

Yield parameter	Weed free	Weed presence for 2 weeks	Weed presence for 4 weeks	Weed presence for 8 weeks
Grain yield (t/ha)	4.78 a	3.17 b	2.24 c	1.72 d
Thousand grain weight TGW (g)	60.12 e	58.34 ef	58.14 ef	55.77 f
Oil concentration (%)	38.24 g	39.23 g	37.65 g	38.78 g
Oil yield (t/ha)	1.83 h	1.24 i	0.84 j	0.47 k

CONCLUSIONS

The results of the present study revealed that even the highly productive sunflower hybrids like ‘PR63A90’ suffer from weed competition. In particular, even a small delay of weed control can cause significant reductions to both the growth and productivity of the crop. Yield losses due to weed presence were from 34 to 64%. It is noticeable that even a delay of weed control for 2 weeks resulted in a yield reduction of 34%. On the contrary, oil concentration was not affected by the presence of weeds, however oil yield was significantly reduced in the plots with a late weed removal, probably due to the seed yield reduction. In particular, later weed removal resulted in oil yield reduction by 32 to 74%. Consequently, the need of an early weed management is imperative and crucial for the successful establishment, growth, productivity and quality of sunflower.

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YIELD CAPACITY OF ELBRUS DURUM WHEAT UNDER THE INFLUENCE OF ORGANO-MINERAL FERTILIZER PRODUCTS

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Abstract

During the period 2014-2017, a field experiment was conducted at the Experimental and Implementation Base of the Department of Crop Production at the Agricultural University of Plovdiv, in which the effect of two organo-mineral products was studied: Megafol (3000 mL/ha) and Megafol protein (3000 mL/ha) on the yield capacity of Elbrus durum wheat. There was also untreated control. The fertilizers were applied in the phases of tillering, shooting-up and ear formation. The experiment was made after the precursor chickpea by the method of fractional plots in four repetitions with a size of 10 m^2 of a harvest plot. As a result of the experience, the following was proved: The organo-mineral fertilizer products tested had a positive effect on the yield capacity of Elbrus durum wheat. The highest grain yield of durum wheat of Elbrus variety was obtained in the variant processed in the phase of tillering with the organo-mineral product Megafol (3000 mL/ha), where the yield capacity increased averagely for the experimental period with 479 kg/ha (13.8%) more than unprocessed control. The new organo-mineral products helped to increase the values of the structural elements of the crop such as: number of spikelets, number of grains and mass of grains per plant.

***Key words**: durum wheat, organo-mineral products, yield.*

INTRODUCTION

Over the last few years, with the admission of Bulgaria to the European Union, there has been a rise in durum wheat production. The area sown with durum wheat in Bulgaria has increased up to 18,000 ha, but the yield of grain per hectare is lower from 15% to 25% compared to common wheat. The positive impact of the use of organo-mineral products and biologically active substances in enhancing the productive capacity of a number of cereals has been demonstrated in experiments conducted abroad (Petr, 2005; Wolber et al., 2006) and in Bulgaria (Delibaltova et al., 2009; Kolev et al., 2011). The published scientific literature presents data on preparations that increase the resistance of plants to various stress factors, such as high and low temperatures (Delchev et al., 2001; Delchev et al., 2011, Kolev et al., 2015).
In this study, we set out to identify the impact of new organo-mineral products on the productivity of Elbrus durum wheat.

MATERIALS AND METHODS

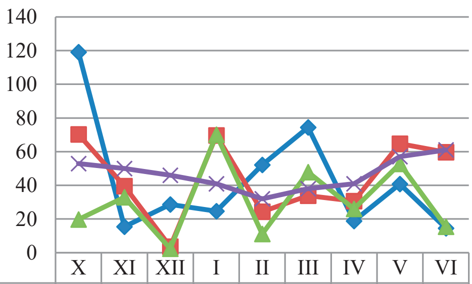
During the period 2014-2017, a field experiment was conducted at the Experimental and Implementation Base of the Department of Plant Growing at the Agricultural University of Plovdiv, in which the effect of two organo-mineral products was studied, namely: Megafol (3000 mL/ha) and Megafol protein (3000 mL/ha) on the production of Elbrus durum wheat. The results of the variants treated with the organo-mineral products tested were compared with an untreated control. Spraying with organo-mineral products was carried out in the phases of tillering, shooting up and ear formation of durum wheat. The experiment was carried out after chickpea precursor, repeated 4 times, with size of the cultivated plot of 10 m^2, on alluvial-meadow soil (FAO, Molic Fluvisols), characterized by an average sandy-clay mechanical composition, humus content of 1-2%, pH 7.7, with presence of carbonates up to 7.4% and absence of salts. In the 0-20 cm soil layer, the contents of the basic nutrients were as follows: N - 15.6 mg/1000 g, P_2O_5 - 32 mg/100 g, K_2O - 47 mg/100 g (Popova & Sevov, 2010).
Durum wheat of Elbrus variety is sown in the optimal period from 20.10 to 05.11, with a sowing rate of 500 germinating seeds/m^2 and mineral fertilization with 120 kg/ha of nitrogen and 80 kg/ha of phosphorus, with all the

phosphorus fertilizer and 1/2 of the nitrogen being imported before sowing and the rest of the nitrogen in the early spring as a further nutrition. During the experiment, all agrotechnical measures of the approved technology for cultivation of durum wheat were observed (Yanev et al., 2008), except for the tested organo-mineral products applied in the three phenological phases of the plant development.

The number of tillers per m^2, the number of ear-bearing stems per m^2, the number of spikelet per ear (pcs), the number of grains per ear (pcs), the mass of grains per ear (g), and the yield of grain (t/ha) were reported. The statistical processing of the data obtained on the surveyed indicators was carried out with the BIOSTAT software (Penchev, 1998).

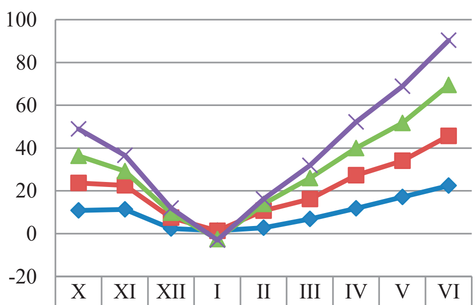
RESULTS AND DISCUSSIONS

The rainfall during the durum wheat growing season was as follows: 2014/2015 - 655.8 mm, 2015/2016 - 388.5 mm and 2016/2017 - 264.2 mm compared to the value of 419.0 mm over a thirty-year period. During the studied years, favourable for the growth and development of durum wheat with good rainfall distribution was the year 2017 (regardless of the less but better distributed rainfall during the critical phenophases of the plant development), and when also higher yields were obtained of all the grain variants. Unfavourable for the development of the plants was the first year, 2014/2015, due to the drought in April, when the structural elements of the production develop, see Figures 1 and 2.



	X	XI	XII	I	II	III	IV	V	VI
2014-2015	119,1	15,4	28,7	24,6	52,1	74,4	18,8	40,8	14,6
2015-2016	70,3	39,6	3,6	69,6	24,4	33,9	30,7	64,7	59,7
2016-2017	19,7	32,9	2,4	70,1	11,1	47,9	26,1	52,7	15,4
Average for 30 years	53	50	46	41	32	38	41	57	61

Figure 1. Precipitation by months, sum mm/m^2



	X	XI	XII	I	II	III	IV	V	VI
Average for 30 years	12,6	7,4	2,2	-0,4	2,2	6	12,2	17,2	20,9
2016-2017	12,7	6,6	2,2	-3,9	3,2	9,7	12,7	17,6	23,7
2015-2016	12,8	11,3	5,1	-0,1	8	9,3	15,5	17	23,3
2014-2015	10,8	11,3	2,4	1,4	2,7	6,9	11,8	17,1	22,4

Figure 2. Monthly temperatures (average)

Number of tillers per m^2

Tillering is a process that begins in the early stages of growth and depends mainly on the sowing density, moisture supply and nitrogen supply of the plants (Garsia del Moral et al., 1991; Simane, 1993). Similar results were observed for this phenophase by Fuccillo et al. (2015). He found that in the case of favourable plant development, if there were differences, they were mainly due to the use of resources such as soil moisture, nutrients and light. The process has a strong connection with the yield results.

Tillering can partially or completely compensate for the differences in the number of plants. It helps for their recovery after a bad winter. According to Garsia del Moral et al. (2003), the tillering and the number of ears per m^2 are positively affected by good moisture supply and low temperatures, while water and nitrogen deficiency severely limit this process. Some researchers, Peterson, (1984), Rickman (1983), report that tillering is highly sensitive to lack of water. It can be reduced to a half if there was a drought during this phase. Other authors consider lower tillering capacity as the main reason for lower yields. As a result of many years of phenological observations (Krasteva et al., 2006), has established that the number of ears per m^2 is related to the tillering capacity of the plants and is formed mainly by the end of March and the beginning of April. According to her, the lower yield at high densities is due to productive tillering capacity, which reduces the possibility of high sowing density. Researchers (Slafer et al., 1993) cite high temperatures as one of the main causes of reduced tillering, especially in dense crops. Under ideal conditions, the varieties would better unleash their potential. Similar results shed light on the causes of reduced yield in dry winters by Lioveras (2004). He indicated as a reason for a reduced yield the reduced number of ear-bearing stems, due to reduced tillering.

The results in Table 1 show that the highest number of tillers per m^2 was reported in plants treated with the product Megafol in tillering phenophase, averaging 473 pcs/m^2.

Table 1. Number of tillers per m^2 and number of ear-bearing stems per m^2 (average 2014-2017)

Phases of growth	Organo-mineral products	Number of tillers per m^2	%	Number of ear-bearing stems per m^2	%
Tillering	Control	408	100	307.5	100
	Megafol	473	115.9	389	126.5
	Megafol protein	444	108.8	357.5	116.3
Shooting-up	Control	396.5	100	304	100
	Megafol	446	112.5	348.5	114.6
	Megafol protein	418.5	105.5	315	103.6
Ear formation	Control	388.5	100	327	100
	Megafol	405	104.2	330	100.9
	Megafol protein	394.5	101.5	328	100.3

In plants treated during the same phenophase with the product Megafol Protein, a better result was reported for the Elbrus variety - 444 pcs/m^2. The results in the phenophase of shooting up are similar, maintaining the same trend - a greater number of tillers per square meter in the Elbrus variety - 446 pcs/m^2, treated with the product Megafol, followed by plants treated with Megafol Protein, respectively 418.5 pcs/m^2. The results obtained in the treatment of plants during the phenophase of ear-formation are lower than the application of these preparations in phenophase of tillering and shooting up. This leads to the conclusion that the two organomineral products Megafol and Megafol Protein have the best effect when administered in the tillering phenophase.

Number of ear-bearing stems per m^2

The results presented in Table 1 show that the number of ear-bearing stems per m^2 in the Elbrus variety varies from 328 pcs/m^2 to 389 pcs/m^2 when treated with the organo-mineral products tested in the different phenophases during the vegetation of the plants. The best results for the Elbrus variety have been reported with the use of Megafol in phenophases of tillering and shooting up. The number of ear-bearing stems is higher, respectively by 81.5 pcs/m^2 when treated in tillering phenophase and by 44.5 pcs/m^2 in shooting up phenophase. The highest number of ear-bearing stems when using the product Megafol Protein is reported in the same phenophases, but the values obtained are lower compared to Megafol.

Number of spikelets

The greater number of spikelets under favourable conditions during flowering and fertilization is a guarantee for the formation of well-grained ears. Extremely favourable weather conditions during this period are a good prerequisite for the formation of a large number of durum wheat spikelets.

The data from the experiment given in Table 2 shows that the application of Megafol in tillering phenophase of Elbrus durum wheat gives the best result according to the criterion number of spikelets, respectively 21.5 pcs. Good results were also obtained in the treatment of plants in the shooting up phenophase, respectively 20.1 pcs. Treatment with Megafol Protein in tillering phase gives the best results - 20.0 pcs for the Elbrus variety. A relatively large number of spikelets are also formed with treatment in phenophase of shooting up, while the least number of spikelets are formed when applying the two tested products in the ear-formation phenophase. The experiment carried out showed that the smallest number of spikelets are in the untreated controls, which allows us to conclude that the use of organo-mineral products Megafol and Megafol Protein in the tillering phase has the most beneficial effect on the number of spikelets in durum wheat of Elbrus variety.

Table 2. Biometrical data (average 2014-2017)

Phases of growth	Organo-mineral products	Number of spikelets	Number of grains in the ear	Grain mass in the ear, g
Tillering	Control	19.4	37.8	1.787
	Megafol	21.5	44.2	2.039
	Megafol protein	20.0	40.2	1.851
Shooting-up	Control	18.8	37.2	1.623
	Megafol	20.1	41.9	1.839
	Megafol protein	19.2	39.4	1.718
Ear formation	Control	17.8	34.9	1.569
	Megafol	18.7	39.7	1.657
	Megafol protein	18.3	35.4	1.631

Number of grains in the ear

This indicator is strongly linked to yield. This has been proven by many researchers. Philip et al., (2018) found that the major factor for the formation of yield in durum wheat was the greater number of grains of the ear. According to Bergman (1991), the increase in yield is due to the increased number of grains in the ear. The indicator is closely linked to the conditions during the formation of spikelets and flowers. Rajkine (1960) reports an established relationship between the number of grains formed and the duration of flowering. The moisture supply during flowering of the durum wheat and the formation of the grain is of great importance both for the number of grains in the ear and for their normal development. Another researcher, Araus et al. (2005), points out that one of the main directions of modern selection is to increase the number of grains in the ear. According to Sayre (1997), the increased yield achieved over the last 30 years is probably related to the increased number of grains. Essential for achieving the productive capacity of the variety, as well as for the yield volume, is the number of grains in the ear of the main tiller. The formation of more grains depends a lot on the climatic conditions during flowering and fertilization.

Table 2 shows that the application of the orano-mineral product Megafol in the tillering phase of durum wheat gives the best result in terms of the number of grains in the ear of Elbrus variety, respectively 44.2 pcs. Good results were obtained in the shooting up phase, respectively 41.9 pcs., while the least increase is obtained by treatment in the phase of ear-formation, which is 39.7 pcs.

Treatment with the organo-mineral preparation Megafol Protein in the tillering phase gives the best results - 40.2 pcs. The smallest is the number of grains in the untreated control in the three tested phenophases of the development of durum wheat of Elbrus variety, respectively 37.8 pcs. during tillering, 37.2 pcs. during shooting up, and 34.9 during ear formation, which allows us to point out that the use of the organo-mineral products Megafol and Megafol Protein has a positive effect on the number of grains in durum wheat of Elbrus variety.

Grain mass in the ear

Another very important indicator of the productive capacity of the variety and the amount of yield is the mass of grains in the ear. Both meteorological conditions and various agro-technical activities play an important role in the period of grain formation.

Table 2 summarizes the data obtained from the implementation of this experiment for this indicator. Regarding the first factor investigated, i.e. organo-mineral fertilizers, the greatest grain mass was reported when applying the ograno-mineral product Megafol during tillering phase, namely - 2.039 g. A tendency to increase the mass of grains in the ear is also observed in the treatment of plants in phenophase of shooting up - 1.839 g. The least increase in the grain mass in the ear was reported when applying Megafol in the ear-formation phase, which was 1.657g.

High values of the indicator were also reported in the second organo-mineral fertilizer tested - Megafol Protein. The largest mass of grains was reported in phenophase of tillering - 1.851 g, followed by phenophase of shooting up 1.718 g, and in ear formation - 1.657 g. The values of the control crops for this indicator are the lowest in all three phenophases tested, which shows the positive effect of the two organo-mineral products Megafol and Megafol Protein on the grain mass of durum wheat, with the highest results reported when treated with Megafol and Megafol Protein during the phases of tillering of the durum wheat of Elbrus variety.

Grain yield

Grain yield is the most important and accurate criteria for the effect of agri-environmental, organizational and technological factors. Thus, the application of organo-mineral products together with the introduction of new higher-yielding varieties, and technologies of durum wheat cultivation are some of the most effective factors in intensifying grain production and meeting consumer needs. To produce bigger quantities of and high quality grain is unthinkable without optimizing the varietal composition, sowing density, fertilization, diseases, pest and weed control, harvesting, storage and processing of durum wheat.

The results of the experiment carried out to determine the influence of the studied factors are shown in Table 3.

Table 3.Grain yield, t/ha

Phases of growth	Organo-mineral products	2015	2016	2017	Average	%
Tillering	Control	3.101	3.332	3.951	3.461	100.0
	Megafol	3.961	3.630	4.429	4.007	115.7
	Megafol protein	3.491	3.589	4.083	3.721	107.5
Shooting-up	Control	3.420	3.500	4.008	3.643	100.0
	Megafol	3.756	3.935	4.215	3.969	108.9
	Megafol protein	3.491	3.683	4.031	3.735	102.5
Ear formation	Control	3.385	3.415	3.972	3.591	100.0
	Megafol	3.542	3.621	4.055	3.739	104.1
	Megafol protein	3.465	3.493	3.996	3.651	101.7

	A	B	A x B	A	B	A x B	A	B	A x B
GD 5%	0.02	2.53	3.91	0.13	2.21	3.15	0.09	3.51	4.65

The highest yield of grain, on average for the study period 2015-2017 was obtained with the variant of Elbrus durum wheat treated with the organo-mineral product Megafol in phenophase of tillering - 3.940 t/ha or with 0.479 t/ha more (13.8%) than the untreated control. By years, the increase in yield is in the range of 0.298 t/ha in 2016 to 0.86 t/ha in 2015. Next is the variant of applying the product Megafol in phenophase of shooting up of the durum wheat from 0.207 t/ha in 2017 to 0.435 t/ha in 2016 or an average over the study period of 0.326 t/ha more than the untreated control.

The grain yield in the case of treatment with the organo-mineral product Megafol Protein is less than that of the Megafol-treated variants, with the best result being reported in phenophase of tillering, from 0.132 t/ha in 2017 to 0.390 t/ha in 2015, with average result for the three-year experimental period being 0.260 t/ha. The control crops achieved the lowest yield results.

The use of the organo-mineral products Megafol and Megafol Protein during the phenophase of ear-formation does not lead to any significant positive changes in the grain yield obtained.

From the data obtained from the conducted experiment we can say that higher yield is achieved with the organo-mineral product Megafol, applied in phenophase of tillering and phenophase of shooting up of durum wheat. The organo-mineral product Megafol Protein produces lower results than Megafol, with the highest yield being observed when applied during the phenophase of tillering of the Elbrus variety.

CONCLUSIONS

The organo-mineral products tested have positively affected the productivity of Elbrus durum wheat.

The highest grain yield was obtained in the period of 2014-2017 by treating with the organo-mineral product Megafol in phenophase of tillering. On average for the experimental period for Elbrus durum wheat, the yield reported was 3.940 t/ha, or with 0.479 t/ha more (13.8%) than the control crops. Next is the variant of applying the product Megafol in phenophase of shooting up of durum wheat, the yield being averagely for three years with 0.326 t/ha more than the untreated control.

The grain yield achieved with the use of the organo-mineral product Megafol Protein was smaller than the variant treated with Megafol, with the best result being achieved in phenophase of tillering, averagely for the experimental period 3.721 or with 0.260 t/ha (7.5%) more than control.

The use of the organo-mineral products Megafol and Megafol Protein during the phenophase of ear formation did not lead to significant positive changes in the grain yield obtained.

The new organo-mineral products helped to increase the number of tillers, number of ear-bearing stems, number of spikelets in an ear, number of grains and grain mass of one plant.

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ESTIMATION OF THE SOWING RATE AND ROW SPACING INFLUENCE ON GREEN BIOMASS QUALITY FOR ALFALFA BY MEANS OF MATHEMATICAL AND STATISTICAL ANALYSIS

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Abstract

The objective of the present study is by means of a mathematical approach (variance, regression and variation analysis) to estimate the yield itself and to determine the row spacing influence on yield. Data analysis and mathematical processing, obtained during the study period (2016-2018), showed that the tested alfalfa varieties produced the best results at standard row spacing of 12.5 cm, regardless the used seed rate. The dispersion analysis of green mass and hay yields registered that the influence in the first year was the strongest (η = 96). In the second and third years, the variety had stronger influence on the yield (η = 95 and η = 99). The strongest factor correlation was the one between variety and row spacing (η = 89; η = 91; η = 94), which confirmed the fact that varieties react differently and have different compensatory capacity with the row spacing increase. The linear regression model between hay yield and total green mass yield for both varieties (Multifolium 1 and Legend) showed a high degree of correlation.

Key words: *alfalfa, yield, analysis of variance evolution.*

INTRODUCTION

Alfalfa quality and durability depend on both, external environmental conditions and internal genetic factors. Alfalfa related research shows that yields have increased by 20% over the last hundred years (Kertikova, 2000), with only 10% of the increase being due to genetic improvements (Riday and Brummer, 2002).
Along with environmental factors, production potential is also influenced by the alfalfa crop age and the intensity of use (Brink et al., 2010).
Successful alfalfa crop production is a basic factor for obtaining maximum production. The optimum sowing rate continues to be the subject of numerous studies because of the fact that significant differences have been found depending on the region environmental conditions. The recommended sowing rate for alfalfa varies from 4 to 40 kg/ha depending on the area, its soil and climatic conditions, and the sowing method, which directly affects seed germination and sprouting.
The present experiment aims at: 1) analyzing the influence of *variety*, *row spacing* and *sowing rate* factors on plant biometric indices by means of factor dispersion analysis, establishing both, their self-action and their interaction; 2) finding a proper mathematical function that describes empirical data distribution on yield.

MATERIALS AND METHODS

The experiment was conducted during the period 2016-2018 in the experimental field of the Crop Production Department at the Agricultural University – Plovdiv. A basic task was to determine the influence of *variety*, *row spacing* and *sowing rate* factors on plant biometric parameters. The experiment was based on the block method in four repetitions (Barov, 1982).
The obtained experimental data were included in a factor variance analysis. The factors *variety* (factor A), *row spacing* (factor B), *sowing rate* and their interaction were examined. The following options and combinations were tested: b1 - row spacing 12.5 cm; b2 - row spacing 25 cm; b3 - row spacing 50 cm; c1 - seed rate 2.5 kg/da; c2 - seed rate 1.5 kg/da (Table 1).
The total yield of alfalfa green mass and hay was formed by the yield obtained from swaths developed under different climatic conditions, both, during the growing season and the experimental years.

Table 1. Green mass and hey total yields for the period 2017-2019

	Multifolium 1		Legend	
	Average for the period 2017-2019		Average for the period 2017-2019	
Options	Total green mass yield	Total hey yield	Total green mass yield	Total hey yield
b1c1	6.75	1.49	7.59	1.79
b1c2	6.48	1.45	7.41	1.73
b2c1	6.13	1.38	7.36	1.72
b2c2	6.14	1.37	7.28	1.70
b3c1	5.96	1.33	6.29	1.47
b3c2	5.95	1.34	6.27	1.46

The regression analysis included: 1) constructing the mathematical model of dependence, i.e. calculating the function parameters and compiling the regression equation; 2) verifying the model statistical significance and the function parameters; 3) determining the actual deviations size from the theoretically expected results and on this basis, estimating the dependent variable state (Y) according to the individual state of the independent variable (X). Experimental data were estimated by the average values $\bar{x}$ of each variety multiplied with the total yield, the error of the average $S\bar{x}$, the standard deviation s and the variation coefficient CV, %. An interesting approach has been used to manage and compare data on some major food products in Bulgaria (Dimova, 2018). This approach has been used for establishing the relation between important agronomic indicators in maize hybrids (Stoyanova and Delchev, 2014), common wheat (Delchev et al., 2015, Stoyanova et al, 2016, Stoyanova et al., 2018) and celery (Kuneva et al., 2016).
The mathematical and statistical empirical data processing was performed using MS Excel.

RESULTS AND DISCUSSIONS

The dispersion analysis of green mass and hay yields (Table 2) showed that in the first year the influence of row spacing on yield was the strongest (η = 96). In the second and third years, the variety had stronger influence on yield (η = 95 and η = 99). The strongest factor correlation was the one between variety and row spacing (η = 89; η = 91; η = 94), which confirmed the fact that varieties react differently and have different compensatory capacity as the row spacing increases.

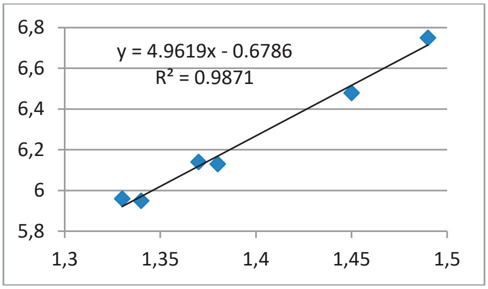
Interaction between sowing rate and row spacing factors (η = 91) was also proven, leading to changes in plant height, leaf weight and number, emergence of multifoliage, with changes in sowing density.
2018 differed slightly from the previous year due to the fact that variety influence was the strongest (η = 98). Row spacing influence came on the second place (η = 95). The correlation between variety and row spacing factors (η = 89) was the strongest, as it was also the case for green mass production.
In 2019, the trends observed over the years were confirmed. Variety had the most significant influence on hay production (η = 99). Row spacing influenced yield (η = 97), and the interaction between sowing and row spacing (η = 94) was also proven. Data analysis and mathematical processing obtained during the study period (2017-2019) showed that the tested alfalfa varieties produced the best results at a standard row spacing of 12.5 cm, regardless the used seed rate. In rarer crops, both varieties formed more leaves, the degree of multiplicity was higher and biomass with a higher protein content was obtained. For successful practice, alfalfa leaf can also be used in a lower seed rate of 1.5 kg/da, as recommended by other authors (Contreras-G et al., 2009), without affecting green mass and hay yield and quality.
Table 3 presents the analysis data of variance for hay yield. The results showed that in 2016 the row spacing (η = 98), followed by the variety effect on yield (η = 97), had the strongest influence on hay production, as in the case of green mass. The interaction between sowing and row spacing (η = 84) was also proven (Table 2).
For both tested cultivars, considering the three mowings and the growing season in general, the best results were obtained with the standard alfalfa cultivation - 12.5 cm, for the case of Multifolium 1 - 6.90 t/da, and 6.79 t/da, and for the Legend variety - 7.67 and 7.76 t/da, respectively, for both sowing rates. Again, the Legend variety exceeded the Multifolium 1 variety in both, green mass and hay production, regardless the swath sequence (Table 1), 2 kg - 0.235 kg of hay were obtained from 1 kg green mass for the Multifolium 1 variety through different swaths, while the Legend yield ranged from 0.220-0.240 kg.

Table 2. Dispersion analysis of green mass yield by years

Sourse of variation	SS	Df	MS	P-value	ETA, Sqd
2016					
Variety (A)	2630343.6	1	2630343.4	0.000	87
Line spacing (B)	9594649.0	2	4797324.1	0.000	96
Sowing rate (C)	9441.36	1	9441.6	0.465	2
Interaction (A×B)	414676.2	2	207228.6	0.000	50
Interaction (A×C)	104868.3	1	104868.03	0.021	20
Interaction (B×C)	996371.2	2	498185.86	0.000	71
Interaction (A×B×C)	1496264.6	2	74632.03	0.024	27
Errors	411250.7	24	17135.44		
2017					
Variety (A)	5666780.3	1	5666780.3	0.000	95
Line spacing (B)	6742886.0	2	3371443.0	0.000	96
Sowing rate (C)	2256.3	1	2256.3	0.672	0,8
Interaction (A×B)	2293728.0	2	1146864.0	0.000	89
Interaction (A×C)	39800.3	1	39800.3	0.085	12
Interaction (B×C)	7214.0	2	3607.0	0.749	24
Interaction (A×B×C)	134304.0	2	67152.0	0.011	31
Errors	295516.0	24	12313.2		
2018					
Variety (A)	9770834.03	1	9770834.0	0.000	99
Line spacing (B)	2690550	2	1345275.0	0.000	98
Sowing rate (C)	573806.25	1	9770834.0	0.000	89
Interaction (A×B)	1094505.6	2	547252.8	0.000	94
Interaction (A×C)	2417.4	1	2417.4	0.362	4
Interaction (B×C)	654450.0	2	327225.0	0.000	91
Interaction (A×B×C)	86822.2	2	43411.1	0.000	56
Errors	67235.3	24	2801.5		

Table 3. Dispersion analysis of hey yield by years

Sourse of variation	SS	df	MS	P-value	ETA, Sqd
2017					
Variety (A)	310620.4	1	310620.4	0.000	97
Line spacing (B)	489887.7	2	244943.9	0.000	98
Sowing rate (C)	1272.1	1	1272.1	0.083	12
Interaction (A×B)	20291.7	2	10145.9	0.000	69
Interaction (A×C)	2952.1	1	2952.1	0.011	24
Interaction (B×C)	49457.1	2	24728.5	0.000	84
Interaction (A×B×C)	6927.1	2	3463.5	0.001	43
Errors	9307.3	24	387.8		
2018					
Variety (A)	551306.3	1	551306.3	0.000	98
Line spacing (B)	333747.2	2	166873.6	0.000	96
Sowing rate (C)	0.03	1	0.03	0.994	0
Interaction (A×B)	136620.2	2	68310.1	0.000	91
Interaction (A×C)	2721.4	1	2952.1	0.035	17
Interaction (B×C)	1147.4	2	573.7	0.363	8
Interaction (A×B×C)	6517.1	2	3258.5	0.008	33
Errors	13039.3	24	543.3		
2019					
Variety (A)	794475.1	1	794475.1	0.000	99
Line spacing (B)	128376.1	2	64188.0	0.000	97
Sowing rate (C)	28448.4	1	28448.4	0.000	88
Interaction (A×B)	58628.7	2	29314.4	0.000	94
Interaction (A×C)	44.4	1	44.4	0.601	12
Interaction (B×C)	30935.4	2	15467.7	0.000	89
Interaction (A×B×C)	4605.4	2	2302.7	0.000	55
Errors	3806.7	24	158.6		



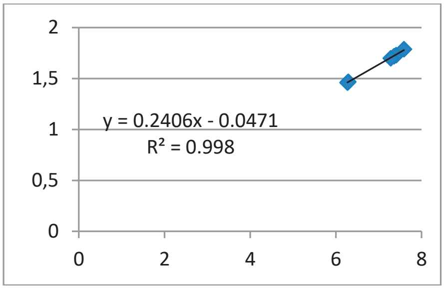


Figure 1. A- Linear regression model between hey yield and green mass total yield for Multifolium1 variety
B- Linear regression model between hey yield and green mass total yield for Legend variety

The visual representation of the correlation between both indicators for the tested varieties is obtained from the graphical presented data (Figures 1 A and B). Based on the empirical values, a point diagram (scatterplot) is drawn. The obtained linear regression models express the influence of hay yield indicator on green mass yield, allowing us to theoretically determine how and in what direction the change in these indicators contributes to yield improvement.

The determination coefficient R^2 indicates the percentage of resultative variable scattering and is explained by the factor variable action.
In our case, $R^2 = 0.9871$, i.e. hay yield depends on 98.7% of green mass yield for Multifolium 1, and for Legend variety it is $R^2 = 0.998$, ie. 99.8%. As green mass yield increases, the total hay yield increases.
We can assume that the linear regression models are reliable.

Table 4. Descriptive Statistics for the Multifolium 1 variety

Indicator	Min	Max	Mean		Std.	Variance
			Statistic	Std. error		
Yield of hay	5.95	6.75	6.24	0.13	0.32	0.100
Yield of green mass	1.33	1.49	1.39	0.03	0.06	0.004

Table 5. Descriptive Statistics for the Legend variety

Indicator	Min	Max	Mean		Std.	Variance
			Statistic	Std. error		
Yield of hay	6.27	7.59	7.03	0.24	0.59	0.351
Yield of green mass	1.46	1.79	1.65	0.06	0.14	0.020

It is logical that the hay yield for both varieties decreases with the row spacing increase, regardless of the sowing rate. For Multifolium 1, this is mathematically proven within all options with row spacing of 25 and 50 cm; for the Legend variety it is proven only within options with row spacing of 50 cm.
Variation analysis for Multifolium 1 and Legend varieties take into account the limits of variation in yields for each individual variety (Tables 4 and 5). Results of the variability between the studied indicators show lowest value at the yield of green mass for Multifolium 1 (x = 1.33), and highest at the yield of hay for Legend variety (x = 7.59).

CONCLUSIONS

The dispersion analysis registered that the examined alfalfa varieties showed best results at standard row spacing of 12.5 cm, regardless the used seed rate. The obtained linear regression models express the influence of the hay yield indicator on the green mass yield, allowing us to theoretically determine how and in what direction the change in these indicators contributes to yield improvement.
In our case, $R^2 = 0.9871$, i.e. hay yield depends on 98.7% of green mass yield for Multifolium 1, and for the Legend variety it is $R^2 = 0.998$, i.e. 99.8%. As green mass yield increases, the total hay yield increases.

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WHEAT PROTEIN FRACTION TO GRAIN QUALITY CHARACTERISTICS OF SOFT ALBANIAN WHEAT

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Abstract

The classical Osborne wheat protein fraction (albumins, globulins, gliadins, glutenins), modified according Maes and chemical - technological characteristics, were determined in ten lines of soft wheat, collected during the summer season of 2014. The solvent, used during the process of extracting proteins from wheat flour, consisted of distilled water, 40% isopropyl alcohol, 3.85% lactic acid and 0.5% potassium hydroxide. Wheat lines investigated in the experiment was carried out as a randomized block design in 3 m² plots with three replicates. The flour obtained from those lines of wheat has the following quality: protein: 15.05%, Zeleny Sedimentation: 47 ml and gluten index (GI): 53.95%. The protein extraction with this procedure was efficient and provided 90-99% protein recovery.

Key words*: soft lines, quality parameters, protein fraction, gluten index.*

INTRODUCTION

Among the large assortment of food products from wheat, the bread is still the most essential food product from wheat. For this reason, the good quality is important and thus, the studies of bread making quality are of a fundamental nature. The quality of wheat could be affected by several variables, including: physical grain properties, protein content and composition, starch and lipid content. Among the quality characteristics, the baking quality of wheat flour is primarily dependent on the quantity and quality of flour protein (Finney, 1987; Dowell et al., 2008). As most of the proteins in flours are gluten and gluten forming proteins play key roles in baking quality of wheat by improving the water absorption capacity, extensibility and elasticity of the dough. Total protein content, as well as protein quality, as measured by Zeleny sedimentation (K-SDS), wet gluten (WG) and Gluten Index (GI), are influenced by environmental and genetical factors (Curic et al., 2001; Dowell et al., 2008; Šekularac et al., 2018). Wet gluten content is determined by washing the dough obtained from flour, with water or other solutions (e.g. NaCl solution), in certain conditions, to remove the starch and other soluble compounds of the sample (Mis, 2000). The rubbery mass that remains after washing is the wet gluten. The gluten proteins, the gliadins and glutenins, constitute up to 80-85% of the total flour protein (Shewry et al., 1995). The gliadins and glutenins constitute each around 50% of the gluten proteins. In 1907,Osborne classified wheat protein according to the basics of solubility: albumins (soluble in water), globulins (aqueous solution of salt), gliadins (aqueous ethanol) and glutenins (dilute acid or alkali). Water-soluble albumins and salt-soluble globulins constitute anywhere from 10% to 22% of the total flour protein (Singh and MacRitchie, 2001). The gliadins constitute anywhere from 30% to 40% of total flour proteins and are divided into four groups, alpha- (α-), beta- (ß-), gamma- (γ-), and omega- (ω-) gliadins, based on their electrophoretic mobility at a low pH (Woychik et al., 1961; Kasarda et al., 1983). Maes (1962) presented a novel approach of fractionating of flour protein based on solubility. The new solvent sequence consisted of: distilled water, 40% isopropyl alcohol, 3.85% lactic acid and 0.5% KOH.

The goals of the study are as follows: (i) to evaluate the wheat protein fraction according Maes (albumin, globulin, gliadins and glutenins) in ten lines of soft wheat and (ii) to determine the content of protein, wet gluten, gluten index and K-SDS as quality parameters of wheat flour.

MATERIALS AND METHODS

Ten lines of soft wheat (L 1, L 2, L 3, L 4, L 5, L 6, L 7, L 8, L 9, L 10) were grown during the year 2013-2014 in the Experimental Didactics Economy (E.D.E) of the Agricultural University of Tirana (latitude 41°19'39"N, longitude 19°49'08"E; average altitude 89 m). Each plot was planted in five rows; the plot size being 5 m x 1.2 m. The protein content (% N x 5.7) was determined by the Kjedahl method (AOAC, Method 979, 09), and lipid content was analysed based on the Soxhlet extraction method utilizing n-hexane (AOAC, Method 4.5.01). The Wet Gluten and Gluten Index values for all flours samples were determined using the Glutamatic system (Petern Instrument AB, Stockholm, Sweden) with the use of the AACC method 38-12.02. Sedimentation value (K-SDS) was determined according to Zeleny (Zeleny, 1947). The fraction of wheat protein was based on solubility using the continuous extraction procedure proposed by Maes (1966) and applied with some modification by Mattern et al. (1968) and Williams and Butler (1970). Solvent sequence consisted of: distilled water, 40% isopropyl alcohol, 3.85% lactic acid and 0.5% KOH. All chemical analyses were performed in three replications and the results were statistically analysed. Pearson's correlation and the analysis of variance (ANOVA) between the obtained results were performed using StatSoftStatistica 10.0 software.

RESULTS AND DISCUSSIONS

Data in Table 1 indicates that the content of total protein was significantly higher in ten lines of wheat, which varied from 13.31% (line 8) to 17.34% (line 4). In wheat, the bread - making characteristics of dough is strongly influenced by protein content and protein quality (Johansson and Svensson, 1999).

Table 1.Chemical parameters in ten lines of wheat

Lines	Protein (%)	IG (%)	K-SDS (ml)
1	14.93±0.16^{ab}	90.80±0.14^{h}	49.50±0.71^{b}
2	15.07±0.06^{ab}	53.65±0.21^{e}	47.50±0.71^{b}
3	14.90±0.11^{ab}	59.85±0.21^{f}	48.00±0.00^{b}
4	17.34±0.03^{b}	19.60±0.14^{a}	42.50±0.71^{a}
5	15.07±0.04^{ab}	92.20±0.28^{i}	51.00±1.41^{b}
6	14.93±0.06^{ab}	25.70±0.00^{b}	51.00±1.41^{b}
7	15.68±2.07^{ab}	42.25±0.21^{d}	47.50±0.71^{b}
8	13.31±0.07^{a}	34.35±0.21^{c}	42.50±0.71^{a}
9	14.41±0.02^{a}	42.80±0.14^{d}	41.00±1.41^{a}
10	13.45±0.06^{a}	78.75±0.21^{g}	49.00±1.41^{b}

*The results are expressed as mean ± SD values, followed by different letters in the same column that are significantly different ($p < 0.05$), according to Tukey's HSD test.

However, high value of grain protein content does not reliable indicator for good bread making quality (Zhu and Khan, 2002), while gluten proteins (content and composition of gliadin and glutenins) are associated with viscoelastic properties of dough and with bread making quality (Shewry and Halford, 2002; Bekes et al., 2004; Delcour et al., 2012). Gluten is the rubbery mass that is left when wheat flour is washed with water to remove starch. Gluten structure has a major importance on dough rheological properties (Pedersen and Jorgensen, 2007). Guten proteins (gliadin and glutenins) are responsible for viscoelastic characteristics of dough and bakery products (Singh and Singh, 2013; Wang et al., 2015). Gluten Index (GI) is a method of analysing wheat protein that provides necessary information for gluten quality and quantity (AACC, 2000). The Gluten Index (GI) value was the highest in Line-1 (92.20%) and the lowest in Line 4 (19.60%). In this study the GI range between 19.60% (L-4) and 92.20% in L-5. According to scale of Oikonomou et al. (2015) we can classify analyzed wheat line in three groups. First group had strong gluten with GI over the 80% (L-1 with GI = 90.80% and L-5 with GI = 92.20%), second group of line L-2, L-3, L-7, L-8, L-9 and L-10 which have normal gluten which gluten index was between 30% and 80% and third group of wheat with weak gluten which GI is lower than 30% L-4 with GI = 19.60% and L-6 with GI = 25.70%) (Table 1). The line 10 with GI = 78.75% can classify in the group with strong gluten if we consider criteria of to Ćurić et al. (2001) who are reported that the optimum value of gluten index is between 75% and 90%. The wheat line (L 1, L 5 and L 10) had high protein content (14.93%, 15.07%, 13.45%) and an optimum level of gluten index (90.80%, 92.20%, 78.75%), belongs the group of wheat which can provide optimum bread making quality (Table 1). According to Šekuralec et al., (2018), in two years of experimental study values of gluten Index (GI) were higher than 80% for all six wheat varieties in both years and GI varied from the lowest 80.50% in Zvezdana to the highest 96.50% in NS 40S. Zeleny sedimentation (K-SDS) is another test used to determine protein quality. In this study K-SDS value varies from 41 ml (line 9) to 51 ml (lines 5, 6). The significant differences among the investigated parameters were established for protein content and GI.Similar data of significat differences for gluten index (GI) among wheat cultivars were found in investigation of Šekularac et al. (2018). The protein composition is strongly influenced by the genetic background (Ćurić et al., 2001).

Because grains were collected from plants grown under equal conditions in the field at the same location during the same growing season, the influence of environmental factors can be ignored. The fraction of wheat protein was based on solubility using the continuous extraction procedure proposed by Maes (1962). Solubility fractions of wheat are presented in Table 2. Data are expressed as the percentage of total protein (protein solubility index - PSI). Compared to the Osborne method, this method investigates the whole spectrum of wheat proteins. The following fractions were understood to be present; in water, there are albumin extractions, in isopropyl alcohol 40% of the fractions are globulins extractions, in lactic acid 3.85% are gliadins extractions and in KOH 0.5% are glutenin extractions. The protein fraction content soluble in water (albumin) and protein fraction content soluble in isopropyl alcohol 40% (globulin) ranged from 18.10% (line 7) to 22.68% (line 3); 10.26% (line 4) to 17.89% (line 5), respectively. According to Stehno et al., (2008), albumin - globulin constitute from 22.29% to 30.81%, in soft wheat. Similar mean values of albumin contents (23.12 g kg^{-1}) for 15 analyzed bread wheat found Branković et al. (2015). In most of the lines of wheat samples it was found that the protein fraction content soluble in lactic acid 3.85% (gliadin) was lower and ranged from 23.30% (line 4) to 42.16% (line 8). Although the albumin and globulin fractions are not known to play a direct role in bread making, they may be necessary for normal baking properties (Peruffo et al., 1996).

Table 2.The content of protein fraction (%) in 10 lines of wheat

Lines	H_2O	Isopropyl alcohol 40%	Lactic acid 3.85%	KOH 0.5%
1	21.81±0.08^{f}	13.49±0.04^{f}	30.66±0.04^{f}	18.37±0.08^{e}
2	20.23±0.06^{d}	15.67±0.07^{h}	26.88±0.03^{c}	20.12±0.06^{c}
3	22.68±0.17^{g}	17.82±0.01^{a}	27.55±0.21^{d}	16.18±0.01^{d}
4	19.50±0.07^{c}	10.26±0.08^{b}	23.30±0.02^{a}	14.98±0.07^{a}
5	19.79±0.08^{c}	17.89±0.06^{a}	25.86±0.04^{b}	19.61±0.11^{b}
6	18.99±0.01^{b}	17.45±0.05^{i}	28.41±0.04^{e}	19.96±0.03^{c}
7	18.10±0.06^{a}	12.38±0.03^{d}	38.45±0.01^{h}	22.89±0.11^{f}
8	20.71±0.11^{e}	15.05±0.01^{g}	42.16±0.03^{j}	19.46±0.01^{g}
9	20.60±0.01^{e}	13.09±0.01^{e}	38.13±0.02^{g}	25.25±0.18^{b}
10	20.90±0.08^{e}	11.65±0.13^{c}	40.16±0.03^{i}	14.85±0.11^{a}

*The results are expressed as mean ± SD values, followed by different letters in the same column that are significantly different ($p < 0.05$), according to Tukey's HSD test.

A higher variation of gliadins was found in ten lines of wheat samples. The protein fraction content soluble in KOH 0.5% (glutenin) ranged from 14.85% (line 10) to 25.25% (line 9). Gliadins and glutenins are recognized as a major wheat storage protein. Glutenin affects the elastic properties of dough where as gliadins affect the viscous properties of dough. Pearson correlation coefficients were calculated between flour quality characteristics and protein fraction.

Table 3. Correlation between chemical parameters and protein fraction content

	Lipids	IG	K-SDS	H_2O	Isop. alc	Lactic ac.	KOH
Protein	-0.071	-0.297	-0.079	-0.369	-0.237	-0.649^{+}	-0.120
IG			0.562*	0.459*	0.209	-0.031	-0.172
K-SDS				-0.028	0.491*	-0.317	-0.229
H_2O					0.209	-0.010	-0.381**
Isop. alc						-0.322	0.119
Lactic ac.							0.340

$^{+}$Correlation is statistically significant at $p < 0.01$ level; *correlation is statistically significant at $p < 0.05$ level; **correlation is statistically significant at $p < 0.01$ level.

Table 3, indicates a significant positive linear correlation between gluten index (GI) and Zeleny sedimentation (K-SDS) (r = 0.592). It was not possible to find a correlation between the variation of the protein fraction and quality characteristics. The only correlation of practical interest found, was between K-SDS and isopropyl alcohol fraction (r = 0.491). According to the previous study conducted by Maes (1966), it was not possible to confirm either the positive correlation between K-SDS and alcohol soluble fraction or the negative correlation between protein content and the lactic acid fraction. This is because the flour quality characteristics, in most cases, are strongly influenced by recessive or incompletely dominant genes (Fortini et al., 1976; Weegels et al., 1996).

CONCLUSIONS

From the results of this research study, all the lines of soft wheat are characterized with high protein content and good bread making characteristics. There were significant variations in means of soluble protein fraction between ten lines of wheat. The correlation between wheat protein fraction and quality parameters, characterizes the flour and the quality of finished the product, and shows a positive correlation between GI– K-SDS (r = 0.592) and K-SDS - isopropyl alcohol fraction (r = 0.491). It was not possible to find a lot of correlations between the variation of protein fraction and quality characteristics due to the fact that the quality characteristics are influenced by genetic background. The protein extraction with this procedure was efficient and provided 90-99% recovery of proteins.

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STUDY REGARDING THE OPTIMIZATION OF GRAIN SORGHUM CULTIVATION TECHNOLOGY IN THE CONTEXT OF SUSTAINABLE AGRICULTURE

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Abstract

Under the current conditions of climate drying, the identification of agricultural practices with the potential to mitigate the impact of climate change on the security of agricultural production is raising more and more interest. In this context, the orientation towards drought resistant crops is emerging as an alternative solution. Known as a culture specific to areas with arid climate and poorly productive soils, grain sorghum (Sorghum bicolor (L.) Moench, var. eusorghum) has become, in the context of increasingly obvious climate change, an alternative to maize, the cultivated area of sorghum increasing annually due to the stability of the productions and their nutritional quality.
The results of the research presented in this paper show that the level of the registered productions increased with the density of plants and the increase of the nutrient doses, the productions registering values of over 8,140 kg/ha in the variant with the density of 30 seeds/square meter on the level of fertilization with $N_{150}P_{80}$ *+ Aminosol. The level of fertilization ensured has led to high values of production quality indices: protein and starch.*

Key words: *grain sorghum, fertilization, density, yields.*

INTRODUCTION

Worldwide sorghum culture has recorded an upward trend in last 10-15 years due his important agronomic features: resistance to drought, diseases and pests, high ecological plasticity and high and constant production.
In 2017, the world Sorghum production reached 63.9 million tons, and the average yield was of 1,427 kg/ha. The main producers of Sorghum are the USA, Nigeria, Sudan, Mexico, Ethiopia and India (Popescu et al., 2018). At EU level, sorghum represent only 0.12% from world area cultivated with sorghum and from the main EU producers of Sorghum we can highlight: Italy, France, Spain, Romania, Austria, Hungary, and Bulgaria.
The area of sorghum cultivated in Romania represent a bit of over 25% of the total EU. The data from FAOSTAT (2018 records) show that the area cultivated in Romania was 15,819 ha with an average production of 4,823 kg/ha. However, the yields obtained on grain sorghum are far below the production potential that the climatic conditions in Romania offer and that is why it is necessary as the cultivation technology to be improved.
Oprea et al., 2017, in a research carried out in South East part of Romania, using ten fertilization levels and two sowing distances between rows had concluded that both sowing distance and fertilization had a statistically significant influence on sorghum's grain yield. The most favourable combination of technological factors, which ensured a maximum yield of 9.22 t/ha, was represented by sowing sorghum at the distance of 70 cm between rows and using a fertilization level N-120 P-60 K-60. Compared to the sowing distance of 50 cm between rows, the sowing distance of 70 cm between rows generated yield increases between 0.21 t/ha and 0.48 t/ha.
Pochiscanu et al., 2017, in a research from Central area of Moldavia focused on the influence of technological measures of quality of grain sorghum yield show that the sowing

density had negative influences on phenological characters and on quality of the grain, but positive on yield. The correlations were positive between fertilization with nitrogen and phosphorus and number of shoots ha^{-1}, yield and grain quality and negative between sowing density and number of shoots ha^{-1} and grain quality.

MATERIALS AND METHODS

The research was carried out at Agricultural Research and Development Station Caracal (ARDS), during the 2019 year in the conditions of a chermozem soil, medium rich in nutrient and with a humus content which varied between 3% to 4%. The soil in the arable layer (0-20 cm) has a lutearic texture with a clay content (particles below 0.002 mm) of 36.2%, an apparent density of 1.42 g/cm^3, a total porosity of 47% and one medium penetration rate (penetration resistance of 42 kg/cm^2).
From the point of view of the hydric features in the superficial layer, the wilting coefficient records the value of 12.3%, the field capacity 24.5% and the hydraulic conductivity is 9.2 mm/h.
The main aim of the research was to establish the most valuable variant of fertilization on the best density on grain sorghum. As experimented genotype we use grain sorghum hybrid Albanus (Concep III treatment) from Euralis Company, an early hybrid, with high tolerance on drought and good capacity for productions. The experiment was placed into rotation of 4 years with previous plant colza. The crop was sowing on 2^{th} of May and the complete emergence of plants was registered 11^{th} of May. The weed control was ensured by the treatment with Dual Gold herbicide in pre emergence and Casper herbicide applied in the first stage of plant development.
The experiment had two factors:
A factor - crop density - with three graduations:

- a1 - 20 seeds/square meter;
- a2 - 25 seeds/square meter;
- a3 - 30 seeds/square meter.

B factor - fertilization - with five graduations:

- b1 - unfertilized variant;
- b2 - $N_{75}P_{80}$;
- b3 - $N_{75}P_{80}$ + Aminosol;
- b4 - $N_{150}P_{80}$;
- b5 - $N_{150}P_{80}$ + Aminosol;

The collected data in the field were analysed using statistical ANOVA program.

RESULTS AND DISCUSSIONS

Climatic conditions (Table 1) during the experiment had an important influence on the evolution of grain sorghum crop. The recorded data certify that the 2019 year was an excessively hot year.
Compared to the normal area, an average temperature of 12.7°C was achieved, with 2.1°C higher than the normal range for the area of 10.6°C. Regarding the months of the warm period of the year (April - September) we find that in no month were temperatures lower than the multiannual average.

Table 1. Climatic conditions registered in 2019 at ARDS Caracal

2019	**Temperature [°C]**			**Solar radiation [W/m2]**	**Precipitations [mm]**	**Wind speed [m/s]**		**Daily ET0**
Month	**avg.**	**max**	**min**	**avg.**	**sum**	**avg.**	**max**	**[mm]**
January	-0.85	9.01	-13.47	41	38.6	1.6	8.5	0.2
February	3.42	17.41	-8.19	90	14.2	1.4	8.2	0.4
March	9.4	25.07	-3.96	156	25.2	1.7	9	2.8
April	12.11	27.16	-0.2	167	44.4	1.7	7.8	2.8
May	17.13	30.88	4.45	215	69	1.7	9.2	2.9
June	22.79	34.13	12.87	269	285.8	0.5	6	2.6
July	23.13	38.81	9.62	263	60	0.4	6.2	4.8
August	25.02	38.71	12.5	242	1	0.5	3.6	4.8
September	20.01	35.02	2.74	167	2	0.8	4.9	3.4
May-September	***21.6***	***38.81***	***2.74***		***417.8***			

The deviations were positive, ranging from 0.4ºC to 3.1ºC. It is noted as extremely hot in July, August and September, with a thermal surplus between 2.3ºC and, respectively, 3.1ºC. Also worth mentioning are the many days in July and August when the maximum temperature has exceeded the value of 38ºC and daily ETO had registered values of over 4.8 mm significantly reducing the water from soil.

During the vegetation period of the sorghum, from May to September, the total of 417.8 mm, numerically representing a sufficient value for a plant with relatively low requirements compared to the vegetation factor of water, but during the period of formation and filling of the grain, the months of August-September as very poor in precipitation, with a deficit of -49.7 mm and respectively -37.6 mm conduct to have resulted in the diminution of the elements of production, especially of the MMB.

Having a good capacity to efficiently capitalize on natural resources, sorghum has produced high yields under ecological conditions slightly favourable to other cereals (Antohe et al., 1981; Drăghici, 1989; 1999; Matei, 2011; 2016). Research has shown that the elements of technology: crop rotation and fertilization (Varvel, 2000; Khalili, 2008), crop density (Schatz et al., 1990) and distance between rows (Fernandez et al., 2012) - significantly influence the production potential of grain sorghum (*Sorghum bicolor* (L.) Moench var. *Eusorghum*).

The growth and development of the plant of grain sorghum was directly correlated with the technological factors studied: density and fertilization. The main morphological determinations made were presented in Table 2.

Table 2. The influence of fertilization and density on the development of the grain sorghum plants

A Factor Sowing density (seeds/sqm)	B Factor Fertilization (kg a.s./ha)	Height cm	Average leaves number	Panicle length cm
20	Unfertilized	106.8	8.6	23.7
	$N_{75}P_{80}$	107.3	9.0	25.4
	$N_{75}P_{80}$ + Aminosol	108.4	9.0	25.7
	$N_{150}P_{80}$	109.0	9.5	26.1
	$N_{150}P_{80}$ + Aminosol	112.0	9.8	26.3
Average		***108.7***	***9.2***	***25.4***
25	Unfertilized	106.6	8.0	25.0
	$N_{75}P_{80}$	107.3	8.5	25.2
	$N_{75}P_{80}$ + Aminosol	109.2	8.8	25.3
	$N_{150}P_{80}$	113.3	8.9	25.6
	$N_{150}P_{80}$ + Aminosol	114.6	9.4	25.6
Average		***110.2***	***8.7***	***25.3***
30	Unfertilized	115.9	7.5	25.0
	$N_{75}P_{80}$	116.5	7.6	25.6
	$N_{75}P_{80}$ + Aminosol	117.4	7.9	25.6
	$N_{150}P_{80}$	118.3	8.0	26.0
	$N_{150}P_{80}$ + Aminosol	119.0	8.0	26.3
Average		***117.4***	***7.8***	***25.7***

In the conditions of 2019, an increase in the size of the plant was observed in the range 106.6 cm to 119.0 cm, depending on the fertilization regime and in the range 108.7-117.4 cm, depending on the density of plants per unit area. Related to the leaves number we can observe that there is a positive correlation with level of fertilization - from 7.5 to 9.8 - and a negative one with plant's density 9.2 on the smallest density to 7.8 on the highest plant's density.

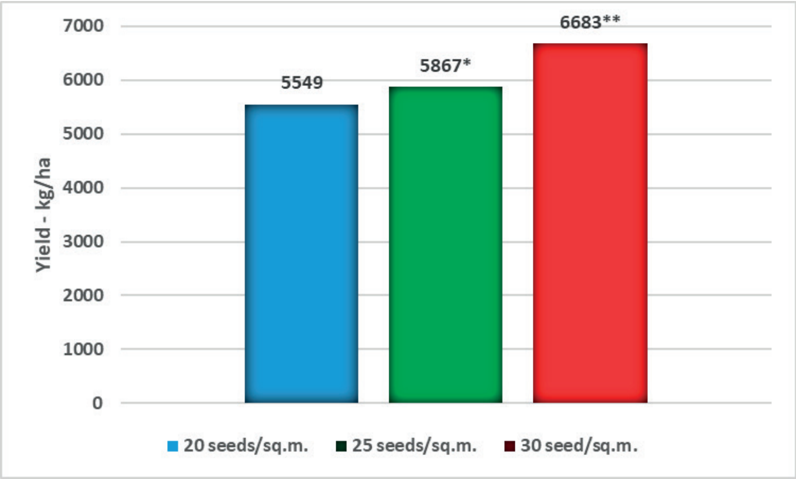
Related the panicle length, the experimented hybrid reacts only in case of fertilization, with small increase simultaneously with amount of doses and had a neutral reaction due the second factor - density - explained by very small differences between registered values.

Croitoru et al. (2018) into an experiment carried out in the condition of sandy soils from South of Oltenia, testing 10 hybrids of grain sorghum has concluded that the production potential for the area ranged between 5,077

kg/ha and 9,646 kg/ha depending of the genotype, the technology of culture and climatic conditions.
The results obtained at SCDA Caracal show that in the conditions of the year 2019 for the Caracal Plain area, sorghum is proving to be a species with real capacities of extension of the cultivated areas due to its adaptability, high production capacity and quality of grain production (Table 3, Figures 1 and 2).

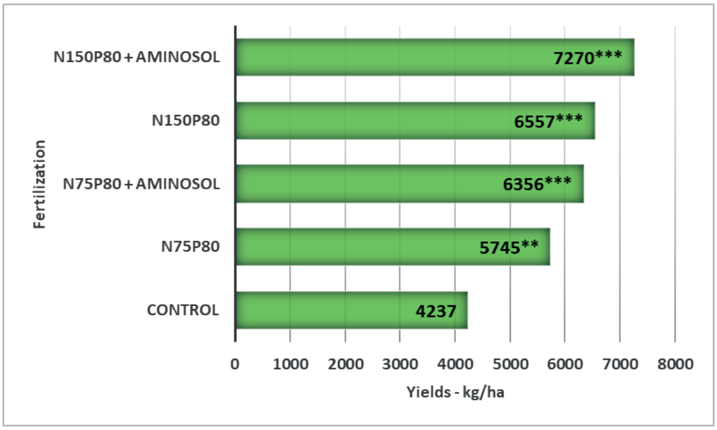
Table 3. The influence of interaction of density (A) and fertilization (B) on yield at grain sorghum

A Factor Sowing density (seeds/sqm)	B Factor Fertilization (kg a.s./ha)	Grain yields			Signification
		kg/ha	(%)	Differences kg/ha	
20	Unfertilized	3,761	62.3	-2,272	OOO
	$N_{75}P_{80}$	5,416	89.8	-617	-
	$N_{75}P_{80}$ + Aminosol	5,964	98.9	-69	-
	$N_{150}P_{80}$	6,023	99.8	-10	-
	$N_{150}P_{80}$ + Aminosol	6,583	109.1	550	-
25	Unfertilized	4,166	69.1	-1,867	OOO
	$N_{75}P_{80}$	5,511	91.3	-522	-
	$N_{75}P_{80}$ + Aminosol	6,190	102.6	157	-
	$N_{150}P_{80}$	6,386	105.8	353	-
	$N_{150}P_{80}$ + Aminosol	7,083	117.4	1,050	**
30	Unfertilized	4,785	79.3	-1,248	OO
	$N_{75}P_{80}$	6,309	104.6	276	-
	$N_{75}P_{80}$ + Aminosol	6,916	114.6	883	*
	$N_{150}P_{80}$	7,261	120.4	1,228	**
	$N_{150}P_{80}$ + Aminosol	8,142	135.0	2,109	***
Average/experience (Control)		**6,033**	**100**	**CONTROL**	**CONTROL**
LSD 5% (kg/ha)		637			
LSD 1% (kg/ha)		1,023			
LSD 0.1 % (kg/ha)		1,548			



LSD 5% - 235 kg/ha; LSD 1% - 594 kg/ha; LSD 0.1% - 1027 kg/ha

Figure 1. Influence of the A factor - plant's density - on the yields at grain sorghum



LSD 5% - 826 kg/ha; LSD 1% - 1472 kg/ha; LSD 0.1% - 2945 kg/ha.

Figure 2. Influence of the B factor - fertilization - on the yields at grain sorghum

The lowest production, of 3,761 kg/ha, was registered at the density of 20 seeds/sqm in the unfertilized version, and the highest value of the production was obtained at the density of 30 seeds/sqm in the version with $N_{150}P_{80}$ + Aminosol, of 8,142 kg/ha.

The average/experiment, used as control, has reached 6,033 kg/ha. Compared to this, it is noted on the 3 densities tested statistically assured increases in production for variants with higher levels of mineral fertilization to which was added also Aminosol, statistically assured increases at the $N_{150}P_{80}$ + Aminosol dose of 1,050 kg/ha at density of 25 seeds/sqm and 2,109 kg/ha at a density of 30 seeds/sqm. The probability of achieving a crop at a high density, of 30 seeds/sqm, is also supported by the average of the productions on the experienced densities (A factor), of 5,549 kg/ha for the density of 20 seeds/sqm, of 5,867 kg/ha for the density 25 seeds/sqm and 6,683 kg/ha at a density of 30 seeds/sqm.

The climate and soil condition of the area also allowed to obtain valuable productions also in the case of the unfertilized variants, sorghum having the capacity to capitalize on the agroproductive properties of the argic chernozem that was experimented, the average of the factor B - fertilization - ranging between 4,237 kg/ha for the unfertilized variant and 7,269 kg/ha for the $N_{150}P_{80}$ + Aminosol variant.

The role of the Aminosol ®, as biostimulator, containing 22 different amino acids and peptides (56-58%), corresponding to 9% N (110.7 g/N/l) organic nitrogen, has been highlighted in ensuring a high productivity of drought plants in drought conditions and the ability of the preparation to form a stronger root system, facilitating the absorption of nutrients from the soil and increasing the plant's resistance to drought. The applied Aminosol indirectly influenced the development of plants through the rhizosphere microbial community, which increased the absorption capacity of the nutrients in the soil and also had a very good influence to the main parameters of the quality of productions.

The quality of the grain sorghum production obtained was another goal of our research and the results of the accumulations of protein and starch (determined using a rapid seed analyser PERTEN-TA 7200) were analysed at the level of the tested variants. The recorded data shows that both factors - density and fertilization level - had influenced the chemical composition of sorghum grains, in terms of quality (Table 4).

The research literature on quality of grain sorghum yields shown that Khalil et al. (1984) had obtain under the conditions of Saudi Arabia values of the protein content which varied between 15.3% and 15.9%.

Other research of related the response of grain protein concentration and yield of sorghum to nitrogen fertilization in rates of 0, 60, 120, 180, 240 and 300 kg $N.ha^{-1}$ was studied in the experimental field of Agricultural University of Plovdiv, Bulgaria in 2017-2018 under non-irrigated conditions by Kostadinova et al. (2019). The main result proved that the fertilizers and climatic conditions had a powerful influence to the quality of grain sorghum yields: rate N-300 significantly increased concentration of grain protein by 15.0% and 21.9%, respectively in 2017 and 2018, compared to N-0 plants. Fertilization N-60 - N-300 proven increased grain protein yield over the N-0. Rate N-180 provided higher grain protein yield of 708 $kg.ha^{-1}$ in 2017 and higher N-240 and N-300 rates showed a downward trend in protein yield within limits 677-708 $kg.ha^{-1}$. In snore favourable in terms of rainfall 2018, the highest grain protein yield 907 $kg.ha^{-1}$ was obtained at N-240. Application of N-300 proven reduced by 80 $kg.ha^{-1}$ the protein yield, compared to N-240 Rates 0-300 kg $N.ha^{-1}$ highly positively correlated with grain protein concentration (0.864**-0.962**) and protein yield (0.839**-0.874**) of sorghum.

Table 4. The influence of interaction of density (A) and fertilization (B) on quality of yield at grain sorghum

A Factor Sowing density (seeds/sqm)	B Factor Fertilization (kg a.s./ha)	Protein			Starch		
		%	Differences	Signification	%	Differences	Signification
20	Unfertilized	9.29	-0.37	OO	64.50	0.78	*
	$N_{75}P_{80}$	9.95	0.29	**	63.80	0.08	
	$N_{75}P_{80}$ + Aminosol	10.06	0.40	**	63.90	0.18	
	$N_{150}P_{80}$	10.09	0.43	**	63.00	-0.72	O
	$N_{150}P_{80}$ + Aminosol	10.60	0.94	***	63.50	-0.22	
25	Unfertilized	9.20	-0.46	OO	64.70	0.98	**
	$N_{75}P_{80}$	9.45	-0.21	O	63.80	0.08	
	$N_{75}P_{80}$ + Aminosol	9.51	-0.15	O	63.60	-0.12	
	$N_{150}P_{80}$	9.67	0.01		63.20	-0.52	
	$N_{150}P_{80}$ + Aminosol	10.12	0.46	**	63.40	-0.32	
30	Unfertilized	8.27	-1.39	OOO	64.60	0.88	*
	$N_{75}P_{80}$	9.36	-0.30	OO	62.90	-0.82	O
	$N_{75}P_{80}$ + Aminosol	9.7	0.04		63.10	-0.62	O
	$N_{150}P_{80}$	9.8	0.14	*	62.90	-0.82	O
	$N_{150}P_{80}$ + Aminosol	9.85	0.19	*	64.90	1.18	**
Average/experience (Control)		**9.66**	**CONTROL**	**CONTROL**	**63.72**	**CONTROL**	**CONTROL**
DL 5%		0.12			0.59		
DL 1%		0.26			0.93		
DL 0.1 %		0.71			2.04		

The protein level accumulated by the Albanus hybrid ranged from 10.60% at the $N_{150}P_{80}$ + Aminosol variant at the density of 20 seeds/sqm and 8.27% at the unfertilized variant at the maximum tested density of 30 seeds/sqm. High doses of nitrogen applied alone or in combination with the Aminosol biostimulator led to qualitative increases in production, quantified in increases in the protein level in grains, statistically assured increases predominantly at the densities of 20 and 25 seeds/sqm.

Research by Kaufman et al. (2013) showed that the protein content in sorghum grains increased with increasing nitrogen rate. Looking at the results that we present we can see the same evolutions of the protein content, starting to lowest level on the unfertilized variant and increasing to the highest level of the fertilizers on all three densities tested, from 20 seeds/sqm. to 30 seeds/sqm.

Regarding the level of starch accumulated under the conditions of experimentation in 2019, we notice the small differences between the variants, the level of the registered values falling between 62.90% for the variants $N_{75}P_{80}$ and respectively $N_{150}P_{80}$ from the density of 30 seeds/sqm and 64.70% at unfertilized variant from the density of 20 seeds/sqm. If we look at the quality data, we can notice a kind of an inverse proportional relationship between the two components: protein and starch.

CONCLUSIONS

Taking in account all the date presented in this paper, we can highlight, the follow important conclusions:

- climatic conditions of 2019 year from ARDS Caracal ensure the grain sorghum to give good productions which varied between 3,761 kg/ha obtained at unfertilized variant on the smallest density to 8,142 kg/ha registered on highest density at maximum level of applied fertilizers;
- both tested factors, sowing density and nutritional regime had very powerful influence on the level of yields, ensuring very significant increase in productions related the Control used;
- for grain sorghum cultivated on argic chernozem, the best variant of plant's density prove to be 30 seeds/square meter, variant where the average production registered was over 6,683 kg/ha;
- the level of fertilization had also a very strong influence to the yields, grain sorghum having the ability to harness very well the nutrients applied and from this point of view, the most valuable variant proved to be $N_{150}P_{80}$ + Aminosol, whatever the density tested;
- the increases in production of the variants treated with Aminosol in comparison with those without treatment, at the same level of fertilizers, shown us that the grain sorghum has a very good capacity to use the minerals applied and conduct to the development of plants through the rhizosphere microbial community, which increased the absorption capacity of the nutrients from soil;
- in terms of quality of productions, we can observe that there is a correlation between protein content and starch content, in sense that on the variant where we observe the highest content of protein, the starch level decrease - on the same density variant;
- a large nutritional space on 20 seeds/square meter conduct to a higher accumulation of protein, of 10.60% at the highest level of fertilizers of $N_{150}P_{80}$ + Aminosol.

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STUDY OF THE EFFECT OF ORGANIC FERTILIZER SIAPTON ON PRODUCTIVITY OF OIL ROSE (*Rosa damascena* Mill.)

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Abstract

*The aimed of the study is to investigate the effect of nourishment and irrigation on the productivity of oleaginous rose (*Rosa damascena* Mill). The study was conducted with a bio-fertilizer based on natural hydrolyzed Siapton proteins. The observations were carried out under irrigated and non-irrigated conditions. The field experiment was performed in the field of experience of the Institute for roses, aromatic and medicinal plants, Kazanlak, Bulgaria in the period 2015-2017. The treatment was done twice with in a period of 20 days, of foliage in the following variants: Siapton treated 1500 ml/ha, Siapton treated 2500 ml/ha, Siapton treated 3500 ml/ha. The biometric analysis shows an increase in mass and color diameter of the treated variants. The correlation relationships obtained indicate the degree of the influence of each indicator in the formation of yields in oleaginous rose under the irrigation conditions considered. The linear regression models are expressing the influence of the indicator of yield, enabling theoretically how to determine and also in which direction it have to be change this indicator contributes to improved yield.*

Key words: *damask rose, organic fertilizer, rose oil, irrigation, correlation.*

INTRODUCTION

Rose production is a traditional industry in Bulgaria. The oleaginous rose (*Rosa damascena* Mill.) Is a crop of priority economic importance to the country. According to the Ministry of Agriculture, about 4,500 ha are occupied by oleaginous rose. Bulgaria is the world-recognized supplier of the highest quality rose oil. Since the beginning of 2020 the law act guarantees the quality and price of Bulgarian oleaginous rose. The requirments for propagating matelia and the varietal of which can be produced rose oil has been advertised.
Extremely rich is the choice of cosmetics and food products created on the basis of rose oil and rose water from Bulgarian rose. Products from oleaginous rose is used in perfumery and cosmetics. The pharmaceutical oleaginous rose industry is included in drugs production for eyes, stomach, skin and dental diseases.
The climatic conditions in the country are favorable for obtaining high yields and quality rose oil. In addition, oleaginous rose plantations (*Rosa damascena* Mill.) Also perform anti-erosion functions (Kovacheva et al., 2010). Climate change towards global warming and drought has created a strain on water resources. Water deficit during crop vegetation impedes growth, development, productivity and quality of yields. Optimizing irrigation and nutrition in the oilseed rose (*Rosa damascena* Mill.) is a condition for improving productivity.
The application of soil absorber Terawet, when creating a new plantation, has a positive effect on the rate of uptake and survival of young plants during the growing season, established Kovacheva (2011). This can save the extra watering needed for young plants when planting. Soil improvers serve as a reservoir of soil moisture and reduce the risk of plant deaths in water shortages (De Boodt, 1990; Johnson and Leah, 1990; Yangyuru et al, 2006). Rose plants require well-aerated soils with a large humus horizon (Pal et al., 2013). Improving the diet influences the formation of vegetative growth of shrubs, Lambev points out in his study (Lambev, 2011). The use of Humus Life Universal in dose of 300 ml/da contributes to a 7% to 11% increase in rose yields.
Production efficiency is related to the application of appropriate modern agrotech-nical approaches in the cultivation of

oleaginous rose (*Rosa damascena* Mill.). Proper agrotechnology is a guarantee for obtaining a specific composition of rose oil and authentic rose aroma. All this involves soil tillage, pesticide treatment, and mechanization of the technological process is particularly important (Mihov et al., 2015; Bozhkov et al., 2017).

In this study, we aim to examine the effect of nourishment and irrigation on the productivity of the oleaginous rose (*Rosa damascena* Mill). Applying mathematical approaches to determine the correlation between qualitative indicators to make a more objective assessment. Using the possibilities of the regression analysis to express the influence between the indicators and the trends for improving the yield of pink color and rose oil.

MATERIALS AND METHODS

The field experiment was performed in the experimental field of the Institute for roses, aromatic and medicinal plants, Kazanlak, Bulgaria. During the period 2015-2017, the impact of a specialized bio-fertilizer on the vegetative growth and productivity of the oleaginous rose was tested. The study was conducted with a bio-fertilizer based on natural hydrolyzed proteins “Siapton”. Observations were recorded under irrigation and non-irrigation conditions. The experiment is based on the Zade method (by the method of long plots), in four variants of five repetitions. Size of the test plot 25 m^2.

The field experiment was conducted under the conditions of leached forest soils. During the vegetation of oleaginous rose, variety "Svezhen", treatments with bio-fertilizer were carried out. The treatment was done by foliage twice with a period of 20 days in the following variants: Siapton treated -1500 ml/ha, Siapton treated 2500 ml/ha, Siapton treated 3500 ml/ha and untreated control. Irrigation is done through a drip irrigation system. From the beginning of the emergence of buttons to the end of flowering, waterings are implemented to maintain the water supply in the soil. By the irrigated variant irrigation rates were done by drip system as the soil moisture contents was maintained minimum in the range of 80 to 85% of the field capacity (FC) in the layer of 0-0.60 m. Dynamics of soil moisture in the layer 0-1.00 m was monitored periodically (once a week) by a weight method.

The bio fertilizer Siapton is a versatile organic fertilizer and biostimulant for leaf and soil applications containing amino acids and peptides. Ingredients: Total nitrogen 9.1%; organic nitrogen 8.7%; Ammonium nitrogen 0.4%; Organic carbon 25%; Total amino acids (of animal origin) 54.4%; Free amino acids 10.0%; Dry matter content 63%.

During the growing season, phenological observations and biometric measurements of the plants were carried out. In the rose plantation, harvesting during flowering is carried out manually. The analysis of the rose oil was recorded using a micro-distillation apparatus - a cleaver. Using gas-chromatophore analysis, the qualitative indicators of variants were established.

Test conditions: Column: capillary length 30 m, diameter 0.32 mm, film thickness of 1.0 μm, oven temp 70°C to 240°C at rise 8°C/min, isotherm at 240°C - 10 min, injector -300°C, detector 300°C. Standards/Validated Methods - Bulgarian State Standard (BSS) ISO 9842-2004, BSS ISO 11024-1.

The mathematical and statistical processing of the empirical data was performed through the SPSS statistical program.

RESULTS AND DISCUSSIONS

In the first and second years of the study, the average daily temperature values were close to those for a multi-year period (1978-2008). In the third year average daily temperatures above the normal were recorded. In terms of size and amount of rainfall, 2015 is favorable for the development of rose plants. Falling rainfall during the period of phenophase budding is in higher quantities, which, in combination with normal temperatures during this period, helps to lay more flower buds in rose bushes.

During the flowering period, precipitation amounts are lower than the norm for many years, but they are sufficient. In 2016, the amount of registered rainfall in May (209 mm) is three times higher than the norm (71 mm). The limited sunshine and the adverse distribution of rainfall have an adverse effect on the quality of rose oil. Meteorologically, the third year is characterized by rainfall and temperatures close to the norm and favoring the development and flowering of rose bushes.

The analysis of biometric indicators shows an increase in the mass and color diameter of the control variants. The increased vegetation growth and the increase in blossom is due to amino acid fertilizer applications.

The statistical analysis is based on the results of biometric measurements on fresh blossom oleaginous roses - blossom diameter (DM) and blossom weight (Mass) and yield of rosy blossom and essential oil. The experimental data were processed by correlation and regression analysis, with the help of which the correlation between the studied parameters was established and evaluated.

Correlation coefficients expressing the relationship between the studied indicators are indicated in the correlation matrices (Tables 1 and 2).

Table 1. Correlation dependencies under non-irrigation conditions

Indicators	Mass	DM	Yield rose oil	Yield of rosy flower from damask rose (*Rosa damascena* Mill.)
Mass	1.00	0.965**	0.917*	0.897
DM		1.00	0.885	0.939*
Yield rose oil			1.00	0.960**
Yield of rosy flower from damask rose (*Rosa damascena* Mill.)				1.00

Table 2. Correlation dependencies under irrigation conditions

Indicators	Mass	DM	Yield rose oil	Yield of rosy flower from damask rose (*Rosa damascena* Mill.)
Mass	1.00	0.934*	-0.042	0.878
DM		1.00	-0.369	0.676
Yield rose oil			1.00	0.232
Yield of rosy flower from damask rose (*Rosa damascena* Mill.)				1.00

A strong positive correlation was found between Mass and DM, and rose oil yield and rose color, respectively, with correlation coefficients - r = 0.965 and r = 0.960. The correlation between mass and production of rose oil is less pronounced; DM and rosy blossom yield, respectively, with ratios - r = 0.917 and r = 0.939.

Regression analysis was performed at the biometric indicator rose blossom under natural moist supply and under irrigation conditions (Figures 1 and 2). In the figures shown blossom weight is X and blossom diameter is respectively Y.

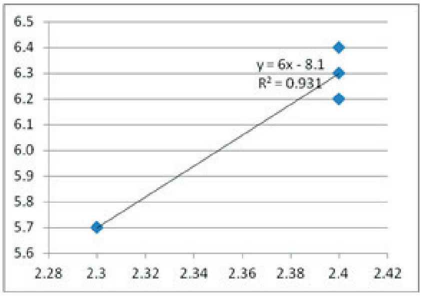

Figure 1. Linear regression model between Mass and DM under natural moist supply

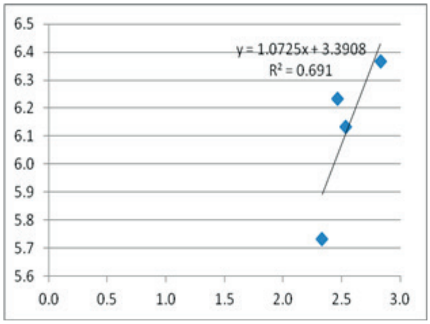

Figure 2. Linear regression model between Mass and DM under irrigation conditions

The correlation coefficient, R^2 is called the coefficient of determination, it shows what percentage of the variance of the resultant variable is explained by the effect of the factor variable. In our case, $R^2 = 0.931$, i.e. 93.1% of the yield under non-irrigation conditions and $R^2 = 0.691$, ie 69.1% of the yield under irrigation conditions. In the figures 3 and 4 shown rose oil yield is X and yield rose blossom is respectively Y.

As can be seen, the coefficient of determination is high, which means that the models describe well-observed values in the oleaginous rose. We can assume that the linear regression model is adequate.

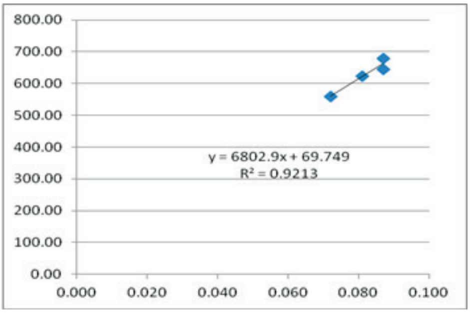

Figure 3. Linear regression model between rose oil yield and yield rose blossom under natural moisture supply

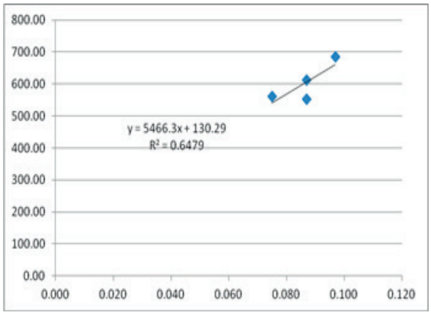

Figure 4. Linear regression model between rose oil yield and yield rose blossom under irrigation conditions

Table 3. Results of statistical processing of experimental data in the regression analysis performed, without irrigation

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std.
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
VAR00001	4	2.30	2.40	2.3750	.02500	.05000
VAR00004	4	558.27	677.70	625.8850	25.12120	50.24240
VAR00002	4	5.70	6.40	6.1500	.15546	.31091
VAR00003	4	.07	.09	.0818	.00354	.00709
Valid N (listwise)	4					

Table 4. Results of statistical processing of the experimental data in the regression analysis performed, under irrigation conditions

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std.	Variance
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
VAR00001	4	2.30	2.80	2.5250	.10308	.20616	.043
VAR00002	4	5.70	6.40	6.1000	.14720	.29439	.087
VAR00003	4	.09	.10	.0928	.00338	.00675	.000
VAR00004	4	552.87	685.97	603.1275	30.55917	61.11834	3735.451
Valid N (listwise)	4						

CONCLUSIONS

The obtain correlation dependences under irrigation conditions, shows that the degree of influence of each indicator in formation of oleaginous rose yields were considered. A strong positive correlation was found between blossom diameter and blossom weight (r = 0.965) and between rose oil yield and rose color (r = 0.960) under non-irrigation conditions. Correlation coefficient under irrigation conditions is the highest between blossom diameter and blossom weight (r = 0.934).
The linear regression models, is expressing the influence of indicator on yield per unit area, allow us theoretically how to determine and in which direction has to be change this indicators to improve yield.

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PROTEIN, LYSINE AND METHIONINE CONTENT IN THE GRAIN OF TRITICALE GROWN UNDER ORGANIC SYSTEM

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Abstract

The present study was conducted to determine the effect of variety, organic fertilizer and predecessor on protein, lysine and methionine contents, and their variation in triticale grains under biological cultivation. During the 2014-2017 period, a three factors experiment was managed on field certified for organic farming in Field Crops Institute - Chirpan, Bulgaria. Three varieties, three doses of organic fertilizer and two predecessors (sunflower and durum wheat) were tested. Based on the data obtained, it was found that the variety has a significant influence on the parameters studied. The highest significant values of the chemical parameters were observed when applying organic fertilizer at a dose of 1,750.0 kg/ha. After a predecessor of durum wheat, the value of lysine was higher and statistically significant compared to predecessor of sunflower. Similar coefficients of variation of the studied parameters were found, which not depended of a variety, a predecessor and an applied organic fertilizer, varying between 4.38% and 5.35% for protein, 4.1% and 5.33% for lysine and 4.31% and 5.22% for methionine.

***Key words**: lysine, methionine, organic farming, protein, triticale.*

INTRODUCTION

The quality of the grain is determined by the content of organic compounds, minerals, vitamins, antioxidants and more. The protein is one of the most important chemical component of the grain, which largely determines its nutritional value as a food crop and feed. In cereals, the content of lysine as a proteinogenic essential amino acid largely determines the biological value of proteins. Triticale has a high nutritional value, contains considerable quantity of protein, fiber, vitamins and minerals (Kruma et al., 2018) and is rich in essential amino acids (Fernandes-Figares et al., 2000). The most important nutritional characteristics of triticale grains are starch, as well as the quantity and quality of the protein (Edel Leon et al., 2008).

The interest in triticale as a source of feed is determine by a higher protein concentration and a better amino acid balance than other forage crops (Myer et al., 2004). On the other hand, the triticale has low gluten content, effective viscoelasticity and the prepared bread has low quality compared to wheat bread (Doxastakis et al., 2002). Myer et al. (2004) have summarized data from various authors for the period 1990-2002 and have found that the protein and lysine levels in triticale grains are higher than those in maize and wheat. Recent studies have showed that the protein content of triticale grains is close to wheat protein (11.4-14.0%) but has a higher amount of lysine (0.33-0.71%) (Fraú et al., 2016). According to Glamočlija et al. (2018), the average total protein content of triticale grains varies from 10.2% to 15.6% of the dry matter. These protein and lysine advantages are major factors for the development of triticale as a commercial crop (Gebre-Mariam and Larter, 1979) and determine its use primarily as a source of feed.

According to Glamočlija et al. (2018), the direction for triticale use depends from the characteristics of the variety, namely higher protein varieties can be use as concentrated feed for poultry, ruminants and non-ruminants, and varieties with high biomass can be use for grazing, silage or hay.

The protein quality is determined by the proportions of essential amino acids, which cannot be synthesized by animals and humans and must be provided through a diet. The lysine and methionine (sulfur-containing amino acid) are the first two essential amino acids, of great importance composing diet for farm animals, as they participate in a numbers of biochemical processes in the animal cell (Bouyeh, 2012). In this regard, although winter cereal grain is poor of lysine, it is an optimal source of sulfur amino acids (Alijošius et al., 2016).

When triticale is used in feed ration, it is more important to consider lysine content than protein (Angelova and Angelov, 1981). The lysine and methionine contents are low in plants, i.e., their levels are insufficiently to guarantee optimal growth for animals and humans (Galili and Amir, 2013). Consequently, the composing of fodder rations entirely from organically grown crops is complicated.

The organic farming is gain in popularity as an alternative to conventional farming systems (Benaragama, 2016), and should be understood as highly specialized farming. Along with the search for technological solutions to increase productivity for cereals, there is a requirement for grain quality. The triticale is a suitable crop for cultivation under biological system due to its stable yield, tolerance to unfavorable conditions, resistance to diseases and high competitiveness against weeds (Kronberga et al., 2013).

In Bulgaria a detailed investigation about the chemical qualities of triticale grain prodused under biological technology was missing so far. Considering the advantages and importance of triticale, the present study was conducted to determine the influence of the variety, organic fertilizer and predecessors on protein, lysine and methionine content, and their variation in the triticale grain under biological breeding system.

MATERIALS AND METHODS

During the period 2014-2017 experiment was managed in field certified for organic farming of Field Crops Institute, Bulgaria (42°11'58"N, 25°19'27"E). The experiment was based on a block method, with a perpendicular arrangement of the degrees of the tested factors in four replicates. The area of the crop plot was 18 m^2 with a seed rate of 550 g s/m^2. The following factors and levels were tested: factor A - variety (Colorit, Boomerang and Respect), factor B - organic fertilizer Lumbrical (applicated in a rate of 0, 1,400.0 kg/ha and 1,750.0 kg/ha) and factor C - Predecessor (sunflower and durum wheat). The organic fertilizer was applied manually before the last pre-sowing treatment of the soil, after which the area was cultivated. Lumbrical is a product of the processing of manure and other organic wastes from red Californian worms (*Lumbricus rubellus* and *Eisenia foetida*). It is applicated for organic farming under EU Regulation 889/2008. According to the FAO classification systems (FAO), the soil of the experimental field of the Field Crops Institute is Pellic Vertisols (Vp.). The contents of protein, lysine and methionine were monitored. The protein content (g/kg DM) was determined as the total nitrogen content by the Kjeldahl method in duplicate (Bremner, 1965), and was calculated using nitrogen conversion coefficient 6.25. The contents of lysine and methionine (g/100 g crude protein) in a protein were determined by Degussa (2001) regression equations. In order to establish statistically significant influences of the studied factors the software BIOSTAT (Penchev et al., 1989-1991) was applied on the data from the three years. Statistica 13 was used to determine the coefficients of variation.

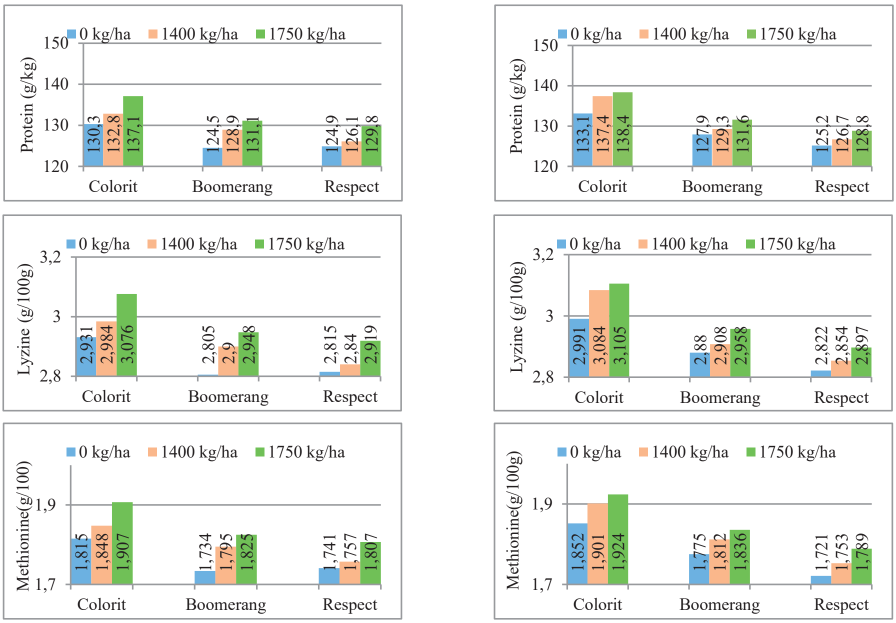
Acording to Table 1, compared to the average of multi-year period 1928-2013 (2009.7°C), the sum of temperatures during the harvest years 2014/15 and 2015/16 were higher, respectively 2,264.1°C and 2,530.4°C. In the third year of the study, the temperature during the growing season was lower (1,843.5°C) than the 86-year period. With regard to precipitation 2014/15 was very humid (578.1 mm), 183.2 mm more than the average for a period 1928-2013. The total amount of precipitation for 2015/16 (323.4 mm) was lower compared to a multi-year period (395.0 mm), and for 2016/17 (375.2 mm) was about the climatically average 1928-2013.

Table 1. Meteorological conditions during triticale vegetation

Period	Months								Σ
	XI	XII	I	II	III	IV	V	VI	
Temperature sums (Σ˚C)									
1928-13	215.9	61.1	-6.2	49.4	188.9	357.9	511.5	630.7	2,009.7
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
Rainfall (mm)									
1928-13	47.3	54.0	44.3	37.7	37.0	45.2	64.1	65.4	395.0
2014/15	36.9	142.3	50.3	61.7	134.9	15.1	58.8	78.1	578.1
±	-10.4	+88.3	+6.1	+24.0	+97.9	-30.1	-5.3	+12.7	+183.2
2015/16	50.2	1.3	73.9	28.3	53.1	26.6	75.0	15.0	323.4
±	+2.9	-52.7	+29.6	-9.4	+16.1	-18.6	+10.9	-50.4	-71.6
2016/17	47.7	5.9	80.1	23.8	51.3	22.6	59.5	84.3	375.2
±	+0.4	-136.4	+35.8	-13.9	+14.3	-22.6	-4.6	+6.2	-19.8

RESULTS AND DISCUSSIONS

The averages date for the period showed differences in the values of the studied parameters, both after the two predecessors and the varieties (Figure 1). A linear increase of values depending on the dose of organic fertilizer was observed due to the higher quantity of macronutrients imported with organic fertilizer. The content of protein, lysine and methionine was highest in grain at Colorit variety after the two predecessor, followed by Boomerang and Respect varieties.



A) predecessor sunflower   B) predecessor durum wheat

Figure 1. Protein, lyzine and methionine content 2014/2017 period

A similar coefficients of variation were found for the three indicators within two predecessors (Table 2). After a predecessor of durum wheat varied between 5.22% and 5.33%, and after sunflower between 4.71% and 4.96%. Rodehutscorda et al. (2016) have reported similar values of the variation coefficients for triticale. The coefficients found in their study were 5.23%, 4.66% and 3.30%, respectively for crude protein, lysine and methionine. The higher variation coefficients after durum wheat are consequence of higher averages values of crude protein, and subsequently of lysine and methionine (Tables 4 and 5). The variation coefficients of the indicators under fertilization were between 4.81% and 5.07%. The results obtained show stability of the studied indicators for all varieties under different agroecological conditions during 2014-2017 period. Base on the date from three years, the analysis of variance showed no significant influence of the interaction between the studied factors on the content of lysine, protein and methionine (Table 3).

Table 2. Crude protein, lysine and methionine coefficients of variation

	Colorit	Boomerang	Respect	Predecessor sunflower	Predecessor durum wheat	Fertilizer Lumbrical
Crude protein (g/kg DM)						
Mean	134.9	128.8	126.9	129.5	130.9	131.4
Min	125.2	119.5	118.7	118.7	118.7	120.8
Max	145.6	139.4	136.5	145.6	143.0	145.6
CV%	4.56	4.54	4.38	4.96	5.35	5.07
Error	1.45	1.46	1.31	1.23	1.34	1.11
Lizyne (g/100 g CP)						
Mean	3.03	2.89	2.85	2.91	2.94	2.97
Min	2.82	2.69	2.68	2.68	2.68	2.72
Max	3.25	3.12	3.06	3.25	3.25	3.25
CV%	4.1	4.33	4.18	4.71	5.33	4.81
Error	0.03	0.02	0.02	0.026	0.029	0.02
Methionine (g/100 g CP)						
Mean	1.87	1.79	1.76	1.80	1.82	1.82
Min	1.74	1.66	1.65	1.65	1.65	1.68
Max	2.02	1.93	1.89	2.02	1.98	2.02
CV%	4.5	4.46	4.31	4.85	5.22	4.96
Error	0.01	0.01	0.01	0.01	0.01	0.015

Table 3. Effect of cultivars, biofertilizing, predecessors and their interaction on protein, lysine and methionine content of triticale grain

Source of variation	df	Protein		Lysine		Methionine	
		η	MS	η	MS	η	MS
A	2	26.03	309.0**	26.05	0.14**	25.20	0.05**
B	2	9.90	117.5^{ns}	9.95	0.05^{ns}	10.10	0.02^{ns}
C	1	1.15	27.31^{ns}	1.22	0.01^{ns}	1.05	0.004^{ns}
A×B	4	0.35	2.06^{ns}	0.33	0.0009^{ns}	0.31	0.0003^{ns}
A×C	2	0.84	9.91^{ns}	0.81	0.004^{ns}	0.66	0.004^{ns}
B×C	2	0.38	4.53^{ns}	0.37	0.002^{ns}	0.36	0.0008^{ns}
A×B×C	4	0.46	2.70^{ns}	0.46	0.001^{ns}	0.34	3.76^{ns}
Error	36	60.9	40.17	60.82	0.02	61.99	0.008

A great, significant effect on the three indicators had the variety, respectively 26.03%, 26.05% and 25.20% of the total variance. Alaru et al. (2003) also have found that the protein level in triticale grain to a great extent dependent of a variety.

Table 4. Protein content (g/kg DM)

<table>
<tr><th colspan="3">A</th><th colspan="3">B</th><th colspan="3">C</th></tr>
<tr><td colspan="2">Colorit</td><td>134.9</td><td colspan="2">0 kg/ha</td><td>127.7</td><td colspan="2">Sunflower</td><td>129.5</td></tr>
<tr><td colspan="2">Boomerang</td><td>128.9 ^{ns}</td><td colspan="2">1,400.0 kg/ha</td><td>130.2 ^{ns}</td><td colspan="2">Durum wheat</td><td>130.9 ^{ns}</td></tr>
<tr><td colspan="2">Respect</td><td>126.9 ^{ns}</td><td colspan="2">1,750.0 kg/ha</td><td>132.8*</td><td colspan="2">-</td><td>-</td></tr>
<tr><td rowspan="3">GD</td><td>5%</td><td>4.3</td><td rowspan="3">GD</td><td>5%</td><td>4.3</td><td rowspan="3">GD</td><td>5%</td><td>3.5</td></tr>
<tr><td>1%</td><td>5.7</td><td>1%</td><td>5.7</td><td>1%</td><td>4.7</td></tr>
<tr><td>0.1%</td><td>7.6</td><td>0.1%</td><td>7.6</td><td>0.1%</td><td>6.2</td></tr>
</table>

Various values of protein in triticale grains under biological breeding system have been cited within the scientific literature. Kronberga (2008) has reported protein content between 10.4 and 13.2%, Straumite et al. (2017) within 9.10 and 11.14 g/100 g^{-1}, and Mikulioniene and Balezentiene (2009) - 8.2%.

On Table 4 the results of ANOVA are presented for independently action of the factors on the protein content in the grain. In the study was established particularity of the varieties for indicator studied. Similar results for the influence of variety have reported by Gulmezoglu et al. (2010).

The data showed statistically no significant values, with lower crude protein content for Boomerang (128.9 g/kg) and Respect (126.9 g/kg) varieties, compared to a control variety Colorit (134.9 g/kg). These results are higher than obtained by Wlcek and Zollitsch (2003), which have establiched a protein value 101.0 g/kg of dry matter under biological system of triticale growing. The highest crude protein content was obtained in the grain (132.8 g/kg) when was applied 1,750.0 kg/ha Lumbrial, and the difference was statistically confirmed compared to a control option–Lumbrial 0 kg/ha. The value for Lumbrial 1,400.0 kg/ha (130.2 g/kg) was not statistically confirmed. Buhedma et al. (2016) also have reported an increase in protein content (%) in triticale grains when applying organic fertilizer, with no significance statistical values. The control values of their study ranged within 12.33 and 12.23%, and from test variants were between 12.59 and 12.79%. Although the protein content in the grain after a predecessor of durum wheat was 1.4 g/kg higher than after sunflower, the difference was no significant. Unlike our research Dimitrova-Doneva (2010) has reported for significant influence of predecessor on the crude protein content for triticale grain.

The content of lysine followed the trends of protein content, regarding the independent influence of the test factors (Table 5).

Table 5. Lysine content (g/100 g CP)

A		B		C	
Colorit	3.029	0 kg/ha	2.874	Sunflower	2.913
Boomerang	2.900 ^{ns}	1,400.0 kg/ha	2.928*	Durum wheat	2.944*
Respect	2.858 ^{ns}	1,750.0 kg/ha	2.984*	-	-
GD 5%	0.009	GD 5%	0.009	GD 5%	0.008
GD 1%	0.123	GD 1%	0.123	GD 1%	0.101
GD 0.1%	0.162	GD 0.1%	0.162	GD 0.1%	0.133

The highest lysine content was obtained for Colorit variety (3.029 g/100 g CP). Wlcek and Zollitsch (2003) have reported a similar content of lysine in the protein in triticale grains (3.1 g/100 g CP), grown under organic farming system. The values of lysine for Boomerang (2.900 g/100 g CP) and Respect (2.858 g/100 g CP) varieties were lower and statistically no significant. Concerning the fertilizer factor the both tested doses of 1,400.0 and 1,750.0 kg/ha had significant statistical effect on the indicator studied. When the fertilizer rates were increased, the lysine content also increased –2.92 g/100 g CP and 2.984 g/100 g CP.

The content of lysine after a predecessor of durum wheat was higher compared to value after predecessor sunflower and statistically significant.

Concerning the methionine content (Table 6) the values were no significantly, both in the tested varieties and after a predecessor of durum wheat. A significant effect on increasing the methionine content was observed when applying 1,750.0 kg/ha Lumbrial - 1.848 g/100 g CP.

Table 6. Methionine content (g/100 g CP)

A		Б		C	
Colorit	1.874	0 kg/ha	1.778	Sunflower	1.803
Boomerang	1.795 ^{ns}	1,400.0 kg/ha	1.811 ^{ns}	Durum wheat	1.822 ^{ns}
Respect	1.768 ^{ns}	1,750.0 kg/ha	1.848*	-	
GD 5%	0.059	GD 5%	0.059	GD 5%	0.048
GD 1%	0.079	GD 1%	0.079	GD 1%	0.064
GD 0.1%	0.104	GD 0.1%	0.104	GD 0.1%	0.085

CONCLUSIONS

The obtained results can be summarized as follows: The variety has a significant effect on the protein, lysine and methionine content; When applying organic fertilizer at a rate of 1,750.0 kg/ha, the highest and statistically significant values of the indicators tested were established; After a predecessor of durum wheat, the protein, lysine and methionine content was higher than after sunflower, but was significant for lysine; A similar coefficients of variation of the investigated parameters were found, which are not dependent of the variety, the predecessor and the applied organic fertilizer.

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INFLUENCE OF TRITICALE STRUCTURAL ELEMENTS ON THE GREEN MASS YIELD IN DEPENDENCY ON THE NITROGEN RATE AND THE HARVESTING PHASE

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Abstract

During the period 2013/2016, at the experimental field of Crop science department at Agricultural University - Plovdiv a field experiment was conducted, using a block method in 4 repetitions and size of the experimental plot 20 m². Two triticale varieties - Musala and Attila were tested. The two varieties were grown at fertilization levels, N_0, N_{120}, N_{160}, N_{200}, N_{240} kg/ha. The green mass yield value was reported in heading and milk maturity phases. Plant height and the yield structural elements have been determined. This research aimed to determine the influence of triticale structural elements on green mass yield, as a source of renewable energy, depending on nitrogen fertilizers and the phase of harvesting. The obtained results show that the structural elements have highest influence on the green mass yield. In milk maturity phase, the structural elements values increase, compared to heading phase. Increasing nitrogen fertilizers, leads to increasing in yield values, and highest yield was reported at N_{200} variant. Attila variety is characterized by higher stems and longer spikes than Musala variety. The differences in leaves number and stem thickness between the two varieties are negligible.

***Key words**: triticale, green biomass yield, structural elements, harvesting phases and nitrogen fertilization.*

INTRODUCTION

Throughout the last few years, interest in green biomass as one of the renewable energy sources has been continuously growing.

The most important indicator when selecting energy crops is the net energy yield from area unit, which is determined primarily by the biomass yield and said biomass convertibility to methane. Nevertheless, crops used for bio-energy are rarely assessed in terms of biomass yields obtained from those crops.

It has been found based on materials published so far on this issue that when using various crops for energy purposes greater attention is paid to the quantity of methane released than to the biomass quantity obtained while the decisive challenge when building biogas plants is the procurement of sufficient quantities of biomass.

According to the studies of Bassu et al., 2013; Bartocci et al., 2020; Cantale et al., 2016; Csikós et al., 2020; Dekić et al., 2013; De Lucia et al., 2013; D'Imporzano et al. 2018; Luca et al., 2016; Lalević et al., 2013; Pathak et al., 2018; Strauß et al., 2019, triticale is one of the crops with proved reputation both in terms of green mass yield and in terms of dry substance of the crop far exceeding the other cereal crops.

That is why our research is focused on establishing the manners of increasing the quantity of green biomass through testing of various nitrogen fertilizer rates and phases of harvesting of two varieties of triticale grown under the conditions in South Bulgaria.

MATERIALS AND METHODS

The research was conducted in the period 2013/2016 at the experimental field of the Crop Science Department of the Agrarian University of Plovdiv by the block method with size of the experimental plot of 20 m² in 4 repetitions. Two triticale varieties were tested, Musala, and Attila, selected in two climatically different regions. The first one was at the Institute of Plant and Genetic Resources - town of Sadovo located in the drier and warmer region of South Bulgaria and the second one was at the Wheat and Sunflower Institute in the town of General Toshevo located in the more humid, cooler and windier region of North Bulgaria.

The crop was planted after rape predecessor. The varieties were grown at the following fertilization levels: N_0, N_{120}, N_{160}, N_{200}, N_{240} kg/ha. The nitrogen fertilizers were introduced in two sessions, one in the autumn during pre-sowing preparation of the soil and another in the spring as nourishment at the earliest opportunity.

The green mass yield was reported in the heading and milk maturity phases by determining the parameters of plant height (cm); spike length (cm); number of leaves per plant (pcs.) and thickness of the stems (cm).

The statistical processing of the data was performed using a SPSS -16.0 software package and the reliability of the differences was determined by Student's t-test.

The soil on which the experiment was conducted is former meadow-swamp soil, slightly saline, sandy clay with power of the humus horizon "A" 25-28 cm. According to its mechanical nature it is a clayish, heavy soil (Popova et al., 2012).

In terms of weather conditions during the years of the experiment, no significant deviations were observed in the values of average 24-hour temperature in the region of Central South Bulgaria compared to the multiannual period. The absolutely minimal temperatures are short-term and do not cause freezing.

The greatest amount of rainfall was registered in 2014/2015 (579.1 mm). The rainfall sum was lower in 2013/2014 (395.5 mm), and lowest in 2015/2016 (336.8 mm).

Regardless of the lowest amount of rainfall compared to the other two years the third year was characterized by the comparatively more regular distribution of such rainfall throughout vegetation and supply of sufficient amount of moisture in the critical phases of the crop's development.

During all three experimental years, the amount of rainfall in the experiment region (Central South Bulgaria) exceeded the rainfall amounts of the multiannual period (321 mm).

RESULTS AND DISCUSSIONS

The results of the experiment regarding the value of green biomass yield indicated that yield changed depending on the meteorological conditions during the years of experiment and depending on the harvesting phases.

The data in Table 1 illustrating the development of the Musala variety indicate that in the heading maturity stage the greatest green biomass yield was obtained during the third year (2016) of the experiment (from 37,400 kg/ha to 47,460 kg/ha).

The relatively lower amounts of rainfall (336.8 mm) and higher temperatures typical for 2016 were favourable for the better development of the Musala variety selected in the region of South Bulgaria.

Yield received throughout the second year of research was 6,890 kg/ha to 9,820 kg/ha lower compared to that from the third year and takes the intermediary place.

The lowest green biomass yield obtained from Musala variety at that stage was during the first experimental year, from 20,420 kg/ha to 30,200 kg/ha.

The reasons include the greater amounts of rainfall (121.5 mm) during the variety heading period accompanied by strong winds of 6-8 m/s by the Beaufort's wind force scale, which led to bending of the plants lowering of yield. The registered degree of bending of the crops by the Eucarpia scale (from 1 to 9) is one or 100%.

With the advance of the development phases of triticale its productivity increased significantly and the green biomass yield obtained in the milk maturity phase considerably exceeded that in the heading phase.

The yield obtained at this stage by years follows the same trend as that in the heading maturity phase. The highest yield was obtained during the third year of the experiment (from 39,200 kg/ha to 51,300 kg/ha), followed by the second year (37,300 kg/ha to 46,300 kg/ha) and the lowest yield was during the first year (from 35,000 kg/ha to 38,840 kg/ha).

In contrast to the Musala variety, the green biomass yield of the Attila variety throughout the years of research followed exactly the opposite trend (Table 2).

The years of the experiment characterized by lower rainfall amounts and higher temperatures (2015 and 2016), which favoured the development of the Musala variety, were less favourable for the development of Attila variety.

That is why with this variety in both phases of harvesting the highest green biomass yield was obtained during the first year of experiment - 2014, the second year by yield was the second year of the experiment - 2015 and the lowest yield was reported in 2016 - the third year of research.

The greater amounts of rainfall and more wind during the first experimental year that had a negative influence on the development of Musala variety did not influence the Attila variety's yield so much.

One of the reasons thereof was its earlier entering into heading maturity phase (15.04.2014) and its thicker and more resistant to bending stem distinguished by its conic shape compared to the Musala variety with typically thinner and easily bending stem.

That is why bending of the plants of Attila variety was significantly weaker (by the Eucarpia scale the crop bending level was 7 (30^0 slope of the stems).

During the first experimental year the green biomass yield obtained from Attila variety exceeded the yield from Musala variety and it varied from 29,280 kg/ha to 39,150 kg/ha at the heading maturity phase and from 38,110 kg/ha to 46,980 kg/ha at the milk maturity phase.

Table1. Yields green mass in phase heading maturity stage and milk maturity stage at variety Musala, kg/ha

Variants of fertilization kg/ha N	Heading maturity stage					Milk maturity stage				
	Years			Mean	%	Years			Mean	%
	2014	2015	2016			2014	2015	2016		
N_0	20420c	27580e	37400d	28470	100,0	35000d	37300e	39200e	37170	100,0
N_{120}	26720b	33010d	40920b	33550	117,8	35760c	43780c	46060c	41870	112,6
N_{160}	26880b	37710b	45280a	36620	128,6	38260b	44580b	47780b	43540	117,1
N_{200}	30200a	38150a	47460c	38600	135,6	38840a	46300a	51300a	45480	122,4
N_{240}	26800b	36330c	43220e	35450	124,5	38230b	42950d	45060d	42080	113,2

*Values with the same letters do not differ significantly

Table 2. Yields green mass in phase heading maturity stage and milk maturity stage at variety Attila, kg/ha

Variants of fertilization kg/ha	Heading maturity stage					Milk maturity stage				
	Years			Mean	%	Years			Mean	%
	2014	2015	2016			2014	2015	2016		
N_0	29280d	24780e	23100e	25720	100	38110e	33680e	31980e	34590	100
N_{120}	34340c	31850d	30170d	32120	124.9	41040d	40710d	39910d	40550	117,2
N_{160}	37040b	35550b	34250a	35610	138,4	43420b	41600b	40560b	41860	121,0
N_{200}	39150a	36760a	35130b	37010	143,8	46980a	45720a	42000a	44900	129,8
N_{240}	36900b	35400c	34020c	35440	137,8	42220c	40990c	40100c	41100	118,8

*Values with the same letters do not differ significantly

For the Attila variety as well, the yield obtained at the milk maturity phase exceeded the yield from the heading phase.

In view of the reasons specified above, during the second and third year of all tested variants and harvesting phases the Attila variety was less productive than the Musala variety.

The productivity of Attila variety during the second experimental year varied from 24,780 kg/ha to 36,760 kg/ha in the heading maturity phase and from 33,680 kg/ha to 45,720 kg/ha, at the milk maturity phase, while during the third year the yield at the heading maturity phase dropped to 23,100 kg/ha, for non-fertilized variants and to 35,130 kg/ha, for the variant fertilized with (N_{200}) and respectively from 31,980 kg/ha to 42,000 kg/ha, at the phase of milk maturity.

Upon monitoring the effect of nitrogen fertilization on the green biomass yield it is evident that both during the years of research and average for the period, for the two varieties and harvesting phases, the lowest yield was obtained in the variant without nitrogen fertilization.

For the Musala variety, it was 28,470 kg/ha at the heading maturity phase and 37,170 kg/ha at the milk maturity phase, while for the Attila variety yield was respectively 25,720 kg/ha; 34,590 kg/ha.

For both varieties and in both phases of harvesting, all fertilized variants exceeded the non-fertilized variants by green biomass yield.
For the Musala variety, average for the research period fertilized variants exceeded the control variant with 17.8% to 35.6% at the heading maturity phase and with 12.6% to 22.4% at the milk maturity phase.
For the Attila variety, a stronger impact was observed of the fertilized variant as to the control variant. All fertilized variants of the variety compared to the control variant in both harvesting phases exceeded by yield the control variant with a higher percentage than that for the Musala variety.
In the Attila variety, the average green biomass yield of the fertilized variants increased compared to the control variant with 24.9% to 43.8%, at the heading maturity phase and with 17.2% to 29.8%, at the milk maturity phase.
With the increase of nitrogen fertilization rates, both during the three years of research and at the two harvesting stages, yield of green biomass increased to the variant fertilized with 200 kg/ha a.s. nitrogen.
For this variant, the average yield obtained for the period at the heading maturity phase with Musala variety was 38,600 kg/ha green biomass and with Attila variety it was 37,010 kg/ha, and at the milk maturity phase yield was respectively 45,480 and 44,900 kg/ha.
For the variants fertilized with the highest nitrogen norm, N_{240}, a drop of yield was observed compared to the previous variant.
The parameters influencing the green biomass yield are the crops' height and the yield-forming structural elements (Figures 1 and 2).
The data in the chart reflect the average values of researched parameters throughout the experimental period.
They indicate that the plant height of Attila variety at the heading maturity phase exceeded the height of Musala variety plants with 10 to 12.9 cm.
The plant height for the variants without nitrogen fertilization was the lowest (108.2 for Musala variety and 118.2 for Attila variety).
With the versions with included nitrogen fertilization the plants of the crops cultivated reacted with different growing intensity for the grown varieties.

The data indicate that with the increase of the nitrogen fertilization norms, the plant height also increased and the highest plants were formed at the nitrogen norm of 200 kg/ha a.s. For this variant, the plants of Musala variety reached a height of 125.5 cm, and the Attila variety reached 138.2 cm.

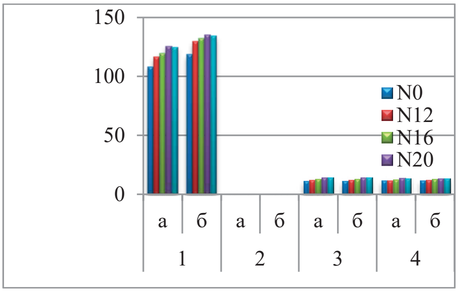

Figure 1. Structural analysis of yield element, mean for the period 2013-2016 at variety Musala
1 - height/plants (cm); 2 - thickness of stems (cm); 3 - number of leaves/plants; 4 - spike length (cm); a - heading maturity stage; б - milk maturity stage

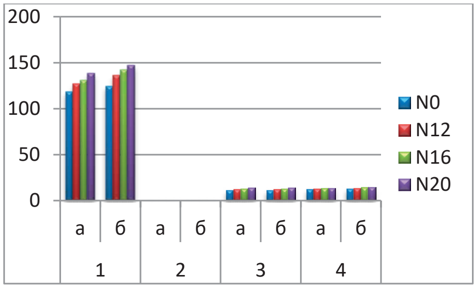

Figure 2. Structural analysis of yield element, mean for the period 2013-2016 at variety Atila:
1 - height/plants (cm); 2 - thickness of stems (cm); 3 - number of leaves/plants; 4 - spike length (cm); a - heading maturity stage; б - milk maturity stage

The Musala variety plants exceeded the control ones with 17.3 cm, while those of Attila variety exceeded the control ones with 20.0 cm, which indicates the greater responsibility of Attila to higher fertilization levels.
For fertilization performed with the highest nitrogen fertilization norms (N_{240}), the height of plants of various varieties was too close to the one reported for the previous variant N_{200}. Most frequently, the values of the two fertilization norms were almost the same.
At the milk maturity phase, the plants developed more vigorously and grew higher. The trend with respect to the plant height and

the activity of different nitrogen norms was preserved.

At this phase, similarly, the plant height values were higher for the Attila variety and exceeded the height of Musala variety plants with 5.6 to 11.6 cm.

Again, the greatest difference in terms of plant height is demonstrated between the control variant and the variant fertilized with N_{200}, 16.6 cm for the Musala variety and 22.6 cm for Attila variety. Analogously, at this phase the lowest plants were those of the variant with no fertilization, 118.7 cm, for Musala variety and 124.3 cm for the Attila variety.

At both harvesting phases, average throughout the research period, the difference in the stem thickness was negligible between the two varieties, with slight prevalence of Attila.

At the milk maturity stage, the plants formed a little thicker stems compared to those at the heading phase.

Stem thickness of the Attila variety varied from 0.49 cm to 0.52 cm at the heading maturity phase and from 0.51 cm to 0.54 cm at the milk maturity phase. The stem of Musala variety was thinner and its thickness varied from 0.47 cm to 0.51 cm at the heading maturity phase and from 0.49 cm to 0.53 cm at the milk maturity phase.

In both varieties, the stems of the plants are a little thicker for the variants without fertilization than those for the variants with fertilization. Depending on the harvesting phase, thickness of 0.51 and 0.53 cm was reported to Musala and for Attila the reported thickness was 0.52 and 0.54 cm.

The stem thickness gradually decreased with the increase of the nitrogen fertilization norm and the stems were thinnest for the highest norm (N_{240}), 0.47; 0.49 cm for the Musala variety and 0.49; 0.51 cm for the Attila variety.

The leaf mass of cereal crops is ultimately formed at the heading phase.

With respect to the leaf number, greater differences were observed between the years of research but between the two varieties studied they were almost none. The plant height is proportional to the leaf number. Plants with higher stems also form a greater number of leaves. That is why, the leaf number is greater for the Attila variety both throughout the years and average for the period of research.

At the heading maturity phase Musal variety formed from 11.39 to 14.21 leaves while Attila formed from 11.54 to 14.38 leaves.

At the milk maturity phase some of the leaves began to dry and their number slightly decreased. At this phase, for Musala variety from 11.30 to 14.12 leaves were reported and from 11.42 to 14.25 leaves for Attila variety.

For both tested varieties and harvesting phases, the least number of leaves was formed in non-fertilized variants. At the heading maturity phase they reached up to 11.39 pieces for Musala variety and up to 11.54 pieces for Attila variety while at the milk maturity phase their number was respectively, 11.30; 11.42 leaves.

With the increase of the nitrogen fertilization norms the number of leaves gradually increased to that of the variant fertilized with N_{200}, where also the highest number of leaves was reported (14.21; 14.12 leaves for Musala and 14.38; 14.25 leaves for Attila variety), which also correlates with the plant height.

For the variant with highest fertilization norm N_{240} the number of leaves for both varieties dropped insignificantly compared to that of the previous variant.

Regardless of the fact that the length of the spike is a genetically determined value, it is at the same time influenced by the climatic conditions and the agricultural technical machinery applied.

It is evident from the figures that in both phases of harvesting, a little longer spikes were formed by the Attila variety. The length of its spikes at the heading maturity phase varied from 12.60 to 13.90 pieces and for Musala it varied from 11.68 to 13.76 pieces.

For both varieties and for both phases the lowest values of length were reported for the non-fertilized variants. At the heading maturity phase the length of the spikes of Attila was with 0.92 cm greater than that of the Musala variety.

The inclusion of fertilization in the following variants had a positive impact on the spike length.

With the increase of the nitrogen fertilization norm, the spike length gradually increased. For both tested varieties the spike length parameter was most distinguished for the variant fertilized $N_{200.}$ In this variant, at the heading maturity phase Musala variety formed spikes of length - 13.76 cm, and Attila variety - 13.90 cm.

In the variant with the highest nitrogen norm (N_{240}) the spike length for both varieties dropped compared to that of the previous variant.

At the milk maturity phase the spike length increased slightly as the spikes were almost completely developed.

At this phase, similarly longer spikes were formed in the Attila variety, from 13.03 cm to 14.80 cm, while in the Musala they were from 11.84 cm to 14.01 cm. In terms of fertilization, the spike length followed the same trend as for the heading phase.

The shortest spikes were those of the variants without nitrogen fertilization - 11.84 cm for Musala and 13.03 cm, for Attila.

With the increase of the fertilizer norms, the spike length also increases as the longest spikes were measured for the variant with nitrogen fertilization norm of N_{200}, 14.01 cm - for Musala and 14.80 cm, for Attila variety.

At this phase as well, for the highest fertilization norm of N_{240} the spike length of both varieties dropped insignificantly than that of the previous variant.

CONCLUSIONS

Due to the selection of tested varieties in different climatic regions, strong variety reaction was observed between them in terms of yield. In 2015 and 2016 the higher yields were obtained from the Musala variety and in 2014 - from Attila.

The higher yield of green biomass was obtained from triticale harvesting at the milk maturity phase.

The lowest yield for both varieties in both phases of harvesting was obtained from the non-fertilized variants and the highest in the variant fertilized with N_{200}, which also correlates with the higher levels of tested parameters.

Attila variety is characterized with a little higher stems and longer spikes compared to Musala variety. The differences in the leaf number and stem thickness between the two varieties are negligible.

At the milk maturity phase, the values of structural elements increased compared to the heading phase, except the number of leaves.

The variants without nitrogen fertilization were with lower values compared to the fertilized variants.

With the increase of nitrogen fertilization norms, parameter values increased up to the variant fertilized with N_{200}. With the highest fertilization norm of $N_{240,}$ the parameter values dropped insignificantly.

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HERBICIDE STRESS AND BIOSTIMULANT APPLICATION INFLUENCES THE LEAF N, P AND K CONTENT OF SUNFLOWER

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Abstract

The aim of the study conducted between 2017 and 2019 is to evaluate the influence of the herbicide stress caused by imitation of "mistaken" treatment of the ExpressSun® sunflower hybrid P 64 LE 25 with imazamox to the leaf N, P and K content of plants before flowering stage. Treatments of the experiment were: 1. Untreated control; 2. Pulsar® Plus - 2.00 l ha^{-1} (25 g/l imazamox); 3. Pulsar® Plus - 2.00 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1} (biostimulant). The herbicide was applied in 4th - 6th true leaf stage of the crop. At treatment 3, biostimulant was applied four days after the herbicide intervention. An increase in the leaf N content after the medicative biostimulant application average for the period was found. No influence of the "mistaken" treatment on the P content in the leaves was recorded. The plants treated "mistakenly" with Pulsar® Plus (2.00 l ha^{-1}) had increased K levels in the leaves. This increased K content was probably due to high abiotic stress. K, in turn, helps the plants to overcome the stressful conditions to some extent.

Key words: *herbicide stress, biostimulants, sunflower, NPK in leaves.*

INTRODUCTION

Bulgaria and Romania are the largest sunflower producers in the EU (Hristov et al., 2019). In order to achieve high yields is necessary to control the weeds effectively in the optimal phases of the crop before the critical period for decreasing the morphological parameters is reached (Tonev, 2000; Simic et al., 2011). In agriculture, the weed control is based mainly on chemical control. A great number of researchers are working on the chemical weed control in sunflower and other crops (Goranovska and Yanev, 2016; Kostadinova et al., 2016; Mitkov et al., 2016; Tityanov, 2016;Yanev, 2015; Tonev et al., 2010a; Tonev et al., 2010b; Tonev et al., 2009a; Tonev et al., 2009b). The main reason for the considerable success of modern herbicides is their selective action (Cobb and Reade, 2010). A selective herbicide is one that kills or retards the growth of weeds, while causing little or no injury to crop species (Carvalho et al., 2009). Two types of mechanisms conferring resistance to herbicides in weeds and crops are currently described. The mechanisms that are related to the specific site of action of the herbicide in the plants are known as target site mechanisms, while the others that involve processes not related to the mechanism by which herbicides kill plants are known as non-target site mechanisms (Hanson et al., 2007). Independently that the modern herbicides are considered selective for the crop plants, in some conditions they can be toxic for the crops and can cause herbicide phytotoxicity (Tonev and Vassilev, 2011). Herbicides have distinct target sites where they act to disrupt biochemical process leading to cell, tissue or plant death. The majority of target sites are specific enzymes that play a key role in a plant metabolic process, which dysfunction has lethal consequences to the susceptible plant. Herbicide selectivity could be derived also from target-enzyme insensitivity “site of action resistance” (Devine et al., 1993).

Herbicide phytotoxicity can occur after long-term effects and herbicidal drift on non-target crops (Vischetti et al., 2002). This undesirable phenomenon can be observed in the low-volume spray with droplet size lower than 150 μm (Hanks, 1995), as well as in conditions of inappropriate wind speed and direction (Thistle, 2004). Herbicides can have a toxic effect on crops in several major cases: 1. In case of technological mistakes - increase in the herbicide dose; 2. Due to insufficient selectivity; 3. By application of the herbicides in unsuitable weather conditions (drought, waterlogging, low temperatures, etc.) as well as in the driftage of the herbicide on another crop (herbicide drift); 4. Application of herbicide by mistake on crop that is sensitive; etc.

The tolerant plants have the ability to detoxify the herbicide, with this process occurring in 3 phases (Yuan et al., 2007; Cole, 1994). In the first phase, the herbicide molecules that enter the cell undergo oxidation or hydrolysis, providing functional groups that are suitable for subsequent conjugation with endogenous metabolites (Barrett et al., 1995). In the second phase, once oxidized herbicides are conjugated with glucose or the metabolite glutathione, these reactions are catalyzed by enzymes including glycosyl transferases and/or glutathione S-transferases (Lamoureux et al, 1991). The third phase of xenobiotic metabolism is the transport and storage of herbicide conjugates in the cellular vacuole (Yuan et al., 2007).

When herbicide detoxification is not effective enough, various functional impairments occur in plants. Herbicide phytotoxicity is most often chronic, but in some cases (eg. drift) it can also be fatal to the crop. The extent of damage can be assessed visually (if visible) or by various physiological and biochemical indicators (Dayan et al., 2015; Dayan and Zaccaro, 2012).

Ability to recover the herbicide-damaged plants depends on the degree of the occurred structural-functional impairment. A number of studies have shown that chronic herbicide phytotoxicity can be overcome (to some extent or completely) by application of biostimulants, foliar fertilizers, growth regulators, herbicide antidotes, etc. (Jablonkai, 2013).

Tanev (1987) demonstrated that the low molecular weight fractions of humic substances extracted from manure exhibit herbicide phytotoxicity antidote properties. Balabanova et al. (2016) show that the biostimulant Terra-Sorb Foliar significantly restores the physiological status of IMI-R sunflower plants treated with the herbicide product Pulsar 40 (imazamox). Soltani (2015) reported a significant increase in yields of oats and winter wheat as a result of the addition of biostimulant after vegetative treatment of the crops with the herbicides glyphosate, topramezone and atrazine.

There is limited information for the influence of the herbicide stress on the nutrient status of the crops. Zaidi et al. (2005) found that nitrogen content in plants decreased with increasing herbicide dose.

The aim of the research is to evaluate the influence of the herbicide stress after imitation of mistaken treatment of ExpressSun® sunflower hybrid with imazamox (Pulsar® Plus) and the medicative biostimulant application on the leaf N, P and K content of sunflower before flowering stage.

MATERIALS AND METHODS

The experiment was situated in the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 3 replications. The size of the experimental plot is 28 m². The study included the following treatments: 1. Untreated weed free control; 2. Pulsar® Plus 2.00 l ha^{-1}; 3. Pulsar Plus® 2.00 l ha^{-1} + Amino Expert® Impuls 2.00 l ha^{-1}.

Pulsar® Plus (25 g/l imazamox) is herbicide for weed control in the Clearfield® Plus Technology of sunflower. The biostimulant's content is as follows: Amino acids - 58.00 g/l; N - 29.37 g/l; MgO - 5.93 g/l; SO_3 - 46.66 g/l, Phytohormones - 0.0035 g/l; Micronutrients: B - 6100 mg/l; Cu - 4600 mg/l; Fe - 4520 mg/l; Mn - 4520 mg/l; Mo - 920 mg/l; Zn - 9040 mg/l. The herbicide was applied in 4th – 6th true leaf stage of the sunflower (BBCH 14-16). The size of the spraying solution was 220 l ha^{-1}. The biostimulant was applied 4 days after the herbicide intervention. The grown sunflower hybrid was P 64 LE 25 - bred to be grown by the Express Sun® Technology of sunflower.

A predecessor of the sunflower in the three years of the study was winter wheat. On the trial field deep ploughing, two times disc harrowing and two times cultivation before sowing were done. The application was performed after sowing before germination of the crop. 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.

To determine the content of nutrient elements in the leaves before flowering stage of the sunflower the last fully developed leaves of the crop were collected. The plant samples were dried at 60°C, weighted and milled. They were mineralized with concentrated H_2SO_4 using H_2O_2 as a catalyst. The total nitrogen content was determined according to Kjeldahl method by distillation in apparatus of Parnas-Wagner (Tomov et al., 2009). Phosphorus was determined colorimetrically (spectrophotometer Camspec E105) (Tomov et al., 2009) and potassium - photometrically (flame photometer PFP-7) (Ivanov and Krastev, 2005).

The herbicide phytotoxicity was determined by the 9-score scale of EWRS (European Weed Research Society) 7 days after the herbicide application as followed:

1. No damage/healthy plant;
2. Very slight symptoms, weak suppression;
3. Slight but clearly visible symptoms;
4. Severe symptoms (e.g. chlorosis) which do not lead to a negative effect on yield;
5. Thinning, severe chlorosis or suppression; yield reduction expected;
6. Severe damage up to complete destruction;
7. Severe damage up to complete destruction;
8. Severe damage up to complete destruction;
9. Severe damage up to complete destruction.

Statistical analysis of collected data was performed by using Duncan's multiple range test by the software SPSS 19. Statistical differences were considered proved at $p < 0.05$.

RESULTS AND DISCUSSIONS

The ecological conditions at the time of the herbicide application and the first days after that had the strongest influence on the retention and adsorption of the foliar herbicides, as well as the subsequent processes that determine their action (Tonev et al., 2007).

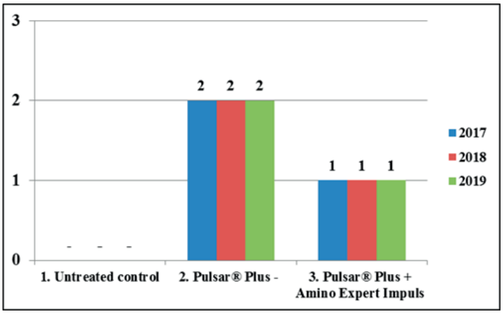

Figure 1. Visual phytotoxicity 7 days after the treatments (Scores)

Sulphonylureas (tribenuron-methyl) and imidazolinones (imazamox) are herbicides inhibiting ALS (Acetolactate synthase), an enzyme that plays an important role of the biosynthesis of the vital amino acids valine, leucine and isoleucine. The visual toxic symptoms in the sensitive or non-target plants are growth retardation or stopping, the leaves lose their turgor, the plants lose their vitality, etc. (Fedke and Duke, 2005).

The visual phytotoxicity at treatment 2 (Pulsar® Plus 2.00 l ha^{-1}) was determined as score 2. Symptoms of yellowing of the plant's leaves were observed. At treatment 3, after the medicative biostimulant application, the phytotoxic symptoms were determined as score 1 and the plants were healthy.

The optimal N levels in the upper fully developed leaves in the beginning of flowering stage (BBCH 61) vary from 3.00 to 5.00% (Bergmann, 1992). After the "mistaken" treatment with Pulsar® Plus at the studied hybrid P 64 LE 25, the nitrogen content in the leaves in the beginning of flowering was diminished in comparison to the untreated weed free control. Increase in the N content in this phenophase after the medicative application of the biostimulant Amino Expert® Impuls was found. This is probably due to the biostimulant that contents aminoacids, macro- and micronutrients. Coupled with the higher tolerance of the hybrid to herbicide stress is probably the reason for the increase of N in leaves. The results are presented in Table 1.

Table 1. Nitrogen content in the leaves before flowering of the sunflower (%)

Treatments	2017	2018	2019	Ave.
1. Untreated control	4.10b	4.44a	5.28a	4.61 a
2. Pulsar® Plus - 2.00 l ha^{-1}	3.88c	3.97b	4.65b	4.17 b
3. Pulsar® Plus - 2.00 l ha^{-1} + Amino Expert® Impuls - 2.00 l ha^{-1}	4.51a	4.82 a	5.12a	4.82 a

All values with different letters are with proved difference according to Duncan's test, $p < 0.05$.

The optimal phosphorus levels in the upper fully developed leaves in the beginning of flowering stage (BBCH 61) vary from 0.25 to 0.50% (Bergmann, 1992).

In the trial the plants had higher phosphorus content in the leaves (Table 2). It was found that the plants treated "by mistake" with imazamox showed higher phosphorus content in the leaves before flowering stage - 0.92% average for the period of investigation. The difference in this result is with proved difference according to Duncan's test, $p < 0.05$ when compared to the untreated control and the variant with applied medicative biostimulant application (treatment 3).

Table 2. Phosphorus content in the leaves before flowering of the sunflower (%)

Treatments	2017	2018	2019	Ave.
1. Untreated control	0.83b	0.88b	0.89b	0.87b
2. Pulsar® Plus - 2.00 l ha^{-1}	0.91a	0.95a	0.90a	0.92a
3. Pulsar® Plus - 2.00 l ha^{-1} + Amino Expert® Impuls - 2.00 l ha^{-1}	0.81b	0.86b	0.89b	0.85b

All values with different letters are with proved difference according to Duncan's test, $p < 0.05$.

The optimal potassium levels in the upper fully developed leaves in the beginning of flowering stage (BBCH 61) vary from 3.00 to 4.50% (Bergmann, 1992).

Table 3. Potassium content in the leaves before flowering of the sunflower (%)

Treatments	2017	2018	2019	Ave.
1. Untreated control	3.57b	3.42b	3.89a	3.63b
2. Pulsar® Plus - 2.00 l ha^{-1}	3.83a	4.26a	3.99a	4.03a
3. Pulsar® Plus - 2.00 l ha^{-1} + Amino Expert® Impuls - 2.00 l ha^{-1}	3.02c	3.16c	3.30b	3.16c

All values with different letters are with proved difference according to Duncan's test, $p < 0.05$.

In the experiment a treatment with lower phosphorus levels under the optimal values was not found. The obtained data is presented in Table 3. Despite the optimal content of potassium in the leaves, it was found that the plants treated "mistakenly" with Pulsar® Plus - 2.00 l ha^{-1} had increased potassium levels in the leaves before flowering - 4.03%, which is with 0.40% higher than the untreated control - 3.63% on average for the period of the study. This increased content is probably due to the fact that the plants of treatment 2 are subjected to high abiotic stress, and potassium in turn helps the plants to overcome the stress conditions to some extent (Nikolova, 2010). It seems the probable reason for the higher potassium content of the leaves of the plants from treatment is the caused herbicide stress.

CONCLUSIONS

After the application of the biostimulant Amino Expert® Impuls for medicative treatment, the plants of treatment 2 overcome the herbicide stress to some extend;

The plants of all variants had optimum nitrogen content in the leaves before flowering, but stressed by "wrong" treatment with Pulsar® Plus had a reduced nitrogen content in the leaves before flowering;

The plants treated "by mistake" with imazamox had higher phosphorus content in the leaves before flowering stage;

Despite the optimum content of potassium in the leaves, plants mistakenly treated with imazamox had increased potassium levels in the leaves before flowering. The probable reason for the higher potassium content of the leaves of the plants is the caused abiotic stress

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RESEARCH REGARDING LAND EVOLUTION AND AGRICULTURAL AREA OF GALATI COUNTY

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Abstract

Agricultural land is distributed on farms of different types and legal forms, which differ depending on the size of the resources held. The result of agricultural production depends on the volume of resources, although the low degree of investment concentration, in the case of family farms, complicates the exploitation process. A general analysis of the structure of vegetable production in Galati County has highlighted a number of deficiencies in recent years, which have created distortions between local demand and supply of agricultural products. In Galați County there have been changes in the structure of crops, with the decrease of the share of cereals and the increase of areas for peas and sunflowers. The research results showed that there were certain structural imbalances related to the supply of agricultural raw materials to the local processing sector. These researches are part of a complex study, which aims to optimize agricultural production in the Galati area and increase the competitiveness of the national agricultural sector.

Key words: *production structure, agriculture, agricultural production.*

INTRODUCTION

Given that, in Romania, rural areas occupy almost 90% of the territory and have about 50% of the total population, to achieve a real picture of agricultural development, it is necessary an analysis aimed at the evolution of land structure and especially of the structure of arable land, in order to identify trends and their medium and long-term effects.

The change in the type of ownership of agricultural land and the formation of a new organization of agricultural producers, have generated excessive fragmentation of agricultural land, the formation of a large number of individual farms, subsistence, a restriction of productive services for agriculture (irrigation, fertilization, mechanization, etc.) and a significant depreciation of the productive quality of the lands (Nicula et al., 2019).

Romania has an important land wealth, 63.3% of the country's surface has an agricultural destination and it is structured on categories of use (arable, pastures and hayfields, vineyards and orchards), being influenced by natural factors but also economic and social-historical factors (people's needs and concerns, tradition, etc.). The evolution of agricultural productions is closely related to the changes that have taken place in the ownership structure of the land fund and of the other means of production, as well as of the climatic variability (Nicula & Stanciu, 2018).

The predominantly cereal character of the production structure represents a negative aspect of the Romanian agriculture.

The share of agricultural area filled with grain is high, even if the cultures don't have very favorable agro-climatic conditions for the development of those species.

The decision regarding the elaboration of the crop structure, in order to achieve an efficient crop rotation, aims for a number of main crops to be established in accordance with the occupation of the arable surface of the farm, respecting the size restrictions, production costs, and labor.

Determination of the crop rotation is achieved using mathematical and economic methods in which the weight of each total area of cultivated crops, is based on the optimality criteria of the chosen variant.

MATERIALS AND METHODS

The material that formed the basis of this paper is information obtained by the usual means, namely: newsletters, statistical yearbooks, statistical briefs. The research methods used in this study are: basic research (pure or basic) and evaluative research.

The preliminary documentation aimed to identify and study the general information sources and, subsequently, the specific information sources, in order to determine the characteristics of the researched area. For this purpose, the databases and statistical bulletins of the National Institute of Statistics of Romania were consulted, in order to observe the time series of some indicators relevant for research, as well as data provided by the County Directorate of Statistics.

RESULTS AND DISCUSSIONS

In order to highlight the most significant aspects regarding the evolution of agriculture in Galati County, the following indicators were used:

- the *structure of the land fund;*
- the *structure of the arable land on groups of crops and cultures.*

The study analysed these indicators both in dynamics and in territorial profile. For these indicators, the analysis referred to the results obtained in Galati County. As it can be seen, from the data presented in Table 1 in 2014, agricultural area of Galati County is estimated at 358.31 thousand ha, of which 292.92 ha arable land (81.75% of the total).

Table 1. The structure of the agricultural land in Galati County, by categories of use and owners, in 2014

Category of land	Total county (ha)	% of agricultural	Private sector (ha)	% of the total county	Public sector (ha)	% of the total county
Arable	292.926	81.75	290,979.48	99.34	1946.52	0.66
Pastures	43.612	12.17	18656.30	42.78	24955.70	57.22
Grasslands	656	0.18	52	7.93	604	92.07
Vineyards	19.397	5.41	18648.22	96.14	748.78	3.86
Orchards	1720	0.48	1702	98.95	18	1.05
Agricultural - total	358.311	100,00	330.038	92.11	28.273	7.89

Source: data processed by the GALAȚI County Directorate of Statistics.

NOTE: Fund land by way of use, county, is the land of the property owners in radius administration, until the completion of the action of cadaster of land made by the National Agency of Cadastral and Real estate Advertising; data series are blocked at the level of 2014.

Analysing the structure of the agricultural land, by the owner of the area, from the total agricultural area of Galati county, 92.11% is private property, whereas the private sector owns 99.34% of the arable land. The sector with state majority, has only a percentage of 7.89% of agriculture area and only 0.66% of the total area of arable land. In Galati County, the private sector holds 42.78% of the area of pastures, 96.14% of the area of vineyards, 98.95% of orchards. Regarding the evolution of the structure of agricultural land by categories of use in Galati county (Table 2), it results that in the period 2010-2014, the total agricultural area was reduced by 0.02% by reducing by 0.04% the arable land areas and the pastures by 0.12%. (58.85)

Table 2. Structure evolution of agricultural land, by categories of use in Galaţi

Categories of use	2010 (Ha)	2014 (Ha)	(±) % 2010
Agricultural area, of which:	358,394	358,311	-0.02
arable	293,043	292,926	-0.04
pastures	43,663	43,612	-0.12
grasslands	656	656	0.00
Vineyards and vinicultural nurseries	19,316	19,397	0.42
Orchards and fruit nurseries	1,716	1,720	0.23

Source: data processed by the GALAȚI County Directorate of Statistics.

From the structure evolution of the main crops, shown in Table 3, there is a high proportion of grains (67.11% of the cultivated surface of Galati in 2014) the share decreasing in 2018

From the data presented in table 3 there are higher weights of maize (39.68%) of the cultivated area, followed by wheat and rye (19.33%) and sunflower (17.58%). Taken as a whole, there is a decrease in the share of most areas cultivated in 2014 compared to 2018, except for the pea crop which registers an increase of 794.08%.

Table 3 The structure evolution of the arable land cultivated with the main crops, in Galati County

Specification	2014 (Ha)	%	2018 (Ha)	%	(+/) % of the
Total cultivated area	283,976	100	275,685	100	-2.92
Grains	190,589	67.11	162,248	58.85	-14.87
Wheat and rye	54,882	19.33	47, 934	17.39	-12.66
Maize	112,674	39.68	96,671	35.07	-14.20
Leguminous	1,829	0.64	9,929	3.60	442.86
Peas	1,048	0.37	9,370	3.40	794.08
Beans	751	0.26	557	0.20	-25.83
Oleaginous plants	68,828	24.24	82,438	29.90	19.77
Sunflower	49,918	17.58	54,058	19.61	8.29
Potatoes	1,436	0.51	1117	0.41	-22.21
Vegetables - total	11,540	4.06	1,0202	3.70	-11.59

Source: data processed by the GALAȚI County Directorate of Statistics.

The structure of crops influences the production results and the profit of the exploitation through the use of the exploitation capital, as well as through the incomes which have consequences on the formation of a part of the production expenses (Stanciu, 2017). In addition, it is considered that the choice of certain crop structures have a determining role regarding *the best placing in the value of the land* (if it ensures consistency between the requirements of plants and conditions offered by the land areas), *the use of other production factors, a better use of potentialities of the exploitation and the natural environment, the size of income and profit*, *the degree of use of labor and the wages* (when they use labour employment) etc. In conjunction with the medium productions by hectare and areas cultivated, in the region of Galati were produced important quantities of plants (Table 4). It can be seen that a significant share is represented by the cultivation of maize, increasing by 32.78% in 2018 compared to 2014.The increasing for share of certain crops in the total plant production, consists of peas (306.63%), followed by sunflower (45.57%) and maize (32.78%).

Table 4. Evolution of total plant production obtained in Galati County

Galati County	2014 (Tons)	2018 (Tons)	(±) % compared to2014
Grains, of which:	739,650	881,848	19.23
Wheat and rye	199,255	179,704	-9.81
Maize	481,538	639,398	32.78
Leguminous, of which:	3,027	8,577	183.35
Peas	1,960	7,970	306.63
Beans	1,055	605	-42.65
Oil plants	152,234	207,407	36,24
Sunflower	101,106	147,179	45.57
Potatoes – total	22,472	15,887	-29.30
Vegetables – total	271,636	276,713	1.87

Source: data processed by the GALAȚI County Directorate of Statistics

The use of agricultural land is determined by the overall evolution of human society, mainly by the demand-supply ratio of agricultural products that manifests itself on the market. The demand for agricultural products is determined by the needs of final or productive consumption, needs directly related to the number of the population, its purchasing power and the degree of integration and development of agricultural raw materials processing industries. The supply of agricultural products is determined by the area in the agricultural circuit and the level of yields per hectare.

The use of agricultural land is influenced by the agricultural-climatic conditions, as well as by the economic, financial, demographic, technical and technological conditions, existing in a country at a given time.

Thus, three ways of using agricultural land are known: *extensive, rational* and *intensive.* Each form involves several stages of manifestation: *appearance, development, maturity, decline* and *disappearance.*

Among the indicators used in assessing the use of agricultural land:

1. The structure of agricultural land by categories of use (formula 1) calculated as a percentage ratio between the area owned by each category of use and the total agricultural area:

$$ITA = \frac{Scu}{SA} \quad (1)$$

a) for arable lands:

$I_{TA\ 2010}$ = (293.04/358.40) *100 = 81.76 %

$I_{TA\ 2014}$ = (292.92/358.31) *100 = 81.75%

When I_{TA} < 67%, land use is extensive; when $I_{TA} \approx$ 67%, the land use is rational, and when I_{TA} > 67%, the land use is intensive. It results that in Galati County, the use of arable land is intensive, without variations in the period 2010-2014

b) for vineyards and orchards:

$I_{TA\ 201\ 0}$ = (19.31/358.39)*100 = 5.38%

$I_{TA\ 2014}$ = (19.39/358.31) *100 = 5.41%

When I_{TA} < 7%, land use is extensive; when $I_{TA} \approx$ 7%, the land use is rational, and when I_{TA} > 7%, the land use is intensive. We can conclude that in Galati county, the use of land with vineyards and orchards is extensive.

c) for natural pastures and hayfields:

$I_{TA\ 2010}$ = (43.66/358.39) *100 = 12.18 %

$I_{TA\ 2014}$ = (43.61/358.31) *100 = 12.17%

When I_{TA} < 25%, land use is extensive; when $I_{TA} \approx$ 25%, the land use is rational, and when I_{TA} > 25%, the land use is intensive. The result is a rather small value, which shows that the cultivated area with pastures and hayfields is very small and that in the whole county, the use of the land with pastures and hayfields is extensive.

2. The share of uncultivated areas in the total arable area (formula 2) shall be calculated as a percentage ratio between the uncultivated arable area and the total:

$$ISn/Sa = \frac{Su}{Sa} \quad (2)$$

$I_{TA\ 2010}$ = (4.95/358.39) *100 = 3.96%

$I_{TA\ 2014}$ = (4,39/358.31) *100 = 5.40%

When $I_{Sn/Sa}$ <10%, land use is extensive; when $I_{Sn/Sa} \approx$ 10–20%, the land use is rational, and when $I_{Sn/Sa}$ > 20%, the land use is intensive. It turns out that in Galati county the use of arable land is extensive.

3. The degree of intensity of agricultural land use (formula 3) is determined as the ratio between the conventional agricultural area (the categories of use are transformed into conventional arable land by applying equivalence coefficients) and the total agricultural area:

$$Gi = \frac{\sum_{i=1}^{n} Si \times ki}{\sum_{i=1}^{n} Si} \quad (3)$$

where: Si represents the area occupied by the use category (arable, pastures, hayfields, vineyards, orchards), and ki represents the equivalence coefficients between the other categories of agricultural use and the arable land (Table 5).

Table 5. Evolution of the intensity degree of agricultural land use

Categories	Coefficients of equivalence	2014		2018	
		(thousand ha)	Si (thousand ha)	(thousand ha)	Si (thousand ha)
Agricultural, of which:	-	358.39	-	358.31	-
arable	1	293.04	293.04	292.93	292.93
pastures	0.2	43.66	8.73	43.61	8.72
grasslands	0.5	0.66	0.33	0.66	0.33
vineyards	8	19.32	154.53	19.4	155.18
orchards	5	1.72	8.58	1.72	8.6
Intensity degree (Gi)%	-	129.71		129.98	

Source: own calculation of the data of GALAȚI County Directorate of Statistics

4. The crop structure of arable land (formula 4) is determined as a percentage ratio between the area owned by each crop and the total arable area:

$$I_{Ci\ /\ Sa} = \frac{Sci}{Sa} \qquad (4)$$

The assessment of this indicator is made according to the share of intensive crops (peas, sunflower, vegetables and tobacco) in the total arable area.

When $I_{Ci\ /\ Sa} < 10\%$, land use is extensive; when $I_{Ci\ /\ Sa} \approx 10\text{-}20\%$, land use is rational, and when $I_{Ci\ /\ Sa} > 20\%$, land use is intensive.

Analysing the crop structure of arable land and considering the crops of peas, sunflowers and vegetables, it results that in Galati County in 2014 (22.01%) the land use is intensive (Table 6).

From the data presented in Table 6 it results that, in Galati County in 2018 compared to 2014, the share is close to intensive crops, respectively a share of 19.61% for sunflower in 2018 compared to the share of 17.58% in 2014 and in vegetables, in 2018, 3.70% to 4.06% in 2014. For peas, the share was of 3.40% in 2018 to 0.37% in 2014.

Table 6. Share evolution of intensive crops in arable land in the county of Galati

Specification	2014		2018		(±) % 2014
	Ha	Ici/Sa (%)	Ha	Ici/Sa (%)	
Total cultivated area	283 976	-	275 685	-	-
Cereals	190 589	67.11	162 248	58.85	-8.26
Wheat and rye	54 882	19.33	47 934	17.39	-1.94
Maize	112 674	39.68	96 671	35.07	-4.61
Leguminous	1829	0.64	9929	3.60	2.96
Peas	1048	0.37	9370	3.40	3.03
Beans	751	0.26	557	0.20	-0.06
Oleaginous plants	68 828	24.24	82 438	29.90	5.67
Sunflower	49 918	17.58	54 058	19.61	2.03
Potatoes	1436	0.51	1117	0.41	-0.10
Vegetables - total	11540	4.06	10202	3.70	-0.36
Total intensive crops	-	22.01	-	26.72	4.7

Source: own calculation of the data of GALAȚI County Directorate of Statistics.

CONCLUSIONS

The structure of land use has changed substantially in recent years, which is why the area of agricultural land of agricultural holdings in the individual sector has increased and is cultivated jointly, in various informal family associations without legal personality.

The cooperative sector has completely disappeared: agricultural production coopera-tives (as well as some state farms) have been transformed into various legal entities, which today control a percentage of 92.11% of agricultural land, including private companies and the remnants of the former agricultural sector of Galati County.

Mutations occurring in the economic and social structure of agricultural units affects not only the aspect of territorial increase, but in particular the increase of the level of production concentration, accompanied by increased efficiency towards small and medium farms.

Increasing production per unit area is considered to be the most important means of increasing agricultural production, although it must be practiced carefully, because excessive use, unbalanced and inconsistent with the requirements of plants is associated with very serious pollution problems of the environment (soil, water, air), obtaining agricultural products with pesticide and nitrate residues, etc.

The potential harvest is determined primarily by the cultivated variety or hybrid, respectively by the genetic dowry possessed by the cultivated plant and which can manifest itself more or less depending on the action of other factors of production. Crop production is directly affected by temperature and precipitation, extreme climatic events (floods, droughts, storms, etc.) and high concentrations of CO_2 in the air.

Due to climatic conditions, one year may be unfavourable to one region in terms of crop production, and at the same time, it may be favourable to another region.

Influenced by the size of the cultivated areas, by the average productions per hectare and by the climatic conditions, the total production in Galati county had a fluctuating evolution. The increase of the efficiency of the agricultural production in Galati County supposes, besides the increase of the yields at the production unit (*hectare*), an improvement of its quality.

During the analysed period, the total production achieved for the main crops in Galati County registered increases in some crops, but also significant decreases in others. Thus, there were increases in total production of cereals for grains (19.23%) legumes for grains (183.35%) and oil plants (36.24%), decreases in total production for wheat and rye (9.81%), beans (42.65%) and potatoes (29.30%).

In the conditions of the functioning of the market mechanisms, possibilities are created for the organization of viable agricultural units, which can produce for commercialization.

Leasing is practiced less in individual farms and more in units with legal personality. The extension of the lease is possible under the application of an agricultural policy favourable to the development of national production, the application of measures provided to support with non-reimbursable funds from the EU young people who have the appropriate training and want to set up farms, access to bank funds and promote technologies modern.

Dissatisfaction with "excessive fragmentation" of property is prevalent among decision-makers and smallholder farmers. In our opinion, the best solution would be to analyse the incomes of rural families with farms of different sizes (including all agricultural and non-agricultural components) and determine the incidence of poverty (which is inability to have a minimum standard of living) from different size categories. farms. This would provide a first indication needed to identify farms that are too small even to meet subsistence needs and should be the subject of the merger.

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RESULTS REGARDING THE EFFECT OF CROP ROTATION AND FERTILIZATION ON THE YIELD AND QUALITIES AT WHEAT AND MAIZE IN SOUTH OF ROMANIA

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Abstract

In conditions of the modernization of agriculture, knowledge and management of the factors that can influence the production and quality are required. An important role belongs to the environmental factors and agrotechnical measures, which together with the genetic characteristics of the varieties or hybrids contribute to the variation of production and quality. The researches were performed during the 2015-2018, in the experimental field of NARDI Fundulea and aimed to study the influence of agrotechnical practices on the yields and quality of wheat and maize. The paper presents the results obtained in long term experiences with fertilizers and rotations, under non-irrigation condition. Yields and the quality is directly influenced by the fertilizers quantity and crop rotation. The results show that 4-year rotations and fertilization contribute with 20-50% to the increase of production and protein content increases with the nitrogen rate applied and less with phosphorus rate, but together (NP) have a significant effect for both wheat and maize. This percentage varied between 9.0-15.5% for wheat and 5.5-9.5% for maize depending on crop year.

Key words: *crop rotation, fertilization, yields and qualities, maize, wheat.*

INTRODUCTION

Crop rotation and fertilization represent basic technological links of modern agriculture, contributing to the increase of the productive potential of plants and implicitly of the soil.
Wheat and maize are among the most important agricultural crops and are the basis for food worldwide (Tack et al., 2015), and production and quality are closely related to the area of culture, plant genetics and technology (Sin, 2007).
Fertilizers, of any type, are applied to maintain the nutritional status of field plants in different crop systems (Kayan et al., 2018). The use of fertilizers is positivewhen we know the degree of soil supply with nutrients and the requirements of the crop plant (Pedersen et al., 2010; Ştefănescu et al., 1997).
Small doses of fertilizers are limiting factors of production, and high doses increase production costs and can cause harm by contaminating the environment with nutrient losses (Theago et al., 2014; Arenhardt et al., 2015, Ladha et al., 2016).
Research shows that crop rotation ensures the harmonization of factors that contribute to the growth and development of crop plants (Picu, 1984; Petcu et al., 2003, Bonciu, 2018).
The quality and level of production are the result of the interaction between the stability of the soil nutrition regime, the technological measures applied and the variation of the environmental factors (Bonciu et al., 2018; Mihăilă et al., 1996; Frye and Thomas, 1991).
The genetic background of the varieties or hybrids contributes to the modification of the quality and production parameters (Stefanescu & Tianu, 2001; Bonciu, 2019).

MATERIALS AND METHODS

Researches regarding the influence of crop rotation and fertilization on the production and its quality at wheat and maize crops was carried out on cambicchernozem from Fundulea, to the non-irrigated, in a stationary experience established in 1967.
The experimental variants studied are: monoculture of wheat and maize, 2-years

rotation (wheat - maize), 3-years rotation (wheat - maize - peas) and the 4-years rotation (wheat - maize - sunflower - peas).

In these rotations, the following fertilization system was applied: unfertilized (N0P0); fertilized with phosphorus at a dose of 75 kg P_2O_5/ha (N0P75), fertilized with nitrogen and phosphorus at a dose of 90 N kg/ha (N90P0), fertilized with nitrogen and phosphorus at a dose of 90 N kg/ha + 75 kg P_2O_5/ha (N90P75) and fertilizers with manure (autumn administration) at a dose of 20 t/ha.

In the experience, all the technological links were respected, the quality determinations were made with the INFRATEC 1225 Grain Analyzer, and the data obtained were processed and statistically interpreted according to the method of analysis of the variance.

The climatic elements have registered important variations during the experimentation period.

Climatic aspects

Research has shown that production potential and quality are also influenced by the degree of favorable weather conditions (temperature evolution, precipitation quantity and distribution). The analysis of the climatic elements (Figure 1) allows appraisals regarding the climatic conditions, with direct implications on the evolution of the crops in correlation with the agrotechnical links applied.

For the study period (2015-2018), the analysis of the recorded rainfall shows their variability, compared to the multiannual average. Precipitation deficits were recorded mainly in the summer/autumn period, influencing the evolution of the crops or in the winter period, with a negative effect on the restoration of the soil water supply.

The 2017 was a humid year, and 2018 a dry year. From a thermal point of view, temperatures were registered with 1.0°C higher than the multiannual average. The variation of temperatures is different from one year to another so that, in 2018, the high temperatures during the summer period associated with the precipitation deficit led to a significant decrease of the harvests.

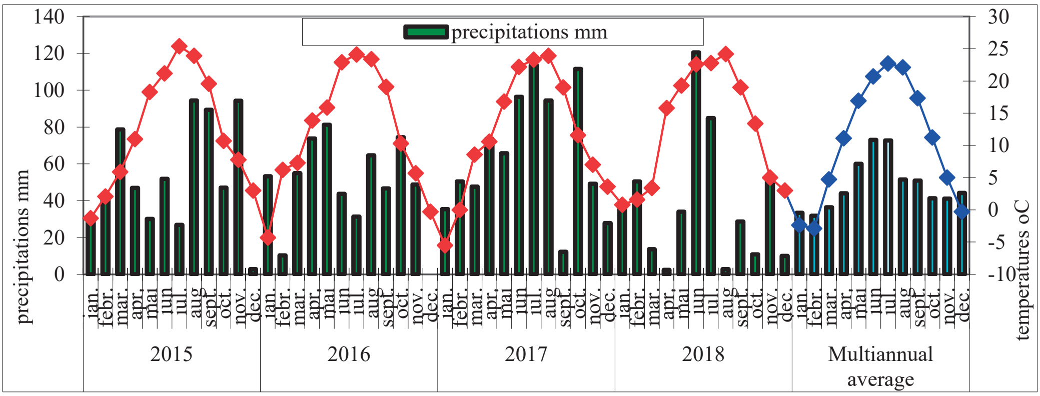

Figure 1. The evolution of the rainfalls and air temperatures, in the agricultural years 1999-2009, at Fundulea

RESULTS AND DISCUSSIONS

The wheat and maize productions obtaining in the period 2015-2018 demonstrate, for both crops, the role of rotation and fertilization with chemical and organic fertilizers, in conjunction with the variation of the climatic conditions of the crop year.

Considering that all the other technological links have been optimally respected and applied, the plants showed their genetic potential at maximum values, as much as they allowed the environmental conditions.

The experimental results obtained in the wheat crop (Figure 2) indicate a significant differentiation of the production, depending on the duration of the rotation and its succession. Thus, in 2018 the lowest level of wheat production is recorded, both within the existing rotations and in the case of monoculture, as a result of adverse climatic conditions. In 2016 and 2017, considered climatically normal, the highest yields are obtained, especially in the 3 and 4 year rotations, where the presence of a leguminous in the rotation is evident through statistically assured production increases.

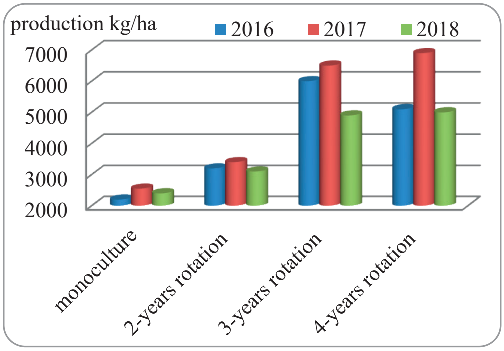

Figure 2. The influence of rotation on wheat yield, on constant nitrogen fertilization at Fundulea, 2015-2018

In maize cultivation (Figure 3), the production differences increased with the reduction of its weight in rotation, this exploiting more effectively the positive effect of placement after wheat and of the presence of leguminous plants in the rotation of 3 and 4 years.

The experimental data show the results of the placement of wheat after peas, within the 3-year rotation, where very significant production increases were obtained, 1.3 t/ha, compared to monoculture (Table 1).

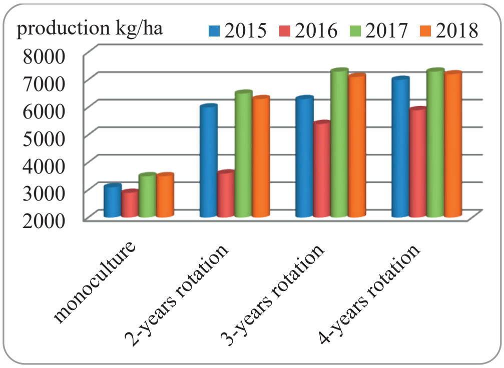

Figure 3. The influence of rotation on maize yield on constant nitrogen fertilization at Fundulea, 2015-2018

In the case of the application of chemical fertilizers, at a dose of 90 kg N/ha + 75 kg P_2O_5/ha, substantial increases of the production increase, between 1.0 and 2.7 t/ha, were obtained, regardless of the rotation used.

The application of manure in doses of 20 t/ha has led to production increases in all rotations, from 0.8 to 2.3 t/ha to wheat, compared to monoculture. Thus, with the monoculture control variant, in the 2-year rotation there was a production increase of 0.8 t/ha and in the 4-year version a production increase of 2.0 t/ha. These production enhancements are important and similar to those from the application of chemical fertilizers with N90P75.

Tabel 1. The effect of rotation and fertilization on wheat yield at Fundulea, 2016-2018

Rotations	N0P0		N90P0		N0P75		N90P75		Manure 20 t/ha	
	t/ha	Dif. ±	t/ha	Dif. ±	t/ha	Dif. ±	t/ha	Dif. ±	t/ha	Dif. ±
Monoculture	2.2	0	3.1	0	2.3	0	2.9	0	2.8	0
2-years rotation	2.4	+0.2	3.6	+0.5	2.6	+0.3	3.9	+1.0	3.,6	+0.8
3-years rotation	3.5	+1.3 ***	3.9	+0.8 ***	3.8	+1.5 ***	5.6	+2.7 ***	5.1	+2.3 ***
4-years rotation	2.9	+0.7 ***	4.0	+0.9 ***	3.2	0.9 ***	5.0	+2.1 ***	4.8	+2.0 ***
LSD 5%	0.3		0.5		0.7		0.9		0.9	

The wheat cultivated in the monoculture to which fertilizers were applied achieved an increase between 0.1 and 0.6 t/ha, compared to the unfertilized variant, but does not represent a good technological variant, due to the production ceiling.

The increase of wheat production, as an average during the agricultural years 2016-2018 (with values between 0.9-2.7 t/ha) showed that crop rotation represents a real technological link, especially the rotation in which leguminous plants are introduced. Their lack of rotation leads to the decrease of the production increase and the conditioning of other technological elements, such as additional fertilization.

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.

For the maize culture, the results obtained during the rotations showed very significant harvest increases, ranging from 0.6 to 1.8 t/ha in rotations of two, three and four years, compared to monoculture (Table 2).
Following fertilization with 90 kg N + 75 kg P2O5/ha the production increase ranged from 1.0 to 2.2 t/ha.

The application of manure in doses of 20 t/ha has led to production increases in all rotations, from 1.2 to 2.1 t/ha to maize, compared with monoculture.
The maize culture made more efficient use of the manure administered, being noticed the rotations of 3-4 years, where the productions exceeded 6.0 t/ha.

Tabel 2. The effect of rotation and fertilization on maize yield at Fundulea, 2015-2018

Rotations	N0P0		N90P0		N0P75		N90P75		Manure 20 t/ha	
	t/ha	Dif. ±	t/ha	Dif. ±	t/ha	Dif. ±	t/ha	Dif. ±	t/ha	Dif. ±
Monoculture	2.6	0	3.0	0	2.8	0	4.6	0	4.2	0
2-years rotation	3.2	+0.6	4.5	+1.5	3.2	+0.4	5.6	+1.0	5.4	+1.2
3-years rotation	3.9	+1.3 ***	5.0	+2.0 ***	4.0	+1.2 ***	6.3	+1.7 ***	6.0	+1.8 ***
4-years rotation	4.4	+1.8 ***	5.1	+2.1 ***	4.6	+1.8 ***	6.8	+2.2 ***	6.3	+2.1 ***
LSD 5%	0.6		0.8		1.0		1.2		1.3	

The protein content of wheat grains registered the highest values in 2018, and they were obtained on agrofonds N90P0 and N90P75, from 3-year rotations (by 12.4% and 13.0%, respectively) and 4-years (by 12.0% and respectively 12.4%).
Among the existing functions in the Windows program - linear, logarithmic, polynomial, power and exponential - the polynomial function has the highest regression coefficient for the connection between agrotechnical measures (rotation/fertilization) and protein content in wheat (Figure 4).

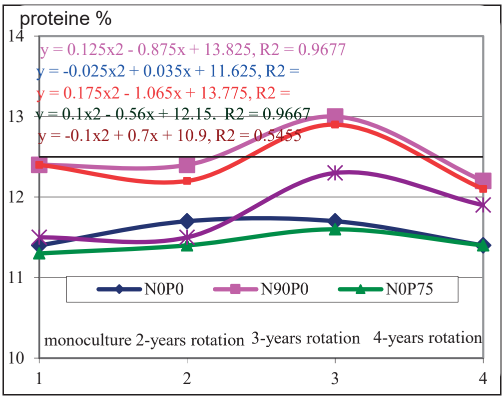

Figure 4. Correlation between agronomic measures and wheat protein content

The increase of the number of years in rotation and the application of nitrogen and phosphorus fertilizers, in economically efficient doses (respectively N90P0 or N90P75), ensure the correlations as being very positive, with regression coefficients between 0.54 and 0.96.
The practice of monoculture, associated with the lack of chemical or organic fertilizers, has resulted in a reduced amount of protein, and the regression coefficient is 0.53.
The percentage of protein in maize is directly correlated with the dose of nitrogen administered (Figure 5).
Thus, applying the dose of fertilizer with N90P0 or associated with phosphorus N90P75 led to increases of 2.0-2.6% compared to the unfertilized variant.

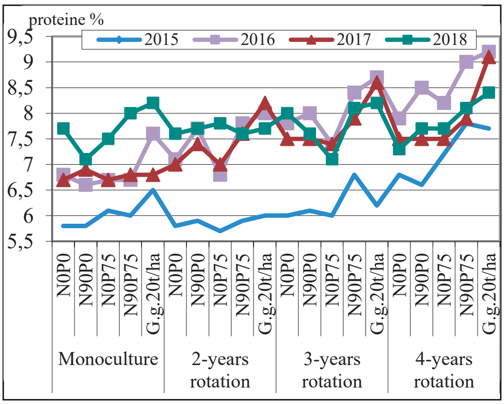

Figure 5. Influence of rotation and fertilization on maize protein content

Application of manure (20 t/ha) resulted in protein values greater than or equal to N90P75,

regardless of rotation.The protein content of the grains registered the highest values in the rotation of 3 and 4 years, compared to monoculture, as a result of a better recovery of nitrogen from fertilizers and by the presence of a leguminous plant in the rotation.
The protein content in 2018 varied slightly, with values between 7.0-8.5%, regardless of rotation or agrofond. The highest values were obtained by applying manure (8.5%).
The percentage of protein in the grain is higher in the less rainy years, with full influence of the hybrid and fertilization. In 2015, the lowest values of the protein were registered (5.6-7.7%).
The influence of the technological links on the fat content is more pronounced in the dry or normal climatic years.
The highest values of fat content (4.1-4.5%) were obtained in 2017, and the lowest (3.4-4.1%) in 2016.
The application of the 3 and 4 years rotation, associated with the fertilization of N90P75 or manure (20 t/ha) registered high increases compared to monoculture and unfertilized (Figure 6).

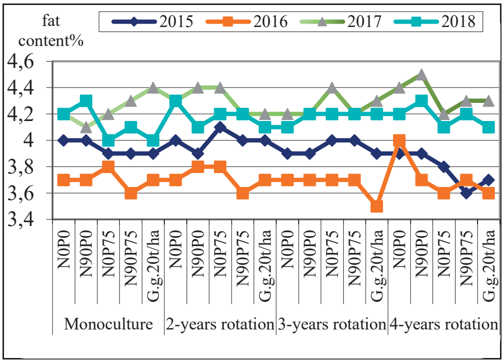

Figure 6. Influence of rotation and fertilization on maize fat content

The lowest values of the starch content were recorded in 2015, considered dry, below the value of 60.0% of the grain content (Figure 7).
The agricultural year 2018 achieved the highest starch contents, between 73-75%, especially when applying fertilizers with N90P75 or manure 20 t/ha.
These high values of starch content confer the qualitative traits necessary for corn to be used as a raw material in the starch industry.

The high fat content of the maize seeds makes from this plant a very good source of oil.

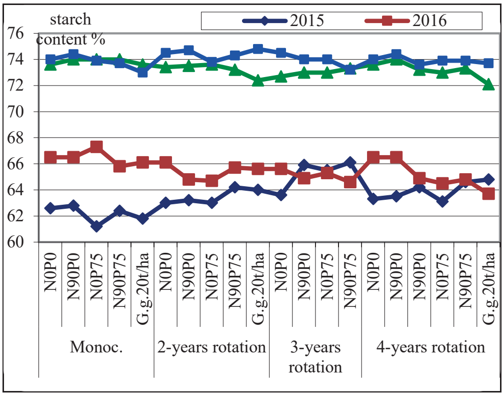

Figure 7. Influence of rotation and fertilization on maize starch content

CONCLUSIONS

Production and quality recorded variations from year to year, both for wheat and maize, depending on crop rotation and fertilization, as well as climatic conditions.
The rotations of 3 and 4 years increased the harvest, both in wheat and maize, compared to the monoculture and the simple rotation wheat / maize that did not have efficiency even under optimum conditions of fertilization.
In the 3-year rotation, wheat production is higher than that of the 4-year variant, due to the beneficial effect of the location of wheat after peas, this influence being enhanced by the unfertilized.
The placement of maize in multi-crop rotations, associated with the application of chemical fertilizers or manure, favors the production of over 6.0 t/ha.
The cultivation of wheat after maize (two-year rotation) and the application of fertilizers bring in yield increases of 0.8 t/ha and after sunflower (4-year rotation), of 2.0 t/ha.
The protein content of wheat groups the variants as follows: very good quality (protein > 13%), good (12-13%) and satisfactory (8-12%).
Thus, the application of fertilization with N90P0, N90P75 or manure (20 t/ha) and rotations of 3 or 4 years, resulted in an increase of the protein content at values of 12-14%,

compared with the unfertilized control and monoculture.

At the maize, the rotation of 3 or 4 years and the application of N90P0 or N90P75 fertilizers resulted in satisfactory values, on average over the whole period, of 5.5-9.5% in protein, 3.9-4.5% in fat and 60.0-75.0% in starch.

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ASSESSMENT OF DIFFERENT SUNFLOWER HIBRIDS UNDER AGRO-CLIMATIC CONDITIONS OF ARDS SIMNIC, ROMANIA

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Abstract

The present study was conducted with the aim to evaluate sunflower hybrids under the agro - climatic conditions of Agricultural Research and Development Station (ARDS) Simnic - Craiova. A set of six foreign sunflower hybrids was sown in a randomised block design with three replications. Data were recorded on seed yield and associated traits such as plant height, head diameter, thousand seed weight and hectolitre mass. Significant differences were observed among the hybrids for seed yield and all the studied traits. Maximum seed yield was produced by hybrid Sumiko HTS (3576 kg/ha), while hybrids Subaro HTS (2700 kg/ha) and SY Bacardi CLP (2633 kg/ha) showed minimum seed yield. A moderate negative correlation, but non-significant existed between seed yield and hectolitre mass. It is concluded that the hybrid Sumiko HTS showed high productivity and was best adapted to the agro-climatic conditions of ARDS Simnic.

***Key words**: head diameter, plant height, seed yield, sunflower (Helianthus annuus L.).*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important crop for Romania, ranking third in area after maize and wheat. It is grown on an area of 1.098,0 thousand hectares in Romania; with a production of 3.080,0 thousand tons sunflower seed and an average seed yield of 2805 kg/ha (MADR, 2018).
Although sunflower has high yield with good adaptation capacity, the seed yield varies widely depending on many factors such as rainfall distribution during the growing season, temperature, time of sowing, plant density, nitrogen nutrition (Anjum et al., 2012; Barros et al., 2004; Ion et al., 2018; Kaleem et al., 2009; Lawal et al., 2011).
According to Sarwar et al. (2013), seed yield of sunflower is greatly affected by hybrid chosen and that's why we have to choose hybrids with characteristics that best suit our needs and production practices.
In Romania is a highly diversified offer of Romanian and foreign hybrids that is changing from one year to another. According to Ion et al. (2010), it is absolutely necessary that sunflower grower to know the yielding traits of sunflower hybrids, especially the foreign ones and the new ones that are less known or even unknown.
Sunflower crop is classified as a low to medium drought sensitive (Rauf, 2008).
The understanding of interrelationships between plant characters in optimal and stress conditions is one of the main problems in breeding plant (Razi and Assad, 1999).
Seed yield in sunflower is a quantitative character that dependent on its own component traits (Arshad et al., 2007).
Plant breeders commonly prefer yield components such as plant height and head diameter that indirectly increase seed yield. Therefore, to get higher seed yields, it is important to know the relationships among yield traits in sunflower (Kaya et al., 2009).
Keeping all this in view, present study was designed to investigate comparative yield of different hybrids of sunflower and relationship between seed yield and its components, so as to choose best hybrid for agro-climatically conditions of Craiova.

MATERIALS AND METHODS

Experiment was conducted in a field research under rain fed conditions in the year 2019.

This field research was performed at the Agricultural Research and Development Station (ARDS) Simnic - Craiova ($44^{0}19'$ N, $23^{0}48'$ E, and 182 m altitude).

Soil of the experimental area is a reddish preluvosol which has the following characteristics for the Ap and Apt surface horizons (0-29 cm; 29-43 cm): the soil reaction is moderately acidic (pH = 5.08-5.33); humus content is low (2.68-2.33%); phosphorus and potassium contents are of 52.2-32.3 mg/kg and 125-104 kg/ha, respectively (Radu et al., 2019).

The experiment organized in a randomised block design with three replications.

Sowing was performed on 4 April 2019.

The crop technology was the regular one for sunflower in Southern-Western Romania, with an application of 250 kg of complex fertiliser (7:21:21).

Weeds were controlled by the help of herbicides, respectively Dual Gold 960 EC applied at a dose of 1.5 l/ha (first decade of April). Also, one manual hoeing was performed.

The year 2019 was not very favourable to sunflower because of the drought during July and August when rainfall was below the multiannual average and because of the high temperatures recorded.

Six foreign sunflower hybrids created by the Company Syngenta Romania, namely Sumiko HTS, Subaro HTS, SY Diamantis, SY Bacardi CLP, SY Neostar CLP and SY Onestar CLP, were tested.

At the maturity, ten plants were selected from each replication in order to determine plant height, head diameter, thousand seed weight and hectolitre weight.

Seed yield was adjusted to an 11.0% moisture basis. Seed yield was measured separately from each replication and expressed as kg per hectare.

The collected data were subjected to One-Way Analysis of Variance (ANOVA) and means separated using the Duncan's multiple comparisons tests at 5% level of probability.

To determine the relationships among the analysed traits, were used the regression and the Pearson correlation coefficients.

RESULTS AND DISCUSSIONS

As shown in Table 1, sunflower seed yield and studied traits were significant ($P < 0.05$) or highly significant ($P < 0.01$) affected by sunflower hybrids.

Table 1. Mean squares (MS) for traits of sunflower evaluated at ARDS Simnic during 2019

Source	DF	MS				
		Plant height	Head diameter	Thousand seed Weight	Hectolitre mass	Seed yield
Hybrids	5	246.50**	3.88**	243.20**	18.00*	347129.60**
Error	12	30.00	0.05	13.33	2.90	16095.00

F-test values are shown: *$P < 0.05$; **$P < 0.01$

Plant height

Analysis of variance showed that the results about the plant height were significant ($p \leq 0.05$) (Table 1).

Multiple comparisons test among means showed that maximum plant height (184 cm) was gained by SY Bacardi CLP which did non-significantly differ from SY Diamantis (179 cm). Minimum plant height was gained by SY Onestar CLP (161 cm) and by SY Neostar CLP (163 cm) - Table 2. These findings are in accordance with Bakht et al. (2006), Killi and Tekeli (2016) and Sarwar et al. (2013), who also reported significant differences for plant height among sunflower hybrids. They reported that sunflower hybrids significantly differed in plant height and this difference might be due to varietal behaviour and environmental conditions.

Table 2. Mean values for seed yield and studied traits of sunflower hybrids evaluated at ARDS Simnic, Craiova during 2019

Hybrids	Plant height (cm)	Head diameter (cm)	Thousand seed weight (g)	Hectolitre mass (kg/hl)	Seed yield (kg/ha)
Sumiko HTS	174 b	13.8 b	52 cd	43 b	3576 a
Subaro HTS	176 b	14.7 a	72 a	45.8 a	2700 d
SY Diamantis	179 ab	12.4 d	48 d	40.3 c	3002 bc
SY Bacardi CLP	184 a	11.6 e	54 bc	46.2 a	2633 d
SY Neostar CLP	163 c	12.9 c	48 d	45.0 ab	3095 b
SY Onestar CLP	161 c	12.2 d	38 b	46.8 a	2876 c

The linear regression between plant height and seed yield of hybrids was non-significant and negative. It means that at increased level of plant height, the seed yield decreased slightly (Figure 1).

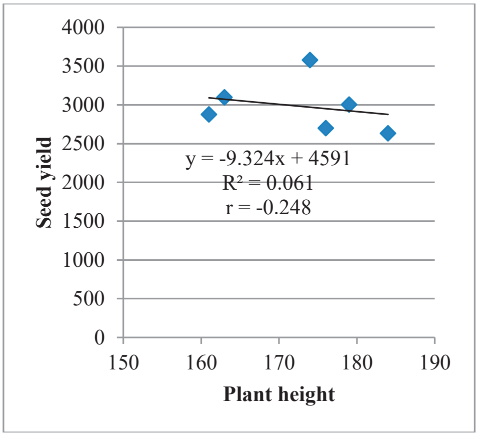

Figure 1. Relationship between plant height and seed yield of sunflower hybrids

Head diameter

Analysis of variance revealed that head diameter showed significant ($p \leq 0.05$) differences among all the hybrids (Table 1). These results are supported by Bakht et al. (2006) and Sarwar et al. (2013), who observed significant differences for head diameter among hybrids.

The largest head diameter (14.7 cm) was recorded in case of Subaro HTS, which was statistically different from all other hybrids, while smallest head diameter was observed in SY Onestar CLP (12.2 cm) and SY Diamantis (12.4 cm) - Table 2.

Previous literature reported head diameter of 9.50-13.30 cm (Alahdadi et al., 2011), 13.1-21.6 cm (Bonea et al., 2010), 14.67-18.87 cm (Fetri et al., 2013) and 13.64-16.29 cm (Iqbal et al., 2017).

The linear regression between head diameter and seed yield of hybrids was positive and non-significantly (Figure 2).

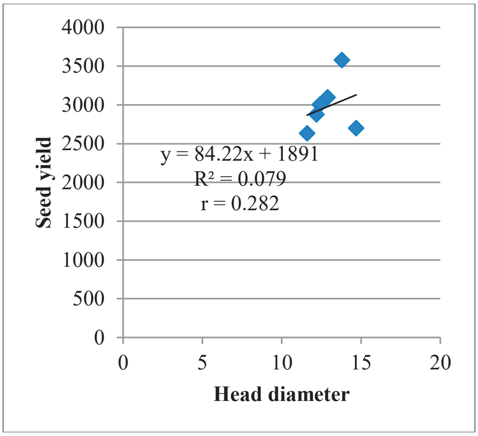

Figure 2. Relationship between head diameter and seed yield of sunflower hybrids

It seems that at increased size of head diameter, the seed yield has increased slightly. Similar results were also reported by Fetri et al. (2013). On the contrary, Iqbal et al. (2017) and Manivannan et al. (2007) reported that the correlation between seed yield and the head diameter was negative and non-significantly. According to Iqbal et al. (2017), decreased the seed yield may be due to less number of fertile florets in large headed plants.

This discordance among results is most likely associated with differences in the environmental conditions and genetic material used in these studies.

Thousand seed weight

Statistical analysis of the data indicated that thousand seed weight was significantly ($p \leq 0.05$) affected by various hybrids of sunflower (Table 1).

The multiple comparisons test among hybrids means showed that maximum thousand seed weight (72 g) was recorded by Subaro HTS, which was statistically different from all other hybrids, whereas minimum thousand seed weight (48 g) were taken by SY Diamantis and SY Neostar CLP (Table 2).

Previous literature reported thousand seed weight of 42-57 g (Bonea et al., 2008), 43.76-52.18 g (Fetri et al., 2013), 50.9-60.5 g (Ion et al., 2015), 48.37-49.11 g (Sarwar et al., 2013).

The linear regression between thousand seed weight and seed yield of hybrids was negative and non-significantly (Figure 3). One the contrary, Bonea et al. (2008), Fetri et al. (2013) and Iqbal et al. (2017), found that relationship between thousand seed weight and seed yield of hybrids was positive.

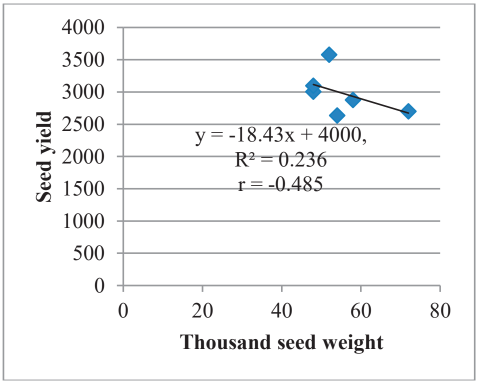

Figure 3. Relationship between thousand seed weight and seed yield of sunflower hybrids

Hectolitre mass

The data regarding hectolitre mass is shown in Tables 1 and 2.

The ANOVA showed that the results about the hectolitre mass were significant ($p \leq 0.05$).

Multiple comparisons test among means showed that maximum hectolitre mass was gained by SY Onestar CLP (46.8 kg/hl), SY Bacardi CLP (46.2 kg/hl) and Subaro HTS (45.8 kg/hl), which were statistically similar with each other.

Minimum hectolitre weight (40.3 kg/hl) was recorded in case of SY Diamantis.

The relationship between hectolitre mass and seed yield of hybrids was moderate negative, but non-significant (Figure 4). On another study carried to ARDS Simnic with ten hybrids of sunflower, Bonea et al. (2008) and Bonea et al. (2013) reported that the seed yield strongly positive correlated with hectolitre mass (r = 0.620 and r = 0.797, respectively).

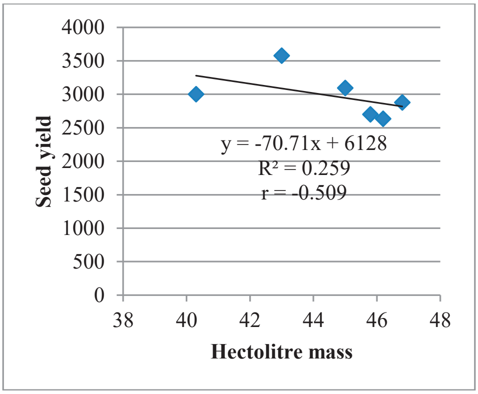

Figure 4. Relationship between hectolitre mass and seed yield of sunflower hybrids

Seed yield

Analysis of variance showed significant ($p \leq 0.05$) differences for seed yield among the hybrids (Table 1).

Comparison of hybrids means shown that the statistically highest seed yield (3576 kg/ha) was obtained in Sumiko HTS, whereas SY Bacardi CLP and Subaro HTS provided least seed yield of 2633 kg/ha and 2700 kg/ha, respectively (Table 2).

According to Hladni et al. (2011), a high seed yield and a high crude protein yield are the two most important criteria for introducing new hybrids into production.

In our study, seed yield level was similar to those obtained previously in Romania and the other countries (Ion et al., 2010; Killi and Tekeli, 2016; Ozturk et al., 2017), but lower or higher than those reported by some other studies (Bonea et al., 2010; Bonea et al., 2012; Bonea et al., 2013; Ion et al., 2015; Ion et al., 2018).

These differences in seed yield values could be explain by their different genetic potential, the agronomic practices used or environment conditions.

CONCLUSIONS

Although all the hybrids included in the experiment performed well under the agro-climatic conditions of ARDS Simnic, but hybrid Sumiko HTS (3576 kg/ha) proved to be the best regarded the seed yield.

The regression results illustrated in the figures confirmed the importance of these components in determining seed yield.

Seed yield was weakly to moderately positively or negatively associate with studied traits.

A moderate negative correlation, but non-significant existed between seed yield and hectolitre mass.

The significant variation in seed yield values and other traits among hybrids under experiment can be due to the genetic potential of the tested hybrids or to climatic conditions and adaptability.

According to the results of this study, sunflower hybrid Sumiko HTS can be recommended for the similar ecological condition of our study region.

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EFFECT OF *Bradyrhizobium japonicum* AND SALICYLIC ACID ON PHOTOSYNTHETIC PIGMENTS CONTENTS AND SOYBEAN GROWTH UNDER PHOSPHORUS AND WATER DEFICIT CONDITIONS

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Abstract

The application of rhizobacteria and salicylic acid is a promising approach in crops production and they can induce resistance against abiotic stress, however, their combined effect is not fully elucidated. A pot experiment was conducted to study the effect of rhizobacteria Bradyrhizobium japonicum applied alone or in combination with salicylic acid (SA) on photosynthetic pigments (chlorophyll a, b, total chlorophyll, and carotenoids) and plant growth of soybean under low phosphorus and drought conditions. The plants exposed concomitantly to low P and temporary water deficit registered the lowest growth and photosynthetic pigments status. Integrated use of rhizobacteria and SA (0.5 mM) increased photosynthetic pigments contents in leaves under normal as well as under water deficit conditions. Their effect was more pronounced in plants subjected to P deficiency and water stress. Results showed that seed treatment with rhizobacteria in conjunction with foliage application of salicylic acid increased plant growth irrespective of soil moisture level. It is inferred that rhizobacteria and SA work synergistically to promote growth of plants under moisture and nutrient deficit conditions.

Key words*: chlorophylls, drought, phosphorus, rhizobacteria, salicylic acid, soybean.*

INTRODUCTION

Drought and insufficiency of phosphorus (P) are major abiotic environmental factors, that often coexist in field conditions and they have significant adverse effects on the growth and productivity of crops. Water deficit stresses are the global issues that affect water and nutrient relations, assimilate production, photosynthesis and respiration in plants. In addition, P-deficiency affects a wide range of physiological, metabolic and biochemical processes in plants (Fredeen et al., 1989; Gan et al., 2002). Legumes, particularly soybean, play an important role in the development of organic and sustainable agriculture. Soybean is the most important source of vegetable oil in the world. However, this species is very sensitive to low P as well as to drought, especially during reproductive development stage (Silvente et al., 2012). This crop is severely affected by drought, resulting in major yield losses particularly in the drought prone areas of Eastern and South Europe countries. In soybean plants, water deficit stress reduces photosynthetic pigments accumulation, biomass production and finally the grain yield (Hossain et al., 2014; Tripathi et al., 2015). Thus, there is a need to find out strategies to maintain the growth and physiology activity of plants grown under unfavorable environmental conditions at more or less acceptable levels.

There is a body of scientific information regarding the beneficial effects of rhizobacteria *Bradyrhizobium japonicum* sp on soybean growth and yields (Abbasi et al., 2013). This bacteria species has many plant growth promoting activities. Along plant growth promoting rhizobacteria, plant growth regulators (PGRs) also have a vital role in maintaining many processes at physiological levels with beneficial repercussions on crops productivity (Asgher et al., 2015). Many reports have documented that exogenous application of plant growth regulators can mitigate the abiotic stress-induced inhibitory effects on plant growth and productivity (Ashraf et al., 2010). Among PGRs, salicylic acid (SA) was demonstrated to be a messenger involved in signal transduction in response to biotic and abiotic stresses (Kim et al., 2018; Raskin, 1992). Salicylic acid acts as an endogen phytohormone from phenol compounds, involved in the antioxidant defense system and regulates various physiological and biochemical parameters of plants such as stomata conductivity (Hayat et al., 2010; Khan et al., 2015; Raskin, 1992), activity of photosynthetic pigments (Hayat et al., 2010), maintenance of tissue water contents and reduced membrane permeability (Farooq et al., 2009) and tolerance to environmental stresses (Kabiri et al., 2014; Khan et al., 2018). Khodary (2004) observed a significant increase in growth, pigment contents and photosynthetic rate in maize, sprayed with salicylic acid. Some researchers demonstrated that the exogenous application of SA at lower concentrations within the range of 0.1-0.5 mM improves photosynthesis, growth and various physiological and biochemical processes, whereas in higher concentrations, more than 1 mM, SA may cause stress in plants (Hayat et al., 2010). Taking into consideration that on one hand, the application of *B. japonicum* strain has contributions to promote plant growth of soybean, and on the other hand salicilic acid may alleviate some negative effects of drought, therefore the main objective of this study was to examine if combined use of rhizobacteria and salicilic acid has synergic effect on photosynthetic pigments contents and plant growth of soybean plants subjected concomitantly to P insufficiency and water deficit conditions.

MATERIALS AND METHODS

A greenhouse experiment was conducted in controlled water conditions to examine the effect of *B. japonicum* application alone or in combination with foliage treatments with salicylic acid on photosynthetic pigments contents and plant growth of soybean (*Glycine max* L. Merrill) under low P supply and temporary water deficit conditions.

Seeds of soybean (cv Horboveanca) were sown in plastic pots (10 L) and filled with soil and sand in a ratio of 3:1. The soil used for the experiment was chernoziom carbonated. Before sowing, the soybean seeds were thoroughly mixed with *B. japonicum* inoculants (10^8 cfu/mL). There were 4 replicate pots per treatment, with two plants per pot. All plants from each treatment were grown under normal soil moisture until the flowering (R1) stage. Normal irrigation was maintained at 70% WHC (water holding capacity). Half of the plants were subjected to water deficit (35% WHC) at full flowering stage for 12 days. Plants were treated with SA (0.5 mM) at the branching and blooming stages. Control plants were not treated with rhizobacteria and salicylic acid. The experiment was in a completely randomized design and four replications were performed for flowering setting pods stage and four for maturity grain stage. Plants were collected after drought stress period and analyzed. Determination of photosynthetic pigments (chlorophyll *a, b*, total chlorophylls and carotenoids) was done using the method of Arnon (1949). Using acetone (80%, v/v) the fresh leaves were homogenized and the supernatant was read at 663, 645 and 452 nm. Values were presented as means with standard errors (SE) from three independent treatments. The differences in the means were determined by the least significant difference (LSD) (P = 0.05) test. Data were statistically analyzed using analysis of variance (ANOVA) by Statistic 7 program.

RESULTS AND DISCUSSIONS

Plant growth regulators, like salicylic acid (SA), are chemical compounds produced in one part of plant and translocated to the other parts, where they play important roles in regulating plant responses to any kind of environmental stresses. Phytohormones play an important role in influencing drought tolerance through modulating several physiological processes and different protective mechanisms. The role of SA has been reported as being critical in modulating physiological responses that lead to the adaptation of plants to unfavorable environments (Nazar et al., 2011; Kang et al., 2013). It is worthy to note that the combined effect of rhizobacteria *B. japonicum* and SA on pigments contents and plant growth in soybean subjected together to P deficiency and moderate drought has not been reported.

Salicylic acid and bacteria strains were found to induce significant effects on various physiological changes in plants. In this study, it was observed that exogenous application of SA enhanced the growth and photosynthetic pigments contents under normal irrigation condition (Figure 1) as well as under drought stress (Figure 2).

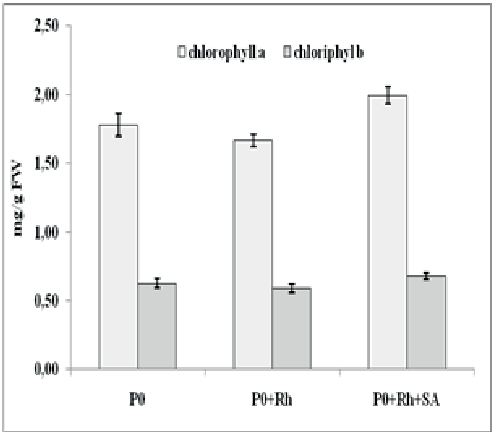

Figure 1. Effect of *B. japonicum* and salicylic acid on content of chlorophyll *a, b* in soybean leaves under low P and normal irrigation, 70% WHC. Data are presented as treatments mean ± SE (n = 4). P0-low phosphorus, Rh-*B. japoniucm,* SA-salicylic acid

The effects of rhizobacteria *B. japonicum* and SA on photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) contents of soybean plant are presented in Figures 1 and 2 and in Table 2. Experimental results revealed that rhizobacteria and salicylic acid had stimulatory effects on photosynthetic pigments contents in soybean plants. The drought decreased significantly the photosynthetic pigments concentrations in all treatments (Figure 2) compared to control plants (70%WHC). It was revealed that the content of chlorophyll *a* and *b* significantly decreased by 94-114% and 47.5-61.5%, respectively (Figure 2), in the leaves of drought stressed plants than in those under normal irrigation (Figure 1). However, plants treated with *B. japonicum* in combination with SA showed lower reductions compared to untreated plants grown under drought stress. Our results are in agreement with those of Mafakheri et al. (2010) and Pospisilova et al. (2011) who reported that exogenous application of SA increased the proline content under the conditions of drought stress. The application of rhizobacteria *B. japonicum* sp alone was also effective in improving the chlorophyll status of leaves. However, the combined use of bacteria strain and SA displayed the better result.

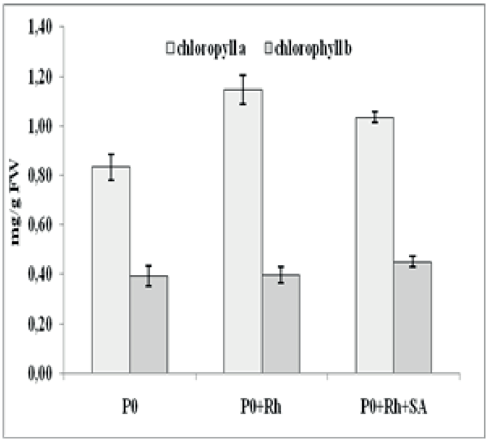

Figure 2. Effect of *B. japonicum* and salicylic acid on content of chlorophyll *a, b* in soybean leaves under low P and water deficit conditions, 35% WHC. Data are presented as treatments mean ± SE (n = 4). P0-low phosphorus, Rh-*B. japoniucm,* SA-salicylic acid

Therefore, the foliar application of plant growth regulator SA on combination with bacteria strain significantly increased the chlorophyll content in soybean plants. The beneficial effect of SA on photosynthetic pigments in our study is in accordance with those obtained by Barakat (2011) on wheat and Saeidnjad et al. (2012) on maize. Similar to our findings, exogenous application of SA increased the chlorophylls content of soybean leaves as reported by Khan et al. (2003). Foliar application of SA (0.5 mM) enhanced pigment content by about 18% for total chlorophyll (Table 1) and by 27.6% for carotenoids (Table 2) at the end of drought stress compared to untreated plants. Hence, in our research, drought stress caused the reduction of photosynthetic pigments but rhizobacteria and SA foliage treatment increased green pigments and carotenoids contents in water stress conditions. Regarding SA effect, Khodary (2004) and Szepesi (2006) found that SA treatment increased the chlorophyll, carotenoids contents, improved the photosynthetic efficiency and enhanced photosynthetic rate, the dry weights and maintained membrane integrity, leading to improvement of plant growth in maize and tomato plants respectively under stress conditions. The experimental results of the present study revealed that total chlorophylls in leaves increased by 11.7% as a result of combined use of rhizobacteria and SA in plants cultivated at normal level of soil moisture (Table 1). However, their application was more efficient in plants subjected to water deficit, and total chlorophylls increased by 18% compared to control plants.

Table 1. Total chlorophylls content (mg/g FW) in soybean leaves in relation to *Bradyrhizobium japonicum* and salicylic application under low phosphorus and water deficit condition. P0-low phosphorus, Rh-*B. japoniucm,* SA-salicylic acid

Treatments	70% WHC		35% WHC	
	Total chlorophylls mg/g	Ration a/b	Total chlorophylls, mg/g	Ration a/b
P0	2.40	2.82	1.23	2.13
P0+Rh	2.26	2.83	1.54	2.87
P0+Rh+SA	2.68	2.94	1.49	2.29

It was documented that carotenoids are involved in processes of reactive species oxygen annihilation, in photosynthetic machinery stabilization, hence contributing to diminish the adverse effects of abiotic factors on plants (Moharekar et al., 2003). Therefore, it was interesting to evaluate the changes of carotenoids contents in leaves in relation to application of rhizobacteria alone or in combination with SA. The application of *B. japonicum* separately or in combination with SA did not significantly affect their concentrations in plants cultivated under both soil moisture levels. Under drought stress, reduction of carotenoids could be related to its protection role in the photosynthetic apparatus, because carotenoids were responsible for scavenging of ROS, preventing lipid oxidation, and ultimately the mitigation of oxidative stress (Moharekar et al., 2003). Applied SA may temporarily lower the level of oxidative stress in plants, which acts as a hardening process, improving the antioxidative capacity of plants and helping to induce the synthesis of protective compounds (such as carotenoids) (Kim et al., 2018; Hayat et al., 2010).

The carotenoids content showed variable results only in plants subjected to temporary water deficit. There were not significant changes of this physiological parameter in plants grown under normal irrigation condition. However, the application of rhizobacteria increased this parameter significantly in leaves of soybean plants grown under water deficit conditions (Table 2). Similar effect was observed in treatment with combined use of rhizobacteria and SA. It is worthy to note that biomass of leaves in that treatment registered higher value than in treatment with bacteria strain separately.

Table 2. Carotenoids contents in soybean leaves under application of rhizobacteria and salicylic acid in plants grown under low phosphorus and water limited conditions, mg/g FW. P0-low phosphorus, Rh-*B. japoniucm,* SA-salicylic acid

Treatments	70% WHC	35% WHC
P0	0.82±0.01	0.322±0.11
P0+Rh	0.68±0.03	0.413±0.23
P0+Rh+SA	0.77±0.02	0.411±0.18

The application of rhizobacteria and plant growth regulators is an effective approach for improving plant productivity. Value of biomass production is considered a summary parameter of PGPR and plant growth regulators efficiency. Generally, plants cultivated concomitantly under low P and water deficit registered the lowest productivity. However, plant growth promoting rhizobacteria applied alone or in conjunction with SA treatment significantly increased plant biomass (Figure 3). Under water deficit conditions, the highest increase (39.1%) in plants growth was recorded in combined treatment of rhizobacteria and SA compared to control. It is necessary to mention that water status of soybean plant was reduced under drought stress, while SA treatments improved leaf water status (data not shown) enabling the plants for efficient water intake resulting in a positive influence on photosynthetic machinery and as a result plant growth enhanced.

Likewise, this beneficial effect was registered in plants cultivated under normal irrigation condition. The rhizobacteria applied alone had less effect on plant dry weights as compared to combined application of bacteria strain and SA. It is necessary to note that drought stress reduced the root dry weights significantly in all plants, however, combined treatment of *B. japonicum* and SA significantly reduced the negative influence of drought stress and low P on roots growth (data not shown).

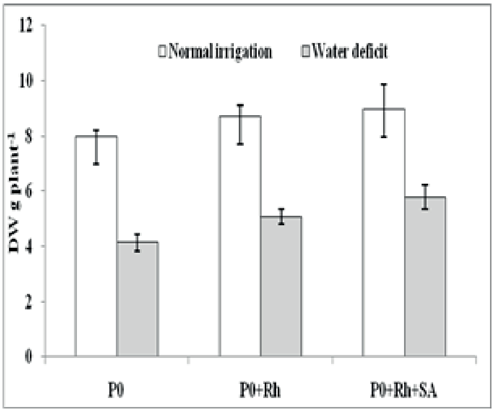

Figure 3. Effects of rhizobacteria and SA on soybean growth under low P and different soil moisture conditions. Data are presented as treatments mean ± SE (n = 4)

Arfan et al. (2007) revealed that foliar application of salicylic acid increased grain yield and performance of spring wheat plants subjected to water deficit conditions. The experimental results proved the role of PGPR and SA in improving the tolerance of plants and suggested that rhizobacteria and SA could be a useful approach to improve photosynthesis and plant growth.

Thus, enhancement of the level of chlorophylls and carotenoids pigments as well as the plant growth demonstrated the synergic interaction assigned to salicylic acid and *Bradyrhizobium japonicum* bacteria.

CONCLUSIONS

The application of rhizobacteria *Bradyrhizobium japonicum* and foliar application of 0.5 mM salicylic acid improved photosynthetic pigments status of soybean plants under normal and temporary water deficit conditions. The combined use of *B. japonicum* strain and SA treatments had synergic effect on growth parameters of soybean under low phosphorus and water deficit conditions. Overall, the results indicated that integrated use of rhizobacteria and salicylic acid could be used as a biological approach to enhance plant tolerance to environmentally unfavorable conditions.

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EFFECT OF PERSPECTIVE BIOSTIMULANTS ON THE ENZYME ACTIVITIES OF NITROGEN METABOLISM AT ALFALFA IN NEW CLIMATE CONDITIONS

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Abstract

Breeding programs related to alfalfa worldwide are aimed at creating varieties with high productivity, improved quality of green mass, resistance to diseases and pests, durability, rapid recovery after mowing and more. In the face of climate change, it is of great importance to use new, environmentally friendly methods to increase plant adaptability as well as the plant product quality. The role of nitrogen in agricultural production has always been one of the most important priorities of agronomic science, since obtaining good yields in practice is mainly related to providing plants with nitrogen. In this study, we investigated the effect of novel biostimulants on the activity of enzymes from nitrogen metabolism in alfalfa. Their application significantly improves plant metabolism, especially in conditions of drought stress. The results obtained are very important with respect to the green strategy in modern agriculture.

Key words*: alfalfa, biostimulants, climate change, enzyme activity, nitrogen metabolism.*

INTRODUCTION

Breeding programs related to alfalfa worldwide are aimed at creating varieties with high productivity, improved quality of green mass, resistance to diseases and pests, durability, rapid recovery after mowing and more. In the selection of varieties with high quality forage techniques are used to directly influence the protein content, fiber (Huset et al., 1991; Kephart et al., 1992; Petkova et al., 2018; Toktarbekova et al., 2020) or by increasing the percentage of leaves in aboveground biomass. Modern varieties and hybrids have high productive potential, which is not fully realized in production conditions. The factors limiting the expression of their productive capacities are various, but among the most important are the environmental conditions and the applied agricultural technology. The use of modern means to improve the adaptability of plants to changing environmental conditions is an important prerequisite for nature conservation in the development of sustainable agriculture. Biostimulants are a variety of chemicals which are applied to plants or soil to improve crop vitality, yield, quality and resistance to abiotic stress. Biostimulants influence by improving plant metabolism leading to increased yields; increasing the resistance of plants to abiotic factors (meteorological factors, treatment with phytosanitary products, etc.); facilitate the assimilation of trace elements and the absorption of nutrients; increase the quality of production and more. (Niewiadomska et al., 2019; 2020). The role of nitrogen in agriculture has always been one of the most important priorities of agronomic science, because obtaining good yields in practice is mainly about securing plants with nitrogen. In most plants, its additional application into the soil is necessary, but in legumes the bulk of the element is secured by the biological fixation of atmospheric nitrogen.

The importance of symbiotic nitrogen fixation, which is a complex of interactions between legumes and soil bacteria of the genus Rhizobium, resulting in the formation of specialized formations (root nodes) has been discussed in a number of scientific studies (Peoples et al., 1995; Herridge et al., 2008; Evers, 2011). Nitrogen fixation takes place in the nodes - bacterroids reduce atmospheric molecular nitrogen to ammonia, which is used by plants to synthesize amino acids and proteins. In turn, the plants supply the bacterroids with carbon to reduce the molecular nitrogen and bring about their growth (Geneva, 2003). The most important are the node bacterias from genus Rhizobium that live in symbiosis with legumes. On average, these bacteria fix from 0 to 500 kg N/ha per year (alfalfa - 500-600 kg N/ha; peas, beans - 50-100 kg N/ha) (Ruselle and Birr, 2004; Kimenov, 1994).

Although node formation is a dynamic process, the size and number of nodes do not fully reflect the degree of symbiotic nitrogen fixation by nodes bacterias and legumes, unless the activity of the enzyme nitrogenesis is directly investigated (EC 1.7.99.2.), which is responsible for carrying out this process (Aranjuelo et al., 2011). The major determinant of the biosynthesis and activity of the nitrogenase are ammonium cations, the end product of nitrogen fixation, which irreversibly inhibit it. Nitrates also suppress the activity of nitrogenase, mainly with the products of its reduction - nitrites, but also with ammonia, which is accumulated as a result of the final reduction of nitrates (Kretovic, 1994). The advantage of legumes is that they can grow on soils with a very low concentration of bound nitrogen, where other plants can not. By increasing mineral nitrogen in the soil (ammonium and nitrate), its absorption from nitrogen-fixing plants increases and symbiotic nitrogen fixation decreases (Campbell, 1999; Ruselle and Birr, 2004).

Nitrogenase activity and nitrogen fixation are highly dependent on photosynthesis and the two processes are directly linked. Nitrate reductase is a key enzyme in regulating nitrate reduction and nitrogen assimilation in crowning plants, including legumes. Nitrate reductase activity in crowning plant cells can be injected by introducing different substrates (Ruselle and Birr, 2004; Hristozkova et al., 2011; Petkova et al., 2018; Nedyalkova et al., 2019).

As a molybdenum-containing enzyme under conditions of molybdenum deficiency, a significant amount of a non-molybdenum precursor of nitrate reductase is continuously synthesized, which is able to rapidly and non-enzymatically attach molybdenum and to form nitrate reductase. Therefore, the formation of a large number of new nitrate reductase molecules is provoked by molybdenum feeding, which enhances nitrogen feeding (Mendel and Haensh, 2002; Kaiser et al., 2005).

In addition to molybdenum, the induction of nitrate reductase activity is provoked by cytokinins, a combination of cytokinins and gibberellins, humic acids, carbohydrates, mainly glucose and sucrose, certain organic acids, ammonium sulfate, organic acids included in the Krebs cycle (Aranjielo et al., 2011; Larina et al., 2019).

No matter how they are obtained from the soil, from nitrogen fixation or from nitrate reduction, ammonium cations pass through a specific tunnel of the symbiotic membrane to the host cells, where they are subsequently converted to glutamine. For the legumes from the temperate climate, typical representatives as peas, lupins, alfalfa and more., it is characteristic that they transport the fixed nitrogen in the form of amides (asparagine and glutamine) (Geneva, 2003). The most important enzyme that catalyzes the incorporation of ammonium cations into glutamate by consuming ATP is glutamine synthetase (EC 6.3.1.2).

This enzyme is located in the uninfected part of the nodes. In the bacteride itself, glutamine synthetase exhibits little or no activity (Udvardi and Day, 1997). Its activity is influenced by the presence of Mg^{2+} and Mn^{2+}, but is characterized by different pH optimizers - pH 8 for Mg^{2+} and pH 5 for Mn^{2+} (Kretovic, 1987; Vasileva et al., 2001). Glutamine synthetase exerts a positive effect on the transcription of the genes responsible for the synthesis of nitrogenase, i.e. the formation of the two enzymes in the cell is interrelated, with the appearance of one affecting the biosynthesis of the other.

An alternative route for the incorporation of ammonium ions into node tubers is through the involvement of an enzyme called asparagine synthetase (EC 6.3.5.4), which catalyzes the ATP-dependent synthesis of aspartic acid by aspartic acid and ammonium ions. Which pathways to incorporate ammonium ions will dominate depends on the plant species, its physiological state and environmental conditions.

In unfavorable abiotic factors, various physiological processes, such as photosynthesis, assimilation of nitrogen, etc., are inhibited. Various techniques are used to overcome adverse conditions, many of which have an unclear mechanism of action (Raza et al., 2019; Niewiadomska et al., 2020). The issue of regulating the biosynthesis, activity, and interaction of nitrogen-fixing enzyme systems in leguminous crops through regulators of these systems (trace elements, phytohormones, and other growth regulators, biostimulants) has not been sufficiently studied. This motivates the present study on the effects of new biostimulants on the activity of enzymes from nitric metabolism in alfalfa.

During 2017-2019 period at the experimental field at the Agricultural university - Plovdiv was conducted a field experiment under the following conditions: alluvial-meadow soil type of pH 6.7-7.1 (H_2O) and medium level of basic nutritive elements.

The investigation was performed by the 4 replications block method in 10 m^2 lots.

The sowing was done in spring 2017 and the plants are treated by generally accepted technology for alfalfa forage production (Yankov et al., 1996).

During the investigation 4 versions were controlled - treated by Tecamin max, treated by AminoBore, treated by Plantafol, treated by Fertigrain Foliar.

Tecamin max contains amino acids 14.4% of which free 12%, organic matter 60%, total nitrogen 7%. It activates the growth and development of crops. The product promotes the restoration of plants after stressful situations - frost, hail, herbicide effect, phytotoxicity. Provides for the transport in plants of mineral nutrients, including trace elements. Increases plant productivity and yield. Improves product quality.

AminoBore is an organic biostimulant containing nitrogen 4%, water soluble boron (B) 9% and free amino acids 5%. It is used in oilseeds, alfalfa, fruits, vineyards and more. (Meets organic farming standard NFU 42-003-2). It promotes faster absorption and movement of boron in the plant, restoration of cultures under stress of abiotic character (cold, drought, hail), stimulation of photosynthesis and fruit formation, overall balanced development of crops.

Plantafol is a NPK 20 20 20 mineral leaf fertilizer enriched with micro elements chelated with an EDTA chelating agent. Its content includes total nitrogen 20% (of which nitrate nitrogen - 4%, ammonium nitrogen - 2% and amide nitrogen - 14%), phosphorus (as diphosphorus pentoxide) - 20%, potassium (as potassium sulfate) - 20%. Of the trace elements, boron (B) - 0.02%, copper (Cu) - 0.05%, iron (Fe) - 0.1%, manganese (Mn) - 0.05% and zinc (Zn) - 0.05% are present.

Fertigrain Foliar - biostimulator for leaf application. Contains amino acids 10%, nitrogen 5%, organic matter 40%, zinc 0.75%, manganese 0.5%, boron 0.1%, iron 0.1%, copper 0.1%, molybdenum 0.02% and cobalt 0.01%. It has a powerful effect of stimulating plant growth and development of the plants due to the unique combination of organic nutrients in the form. Free amino acids and the most important trace elements in the form of chelates are the starting components for protein and enzyme biosynthesis.

The treatment of each swath was performed at stage by 2 l/ha of the preparations.

Plant material

Mnogolistna 1. The variety is representative of the newest generation of multifaceted alfalfa. Over 50% of the leaves of the plants hold from 5 to 7 petals on a single leaf handle.

Legend**.** The variety is registered by the US company Land O'Lakes. It is part of the new generation of so-called multifaceted alfalfa with more than 3 leaf handles, and has better in vitro digestibility than standard three-leaf sorts.

Used preparations

Four leaf treatments were used, with different contents and a combination of active substances at a dose of 300 ml/ha twice. The products are Tecamine Max, Amino Boron, Plantafol and Fertigrain Foliar.

Samples for enzymatic analyzes (roots with nodes and aboveground part) were collected in the budding and flowering phases when nitrogen fixation was most intense. The activity of four key enzymes of nitrogen assimilation - nitrogenase, glutamine synthetase, asparagine synthetase and nitrate reductase - was investigated.

Nitrogenase activity (EC 1.7.99.2.) - was determined by the method of Hardy et al. (1973) with modification (Popov et al, 1985).

The activity of glutamine synthetase (EC 6.3.1.2.) was determined by orthophosphate separated from ATP, which was determined by the Sumner method (Evstigneeva et al., 1980).

Asparagin synthetase activity (EC 6.3.5.4.) - Determined by the same procedure as for the determination of the enzyme glutamine synthetase, except that glutamate is replaced by aspartate in the incubation mixture.

Nitrate reductase activity (EC 1.6.6.2.). Nitrate reductase catalyzes the reduction of nitrates to nitrites. The method for determining the amount of nitrite is based on the color complex formed by the interaction of the nitrite ions with sulfanylamide in acidic acid and with N-(1-naphthyl)- ethylenediamine (Berova et al., 2013).

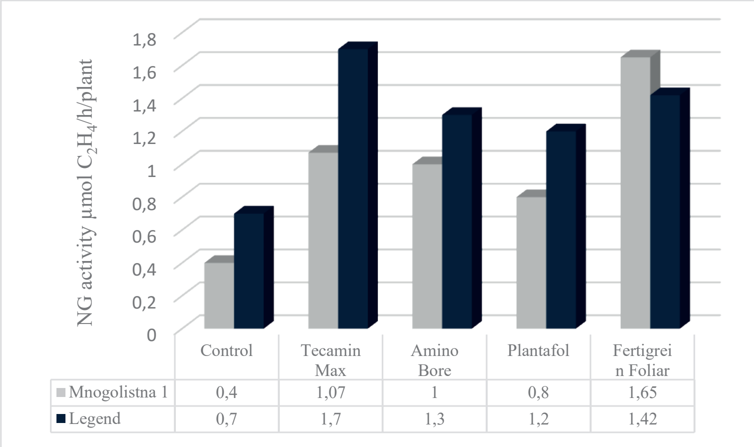
Statistical processing

The obtained data were mathematically processed by the method of variance analysis using the SPSS program, and the Dunkan multivariate test with the smallest significant difference (LSD) - 0.05 (5%) was used to determine the differences between the tested variants (Dunkan, 1955). Correlation analysis was performed with the SPSS program.

RESULTS AND DISCUSSIONS

The growing conditions of the legumes have a strong influence on the symbiotic nitrogen fixation and the factors that accompany this complex process. At the beginning of our study, a neutral soil profile response was found that was optimal for the flow of nitrogen fixation in alfalfa (Koshkin, 2005). This environment is suitable for the formation of large pink nodes on the roots that develop when there is nitrate deficiency in the soil. The content of mineral nitrogen (NO_3^- and NH_4^+) in our test field is low. These values define soil nitrogen storage as “low storage”. In this case, it is favorable for biological nitrogen fixation, since the increase of the mineral nitrogen in the soil suppresses the activity of the nitrogenase and decreases the part of the "biological" and increases the part of the absorbed nitrogen from the soil. With this low nitrogen stock, it can be considered that the more active growth and biomass accumulation of the treated plants is due primarily to the effects of the products on nitrogen fixation, especially since the soil stock with phosphorus and potassium is very good and promotes effective nitrogen fixation. The same is true for the content and ratio of calcium and magnesium, which is why no additional amounts of nitrogen, phosphorus, potassium, calcium and magnesium have been imported, especially in the conditions of marked droughts that have been observed in the recent years.

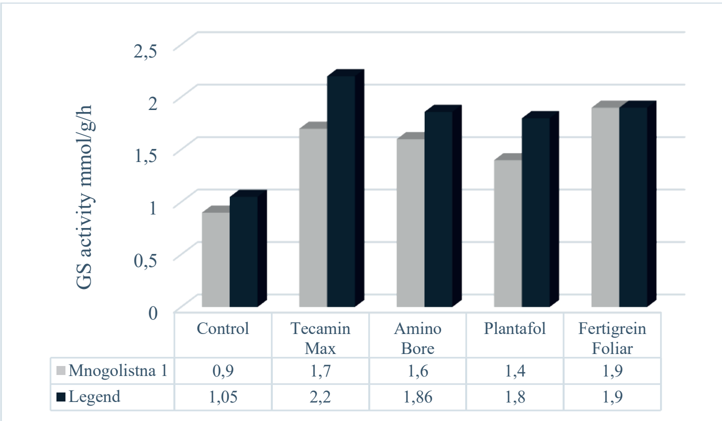
In order to establish the relationship between nitrogen fixation activity and the peculiarities of nodes, numerous studies have been carried out on their morphological characteristics, namely: size, volume, weight, quantity, characteristic location on the root system and color. The results are controversial, but larger nodes, predominantly along the main root, pink-colored, are thought to have higher activity. Research data show a higher nitrogen content in these nodes, which confirms the thesis that more active fixation of atmospheric nitrogen by the bacteria present in them (Jimotudis, 2008). Although node formation is a dynamic process, the size and number of nodes do not fully reflect the degree of symbiotic nitrogen fixation by node bacterias and legumes, unless the activity of nitrogenesis is investigated (EC 1.7.99.2.). The results of our studies indicate increased Nitrogenase activity when treated with different products compared to controls in both studied varieties. Comparing the effect of the different preparations can be seen, that Tecamin Max provokes highest Nitrogenase activity at Legend variety and Fertigrein Foliar at Mnogolistna 1 (Figure 1). The assimilation of ammonia, obtained after the atmospheric nitrogen reduction or the reduction of nitrates is carried out with the formation of primary amino acids, amines, etc. In leguminous crops, glutamine and asparagine synthesis reactions catalyzed by glutamine synthetase and asparagine synthetase play a major role in this assimilation. Glutamine synthetase is the main enzyme for the absorption of fixed nitrogen in lucerne plants. It is located outside the cterroid. In the bacteride itself, glutamine synthetase exhibits little or no activity. Treatment of multifolium alfalfa varieties with preparations containing metallic cations (Mg, Mn, Mo, B, Co) results in a glutamine change - and aspartic synthetases are actively involved in the vegetative mass.



	Control	Tecamin Max	Amino Bore	Plantafol	Fertigrein Foliar
Mnogolistna 1	0,4	1,07	1	0,8	1,65
Legend	0,7	1,7	1,3	1,2	1,42

Figure 1. Nitrogenase activity (µmol C_2H_4/h/plant) in alfalfa roots

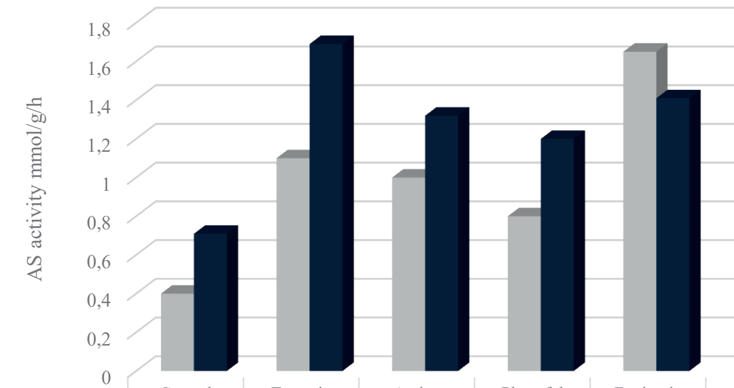
Glutamine synthetase activity in the untreated plants is highest in the Legend variety. Treatment with the preparation is recommended to show sharply glutamine synthetase activity in both varieties, the highest being in Legend (Figure 2).



	Control	Tecamin Max	Amino Bore	Plantafol	Fertigrein Foliar
Mnogolistna 1	0,9	1,7	1,6	1,4	1,9
Legend	1,05	2,2	1,86	1,8	1,9

Figure 2. Changes in glutamine synthetase activity (µmol/g/h) in alfalfa roots

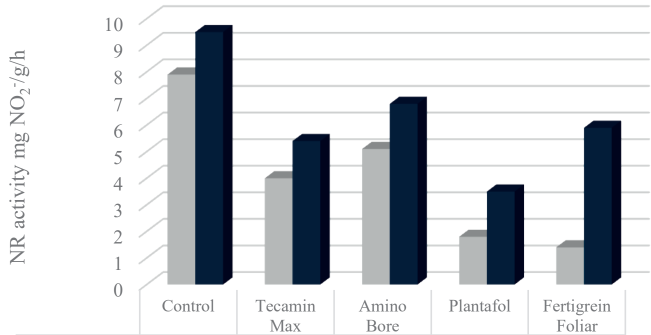
Taken separately, both varieties show similar effects after treatment with the preparations. In all variants of treatment, glutamine synthetase activity increased from 157.1 up to 219.9%. This activity is highest when treated with Fertigrein Mnogolistna 1 variety, and with Legend when treated with Tecamin Max. The results for asparagine synthase activity are similar as the one at glutamine synthase. At Mnogolistna 1 variety, again the highest values were observed with FertigreinFoliar treatment, the excess being 277.8% over the control. Treatment with TecaminMax also resulted in an increase of 147.7% over the control variant (Figure 3). Asparagine synthesis activity data at the Legend variety were also unidirectional, with the highest activity when treated with Tecamin Max (205% compared to the control).



	Control	Tecamin Max	Amino Bore	Plantafol	Fertigrein Foliar
Mnogolistna 1	0,4	1,1	1	0,8	1,65
Legend	0,71	1,69	1,32	1,2	1,41

Figure 3. Changes in asparagine synthetase activity (µmol/g/h) in alfalfa roots

The nitrates absorbed by the plants are reduced further in the roots and then in the leaves. Leguminous plants assimilate nitrates mainly through the roots. They contain an energy-dependent system with high affinity for nitrate absorption. Nitrate reductase activity in leaves varies differently from nitrogenase, glutamine synthetase and asparagine synthetase activity. It is significantly reduced when treated with the preparations to almost the same extent in both varieties. The lowest Nitrate reductase activity is reported at Mnogolistna 1, treated by Fertigrain Foliar (Figure 4). Although we are unable to account for the amount of ammonia involved in glutamine synthetase and asparagine synthetase for nitrogen fixation and for nitrate reductase activity and related nitrogen from the soil, the results show that glutamine synthetase activity is high, where the proportion of symbiotic nitrogen is higher. The preparations exert their stimulating effect on the activation of the nitrogenase activity in comparison with the nitrate reductase, which indicates that the treatment primarily stimulates nitrogen fixation. On the other hand, the preparations increase the activity of nitrogenase and glutamine synthetase, the two most important enzymes of nitrogen fixation. Similar results were obtained by Hristozkova et al. (2009) in the treatment of alfalfa with Agroleaf®. With these data, we support the hypothesis of the interconnection between glutamine synthetase and nitrogenase, i.e. the formation of both enzymes in the cell is related - the appearance of a higher activity of one influences the biosynthesis and activity of the other (Kretovic, 1994; Kaiser et al., 2005; Aranjuelo et al., 2011). According to a number of authors (Kretovic, 1994; Kaiser et al., 2005; Aranjuelo et al., 2011) nitrate reductase is inhibited by glutamine, asparagine and other amino acids. Our data support this hypothesis with increased glutamine- and asparagine synthetase activity after treatment with these preparations. With the results obtained, we confirm the hypothesis for the interaction between glutamine synthetase and nitrogenase - the formation of both enzymes in the cell is related - higher activity of one influences the biosynthesis and activity of the other (Kretovic, 1994; Kaiser et al., 2005; Aranjuelo et al., 2011). According to a number of authors (Kretovic, 1994; Kaiser et al., 2005; Aranjuelo et al., 2011), nitrate reductase activity is inhibited by glutamine, asparagine and other amino acids. Our data show increased glutamine- and asparagine synthetase activity after treatment with these preparations.



	Control	Tecamin Max	Amino Bore	Plantafol	Fertigrein Foliar
Mnogolistna 1	7,9	4	5,1	1,8	1,4
Legend	9,5	5,4	6,8	3,5	5,9

Figure 4. Nitrate reductase activity changes (μg NO_2^-/g/h) at multifoliumf alfalfa varieties

CONCLUSIONS

If the effect of the products, applied to both alfalfa varieties on the activity of the main enzyme systems in the stages of active nitrogen fixation are systematized, the following effects can be considered:

Increase in nitrogenase, glutamine synthetase and asparagine synthetase activity after treatment with Tecamin Max, Amino Bore, Plantafol and Fertigrain Foliar.

Positive dependency between nitrogenase, glutamine synthetase and asparagine synthetase activity.

Variety difference in the effect of the tested products, namely: Tecamin Max increases the nitrogenase, glutamine synthetase and asparagine synthetase activities to a higher degree than the other products in the Legend variety, and Fertigrein Foliar has the highest effect at Mnogolistna 1 variety.

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LOSSES AT MECHANIZED HARVESTING OF BULGARIAN PEANUT VARIETIES

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Abstract

An experiment was conducted to determine the share of fruit losses in mechanized harvesting of the established Bulgarian varieties of peanuts called Kalina, Kremena and Tsvetelina. The results show that total fruit losses range from 9.7% for Tsvetelina to 30.6% for Kremena. The fruit losses at digging the plants were 10.8% for Kalina, 6.3% for Kremena, and 12.6% for Tsvetelina. The losses at picking the plants up were 18% and 15.6% for Kalina and Kremena respectively, while for Tsvetelina they decreased to 3.4%. In order to reduce losses from mechanized picking of peanuts, selection of varieties with increased strength of gynophores is recommended.

***Key words**: peanuts, loss, mechanized harvesting.*

INTRODUCTION

Peanuts are not a major culture for Bulgaria, but they have significant economic potential (Bencheva, 2002). Until recently, they were grown mainly in small-scale farms with lack of mechanization and much manual labor mainly for harvesting the crop (Bencheva et al., 1997; Georgiev et al., 2011). Recently, many farmers are moving to growing peanuts over large areas and introducing mechanization to carry out all technological operations (Bencheva et al., 2008). The results show that Bulgarian peanut varieties scatter some of their fruits into the soil or on its surface at mechanized harvesting. These losses are a significant problem because they require subsequent manual harvesting of the scattered fruits (Ince and Guzel, 2003).
Peanuts require two-phase harvesting technology. The first phase involves digging the roots, separating soil from plants and placing them on the surface. The second phase is carried out after drying the plants and consists in picking them and threshing the fruits. During both phases, the fruits and their gynophores are subjected to dynamic tensile or shock loads, therefore some of them are torn off and scattered on the soil (Georgiev et al., 2011).
The purpose of the study is to determine the share of fruit losses of approved by the practice Bulgarian peanut varieties in the two phases of their mechanized harvesting.

MATERIALS AND METHODS

The losses from mechanized harvesting of approved Bulgarian varieties of peanuts Kalina, Kremena and Tsvetelina were analyzed.
They are selected at the Institute of Plant Genetic Resources - Sadovo - Bulgaria (Georgiev, 1992).
The Kalina variety was recognized and entered in the variety list of Bulgaria in 1992, Kremena in 2003, and Tsvetelina in 2008.
A three row digger KSM 4-36-38 was used to root up the plants and lay them on the soil surface. After reaching 12% humidity, the plants were picked and threshed with peanut harvester Lilystone 1518.
Determination of losses was performed using an existing method (Afshin et al., 2014), which is ased on measurements from one square meter and on the following formulas:

$$A = B+C \quad (1)$$

where:
A is the commercial yield per a square meter, kg;
B - the mass of fruits remaining on the plants, kg;

C - the mass of the fruit left in the soil and scattered on the surface after two phases of plants harvesting, *kg*.

Losses of fruit in the soil or on the soil surface (Photo 1) after digging were determined as a share of the commercial yield:

$$D = 100\frac{C}{A} \qquad (2)$$

where D are the losses from the plant digging, %;

Fruit losses from their mechanized picking up were calculated by the formula:

$$E = 100\frac{F}{A} \qquad (3)$$

where:

E losses of stem picking,%;

F is the mass of the fruits over the soil surface, kg.

Measurements were made in four replications.

Photo 1. Fruit scattered on the soil surface after the first phase of the peanut harvesting

RESULTS AND DISCUSSIONS

The results obtained (Table 1) show that during the first phase of mechanized harvesting, the most peanut fruit is lost by the Kremena variety - 12.6%, followed by the Kalina variety - 10.8% and the Tsvetelina variety - 6.3%. The first harvesting phase accounts 41.2% of the total losses in the Kremena variety. For Tsvetelina they are 64.9% and for Kalina they are 40.9% (Figure 1). The losses from mechanized selection of the Tsvetelina variety are 3.4% or 35.1% of the total losses. For the Kremena variety the losses were 18.0% or 58.8% of the total and for Kalina they were 15.6% or 59.0% of the total losses. As an absolute value, the total losses of the Kremena variety are the largest and reach 30.6%. For Kalina they are 26.4% and for Tsvetelina - 9.7%.

Table 1. Fruit loss in mechanized harvesting of Bulgarian peanut varieties, %

Variety	Loss of fruit at first phase	Loss of fruit at second phase	Total losses
Kalina	10.8	15.6	26.4
Kremena	12.6	18.0	30.6
Tsvetelina	6.3	3.4	9.7

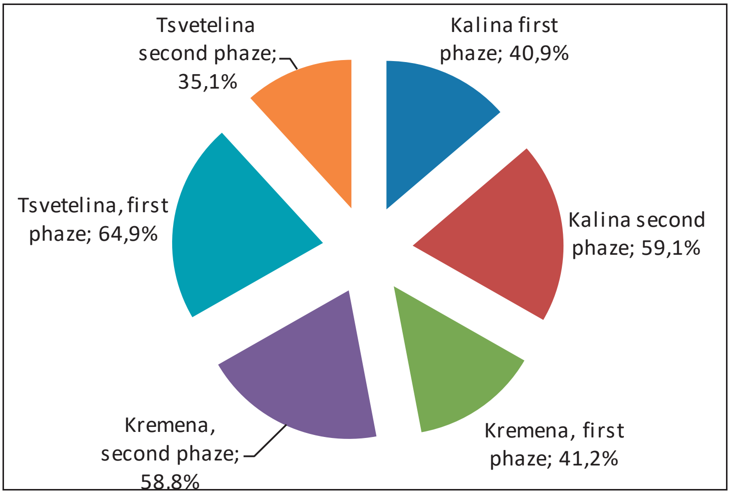

Figure 1. Proportion of fruit losses during the two harvesting phases

The results obtained are similar to studies by other authors from abroad, although they have used other harvesters and there are differences in soil and climatic conditions of investigations.

Losses from 5.3 to 35.2% are reported in the US (Beasley, 1970) and from 3.1 to 47.1% in Brazil (Santos et al., 2013).

The losses in manual and mechanized picking of peanuts are compared (Afshin et al., 2014). Manual harvesting causes losses of 3.5% and mechanized of 20.2%.

It has been determined that large losses in mechanized harvesting increase the price of production due to the re-harvesting of the scattered fruits, which is conducted manually. In all the studies mentioned above is reported that the losses of fruit is mainly due to the low strength of the peanut gynophores.

The need for selection of varieties with gynophores that are more resistant to dynamic impacts is emphasized (Zerbado et al., 2017).

CONCLUSIONS

The losses from the scattering of fruits at mechanized harvesting of peanuts from established Bulgarian varieties range from 9.7% to 30.6%.

The old Kalina and Kremena varieties cause the most losses and are therefore not recommended for mechanized harvesting, but the Tsvetelina variety allows mechanized harvesting with acceptable losses. The results obtained are close to those of other authors from abroad, despite differences in soil and climatic conditions and harvesters used. Therefore, selection of varieties with increased strength of gynophores is recommended in order to reduce the losses from mechanized harvesting of peanuts.

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INFLUENCE OF NPK FERTILIZATION ON GRAIN YIELD AND SOME COMPONENTS OF DURUM WHEAT (*Triticum durum* Desf.)

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Abstract

*The aim of the study was to determine the influence of different levels of mineral fertilizing, individual and combined, on grain yield and some structural elements of productivity. In the field experience for the period 2016-2018 the following mineral fertilization levels were used: N and P - 0, 40, 80, 120 and 160 kg/ha; K - 0 and 80 kg/ha. A maximum increase in the values of the studied parameters was established at the following fertilization rates: number of grains per spike at $N_{160}P_{160}$; grain weight at $N_{80}P_{160}$; length of spike at $N_{160}P_{120}$; spikelets number per spike at N_{120}; plant height at N_{120}. Highest grain yield was reported at $N_{160}P_{80}$. Correlation analysis showed a strong and proven connection between grain yield and the traits surveyed. The strongest correlation connection was between the number of grain per spike and the grain weight (r = 0.789**).*

Key words: *grain yield, NPK fertilizing, triticum durum, yield components.*

INTRODUCTION

Durum wheat is cultivated in our land even before the establishment of the Bulgarian state. The genotype comes from Abyssinia and North Africa (Semkova, 2013). In Bulgaria is transported from Minor Asia and Greece (Lalev et al., 1995.). *Triticum durum* is important raw material for the food industry. It is an important sours of human nutrition and serves as the raw material of numerous food such as couscous (North Africa), pasta (Europe) and bulgur (Middle Eastern) in the alimentation of the world population (Hannachi et al., 2013). Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is cultivated over more than 13 million hectares worldwide and Italy is main European producer with 3.5 milion tons per year (Ferrise et al., 2010).

With the nascency of agriculture the man has begun to apply fertilization as a mean for management of productions. The reutilization is a major factor and indicator for the efficiency of the agricultural production (Nogalskaetal, 2012). Nitrogen is a major nutrient needed to attain optimal grain yield in all environments (Mahjourimajd et al., 2016). Other scientists also have investigated the active influence of the nitrogen fertilizer. (Kostadinova & Panayotova, 2002), have found that its annex has the greatest importance on yield. Studies by Gauer et al., 1992 and Ehdaie and Waines, 2001, also confirms that N is the major nutrient influencing grain yield. On the other hand, to increase crop yield, nitrogen fertilizers have been indiscriminately used, which possesses an immense threat to environment and human health by polluting the air, water and soil (Savci, 2012). Studies of Eichner (1990), Bouman (1994) and Cole et al. (1996) have shown increasing N_2O emissions with an increasing application rate of N fertilizers. The global demand for N has been increasing and was predicted 112 million tons in 2015, indicating the reliance of world food and fiber production on N inputs (FAO). However, adequate food supplies at world level seem difficult to maintain without fertilizer application (Tilman et al., 2002). These preconditions require nitrogen fertilization to be well balanced with the phosphorus and potassium content of the soil and provide the necessary quantities to form the planned yields, including nitrogen losses, in case of leaching (Lalev et al., 1995). Making accurate N fertilizer recommendations can improve fertilizer efficiency, reducing unnecessary input cost to wheat producers (Arregui et al., 2006). The aim of the study was to determine the influence of different levels of mineral fertilizing, individual and combined, on grain yield and some structural elements of productivity of durum wheat cultivar Progress.

MATERIALS AND METHODS

The test was conducted at the Field Crops Institute - Chirpan, Bulgaria (42° 11'58 "N, 25 ° 19'27" E) (Figure 1).

Figure 1. Location of Chirpan, Bulgaria

In 1966 along term field trial was started to conduct, investigating the influence of different levels of mineral fertilization and the rates of nitrogen, phosphorus and potassium fertilizers on the yield and the quality of durum wheat (*Triticum durum* Desf.). The data presented are for the period 2016, 2017 and 2018. The experiment was based on the block method, in four replications, in two-sided rotation cotton - durum wheat. Five normsof nitrogen and phosphorus fertilization were applied (kg/ha) - N_0, N_{40}, N_{80}, N_{120}, N_{160}, P_0, P_{40}, P_{80}, P_{120}, P_{160}, nine combinations of nitrogen-phosphorous fertilization (kg/ha) - $N_{80}P_{40}$, $N_{80}P_{80}$, $N_{80}P_{120}$, $N_{80}P_{160}$, $N_{120}P_{80}$, $N_{120}P_{120}$, $N_{160}P_{80}$, $N_{160}P_{120}$, $N_{160}P_{160}$, and complex application of $N_{120}P_{120}K_{80}$. For control was used $N_0P_0K_0$. The phosphorus and potassium fertilizers were incorporated in the main soil tilling in the autumn, and the nitrogen fertilizer was introduced early in the spring, during the tillering.

The soil of the experimental field of the Field Crops Institute is PellicVertisols (Vp.). The soil is characterized by humidity horizon of 80-115 cm, the structure is crumbly-grained to prismatic in its lower part. In the one-meter layer the humus stock is about 300 t/ha. The soil reaction ranges from pH 6.5-7.4.

The following traits were examined: plant height (cm); number of grains per spike; grain weight (g); spike length (cm); number of spikelets per spike; grain yield (kg/ha).

In the first year, rainfall was higher than the multi-year period (Figure 2). In December the rainfall for the multi-year period was 54.0 mm compared to 2015 (1.3 mm). In June, the rainfall for the multi-year period was 65.4 mm, which was 50.4 mm more than that in 2016. Average temperatures for February (8.1°C), April (14.7°C) and June (22.7°C) were higher than these of the multi-year period, respectively: 2.2°C, 12.2°C and 20.9°C.

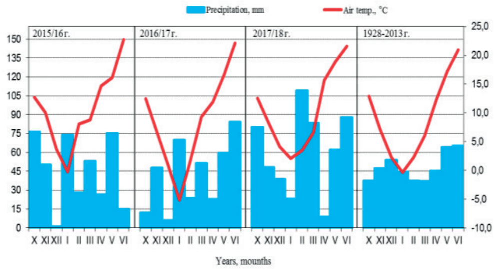

Figure 2. Precipitation and temperature during durum wheat vegetation

In the second year, December had much lower rainfall value (5.9 mm) compared to the multi-year period (54.0 mm). The months of March and June were wet, 14.3 mm and 18.9 mm above the climate norm.Average temperature in January was lower by 4.8°C in comparison to the multi-year period. March and June were warmer, with 3.3°C and 1.2°C, respectively.

In the third year of the study the months of December (with 42.5 mm), February (with 71.3 mm), March (with 46.4 mm) and June (to 22.5 mm) were with higher amounts of rainfall than the climate norm. April was characterized by much lower value (8.7 mm), at average of 45.2 mm for the multi-year period. The average daily temperature was higher than the climate norm: December with 1.9°C, January with 1.7°C and April with 3.5°C.

Analysis of the variance (ANOVA) was applied to establish statistically significant differences (LSD) with the least significant difference of 0.1%. Data correlation was determined with the software Statistica 13.0 (TIBCO, Software, 2018).

Abbreviations:GY - grain yield; NGS - number of grain per spike; GW - grain weight; LS -

length spike; SNPS - spikelets number per spike; PH - plant height.

RESULTS AND DISCUSSIONS

Table 1 presents date of the grain yield (kg/ha), the number of grains per spike and the grain weight (g) averaged over the test period.

Without fertilization, durum wheat achieved an average GY of 1,899 kg/ha. The analysis of dispersion showed that the values of GY with the introduction of P fertilization was not significant compared to the control variant. The tested rates had a negative effect, as a result of which the GY decreased and was lower than the control variant. The lowest GY was obtained at a rate of P_{40} - 1,682 kg/ha, which was 217 kg/ha less than the variant without fertilization. The highest value was obtained with P_{120} - 1,868 kg/ha, which was 31 kg/ha under control variant. In comparison to this study, Grant and Bailey (1998) have reported positive impact of the use of P fertilizers, but with negligible effect, evenin the case of increased norms. François et al. (2009) also have found a higher grain yield under the influence of P, but the difference was not statistically significant.

Table 1. Grain yields (kg/ha), number of grain per spike and grain weight (g) average 2016-2018

Fertilization rates, kg/ha		GY, kg/ha	% St	NGS	% St	GW, g	% St
$N_0P_0K_0$		1,899	100.0	23.5	100.0	1.24	100.0
P_{40}		1,682^{ns}	88.6	30.1***	128.1	2.06***	166.13
P_{80}		1,650^{ns}	86.9	34.8***	148.1	1.95***	157.26
P_{120}		1,868^{ns}	98.4	34.5***	146.8	1.84***	148.39
P_{160}		1,790^{ns}	94.3	36.8***	156.6	1.97***	158.87
N_{40}		2,535*	133.5	35.0***	148.9	2.10***	169.36
N_{80}		3,057***	161.0	36.9***	157.0	2.45***	197.58
N_{120}		3,150***	165.9	40.2***	171.1	2.28***	183.87
N_{160}		3,076***	162.0	**41.6*** **	**177.0**	2.40***	193.55
$N_{80}P_{40}$		3,296***	173.6	36.3***	154.5	2.06***	166.13
$N_{80}P_{80}$		3,294***	170.8	35.1***	149.4	1.91***	154.03
$N_{80}P_{120}$		3,199***	168.5	37.1***	157.9	2.00***	161.29
$N_{80}P_{160}$		3,128***	164.7	39.6***	168.5	**2.47*** **	**199.19**
$N_{120}P_{80}$		3,341***	175.9	38.6***	164.3	2.22***	179.03
$N_{120}P_{120}$		3,344***	176.1	38.4***	163.4	2.26***	182.26
$N_{160}P_{80}$		**3,544*** **	**186.6**	40.9***	174.0	2.40***	193.55
$N_{160}P_{120}$		3,199***	168.5	40.0***	170.2	2.46***	198.39
$N_{160}P_{160}$		3,511***	184.9	42.1***	179.2	2.23***	179.84
$N_{120}P_{120}K_{80}$		3,067***	161.5	40.1***	170.6	2.39***	192.74
Average		2,823	148.7	36.9	157.0	2.14	172.58
LSD	*5%*	*480*	*25.3*	*3.4*	*14.5*	*0.21*	*16.94*
	1%	*642*	*33.8*	*4.6*	*19.6*	*0.28*	*22.58*
	0.1%	*845*	*44.5*	*6.0*	*25.5*	*0.37*	*29.84*

ns: no significant; *, **, ***significant at P = 5%, P = 1% and P = 0.1%

The application of only N fertilizer increased GY. There was a trend for higher values of the trait when the rate was increased to N_{120}. The lowest GY was obtained at rate of N_{40} - 2,535 kg/ha, which was 33.5% above the control. This variant had lowest statistical significant of influence. The remaining three fertilization rates showed slight differences in GY: N_{120} - 3,150 kg/ha, N_{160} - 3,076 kg/haand N_{80} -3,057 kg/ha. Ali et al. (2019) also have found that, with the increasing of the nitrogen rate in the range of 0, 30, 60 and 90 kg N ha, the GY decreased (4.4, 4.5, 4.1 and 4.1 t/ha). Karam et al. (2009) have reported similar results at rate N 100 kg/ha - 3,010 kg/ha, but with increasing of the nitrogen rate to 150 kg/ha, GY was significantly higher than in our study - 3,730 kg/ha.

The combined fertilization with NP in rate of N_{80} led to reduction in GY with increasing of the P rate. The highest value was obtained at the variant with lowest P rate - $N_{80}P_{40}$ - 3,296 kg/ha. The lowest grain yield was reported at the highest P rate - $N_{80}P_{160}$ - 3,128 kg/ha. In a study by Panayotova et al. (2018) the same trend has observed.

The application of $N_{120}P_{80}$ and $N_{120}P_{120}$ led to negligible differences in the values between the two variants. The GY was, respectively 3,341 kg/ha and 3,344 kg/ha. The data obtained were in contradiction with the study of Almaliev et al. (2014), which have found that the combined fertilization of $N_{120}P_{80}$ is most effective. However, both of variants have the highest statistically influence.

The variant $N_{160}P_{80}$ produced the highest grain yield on average of the three years of research - 3,544 kg/ha, which was 86.6% more than the variant without fertilization. With increasing of the fertilizer rate a decrease in the values of the trait was observed. The GY from highest fertilizer rate in the study ($N_{160}P_{160}$) was 3,511 kg/ha. Like a results have reported Dechev and Penov (2009) under similar fertilization rates.

The incorporation of the K fertilizer resulted in reduction of the values of the studied traits. The obtained GY of 3,067 kg/ha was close to that of nitrogen fertilization. This result is contrary to the results of Plana et al. (2008).

According to Garcia et al. (2013) and Slafer et al. (2014) grain number is the main determinant of yield in grain crops.

P fertilization had a positive effect and increased the values in all four rates. However, the largest NGS was obtained when fertilized with P_{120} - 36.8.

Morino et al. (2009) have found that the increasing of the nitrogen rate increases the NGS. As a result of this regularity, the lowest values in our study were reported at a rate of N_{40} - 35.0, and the highest value was at the variant N_{160} - 41.6 or 77.0% greater than the control. Contrary to these results is the study by Álvaro et al. (2008), which have reported a larger NGS (37.7-37.9), obtained atlower fertilization rate (35 kg N/ha).

The value of variant $N_{160}P_{160}$ was with large number of grains (42.1) among of the different combinations. The fallowing variants were with similar values - $N_{80}P_{160}$ - 39.6, $N_{120}P_{80}$ - 38.6 and $N_{120}P_{120}$ - 38.4. The inclusion of K fertilizer had a positive effect on the values of the studied trait. With the application of $N_{120}P_{120}K_{80}$ was reported 40.1 NGS.

Without fertilization, the obtained value of GW was 1.24 g. The application of fertilizer had a positive effect on the GW. However, the highest value of the trait was in the rate of P_{40} - 2.06 g. The increasing of the dosage had the opposite effect and all variants with higher P rate had lower GW. The application of fertilizer had a positive effect on the GW.

Under the influence of N fertilization, the GW increased compared to the variant without fertilization, as the highest value was recorded at the rate of N_{80} - 2.45 g.

Of the combined NP fertilization, the largest increase in GW was observed at a rate of $N_{80}P_{160}$ - 2.47 g, which was 99.19% above the control. However, the differences between $N_{160}P_{120}$ (2.46 g) and N_{80} (2.45 g) were negligible. Considering the other norms, its hould be noted that fertilization with N_{120} (2.28 g) showed higher values than the combined - $N_{120}P_{80}$ (2.22 g), $N_{120}P_{120}$ (2.26 g) and $N_{160}P_{160}$ (2.23 g). In a study by Kolev et al. (2010) authors have reported a higher GW on the same variety at fertilizer rate $N_{120}P_{80}$ (2.45 g), in the conditions of Central South Bulgaria. Fertilizing with $N_{120}P_{120}K_{80}$ showed a GW of 2.39 g.

The value of LS at P_{40} was not statistically proven, this variant remains with the lowest value - 6.1 cm - 17.3% compared to the control (Table 2). The longest spike was obtained for the variant $N_{160}P_{120}$ (8.3 cm), but the difference between variants N_{160} (8.2 cm), N_{120} (8.1 cm) and $N_{160}P_{160}$ (8.1 cm) was small. When the K fertilizer ($N_{120}P_{120}K_{80}$) was included, the trait values decrease - 8.0 cm.

The NSPS averaged over the test period ranged between 16.8 ($N_0P_0K_0$) and 21.7 (N_{120}). The obtained valuesfor N_{12}exceeds the se of the variant without fertilization with 29.2%. The application of 120 kg/ha N showed better results than high nitrogen and phosphorus levels, although with some differences. Combined fertilization with $N_{120}P_{120}K_{80}$ increased the value with 23.8% compared to the control NSPS (20.8).

The difference between the untreated control and the separately applying of P_{40}, P_{80} and P_{120} on PH averaged over the test period was not statistically proven. The rate of 160 kg/ha P showed little significant influence.

N fertilization showed a high, significant effect on PH. The values for the N_{120} rate were close to these of the combined fertilization $N_{160}P_{120}$ - 102.0 and 102.8 cm, respectively.

N fertilization increased the values of the trait to a greater extend in comparison with P fertilization. The highest NSPS was obtained for N_{120} - 21.7. This variant had the highest average trait of the three years of the study. The highest increase over the control was by 29.2%. At the higher N_{160} rate, the NSPS decreased - 20.7.

Table 2. Leight spike (cm), spiklets number per spike and plant height (cm) average 2016-2018

Fertilization rates, kg/da		LS (cm)	% St	NSPS	% St	PH (cm)	% St
$N_0P_0K_0$		5.2	100.0	16.8	100.0	74.9	100.0
P_{40}		6.1 ns	117.3	18.7*	111.3	83.5 ns	111.5
P_{80}		6.5*	125.0	19.7**	117.3	85.2 ns	113.8
P_{120}		6.6*	126.9	19.6**	116.7	85.2 ns	113.8
P_{160}		6.7*	128.9	20.0***	119.1	86.9*	114.8
N_{40}		7.1**	136.5	20.4***	121.4	95.1***	127.0
N_{80}		7.7***	148.1	20.4***	122.0	100.7***	134.5
N_{120}		8.1***	155.8	**21.7*****	**129.2**	102.0***	136.2
N_{160}		8.2***	157.7	20.7***	123.2	100.4***	134.1
$N_{80}P_{40}$		7.5***	144.2	20.5***	122.0	101.4***	135.4
$N_{80}P_{80}$		7.2**	138.5	19.8***	117.9	101.4***	135.4
$N_{80}P_{120}$		7.5***	144.2	20.7***	123.2	99.9***	133.4
$N_{80}P_{160}$		7.7***	148.1	21.0***	125.0	97.3***	129.9
$N_{120}P_{80}$		7.9***	151.9	20.3***	120.8	**104.4*****	**139.4**
$N_{120}P_{120}$		7.7***	148.1	20.4***	121.4	99.4***	132.7
$N_{160}P_{80}$		8.0***	153.9	20.7***	123.2	99.4***	132.7
$N_{160}P_{120}$		**8.3*****	**159.6**	21.2***	126.2	102.8***	137.3
$N_{160}P_{160}$		8.1***	155.8	21.2***	126.2	99.6***	133.0
$N_{120}P_{120}K_{80}$		8.0***	153.9	20.8***	123.8	99.7***	133.1
LSD	*5%*	*1.2*	*23.1*	*10.1*	*10.1*	*10.6*	*14.2*
	1%	*1.6*	*30.8*	*13.7*	*13.7*	*14.2*	*19.0*
	0.1%	*2.2*	*42.3*	*17.9*	*17.9*	*18.7*	*25.0*

ns: no significant; *, **, ***significant at P = 5%, P = 1% and P = 0.1%

The NSPS varied between 19.8 and 21.2 with combined NP fertilization. The highest NSPS was obtained for variants $N_{160}P_{120}$ and $N_{160}P_{160}$. The complex application of $N_{120}P_{120}K_{80}$ increased the NSPS (20.8) with 23.8% in comparison to the variant without fertilization.

Table 3 presents the correlation coefficients between GY and the studied traits. The GY was in a positive and significant correlation with all tested parameters.

The positive correlations of grain yield with most of the traits suggests that improving one or more of the traits as a result of fertilization could result in high grain yield for durum wheat (Tsegaye et al., 2012).

A strong and proven positive relationship was observed between GY and GW (r = 0.727**). The GY was in a well-expressed positive correlation with the NSPS (r = 0.706**).

A strong and significant correlation was also established between GY and NGS (r = 0.706**). Similar results have reported by Moshin et al. (2009) and Kiliç and Yağbasanlar (2010).

Dogan (2009) also have reported a strong correlation relationship between GY and GW. The strongest correlation relationships in analysis were established between the NGS and the GW (r = 0.789**), as well as between the NSPS and PH (r = 0.767**).

Table 3. Correlation coefficients between the surveyed traits

	GY	*NGS*	*GW*	*LS*	*SNPS*	*HP*
GY	1					
NGS	0.706427**	1				
GW	0.726912**	0.789379**	1			
LS	0.317208*	0.384311**	0.142561	1		
SNPS	0.43862**	0.740955**	0.58123**	0.525163**	1	
PH	0.661388**	0.67464**	0.542148**	0.59104**	0.767041**	1

*5%; **1%; n = 57

CONCLUSIONS

Combined fertilization with $N_{120}P_{80}$ has the highest effect on GY - 86.6% in comparison to the control variant.

Under the influence of $N_{160}P_{160}$ the highest NGS was reported by 42.1% more than the variant without fertilization.

The GW showed the highest values in comparison to the control variant at $N_{80}P_{160}$ of 99.2%.

The LS was most influenced by fertilizing with $N_{160}P_{120}$. This variant exceeds control with 59.6%.

SNSP showed the highest values fertilizing with N_{120}. Under this variant was reported 29.2% an increase over the control variant.

The highest plants were measured by fertilizing with $N_{120}P_{80}$ by 39.4% more than the variant without fertilization.

A hight and significant correlation between GY and the indicators surveyed was established. The strongest correlation relations were between the NGS and the GW (r = 0.789**).

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INFLUENCE OF THE APPLICATION TIME ON THE HERBICIDES EFFICACY AGAINST THE WEEDS IN MAIZE (*Zea mays* L.)

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Abstract

Maize is the third most important crop in Bulgaria. Weed infestation is one of the key factors responsible for the lower productivity and poor quality of the maize production. Reduction in grain yield ranges from 33 to 50% or even more depending on the intensity and nature of the weed flora. During 2018 and 2019 cropping seasons field trials were conducted in the region of Plovdiv, Bulgaria. The trials were situated on the base for training and implementation of the Agricultural University - Plovdiv. The experiments were carried out in non-irrigated conditions. The aim of the resent research is to establish the suitability of applying some new herbicide products and to investigate their efficacy in different application timings: pre-emergence (BBCH 00) and early post-emergence (BBCH-13). Several herbicides and mixtures were tested: mezotrione 50 g/l + terbutilazine 326 g/l SC - 180 ml/da; mezotrione 50 g/l + terbutilazine 326 g/l SC - 230 ml/da; mezotrione 75 g/l + terbutilazine 375 g/l + clomazone 40 g/l - 150 ml/da; mezotrione 75 g/l + terbutilazine 375 g/l + clomazone 40 g/l - 200 ml/da; S-metolachlor 312.5 g/l + terbutilazine 187.5 g/l - 350 ml/da. The obtained data was compared with untreated control. The experimental field was naturally infested with Sorghum halepense (L.) Pers., Setaria viridis L., Chenopodium album L., Xanthium strumarium L., Amaranthus blitoides L., Datura stramonium L., Solanum nigrum L. and Portulaca oleracea L. After evaluating the efficacy of the herbicides we concluded that during the testing period when the soil was very dry the efficacy of the herbicides applied during the early post emergence (BBCH-13) showed higher efficacy against the weeds existing on the field.

Key words: *weed infestation, herbicide, efficacy.*

INTRODUCTION

Maize (*Zea mays*) originated in central Mexico in around 5,000 BC. The crop was introduced to Europe in the sixteenth century, from where it spread to Africa and Asia. It is now one of the most widely-grown crops around the world in both temperate and tropical regions. It is among the 10 most important world crops by value.

Production of maize (*Zea mays* L.) is increasing globally, and this trend is evident throughout the Europe. We may expect this trend to continue in the future (Tatsumi et al., 2011), with maize also being the most dominating crop for biogas production (Amon et al., 2007). Weed management had a major effect on success of maize growth because the competition ability of maize is relatively low (Ghanizadeh et al., 2014). With respect to weed control, due to its sowing period in Europe (Mars Bulletin 2012), this crop is very often characterised by a complex plural specific weed flora, composed of grass and broadleaved weeds

Maize (*Zea mays* L.) is main grain-forage crop with adaptive ability to different geographical and climatic conditions. That is the reason for the successful growing of this crop in many regions around the globe. In Bulgaria it is strategical field crop. Maize has the highest energy value in comparison to the others forage crops (Tomov & Yordanov, 1984). One of the main negative factors for agricultural production are the weeds. They decrease the yields and the quality of maize. In Bulgaria, economically the most important weeds at this crop are *Amaranthus retroflexus* L., *Datura stramonium* L., *Xanthium strumarium* L., *Solanum nigrum* L., *Chenopodium album* L., *Abutilon theophrasti* L., *Sinapis arven*sis L., *Echinochloa crus-gali* L., *Setaria viridis* L., *Sorghum halepense* L., *Convolvulus arvensis* L., *Portulaca oleracea* L., *Cynodon dactilon* L. and *Cirsium arvense* L. (Hristova et al., 2012; Kalinova et al., 2012). The maize grain yield can decrease from 24% to 96.7% (Mukherjee and Debnath, 2013; Oerke & Dehne, 2004; Zhalnov & Raikov, 1996). The monoculture growing of maize can lead to increase of the population of *Sorgum. halepense* L., *Cirsium arvense* L., *Cynodon dactilon* L. and other perennial weed species. The most efficient and economically most effective and environmentally safest is the integrated weed control. It includes application of different weed control methods - mechanical, chemical, cultural, biological etc. (Tonev, 2013). The chemical method is the most often used by the farmers. The method is highly effective, fast and easy to apply. The proper herbicide application reduces the weed management costs up to 60%. The fuel cost as well the soil erosion are also decreased (Valcheva, 2011). The aim of the resent research is to establish the suitability of applying some new herbicide products and to investigate their efficacy in different application timings: pre-emergence (BBCH 00) and early post-emergence (BBCH-13). The effect of chemical weed control with reduced herbicide rates (pre-emergency, pre-emergency + post-emergency, post-emergency.) on weed population density.

MATERIALS AND METHODS

During the 2018 and 2019 a field experiment was carried out in the experimental field of the Department of Agriculture and Herbology of the Agricultural University - Plovdiv, Bulgaria. The studied maize (*Zea mays* L.) hybrid was "P 9241" (FAO 370) from Corteva. The trial was conducted by the randomized block design in 4 replications. The size of the treated plot was 28 m^2. Several herbicides and mixtures were tested.

The variants of the trial were:

CLIK PRO (mezotrione 50 g/l + terbutilazine 326 g/l) SC - 180 ml/da;

CLIK PRO (mezotrione 50 g/l + terbutilazine 326 g/l) SC - 230 ml/da;

TONALE (mezotrione 75 g/l + terbutilazine 375 g/l + clomazone 40 g/l) - 170 ml/da;

TONALE (mezotrione 75 g/l + terbutilazine 375 g/l + clomazone 40 g/l) - 200 ml/da;

GARDOPRIM GOLD (S-metolachlor 312.5 g/l + terbutilazine 187.5 g/l) - 350 ml/da.

The obtained data was compared with untreated control.

The herbicides were applied in two timings:

- Pre emergency (BBCH 00)
- Early post emergency (BBCH-13)

The herbicides treatment was carried out by a dorsal sprayer with a working solution of 250 l/ha. The experiments were carried out in non-irrigated conditions.

The experimental field was naturally infested with *Sorghum halepense* (L.) Pers., *Setaria viridis* L., *Chenopodium album* L., *Xanthium strumarium* L., *Amaranthus blitoides* L., *Datura stramonium* L., *Solanum nigrum* L. and *Portulaca oleracea* L.

RESULTS AND DISCUSSIONS

The results were compared with untreated control. The experiments were carried out with different rates of the herbicides and in different timings of application. The reporting of herbicide efficacy and selectivity was made on the twenty-eight, forty -third and seventy days after treatment with herbicides. In all variants during the evaluation dates, we have not found visual manifestations of phytotoxicity on tested hybrid of maize P 9241. The efficacy is evaluated based on EWRS scale. The weeds *Amaranthus retroflexus* L., *Solanum nigrum* L., *Datura stramonium* L. and *Chenopodium album* L. were controlled 100% from all herbicides and rates tested in the experiment.

The efficacy against *Setaria viridis* L. is from 80 to 90% in different herbicides and rates.

The efficacy against *Xantium strumarium* L. is 100% in tested herbicides and rates, but the efficacy from the standard product (S-metolachlor 312.5 g/l + terbutilazine 187.5 g/l) is very low - 5 % only. The tested herbicides in different rates cannot control successfully the weed *Portulaca oleracea* L. - the efficacy 35 days after the application is from 40-60% only. The efficacy against the weed *Sorgum halepense* L. from seeds is almost 100% from all tested herbicides and rates, but they do not control at all the weed *Sorgum halepense* L. from rhizomes. The efficacy from all herbicides against *Cynodon dactylon* L. Pers. and *Convolvulus arvensis* L. is 0%. For reference - the total amount of rainfall for April were 71 l/m² in 2018 and 74.3 l/m² in 2019. They were evenly distributed in the different days of the month. During the 7 days before, 7 days after and during the herbicide treatment, no temperature values were reported that could contribute to a wrong assessment of the efficacy, selectivity and phytotoxicity of the studied herbicides.

Picture 1. Untreated control

Picture 2. CLIK PRO (mezotrione 50 g/l + terbutilazine 326 g/l) SC - 230 ml/da

Picture 3. TONALE (mezotrione 75 g/l + terbutilazine 375 g/l + clomazone 40 g/l) - 200 ml/da

Climatic characteristic of the experimental season of maize.

On Figure 1 are presented the average minimum and maximum monthly temperatures as well as the precipitation during the vegetation of maize. The meteorological data is provided by the department of Botany and Agro meteorology at the Agricultural University of Plovdiv, Bulgaria. According to the meteorological data during the vegetation we can determine how weather conditions affect the growth and development of the plants.

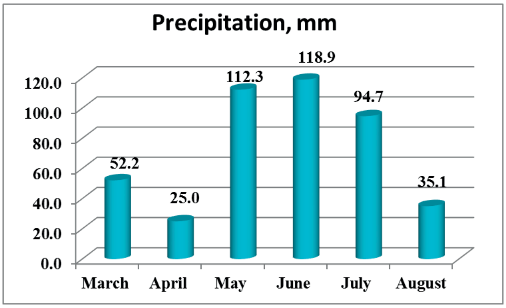

Figure 1. Average monthly precipitation during the vegetation of maize (mm)

The sum of the monthly precipitation during the vegetation is total of 438.2 mm, which is a prerequisite for relatively good moisture storage and normal flow of the vegetation. The average minimum and maximum monthly temperatures were very appropriate for germination after sowing. The maize requires minimum temperature of 10-11°C for good of germination. After germination and during the whole vegetation the plants have optimal temperatures for growing and development as the optimal temperatures during the flowering period are in the rage of 25-32°C.

CONCLUSIONS

The experimental conditions for the growing season of 2018 and 2019 were favorable for the growth, development and realization of the productive abilities of the maize hybrid "P 9241" (FAO 370) from Corteva grown in the study.

The temperature values and precipitation during the 7 days before, 7 days after and during the herbicide treatment were suitable for appropriate assessment of the efficacy, selectivity and phytotoxicity of the active substances;

After evaluating the efficacy of the herbicides we concluded that during the testing period when the soil was very dry the efficacy of the herbicides applied during the early post emergence (BBCH-13) stage showed higher efficacy against the weeds existing on the field then the pre-emergency (BBCH 00) used herbicides. We propose to the farmers to apply the herbicides on maize yearly post.

The efficacy of all applied herbicides to against against *Cynodon dactylon* L. Pers. and *Convolvulus arvensis* L. was 0%. In order to control those weeds we recoment a tank mixute of herbicides with active ingredient flyroxipyr.

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USING LIVE BACTERIAL BIOPREPARATIONS ON SEED AND PLANTING MATERIAL OF TOMATOES AND PEPPERS IN THE RESEARCH AND DEVELOPMENT STATION FOR VEGETABLE BUZAU

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Abstract

The excessive use and increasing doses of chemical fertilizers has led to the emergence of phenomena such as pollution of soil, groundwater, the environment, as well as the health of humans and animals. Excessive consumption of chemical fertilizers has led to the acidification of soils but especially to their degradation and, implicitly, affect the flora and bacterial activity in its structure. Farmers wishing to obtain crops and produce as much as possible did not take into account the negative effects that the chemization has, their activity being focused on maximizing the profit and less on the protection of the soil and the environment. Thus, in order to help stop soil pollution and its degradation, the Romanian producers came to the market with new products and innovative technologies in the agricultural field, namely organic fertilizers (biofertilizers) and organic plant protection products (bioinsecticides). These products are formulated either in liquid or granulated (lyophilized) form, these products containing in their composition live bacterial cultures. The bacteria in the composition of these products already exist in the soil structure. Biofertilizers have the role of ensuring a recolonization of the bacterial flora of the soil, the bacterial activity in the soil having the effects of soil ecology, the decomposition of complex compounds, the fixation of atmospheric nitrogen in the soil as well as a support for the growth, development and stopping of pests or diseases attacks on cultures. The use of these products in the research and development stations in agriculture in Romania has led to much better results compared to the classical chemical fertilizers (increasing agricultural production, increasing mineral elements in soil, looser soil, soft texture, growing plants, stopping the attacks of certain pests, diseases, etc.).

Key words: *biofertilizers, bioinsecticides, organic farming, sustainability, bioeconomy, live bacterial biopreparations.*

INTRODUCTION

The approaches of the agricultural specialists on biological, ecological control and using genetic methods of pest control and soil fertilization involve certain processes to improve genetic resources in order to reduce chemical fertilizers with the help of microbiological means used in both conventional agriculture and the ecological one (Andrieş et al., 2007).
The use of bacterial biopreparations is aimed at microbiologically protecting agricultural crops and replacing classical chemical fertilizers with live bacterial preparations for the purpose of soil colonization. Modern agriculture is an important, strategic area for both human and animal safety. The problems of today's agriculture and the safety of agri-food products have become more and more accentuated, the human demand for food becoming more and more (Larkin, 2010).
One of the obstacles facing the use of bacterial biopreparations in conventional agriculture is the reluctance of farmers regarding the effect of these bacterial biopreparations on harmful organisms in agricultural crops but especially on replacing chemical fertilizers with these bacterial biopreparations meant to replace excessive chemization (Matei et al., 2010).

Agriculture is based on the use of different chemical means on the soil in order to increase fertility as well as to fight diseases and pests in agricultural crops. To all these aspects were applied widely chemical fertilization products as well as plant protection products, products that contributed to the deterioration of the living conditions of both soil and human and animal structures (Tomoioagă, 2013).

In parallel with combating pests from agricultural crops and soil fertilization processes, the use of chemical fertilizers and plant protection products causes serious imbalances in soil and crop ecosystems. Pesticides and chemical fertilizers applied in agricultural crops reduce the bacterial flora of the soil, the change of soil pH towards its acidification, genetic changes of pests but especially the increase of the resistance of pests to the application of pesticides and chemical fertilizers. The use of these excess chemicals has had negative effects on humans and animals, effects such as decreased human immunity, other diseases, etc. (Volosciuc, 2009b).

In order to reduce the irreversible effects on soil, agricultural crops and humans and animals, agricultural researchers have developed a series of ecological products, based on live bacterial cultures have allowed the rebalancing of soil ecosystems, agricultural crops as well as reducing pollution on agri-food products. Bacterial biopreparations used as biofertilizers, bioinsecticides or biofungicides have the effect of ecologically controlling pests in agricultural crops, without side effects for soil, environment, plants, humans and animals (Volosciuc, 2009a). The substitution of chemical fertilizers with organic fertilizers based on bacterial preparations will lead to a greening of the soil, to the colonization of the soil fauna in order to support the growth of mineral elements in the soil in order to support the growth and development of plants and the increase of agricultural production (Voloșciuc et al., 2015).

MATERIALS AND METHODS

The researches were carried out within the Research-Development Station for Vegetable Growing Buzau. Here, together with the team of researchers from Research and Development Station for Vegetable Growing Buzau, a series of experiments were carried out regarding the action of bacterial biopreparations on the seed material, the propagating material, the soil and the plants (2018-2019).

Following the inoculation of the seed material in different solutions, three categories were delimited: control batch - germination seed material, chemical lot - seed seed inoculated with a solution based on complex 16:16:16 0.200 ml/l water, seed germination material and biological lot - seed material inoculated in solution of *Azospirillum lipoferum* 0.100 ml/l water, germinated seed material (SCDL Buzau).

Following the use of direct observation measurements, biometric measurements and determinations made in tomatoes and peppers, a series of quantitative differences (number of germinated seeds) as well as qualitative (height, vigor, color, etc.) were identified.

RESULTS AND DISCUSSIONS

Following the inoculation of the seed material with solution based on chemically complex and *Azospirillum lipoferum*, on the seeding material different structural changes were identified regarding the color, height, number of leaves, number of absorbent brushes, etc. the first research was performed on tomato seed material. Following inoculation of the seed material in the two solutions followed the process of seeding in the alveoli.

Between the three batches (control batch, chemically inoculated batch, biological inoculated batch) there was a difference between the germination period. Thus, in the control-uninoculated group, the germination of the seed material lasted 11 days. The germination of the chemically inoculated lot lasted 8 days, the germination of the biologically inoculated lot in the solution of *Azospirillum lipoferum* (solution provided by the researchers from Romvac Company S.A.) was 4 days (SCDL Buzau).

In order to prove the efficacy of the bacterial biopreparate offered by Romvac Company S.A., the researchers from SCDL Buzau made both a series of photographs of the tested lots and determinations related to the number of leaves, the number of absorbent perch, height, stem thickness or color, as follows (Figures 1-3).

Figure 1. Germinated seed material - control batch, uninoculated

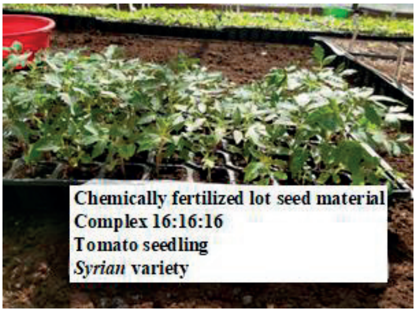

Figure 2. Germinated seed material - chemically inoculated batch

Figure 3. Germinated seed material-batch in Organic Focused: *Azospirillum lipoferum*-Rom-Agrobiofertil NP solution, Romvac Company S.A.

The second research was carried out in SCDL Buzau, with the same team of researchers. The culture tested was the pepper culture, the arum variety. As in tomato culture, the same parameter was monitored, the researchers performing the same treatment for pepper culture. The germination of the seed material from the control-uninoculated group lasted 13 days. In the inoculated chemical lot with the same amount of solution as in the tomato culture, the germination of the seed material lasted 10 days. In the lot where the seed material was inoculated with solution of *Azospirillum lipoferum*, solution taken from the set of Rom-Agrobiofertil NP product (Romvac Company S.A.), the germination time was 7 days. The germination period (the shortest of the three batches) of the seed material and the emergence of the seeding material showed that the activity of the bacterial biopreparate led to the stimulation of the germination processes in the seed structure as well as the acceleration of the occurrence and support of the growth of the seeding material (Figures 4-6).

Figure 4. Germinated seed material - control batch, uninoculated

Figure 5. Germinated seed material- chemically inoculated batch

Figure 6. Germinated seed material - organic inoculated lot: solution of *Azospirillum lipoferum*-Rom-Agrobiofertil NP, Romvac Company S.A.

Following the biometric determinations carried out by the researchers, the following aspects related to the plant structure of the two cultures were also found. Thus, the following increases have been recorded in tomato and pepper planting material (Tables 1 and 2).

Table 1. Biometric determinations of planting material, tomato crop, Syriana variety

Lots	Plant height (cm)	No. root hairs	No. leaves
Control, uninoculated (M)	4	8	3
Chemical inoculated (IC)	9	13	5
Biologically inoculated (IB)	13	17	11
Growth (%) IC vs M	125.0%	62.5%	66.7%
Growth (%) IB vs M	225.0%	112.5%	266.7%
Growth (%) IB vs IC	44.4%	30.8%	120.0%

Table 2. Biometric determinations of planting material, pepper culture, Arum variety

Lots	Plant height (cm)	No. root hairs	No. leaves
Control, uninoculated (M)	2	3	2
Inoculat chimic (IC)	4	7	5
Biologically inoculated (IB)	7	11	8
Growth (%) IC vs M	100.0%	133.3%	150.0%
Growth (%) IB vs M	250.0%	266.7%	300.0%
Growth (%) IB vs IC	75.0%	57.1%	60.0%

From both tables we can see that between the three groups there are major differences in the first determinations made in the experiments. This aspect represents an advantage in using bacterial preparations over chemical fertilizers. The use of certain bacterial preparations on the seed material has led to changes in the seed material as well as on the plant itself.

CONCLUSIONS

Following the application of the treatment with *Azospirillum lipoferum* solution from the set Rom-Agrobiofertil NP (set provided by Romvac Company S.A.) on the seed material of tomatoes and pepper, there were numerous differences.

The foundation of bacterial biopreparations and the use of biological production and protection systems represented a very important step in combating pests in agricultural crops as well as converting the chemization to organic fertilization, biological fertilization.

Among the new methods of combating or, according to the modern scientific language, of managing the density of harmful organisms, biological ones are more efficient, through biological agents and which include a wide range of processes. They include the introduction and acclimatization of live bacterial biopreparations (bacteria, viruses, fungi, etc.) in order to combat pests in agricultural crops as well as to replace the classic chemical fertilizers with certain organic, environmentally friendly biological, organic fertilizers that have the role of ecologize the soil, to support a high production of agricultural crops, as well as to support the growth and development of plants.

In the cultures in which the experiments were performed in SCDL Buzău, major differences were observed between the two groups. The effects of *Azospirillum lipoferum* solution on the seed material proved to be beneficial. The inoculation of the seed material hastened the germination process of the seed material. The pre-germination planting material proved to be higher, with a much better developed root system, which means that the seedlings will benefit from a supply of mineral elements necessary for their growth and development.

At the same time, the height of the plants showed fluctuations between the three groups, the most vigorous plant, with a much more intense color and more resistant to the pedoclimatic factors being that of the group fertilized with the organic solution of *Azospirillum lipoferum.*

The use of a bacterial biopreparate in the agricultural field, from the self-inoculation of the seed material to the fertilization of the crop itself will lead to the appearance of significant growths of the plants but especially to the ecology of the soil, the decomposition of the complex compounds as well as the mineral elements that the plants need in their growth and development. A large number of elements, the resistance of the plants to the attack of some pests as well as the increase of the agricultural productions denotes the beneficial effect that the bacterial biopreparations have on the soil and, implicitly on the plants.

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MODIFICATION OF PHYSIOLOGICAL INDICATORS WHEN TREATING OIL-BEARING RAPESEED WITH SEVERAL FOLIAR FERTILIZERS, SOWN AT DIFFERENT TIME PERIODS

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Abstract

To determine the changes in the physiological parameters, a field experiment was implemented by plot method, repeated 4 times, with the size of the experimental plot of 20 m^2. The study was conducted in the area of the Training, Experimental and Implementation Base of the Plant Growing Department at the Agricultural University - Plovdiv. The purpose of this study is to provide data on the physiological parameters of winter oil-bearing rapeseed sown at different sowing times, treated with several foliar fertilizers. To achieve this, the following parameters were monitored: net photosynthesis rate (A), transpiration intensity (E), and stomata conductance (gs) determined by the portable photosynthetic system LCA-4 (Analytical Development Company Ltd., Hoddesdon, England). From the results obtained, it can be seen that the high values of net photosynthesis combined with moderate to high levels of transpiration in the tested variants were reported in the variants treated with Fertiactyl Starter.

Key words: *rapeseed, physiological indicators, net photosynthesis.*

INTRODUCTION

Drought is a factor that strongly influences plants throughout the various stages of their development (Yordanov et al., 2000; Wentworth et al., 2006). The effects of drought on the growth of different plants, the water-photosynthesis relationship, and adaptation mechanisms have been the subject of research by a number of scientists (Van den Boogard et al., 1997; Lutts et al., 2004; Zlatev and Cebola Lidon, 2012). One way of stimulating plant development and overcoming adverse climatic conditions in order to improve the values of these indicators is by using foliar fertilizers (Ivanova and Todorov, 2020; Todorov and Ivanova, 2020).

So a task was set: to track the physiological indicators, net photosynthesis, stoma conductance and transpiration intensity of rapeseed sown at different sowing times treated with several foliar fertilizers.

MATERIALS AND METHODS

The study was conducted in the period 2012-2015 in the area of the Training, Experimental and Implementation centre of the Department of Crop Production at the Agricultural University - Plovdiv.

The experiment was based on a block method, repeated 4 times, with size of the experimental plot of 20 m^2, by using Visby hybrid, originating in Germany.

Experiment variants:

- Factor A - sowing dates
- Factor B - foliar fertilizers
- Factor C - treatment phases

I. Sowing date 1-10.IX

- Untreated variant
- Spraying with Lactofol B - 400 ml/da - phenophase - 2-4 leaves.
- Spraying with Litovit® - 200 g/da - phenophase - 2-4 leaves.
- Spraying with Fertiactyl Starter - 300 ml/da - 2-4 leaves.

II. Sowing date 1-10.X

- Untreated variant
- Spraying with Lactofol B - 400 ml/da - phenophase - 2-4 leaves.
- Spraying with Litovit® - 200 g/da - phenophase - 2-4 leaves.
- Spraying with Fertiactyl Starter - 300 ml/da - 2-4 leaves.

III. Sowing date 10-20.X

- Untreated variant

- Spraying with Lactofol B - 400 ml/da - phenophase - 1-2 leaves.
- Spraying with Litovit® - 200 g/da - phenophase - 1-2 leaves.
- Spraying with Fertiactyl Starter - 300 ml/da - 1-2 leaves.

IV. Sowing date 20-30.X

- Untreated variant
- Spraying with Lactofol B - 400 ml/da - phenophase - 1-2 leaves.
- Spraying with Litovit® - 200 g/da - phenophase - 1-2 leaves.
- Spraying with Fertiactyl Starter - 300 ml/da - 1-2 leaves.

Rapeseed is grown after wheat as a precursor by conventional growing technology.

Net photosynthesis (A, μmol m^{-2} s^{-1}), stoma conductivity (gs, mol m^{-2} s^{-1}), and transpiration intensity (E, mmol m^{-2} s^{-1}) of rapeseed grown at different sowing dates and treated with different foliar fertilizers were studied.

The reading was performed in the mass flowering phase with a portable photosynthetic apparatus LCA-4 (Analytical Development Company Ltd., Hoddesdon, England).

During the experiment, mainly meteorological factors (air temperature and precipitation) influence the growth and development of the crop, as well as their combination and distribution during vegetation.

The data characterizing these factors in the study area are shown in Figures 1 and 2. They show that during the experimental period no significant deviations from the values of the average monthly temperatures in the area of the experiment are observed in comparison to the multiannual period.

Greater differences are observed in terms of moisture, but the study period is characterized by sufficient moisture in the critical phases of the crop development, with the exception of the sowing-germination period during the first two years.

During the experimental period, an absolute minimum temperature affecting the number of wintering plants and the sowing density was recorded in December and January (Figure 3).

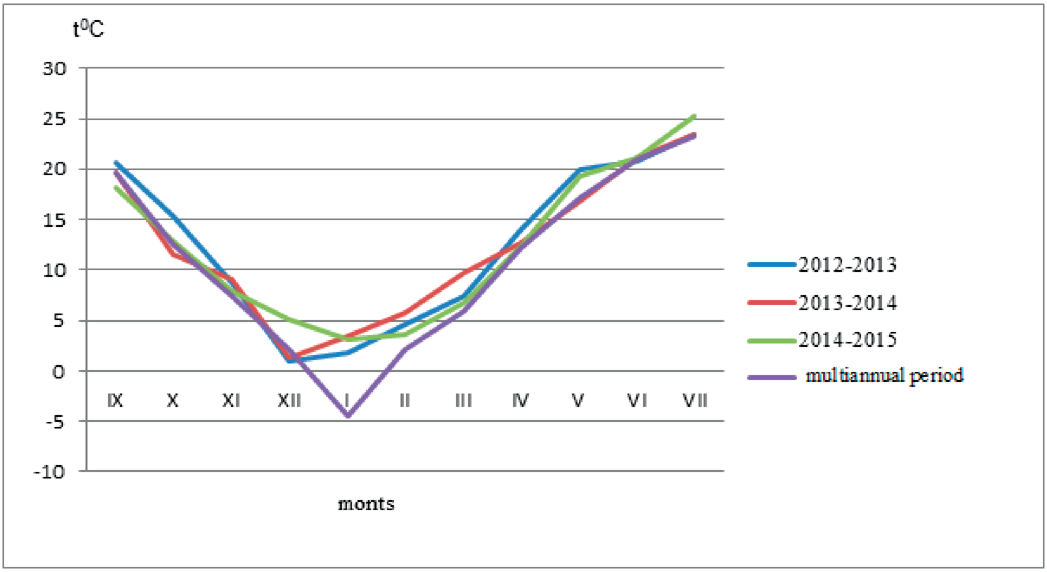

Figure 1. Average monthly temperatures in the region of the Training, experimental and implementation base

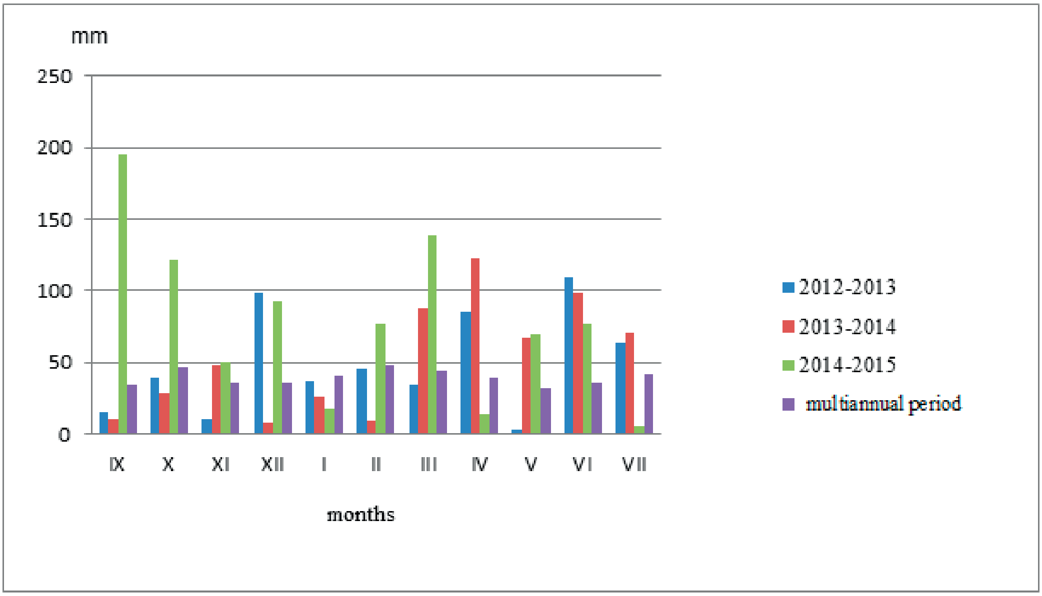

Figure 2. Quantity of rainfall during the years of survey in the region of the Training, experimental and implementation base

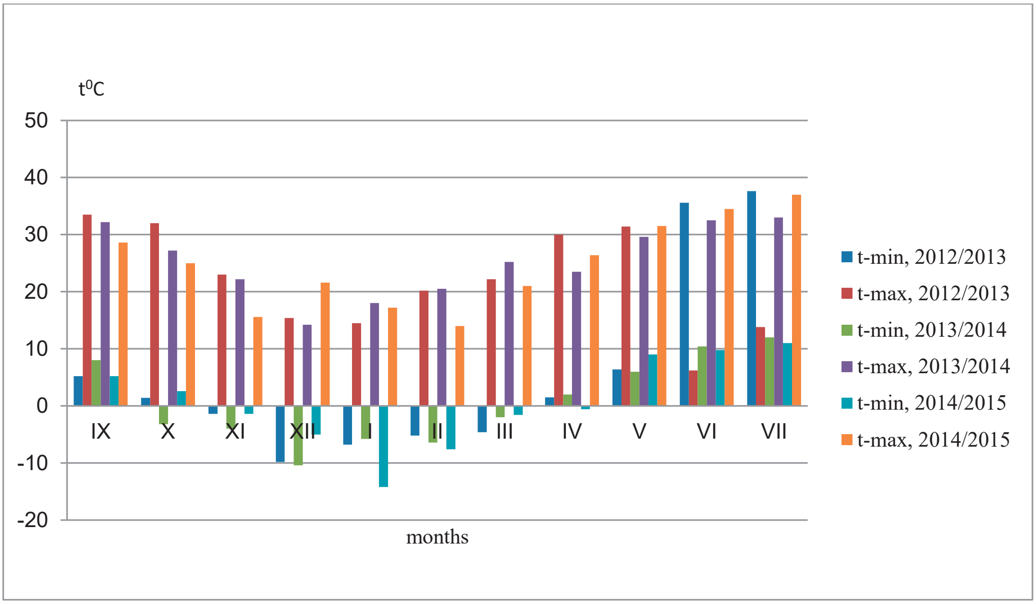

Figure 3. Absolute minimum and maximum temperatures by months 2012/2015

RESULTS AND DISCUSSIONS

The results from the measurements of the net photosynthesis are presented in Table 1.

The results obtained show that the net photosynthesis rate is highest with sowing dates 1-10.IX - from 15.50 to 22.56 µmol m^{-2} s^{-1}, followed by sowing dates 1-10.X - from 14.17 to 18.33 µmol m^{-2} s^{-1}; sowing time 20-30.X - from 13.65 to 17.40 µmol m^{-2} s^{-1} and 10-20.X - from 12.02 to 17.20 µmol m^{-2} s^{-1}.

With regard to the treated variants, the highest net photosynthesis values were reported with Fertiactyl Starter from 17.20 to 22.56 μmol m^{-2} s^{-1}, followed by the Lactofol B treated variants - from 16.48 to 22.26 μmol m^{-2} s^{-1}; Litovit ® - from 15.63 to 17.44 μmol m^{-2} s^{-1}, and control variant from 12.02 to 15.50 μmol m^{-2} s^{-1}.

The Fertiactyl Starter treated variants had the highest percentage increase in net photosynthesis compared to the control variant - from 127.5 to 145.5%, followed by the Lactofol B treated variant - from 119.9 to 143.6%, and Litovit® - from 112.5 to 130.0%.

Table 1. Net photosynthesis rate in rapeseed

	A μmol m^{-2} s^{-1}							
	1-10.IX		1-10.X		10-20.X		20-30.X	
Control	15.50	% 100.0	14.17	% 100.0	12.02	% 100.0	13.65	% 100.0
Fertiactyl Starter	22.56	145.5	18.33	129.4	17.20	143.1	17.40	127.5
Litovit ®	17.44	112.5	16.46	116.2	15.63	130.0	15.63	114.5
Lactofol B	22.26	143.6	16.99	119.9	16.48	137.1	16.99	124.5
LSD 5%	0.33		0,31		0,29		0,30	

On the basis of the mathematical processing carried out, during the three years of the experiment, at all sowing dates, statistical proof regarding net photosynthesis at LSD of 5% was found between leaf-treated variants and the control variant.

Depending on the sowing date and the foliar fertilizers used, changes are also observed in the indicator stoma conductivity (Table 2).

The results in the table show that at the first sowing dates (1-10.IX) the highest values were reported in the Fertiactyl Starter treated variant (0.11 mol m^{-2} s^{-1}) followed by the control variant (0.09 mol m^{-2} s^{-1}), then Lactofol B and Litovit ® (0.07 mol m^{-2} s^{-1}).

The differences between the variants tested for the other sowing dates are insignificant: for sowing time 1-10.X from 0.06 mol m^{-2} s^{-1} for the Lactofol B treated variant to 0.09 mol m^{-2} s^{-1} in the Litovit® treated variant.

At sowing dates 10-20.X the results of the control variant reported are the lowest 0.06 mol m^{-2} s^{-1} while the other variants do not report differences (0.08 mol m^{-2} s^{-1}).

The combination of climatic factors and foliar fertilizers used during the last sowing date 20-30.X are the preconditions for the highest values to be reported for the control variant (0.10 mol m^{-2} s^{-1}), while for the other variants no differences are observed (0.08 mol m^{-2} s^{-1}).

Table 2. Stoma conductivity

	gs mol m^{-2} s^{-1}			
	1-10.IX	1-10.X	10-20.X	20-30.X
Control	0.09	0.07	0.06	0.10
Fertiactyl Starter	0.11	0.08	0.08	0.08
Litovit ®	0.07	0.09	0.08	0.08
Lactofol B	0.07	0.06	0.08	0.08

Transpiration intensity data are presented in Table 3. The results in the table show that at the first sowing date 1-10.IX the highest values were reported for the control variant (1.53 mmol m^{-2} s^{-1}), while the lowest - for the Litovit® treated variant (1.10 mmol m^{-2} s^{-1}). In the other variants, the reported values are as follows: for Lactofol B (1.20 mmol m^{-2} s^{-1}) and Litovit ® (1.10 mmol m^{-2} s^{-1}).

Table 3. Transpiration Intensity

	E mmol m^{-2} s^{-1}			
Control	1-10.IX	1-10.X	10-20.X	20-30.X
	1.53	1.03	1.15	1.38
Fertiactyl Starter	1.17	1.55	1.37	1.40
Litovit ®	1.10	1.54	1.42	1.42
Lactofol B	1.20	1.16	1.46	1.44

For other sowing dates, higher values were observed for the variants treated with foliar fertilizers compared to the control variant. This is probably due to better-developed plants that form a larger leaf area, leading to increased transpiration.

The highest values of net photosynthesis combined with moderate to high levels of transpiration in the variants tested were reported with Fertiactyl Starter treatment. In this variant, water loss is moderate to large, which provides enough CO^2 for the highest photosynthesis, which is a prerequisite for obtaining high yields.

The variants with the highest photosynthesis support transpiration at intermediate levels, indicating that these plants can regulate both water consumption when needed and maintain a high rate of photosynthesis in low to moderate water deficits.

The other variants treated with Lactofol B, Litovit® have lower net photosynthesis values combined with moderate to high transpiration levels, which is a prerequisite for obtaining lower seed yields.

The lowest net photosynthesis values combined with low to high transpiration levels were observed in control variants. In these variants, water loss is high and photosynthesis is inefficient, and under water shortages this could have a severe negative effect on productivity.

CONCLUSIONS

1. Net photosynthesis is higher in the variants treated with foliar fertilizers: Fertiactyl Starter - from 17.20 to 22.56 μmol m^{-2} s^{-1}; Lactofol B - from 16.48 to 22.26 μmol m^{-2} s^{-1}; Litovit® - from 15.63 to 17.44 μmol m^{-2} s^{-1}, compared to the control variant - from 12.02 to 15.50 μmol m^{-2} s^{-1}.
2. The differences between the variants in relation to the indicator stoma conductivity are insignificant and range from 0.06 to 0.11 mol m^{-2} s^{-1}.
3. The transpiration intensity for the first sowing date is from 1.10-1.53 mol m^{-2} s^{-1}, while in the other sowing dates it varies 1.03-1.55 mmol m^{-2} s^{-1}.
4. The highest values of net photosynthesis combined with moderate to high levels of transpiration in the variants tested were reported with Fertiactyl Starter treatment.

In this variant, water loss is moderate to large, which provides enough CO_2 for the highest photosynthesis, which is a prerequisite for obtaining high yields.

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EFFICACY OF TREATMENTS IN CONTROLLING CERCOSPORIOSIS (*Cercospora beticola* Sacc.) IN SUGAR BEET

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Abstract

Experiments were made in the experimental field from the location Sanzieni, Covasna County, under the conditions of the year 2018 and 2019 and were designed to determine effectiveness of the treatments in combating the pathogen Cercospora beticola of the sugar beet. The biological material was represented by the Damian, Matti, Vangelis and Tatry varieties. Different treatment schemes were tested and efficacy was calculated. The application of the treatments reduced significantlythe attack of cercosporiosis in all beet varieties in the experimental versions. The highest efficacy value of 95.44% was calculated at the Damian variety in 2019 in the Sfera 535 SC (0.35 l/ha) + Bravo 500 SC (1.5 l/ha) + Amistar Gold (0.5 l/ha) experiment.

Key words: *sugar beet, micromycete, control, efficacy.*

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) represents for many states the most important crop for obtaining sugar (Toth & Cristea, 2018). The attack of beet-specific pathogens reduces considerably the roots and sugar production. Cercosporiosis or the leaf spot disease of the beet, caused by the attack of the micromycete *Cercospora beticola*, is considered the most common disease of the sugar beet leaf, wherever this plant is cultivated (Radulescu & Bulinaru, 1957). The attack of the fungi influences the plant growth and production through the fact that it leads to drying of the leaves, and the plants react to this loss by issuing new leaves, which happens at the expense of dry matter content. As a result, a lower accumulation of dry matter in the affected plants takes place and the extractable sugar yield drops compared with infection-free plants. Loss caused by the attack of the pathogen *Cercospora beticola* led to a 42% reduction of raw sugar and to 32% reduction of the root weight (Smith & Martin, 1978). Kelber (1977) estimated a 0.3% increase in yield loss with 1% increase disease severity (Kelber, 1977). Integrated control of pathogen *Cercospora beticola* attack envisages the resistance of the varieties (Rosi, 1995; Doncila, 1995a) the corresponding rotation, crop hygiene, the application of fungicide treatments, paying attention to avoiding the appearance of fungus resistance to administered substances (Shane & Teng, 1992; Wolf & Verreet, 2002; Cioni & Zavanella, 2004; Dovas et al., 1976; Georgopoulos & Dovas, 1973; Ruppel, 1975). Control of cercosporiosis attack envisages forecast of attack and warning of the treatment (Cristea & Gheorghies, 2001). Controlling sugar beet seed is of particular importance, as well, knowing that *Cercospora beticola* is present also on clusters, (Cristea, 2005; Milosecic et al., 2006) as seed pathogens have an important role in the epidemiology of the agricultural plant's disease.

MATERIALS AND METHODS

The research took place in the experimental field within the S.C. Agromiki Sanzieni, Covasna county and consisted in monitoring the influence of some fungicides to combat the pathogen *Cercospora beticola*. The presented data include observations and experimental results for two years 2018 and 2019. The used biological material was represented by the varieties: Damian, Matti, Vangelis and Tatry. Experiments were placed in three repetitions by randomized block scheme. In 2018, the following treatments were monitored: *Experiment 1*: Yamato (1.5 l/ha) + Bravo500 SC (1.5 l/ha) + AmistarXtra 280 EC (0.5 l/ha);

Experiment 2: Bravo 500 SC (1.5l/ha) + Yamato (1.5 l/ha) + AmistarXtra 280 EC (0.5 l/ha).
Experiment 3: AmistarXtra 280 EC (0.5 l/ha) + Bravo 500 SC (1.5 l/ha) + Yamato (1.5 l/ha).
In 2019, these treatments were monitored: *Experiment 1*: Amistar Gold (1.0l/ha) + Sfera 535 SC (0.35 l/ha)+ Bravo 500 SC (1.5 l/ha); *Experiment 2*: Bravo 500 SC (1.5 l/ha) + Amistar Gold (1.0l l/ha)+ Sfera 535 SC (0.35 l/ha); *Experiment 3*: Sfera 535 SC (0.35 l/ha) + Bravo 500 SC (1.5 l/ha) + Amistar Gold (1.0 l/ha).
The seed was treated with the insecticide Cruiser 600 in dose of 60 g/UG and the fungicide Tachigaren in dose of 10 g/UG in all variants. Observations on the frequency (F%) and intensity (I%) of the attack were made, based on which the degree of damage (GA%)was calculated, by the formulas: F = n x 100/N, where N = number of observed plants (%), n = n° of characteristic symptoms plants (%), I = Σ (i x f)/n (% 0 where, I = percentage for, f = number of plants (organs) with the respective percentage, n = total number of attacked plants (organs).
Intensity was noted in percent.
The degree of attack was calculated by the formula:

$$DA\ (degree\ of\ attack) = \frac{FxI}{100}\ (\%),$$

where: F = attack frequency (%),
I = attack intensity (%),
DA = rate was calculated by:

$$DD\ (degree\ of\ damage) = \frac{FxI}{100}\ (\%)$$

where:
F = attack frequency (%),
I = attack intensity (%).
The efficacy of the treatments was calculated by the formula of Abbott

$$E = \frac{GAm - GAv}{GAm} x100$$

where DAm = control level, DAv = variant attack level.

RESULTS AND DISCUSSIONS

Experiments placed in the experimental field from the location Sanzieni, Covasna county were designed to monitor the action of some fungicides in combating micromycete *Cercospora beticola* in sugar beet, in 2018 (Table1) and in 2019 (Table2).

Table 1. Scheme of Applying Treatments for Sugarbeet in Controlling the Attack of *Cercospora beticola* at SC Agromiki SRL, Location Sanzieni, Covasna County, 2018

Treatment No.	Product	Active Substance	Dosis (l/ha)	Date
Experiment 1				
1	Yamato	thiophanate-methyl 233 g/l+tetraconazole 70 g/l	1.5	30 June
2	Bravo 500 SC	chlorothalonil 500 g/l	1.5	16 July
3	AmistarXtra 280 EC	azoxystrobin 200 g/l +ciproconazole 80 g/l	0.5	4 August
Experiment 2				
1	Bravo 500 SC	chlorothalonil 500 g/l	1.5	30 June
2	Yamato	thiophanate-methyl 233 g/l+tetraconazole 70 g/l	1.5	16 July
3	AmistarXtra 280 EC	azoxystrobin 200 g/l +ciproconazole 80 g/l	0.5	4 August
Experiment 3				
1	AmistarXtra 280 EC	azoxystrobin 200 g/l +ciproconazole 80 g/l	0.5	30 June
2	Bravo 500 SC	chlorothalonil 500 g/l	1.5	16 July
3	Yamato	thiophanate-methyl 233 g/l+tetraconazole 70 g/l	1.5	4 August

Table 2. Scheme of Applying Treatments for SugarBeet in Controlling the Attack of *Cercospora beticola* at SC Agromiki SRL, Location Sanzieni, Covasna County, 2019

Treatment No.	Product	Active substance	Dose (l/ha)	Date
Experiment 1				
1	Amistar Gold	azoxystrobin 125 g/l + difenoconazole 125 g/l	1.0	17 June
2	Sfera 535 SC	trifloxystrobin 375 g/l+cyproconazole 160 g/l	0.35	3 July
3	Bravo 500 SC	chlorothalonil 500 g/l	1.5	27 July
Experiment 2				
1	Bravo 500 SC	chlorothalonil 500 g/l	1.5	17 June
2	Amistar Gold	azoxystrobin 125 g/l+difenoconazole 125 g/l	1.0	3 July
3	Sfera 535 SC	trifloxystrobin 375 g/l+cyproconazole 160 g/l	0.35	27 July
Experiment 3				
1	Sfera 535 SC	trifloxystrobin 375 g/l+cyproconazole 160 g/l	0.35	17 June
2	Bravo 500 SC	chlorothalonil 500 g/l	1.5	3July
3	Amistar Gold	azoxystrobin 125 g/l+difenoconazole 125 g/l	0.5	27 July

Our observations conducted on the influence of the treatment (Table 3) on the attack of the pathogen *Cercospora beticola* under the conditions of the year 2018, show that the highest frequency of attacks was recorded at the Matti variety, where F = 70% in the second experiment, and the lowest incidence value was calculated for the Tatry variety in the third Treatment experiment (F = 46%). In the case of the Vangelis variety attack frequency values were between 56% in the third experiment and 62%, respectively, 64% in the second and third treatment experiments. In the case of the Tatry variety attack values of the pathogen *Cercospora beticola* were the lowestones in the second and third experiment, similarly, the frequency value was 56% in the first treatment experiment. Values of the micromycete attack frequency were high, of 100% for the Matti and Tatry varieties and 98% for the Damian variety and 96% for the Vangelis variety. Regarding the intensity of the attack the pathogen under the conditions of the year 2018, data from the same table show that the values were relatively reduced, of 1.84% for the Tatry variety in the third experiment and of 3.4% for the Matti variety within the same treatment experiment. Values of attack intensity of cercosporiosis were around 6% for the analyzed varieties. Consequently, the differences between the varieties were due to the frequency variations attack in the analyzed treatment variants. Thus, under the conditions of the year 2018 subunit values of the attack for the variety Tatry in the third treatment experiment and for the Damian variety in the first treatment experiment were obtained. Application of fungicides continues to be an important instrument in controlling the cercosporiosis in sugar beet (Skaracis et al., 2010; Cristea, 2005). Depending on environmental conditions, resistance of varieties, crop protection may require 1-2 up to 6-7 sprinklings per season (Skaracis et al., 1996; Merrigi et al., 2000).

In the experimented treatment scheme (Table 2), under the conditions of the year 2019, values of the attack frequency were reduced comparatively to that from the previous year. Therefore, the lowest frequency value of 37% in the third treatment experiment was determined for the Matti variety, followed by the Damian variety in the same experiment with the treatments. Frequency values were at about 50% in the case of Vangelis variety in all experimented treatment variants in 2019. Values of the attack intensity were varied, being higher for the Damian, Vangelis and Tatry varieties in the first treatment experiment, for the same varieties in the second and for the Vangelis and Tatry varieties in the third treatment variant. The lowest attack values were calculated in the variants from the third treatment experiment for the Damian and Matti varieties with DA = 0.46% and, respectively, 0.59%. Similarly, the Matti variety registered the lowest attack grade values and in the variants of the first and second experiments, as well. In the witness variants the attack grade value was higher under the conditions of the year 2019, reaching 12.3% for the Vangelis variety.

Table 3. Influence of treatment and application scheme on the attack of *Cercospora beticola* in sugar beet, location Sanzieni, Covasna County, 2018-2019

Experiment		Year 2018/Variety				Year 2019/Variety			
		Damian	Matti	Vangelis	Tatry	Damian	Matti	Vangelis	Tatry
I	Frequency	48	58	64	56	42	40	53	49
	Intensity	1.92	2.9	2.56	2.24	4.1	2.9	4.2	3.8
	Degree attack (%)	0.92	1.68	1.63	1.25	1.72	1.1	2.2	1.86
II	Frequency	66	70	62	58	43	39	51	47
	Intensity	3.3	2.8	2.48	2.32	3.7	1.9	4.9	2.8
	Degree attack (%)	2.17	1.96	1.53	1.34	1.6	0.74	2.5	1.3
III	Frequency	58	68	56	46	38	37	54	49
	Intensity	2.3	3.4	2.24	1.84	1.2	1.6	3.5	2.4
	Degree attack (%)	1.34	2.31	1.56	0.84	0.46	0.59	1.8	1.1
Control	Frequency	98	100	96	100	100	87	98	100
	Intensity	5.88	6.0	5.7	6.0	10.1	8.6	12.6	9.5
	Degree attack (%)	5.76	6.0	5.52	6.0	10.1	7.49	12.3	9.5

Table 4. Treatment Efficacy on the Attack of *Cercospora beticola* in Sugar Beet, Location Sanzieni, Covasna County, 2018-2019

Year		2018		2019	
Variety	Scheme Treatment/ Control	DA (%)	E (%)	DA (%)	E (%)
Damian	I	0.92	84.02	1.72	82.97
	Control	5.76		10.1	
Matti	I	1.68	72.00	1.1	85.31
	Control	6.0		7.49	
Vangelis	I	1.63	77.35	2.2	82.11
	Control	5.52		12.3	
Tatry	I	1.25	79.16	1.86	80.42
	Control	6.0		9.5	
Damian	II	2.17	62.32	1.6	84.15
	Control	5.76		10.1	
Matti	II	1.96	67.33	0.74	90.12
	Control	6.0		7.49	
Vangelis	II	1.53	72.28	2.5	79.67
	Control	5.52		12.3	
Tatry	II	1.34	77.66	1.3	86.31
	Control	6.0		9.5	
Damian	III	1.34	76.73	0.46	95.44
	Control	5.76		10.1	
Matti	III	2.31	61.5	0.59	92.12
	Control	6.0		7.49	
Vangelis	III	1.56	71.73	1.8	85.36
	Control	5.52		12.3	
Tatry	III	0.84	86.00	1.1	88.42
	Control	6.0		9.5	

Regarding the efficacy of the treatments applied in the mentioned variants (Table 4) it can be noticed that in the year 2018 the highest value was registered at the third treatment experiment for the Tatry variety, followed by the treatments applied in the first treatment experiment for the Damian variety. Under the conditions of the year 2019 and for the applied treatment scheme (Table 2) efficacy in controlling the disease was higher, reaching 95.44% for the Damian variety in the third treatment experiment and 92.12% for the Matti variety, when the same treatments were applied. Efficiency calculation of the treatments in controlling the plants' pathogens presents agreat importance in establishing schemes to combat them (Jalobă et al., 2019; Alexandru et al., 2019; Doncila, 1995b).

CONCLUSIONS

Application of the treatments and the variety behavior continue to represent very important steps in controlling cercosporiosis in sugar beet. After applying treatment in vegetation amid seed treatment, the degree of damage decreased significantly in all experimental variants in regard to the witness variant. Under the conditions of experimental years attack frequency had superior values to the intensity for all studied varieties.

Under the conditions of the year 2018, for the Damian variety, in the treatment experiment 1, the highest efficacy value was registered, E = 84.02% and the Tatry variety in the third treatment experiment with E = 86%. Under the conditions of the year 2019, the highest efficacy value was registered for the Damian variety in the third experiment, followed by the Matti variety in the same treatment scheme with E = 92.12% and with E = 90.12% in the second treatment variant.

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EFFICACY OF TRIAZOLE FUNGICIDES ON *Pyrenophora teres* ON WINTER BARLEY

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Abstract

Net blotch of barley (Pyrenophora teres Drechsler (anamorph Drechslera teres (Sacc.) Shoemaker)) is common foliar disease of winter barley in Bulgaria. The paper reports results of field experiment performed with triazole fungicides on winter barley variety Nives during 2017-2018 in Agricultural University in Plovdiv. Epoxiconazole 125 g/l was applied in full dose in two applications. Combination of tebuconazole 107 g/l and bromuconazole 167 g/l was used in tree doses of two applications. The aim of the current study was to obtain efficient disease control and to identify the impact of different triazole fungicides. The fungicide efficiency was assessed at the end of April. The trial results show that biologically effective control of barley diseases could be achieved with timely use of lower fungicide dose due to two triazole synergism.

***Key words**: winter barley, Pyrenophora teres, triazole fungicides.*

INTRODUCTION

Winter barley (*Hordeum vulgare* L.) is the second most important and significant grain fodder crop after the corn in Bulgaria, with growing area of 112.26 thousand ha (Bulletin № 367, 2019).

One of the most widely distributed and harmful phytopathogens of barley is *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* (Sacc.) Shoem.).

It is the causal agent of net blotch which is one of the most important diseases in barley resulting in 40-100% yield lost worldwide (Afanasenko, 2009; Rajaa & Brahim, 2009; Lai et al., 2007). The time and level of disease infection in the field depends on the susceptibility of the barley variety, therefore the resistance of varieties has also importance in the control of plant diseases. *Pyrenophora teres* is most effectively controlled using a combination of cultural and chemical means, in addition to host resistance (Liu et al., 2011).

In recent years, intensive cereal cultivation with limited crop rotation and control with products with same active ingredients have increased the occurrence of net blotch in barley. Chemical control measures are needed to avoid yield reduction by disease infections.

To achieve economic profitability, it is important to deploy integrated pest management. Inclusion of new active ingredients and still unused combinations in plant protection program is important to avoid or to reduce fungicide resistance risk.

Triazole-based fungicides are economically important agricultural chemicals as they are widely used on different crops (Buchenauer, 1987; Filipov & Lawrence, 2001). They inhibit the C14 demethylation step in fungal ergosterol biosynthesis, known as demethylation inhibitors (DMIs) (Gisi et al., 2000) as are major systemic group of fungicides, currently used to control cereal diseases. Fungicide resistances in *Pyrenophora teres* to different active ingredients have been reported (Sheridan et al., 1985; Campbell & Crous, 2002).

Three triazole fungicides, namely tebuconazole, bromuconazole and epoxiconazole, deserve particular attention. Tebuconazole and epoxiconazole are widely used in cereals with high efficacy against main diseases. Bromuconazole is used as a broad-spectrum fungicide, with preventative and curative action, for control of diseases caused by Ascomycetes, Basidiomycetes and Deuteromycetes (Menegola et al., 2005). For Bulgaria it is a new molecule and important tool for DMI alternance and resistance management.

The aim of the study was to find out the efficacy of different triazoles at different disease control intensities on winter barley varieties.

MATERIALS AND METHODS

Field trials on disease control of spring barley were conducted with four replicates in a randomized design16.5 m² plots at Agricultural University - Plovdiv during the season of 2017-2018. Two winter barley varieties with different resistance levels were used: Dimitra (moderately resistant to net blotch), Nives (susceptible to net blotch). There were tested two formulated products: combination of tebuconazole 107 g/l and bromuconazole 167 g/l (TEB+BRO) in three different dose rates and epoxiconazole 125 g/l (EPO) in full dose rate used as a standard. Fungicide applications were at stages BBCH 32-33 (T1) and BBCH 65-69 (T2) (Table 1). Treatment dates were 17 March 2018 and 26 April 2018.

Disease infection was scored as the percent of leaf area infected by *Pyrenophora teres* and at BBCH 71-75. The three top leaves of the plant were assessed separately on three adjacent tillers at 10 randomly selected places on each plot. The infection level was expressed as an infection score on flag leaf and second leaves (L-2; the first leaf under the flag leaf). The lesions of net blotch were determined according description of Tekauz (1985).

Registered products in winter barley against *Pyrenophora teres* were collected from Bulgarian Food and Safety Agency list of authorized plant protection products (Fungicides, 2020).

RESULTS AND DISCUSSIONS

In Bulgaria registered triazoles in winter barley to control *Pyrenophora teres* are triadimenol, tebuconazole, epoxiconazole, flutriafol, metconazole, prothioconazole and cyproconazole. Benchmark DMI fungicides contain epoxiconazole (0.125 g ai/Ha) or tebuconazole (0.25 g ai/Ha) straight or in combination with different active ingredients. There is only one recently registered product with the molecule bromuconazole. It is in combination with tebuconazole. It is currently registered on wheat for control of powdery mildew, rust and leaf spot diseases with high efficacy and long lasting effect.

Hot and dry wheather during winter and early spring limited development of *Pyrenophora teres* on the variety Dimitra when only slight damage by net blotch infection was observed (Figure 1).

The highest infection levels were observed on the variety Nives (Figure 2).

Figure 1. Dimitra variety on 22 April 2018

Figure 2. Untreated control (Nives variety)

Net blotch of barley symptoms have occurred on the leaves of the plants. Small circular to elliptical brown spots appeared on leaves and enlarge into, narrow, dark brown and transverse striations, forming the characteristic netlike pattern. The affected part of the leaf turned brown and the adjoining tissue became chlorotic and areas of dead tissue were formed. Severely infected leaves became completely necrotic and dry up. The fungus produces pseudothecia on infected tissues when the plant has matured.

The most significant damage occurred in April on second leaves.

Epoxiconazole (EPO) has moderate effect against *Pyrenofora teres* in barley. Significant synergism has been demonstrated for the combination of tebuconazole with bromuconazole (TEB+BRO). Reduced dose showed slightly higher control of the disease. The best effect was achieved with the highest dose rate of TEB+BRO (Figure 3). High protection of flag and second leaves was provided even with the first treatment (Figure 4).

Treated plots with the combination of tebuconazole and bromuconazole showed better vigor.

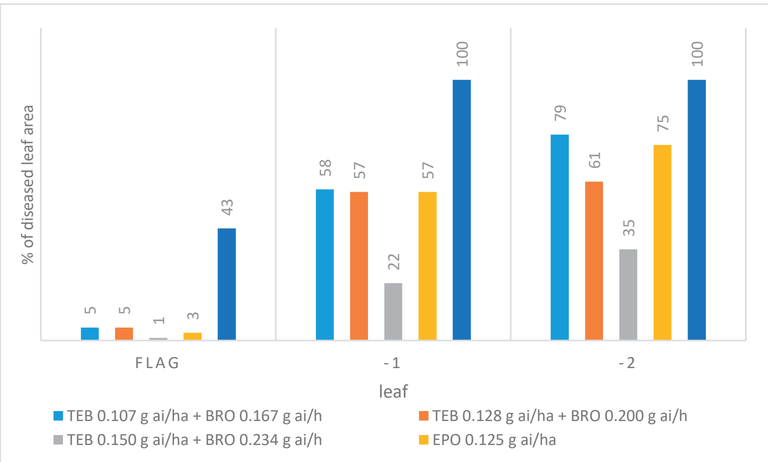

Figure 3. Efficacy of epoxiconazole, tebuconazole and bromuconazole against *Pyrenophora teres*

Figure 4. Treated Nives variety plots

CONCLUSIONS

High disease pressure and non-inclusion of active ingredients in EU requires studies of new uses of the registered products. It was compared the control of net blotch in barley of 2 formulated products - reference product with the active ingredient epoxiconazole and new triazole combination for Bulgarian market containing tebuconazole and bromuconazole.
The weather conditions during 2017-2018 were unfavorable to the development of the infection in resistant variety Dimitra, while Nives variety showed high susceptible to *Pyrenophora teres*.
Sufficient control and better effect was achieved with the triazole combination (tebuconazole and bromuconazole).

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EFFECT OF LIGHT INTENSITY ON DRY MATTER ACCUMULATION OF BARLEY FODDER IN A VERTICAL FARMING GROWTH MODULE

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Abstract

Considering the increase in forage requirement in livestock, lack of same quality products throughout the year, fertilizer and chemical costs, insufficient water resources and environmental restrictions caused by climate change; studies providing solutions for forage production are getting more attention. Vertical farming which is the method of growing crops in vertically stacked layers under controlled environment is one of the promising techniques to protect environmental resources, provide continuous and sustainable forage production. Effect of light intensity on dry matter accumulation and physiology of barley fodder in a vertical farming growth module was investigated in the present study. The experiment was consisted of eight micro chambers represented the growth modules of vertical farming system placed in fully controlled growth chamber. There were four different light intensity, two repetitions each of 40, 100, 160 and 220 μmol/m^2.s. Plants were sampled every day during all experimental period (8 days). Leaf area and fresh/dry weight of root and leaves were determined. Furthermore, chlorophyll a, chlorophyll b and carotenoid contents of leaves were analyzed. Module based water use of each light treatments were calculated. In line with the results, higher light intensity was found to affect the dry matter accumulation positively; since the physiological properties of barley fodder growing under 160 and 220 μmol/m^2.s. light intensity are almost the same, it is recommended to use 160 μmol/m^2.s. light intensity in terms of energy saving. Based on the data obtained from the present micro-level lab-scale study revealed that the effect of factors such as temperature, humidity, water use and seeding density should also be examined in order to provide the best growing conditions for the future studies.

***Key words**: barley fodder, growth parameters, light intensity, vertical farming.*

INTRODUCTION

Undoubtedly, livestock industry is one of the important sectors affected by changing climate conditions. Considering the increase in forage requirement in livestock, lack of same quality products throughout the year, economical reasons, insufficient water resources and extreme weather conditions caused by climate change; studies providing solutions for forage production are getting more attention (Kumar, Mathur, Karnani, Choudhary & Deepika, 2018).

Nowadays the majority of the forage needed is supplying by maize silage (Acosta Aragón, Jatkauskas, & Vrotniakiene, 2012; Şahin & Zaman, 2010). In addition to the hungar and food security problem posed by organizations such as FAO (2019) and European Union (2019), 43% of wheat and maize, which have an important place in human nutrition, are used for animal feeding (Germer et al., 2011); thus alternative feed sources will play an important role both in obviation the forage shortage in livestock and ensuring food security. At this point, the use of barley fodder as forage has recently been a matter of debate. Therefore, studies about this subject have gained speed (Del Castillo, Del Carmen Moreno Pérez, Magaña & Gómez, 2013; Dung, Godwin & Nolan, 2010; Gebremedhin, Deasi & Mayekar, 2015; Islam, Nabilah & Md Ali, 2016; Karaşahin, 2017)

Taking into account the advantages of vertical farming, growing barley fodder in these systems will increase the yield and reduce the impact of agriculture on the environmental burden (Banerjee & Adenaeuer, 2014; Despommier, 2009; Despommier & Ellingsen, 2008). On the other hand, this yield is closely related to environmental conditions such as light intensity, temperature, humudity and amount of carbon dioxide received by the plant.

Light which an important component for the success of vertical farming systems, is provided artificially in these systems (Al-Kodmany, 2018). The light intensity used in in-door systems is generally between 50-200 µmol/m^2.s and the lighting time is between 18-20 hours (Kalantari, Mohd Tahir, Mahmoudi Lahijani, Kalantari, 2017).

Physical energy from the sun or artificial light is converted into chemical food energy by photosynthesis by plants containing chloroplasts (Kacar, Katkat & Öztürk, 2013). Light is a crucial source to complete growth and development in the plant. Although the general opinion is that as the light intensity increases, the rate of photosynthesis increases, this does not apply to all situations. Photosynthesis rate can be limited by other factors (Kadioğlu, 2016). Therefore, these conditions need to be optimized optimally to save more energy by using less energy.

The aim of this study, thus, investigate the effect of light intensity on dry matter accumulation and physiology of barley fodder in a vertical farming growth module.

MATERIALS AND METHODS

A micro chamber experiment was carried out in Department of Field Crops, Ege University, Turkey. The experiment was consisted of eight micro chambers represented the growth modules of vertical farming system placed in fully controlled growth chamber. There were four different light intensities (40, 100, 160 and 220 µmol/m^2.s) and two replications. Each pots contained 16.8 g seeds of the barley (*cv.* Lord, *Hordeum vulgare* L.) and these were germinated in micro chambers for eight days and treated 20 hours light during each day. In this process, other environmental conditions such as temperature (25°C) and relative humidity (70%) were kept constant. The pots were weighed in everyday in order to estimate evapotranspiration and were irrigated as considering to water loss.

The barley sprouts were sampled in every day during all experimental period (8 days). The sampled barley sprouts were separated as leaf and root. The leaves and roots were weighed to calculate fresh weights and then leaf area of each pots were estimated by help of digital imaging based on pixel counting method. Furthermore, dry weights of leaves and roots were determined by drying in 105°C oven in a day. Chlorophyll a, chlorophyll b and carotenoid analysis were performed according to Arnon (1949) and Jayaraman (1988) by using Cary 50 brand UV/VIS spectrophotometer.

Statistical analyses were made using the Excel program and the differences between the values were grouped according to the LSD test comparison method.

RESULTS AND DISCUSSIONS

Despite the plant germination and increased fresh weight, there was almost no change in dry matter (Figure 1a). For this reason, the dry matter rate has decreases day by day (Sneath & Mclntosh, 2003; Fazaeli, Golmohammadi, Tabatabayee & Asghari-Tabrizi, 2012). In addition, although the amount of dry matter is high at 160 and 220 µmol/m^2.s light, the difference is very small (Figure 1). There was no significant increase in dry matter from the first day to the 8th day. In contrast, evapotranspiration increases day by day in direct proportion to the intensity of the light (Figure 1b). Photosynthesis starts with the formation of chloroplasts, but since the harvest cycle is short, dry matter accumulation is very low (Dung, Godwin & Nolan, 2010). The water content increased by 72-78% in the form of inverse proportion for high light intensity and low light intensity (Figure 1c). Karaşahin (2017) concluded that low light intensity has less water content. This data does not match our study.

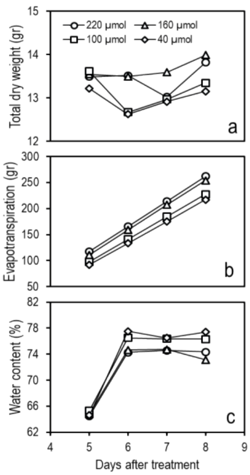

Figure 1. Changes in the dry matter, evapotranspiration and water content of the barley plant during the application of different light intensity

As the light intensity decreases, the leaf area of the barley increases (Figure 2a). If the light intensity is not at the desired level, the plants expand the leaf areas and send less photo-synthesis products and root development slows down (Kacar, Katkat & Öztürk, 2013). Total chlorophyll and carotenoid amount in barley grown under 220 µmol/m^2.s light intensity was higher than barley grown in other light intensities (Figure 2c, d).

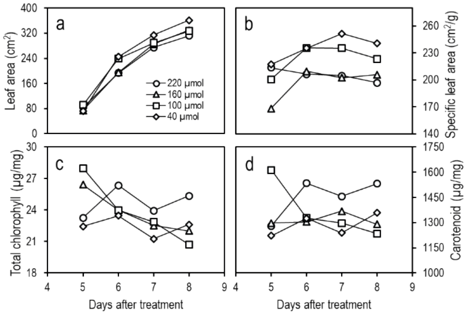

Figure 2. Changes in the leaf area, specific leaf area, amount of total chlorophyll and carotenoid the barley plant during the application of different light intensity

From the general framework, an inverse relationship was found between the light intensity and the specific leaf area, and between the specific leaf area and evapotranspiration (Figure 3).

A positive correlation was determined between evapotranspiration and total dry matter (Figure 4). Similarly, a positive correlation was determined between total chlorophyll and light intensity, and between carotenoid amount and light intensity (Figure 3).

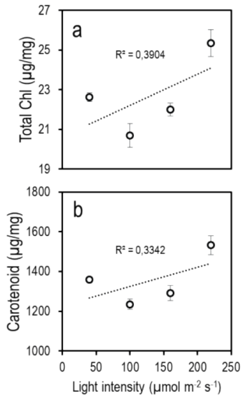

Figure 3. Correlation between total chlorophyll and light intensity, and between carotenoid and light intensity

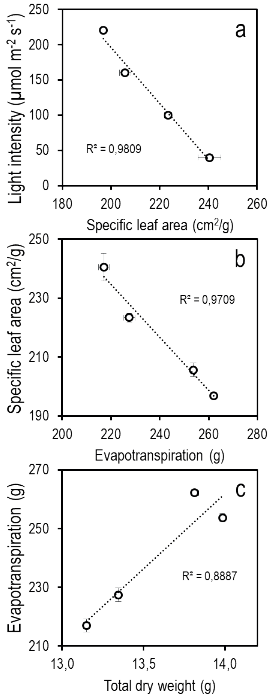

Figure 4. Correlation, between light intensity and specific leaf area, between specific leaf area and evapotranspiration, between evapotranspiration and total dry matter

CONCLUSIONS

The main aim of the study was to determine the effect of different light intensity on dry matter accumulation and physiology of barley fodder, which will be grown in the vertical farming growth module. In this context, a slight increase in the luminous intensity of 160 and 220 µmol/m^2.s. was observed in the accumulation of dry matter. On the other hand, this dry matter content remained almost the same as on the first day. It can be said that the plant almost does not photosynthesize or the rate of photosynthesis is very low. Despite the increase in total chlorophyll, it is recommended to conduct studies to determine the process in which photosynthesis accelerates since the dry matter does not increase almost compared to the first day. In addition, studies aimed at optimizing environmental factors such as irrigation, seed frequency, humidity, and temperature and increasing the dry matter content are thought to be important for the future of barley feed.

There is almost no difference between dry matter and other physiological contents of barley under light intensity of 160 and 220 µmol/m^2.s. For this reason, it is recommended to use 160 µmol/m^2.s light intensity in terms of energy saving.

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STUDY OF THE EFFECT OF THE PERIOD OF REPLAINING ON THE MORBIDITY RATE IN VIRGINIA TOBACCO

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Abstract

*The present work studies the influence of the vegetation period in Virginia tobacco on the spread of the viruses PVY-Complex (%), TMV (%), TSWV (%), CMV (%). To establish this relation, a correlation analysis was applied and the proven effects were represented by linear regression models. In 2014, with an increase of the period from replanting, the incidence of PVY-Complex (0.997**), TMV (0.860*) and TSWV (0.830*) increased significantly. The effect of the replanting on plant morbidity rate is similar (0.808**) also in 2015. In 2016, the very strong impact of the days from replanting on CMV (0.962**) and TSWV (0.907 **) viruses was proven. In 2017 the increase in the period from replanting leads to an increase in the percentage of diseased plants from PVY-Complex (0.946**), CMV (0.940**) and TSWV (0.976**).*

Key words: *Virginia tobacco, viral diseases, correlation analysis.*

INTRODUCTION

Tobacco production is among the major branches of agriculture in Bulgaria. Tobacco is cultivated in more than 100 municipalities, and in a large part of them it is the main livelihood of the local population (Bozukov, 2014). Four varieties are cultivated in the country - Oriental tobacco - Basma and Caba Kulak and big-leaved tobacco - Flue-cured - Virginia and Air Cured-Burley, occupying an area of 9963 ha (MZH, 2016).

Tobacco cultivation should take into account the complicated interactions between the geno-type and agro-ecological conditions as well as the presence of different phytopathogens (Bojinova & Djulgerski, 2006). Some of them are viral diseases and the damage to the crop is significant (Maiss, 2004).

One of the most common viruses causing serious economic losses is the genus Potyvirus. The three potyviruses PVY (Potato virus Y), TEV (Tobacco etch virus) and TVMV (Tobacco vein mottling virus) often occur as a tobacco viral complex (Greenwell, 2011; Dietrich & Maiss, 2003; Yonchev, 2014).

The disease that they cause in tobacco in Bulgaria is called Sipanitsa (Yonchev, 2014). Another economically important viruses are TMV (Tobacco mosaic virus) and TVMV (Tomato mosaic virus) of the genus Tobamovirus (Stoimenova, 1995).

They are spread in all the countries where tobacco is grown. The illness it causes is called a simple tobacco mosaic virus. CMV (Cucumber mosaic virus) is a virus also spread worldwide which causes considerable damage to tobacco production (Srivastava & Bhaskar, 1987).

In Africa it can be found in Zambia and Morocco (Thottapilly, 1992). The disease it causes with tobacco is called cucumber mosaic virus.

Tomato spotted wilt virus is a disease which is a serious problem for tobacco production in many countries around the world. In Bulgaria, the disease was first found in 1952 in Gotse Delchev and Sandanski tobacco regions, and in the first years of its occurrence it caused 30 to 50% losses, and in separate years, such as 1956, 1969, 1977, 1983 and 1984. The viral disease covers up to 70% of the areas with oriental tobacco and 80-100% with large-sized tobaccos (Gabrovska, 1984; Kovachevski et al., 1999).

The aim of the present study is to show the influence of the period from the replanting of Virginia tobacco on the spread of economically important viral diseases in this tobacco group in the region of Plovdiv.

MATERIALS AND METHODS

The areas are routinely studied, with virus spreading evaluated on the basis of the characteristic symptoms of the disease. Reports are made based on the percentage of attacked plants.

The subject of research in the present work is tobacco from Virginia variety group. The data on the basis of which the study was conducted were obtained during the period 2014-2017. The study has taken into account the degree of influence of the following viruses: PVY-Complex (%), TMV (%), TSWV (%), CMV (%) on Virginia tobacco in the period from 20th to 107th days after the replanting.

The correlation analysis makes it possible to determine the degree and direction of impact of a given indicator on another by determining the value of the corresponding correlation coefficient. In the present study the Pearson-Brave coefficient was calculated. It is calculated according to the formula:

$$r = \frac{\sum_{i=1}^{n}(Y_i - \bar{Y})(X_i - \bar{X})}{\sqrt{\sum_{i=1}^{n}(Y_i - \bar{Y})^2 \sum_{i=1}^{n}(X_i - \bar{X})^2}}.$$

The determination coefficient gives information about how much of the change in a given indicator is due to the change of another indicator.

The regression analysis is a mathematical method that renders an established relation between two or more variables in an analytical form. To implement it, it is necessary for the dependent variables to have a near-normal distribution, the experimental data have to be random, the observations have to be independent. The reliability of the compiled regression models is verified by various methods. In this study, the coefficient of significance of each model was calculated; F-test was also carried out.

The mathematical processing of the experimental data in the present study was accomplished through the tools provided by the MS Excel operating system and the IBM Statistics SPSS 25 (Meyers et al., 2013; Hilton & McMurray, 2017; McCormick, 2017).

RESULTS AND DISCUSSIONS

The results of the performed correlation and regression analyzes are given in Table 1. It was previously proved that the experimental data meet the conditions for the application of these analyzes mentioned above in the report.

In Virginia tobacco, the infection of PVY-Complex (0.997**), TMV (0.860*), TSWV (0.830*) in 2014 significantly increased with an increase in the period from replanting. The effect of the replanting on the plant infection is similar (0.808**) in 2015. In 2016, the period from replanting had very strong impact on the virus CMV (0.962**), TSWV (0.907**). For 2017, it has been shown that the percentage of diseased plants by PVY-Complex (0.946**), CMV (0.940**) and TSWV (0.976**) increased with the increasing of the period from replanting.

The regression models presenting in an analytical form the described effects of the period from replanting on the morbidity rate of the plant are given in Table 1. All compiled models are statistically significant at a significance level equal to 0.05.

Table 1. Correlation coefficients and regression models presenting the effect of the period from replanting on the spread of some viral infections in tobacco

Year	Virus Name	Correlation Coefficient	Regression Model	F-test	Sign. of model
2014	PVY-Complex (%)	0.997**	y=0.272x-3.394	930.386	0.000
	TMV (%)	0.860*	y=0.005x-0.186	14.245	0.013
	TSWV (%)	0.830*	y=0.014x+2.219	11.068	0.021
2015	Infection (%)	0.808**	y=1.614x+0.883	13.193	0.008
2016	CMV (%)	0.962**	y=0.069x-1.388	61.395	0.001
	TSWV (%)	0.907**	y=0.038x-0.599	23.160	0.005
2017	PVY-Complex (%)	0.946**	y=0.344x-5.027	33.845	0.004
	CMV (%)	0.940**	y=0.035x-0.98	30.103	0.005
	TSWV (%)	0.976**	y=0.044x-0.664	79.722	0.001

**Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level.

The graphs shown in Figures 1-4 visualize the effect of the period from replanting on the morbidity rate of the plant of viral infections.
The determination coefficients are given on each of the graphical images. Considering these, it can be claimed that the period of replanting has a very strong impact on the morbidity rate of the plant of the corresponding viral infections. In 2014, it has the strongest influence on PVY-Complex (99%), and the trend is continuous growth of the percentage of diseased plants. The other two viruses have comparative stability.
The analysis of the experimental data for 2015 showed the strong impact of the period only on the morbidity rate of CMV/PVY-Complex (65%) (Figure 2). The curve demonstrates strong growth of the diseased plants during 30th-40th days as well as during 60th-70th days. In the remaining period, there is no dynamic in the spread of the diseases.

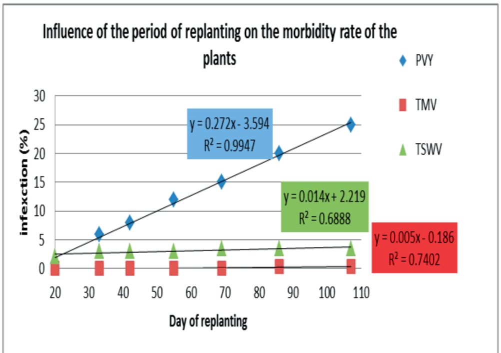

Figure 1. Influence of period of replanting in Virginia tobacco on morbidity rate in 2014

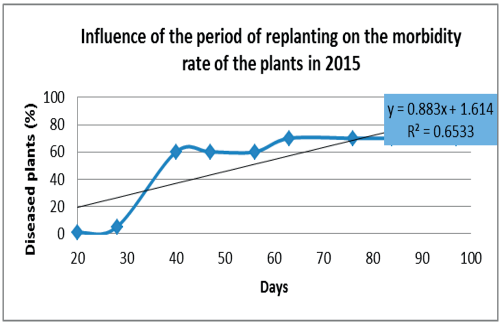

Figure 2. Influence of the replanting period in Virginia tobacco on the morbidity rate in 2015

In 2016, the strong impact of the replanting period on the spread of CMV (92%) and TSWV (82%) was demonstrated. Both viruses show a strong increase in the percentage of diseased plants between 35th and 55th days. In CMV, however, this trend continued after 60th day and no significant changes were observed in TSWV (Figure 3).

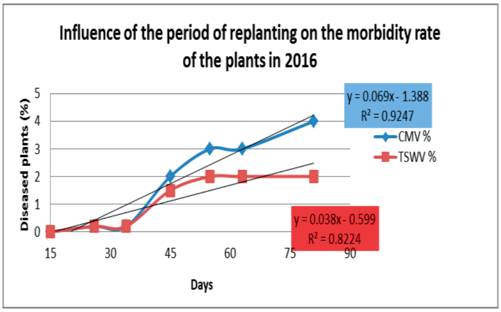

Figure 3. Influence of the replanting period in Virginia tobacco on the morbidity rate in 2016

In 2017 the strong impact of the number of days after planting on PVY-Complex (89%), CMV (88%) and TSWV (95%) was demonstrated (Figure 4). The most dynamic is the change in the percentage of diseased plants from PVY-Complex in the 20th-60th day period. After this period, relative stability in the spread of the disease is observed. The other two viruses did not show peaks or falls in the percentage of diseased plants.

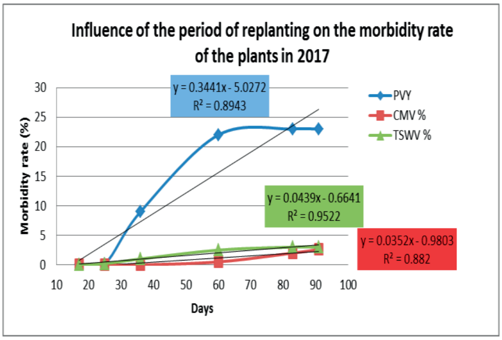

Figure 4. Influence of the replanting period in Virginia tobacco on the morbidity rate in 2017

CONCLUSIONS

The period of replanting has a very strong impact on the morbidity rate of the plants of the relevant viral infections.
In 2014, there is a continuous growth in the percentage of plants with symptoms of

Sipanitsa. There is comparative stability in the spread of the other two diseases.

In 2015, there was a strong dynamics in the development of the cucumber mosaic virus and Sipanitsa, in the period of 30th-40th days and 60th-70th days from replanting. In the remaining period there is no dynamics in the spread of the diseases.

In 2016, the invasion of CMV and TSWV is in dynamics, particularly strong in the cucumber mosaic virus.

In 2017, the greatest dynamics was observed in the spread of the potiviruses. The other two CMV and TSWV viruses did not show peaks or drops in the percentage of diseased plants.

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DEVELOPMENT AND PRODUCTION OF FRESH AND DRIED LEAF BIOMASS OF LEMON BALM (*Melissa officinalis* L.) UNDER ORGANIC FERTILIZERS TREATMENT

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Abstract

The study was conducted at the Demonstration Center for Organic Agriculture at the Agricultural University - Plovdiv, Bulgaria during the 2015-2018 period. A field trial has been set up in three replications with a plot size of 25 m^2 in Lemon balm (Melissa officinalis L.). The aim is to determine the impact of Amalgerol, Lithovit and Tryven organic fertilizers on the growth, development and productivity of Lemon balm. The treatment with the organic fertilizers leads to an increase in values of the researched indicators. Fresh biomass weight when treated with Tryven goes to 250.05 g, when the control variant is only 143 g. Fresh leafs weight reaches higher values at all fertilized variants. Amalgerol treatment leads to - 31.07 g for dry leafs weight and Tryven to 37.72 g, compared to the non treated variant - 22.73 g. Statistically proven - highest yield of green fresh biomass was obtained in the variants treated with Tryven - 989.2 kg/da (7.5% above the control) and Amalgerol - 980 kg/da (6.5% above control), respectively. The yield of the fresh leafs increase with 21.1% compared to the control (up to 541.6 kg/da) when treated with Lithovit and 12.3% (to 502.2 kg/da) when treated with Amalgerol. Highest dry leafs yield was obtained when Tryven was applied - 149.2 kg/da (2.1% above the control).

***Key words**: organic fertilizers, fresh biomass, dried leaf biomass, Melissa officinalis L.*

INTRODUCTION

The biological form of agriculture helps to maintain ecological balance and optimization of biological processes in agriculture. The biological system (agroecosystem) is considered to be a living organism having mutually connected components in dynamic interrelations (Vlahova, 2015). Soil and environmental protection is a fundamental task of organic farming. Lemon balm (*Melissa officinalis* L.) is a valuable essential oil and medicinal plant, with 450,000 kg of leaves and branches of the uterus being produced and exported annually in Bulgaria. The areas in the country are increasing, with a total of 518 tonnes being produced in 2016, of which 21 tonnes are in the biological control system. The leaves and tips of the stem in dry form, etc. Herb (Herba and Folia melissae) is the most commonly used part of humans for tea, extracts, sweets and more. Vegetable mass contains up to 0.3% essential oil, tannins, phenolic acids, triterpenic acids (oleanolic, ursolic), vitamin C. The essential oil of *M. officinalis* L., obtained from fresh or dried plants, leaves or tips of the plant, is characterized by a fresh lemon odor and light yellow color. Its viscosity is lighter than that of water (Anonymous, 2003). It is desirable that this value should not be less than 0.05% (Baytop, 1984). Janina M. (2003) reported that most essential oil (0.14%) was obtained from plants harvested at the beginning of flowering grown in Canakkale under ecological conditions. According to Lin et al., (2012) the lemon balm contains antioxidants, also geranium and neral (Masakova et al., 1979). According to Weiss (1974), lemon balm has a well-pronounced sedative and antispasmodic action, anti-inflammatory (Bounihi et al. 2013), mild sleeping pills (Braun et al., 1974) and anti-diabetic effects (Chung et al., 2010). Substances having antiviral activity have been found in the lemon balm (Allahverdiyev et al., 2004). Fresh leaves are torn off, shaded or dried at 35-40°C. When dried in the sun, the leaves darken and deteriorate. Weed control is an important element of cultivation technology (Tonev et al., 2019). The best time to harvest the above ground mass is before flowering, when the leaves are well developed, because at a later stage the content of the active ingredients decreases. Crushing the raw material causes a sharp deterioration in the quality of the drug. Drying the leaves at 30 and 45°C leads to a 16% and 23% loss of essential oil, respectively, while drying at higher temperatures of 60°C causes significant losses (65%) (Argyropoulos et al., 2014). The application of leaf fertilizers affects the growth of the plant as well as the quality of the essential oil (Keshavarz et al., 2018). The purpose of the study is to monitor the vegetative development and productivity of fresh and dry leaf mass of *Melissa officinalis* L. when treated with leaf fertilizers for organic farming.

MATERIALS AND METHODS

The study was conducted at the Demonstration Center for Organic Agriculture at the Agricultural University - Plovdiv, Bulgaria during the 2015-2018 period. A field trial has been set up in three replications with a plot size of 25 m^2 in lemon balm (*Melissa officinalis* L.). Factors of reading: Factor A: Vegetation year, A1 - 2015-2016, A2 - 2016-2017. A3 - 2017-2018; Factor B: Organic fertilizers: B1 - Control - no application of foliar fertilizers, B2 - Amalgerol - at a dose of 300 ml/da, once in the beginning of May, B3 - Lithovit - at a dose of 300 g/da once in the beginning of May, B4 - Tryven - at a dose of 300 ml/100 l once in the beginning of May. Statistical processing of the data was carried out with the program SPSS for Microsoft Windows (SAS Institute Inc. 1999). The mowing was done before flowering began. The leaves were removed manually, drying was carried out at room temperature in the shade to avoid darkening and deterioration of the quality of the leaves. The following indicators were monitored: phenological development; growth of plants in height - on the 7th and 14th days after application of organic fertilizers; number of stems per plant (rhizome) - measured 10 plants from each plot in three replications for each variant; number of branches per stem; number of leaves per plant - lists all the leaves of all the stems of a single plant; weight of fresh mass of one plant, g - fresh mass of 10 plants of each variant and repetition; weight of fresh leaf from a plant, g - manual separation of leaves from each plant; weight of the dry leaf mass of the plant, g - drying of the fresh leaf mass of each plant at room temperature in the shade; yield of fresh and dry leaves, kg/da. Organic leaf fertilizers used: Lithovit - nanotechnological product. Increases yield, quality and storage properties. Contains 79.19% - $CaCO_3$, 4.62% $MgCO_3$, 1.31% Fe; Amalgerol® is rich in hydrocarbons and natural plant growth hormones. Contains seaweed extracts, distilled paraffin oil, vegetable oils, distilled herbal extracts. Stimulates plant growth, improves the quality and quantity of production in oilseeds, etc.; Tryven - contains N total 24.4%, ammonium nitrogen (N) 2.60%, nitrate nitrogen < 0.01%, urea nitrogen 4.47%, organic nitrogen 17.3%, P_2O_5, water-soluble 17.2%, K_2O water-soluble 7.42%. Designed for use by foliar nourishment, especially for crops with large foliage in order to achieve great vegetative growth.

RESULTS AND DISCUSSIONS

1. Analysis of agrometeorological conditions during the study period.

The soils in the area of the experiment are alluvial-meadow. They have low availability with N and P and good with K (Popova et al., 2010). Meteorological conditions during the study period are relatively favorable for the development of the lemon balm (Figure 1). The 2015-2016 growing season is characterized by being warm and well stocked with moisture. Long-term rainfall was reported in October (70.3 mm/m^2). Precipitation values in May (64.7 mm/m^2) are twice as long as the long term (32 mm/m^2). These conditions, in turn, allow for a good yield of fresh vegetable mass.

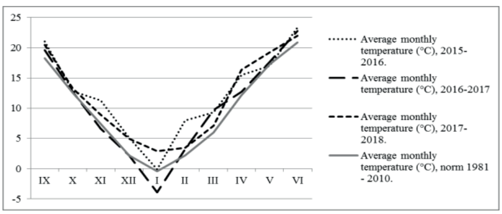

Figure 1. Daily average air temperatures of ten days (°C) for the period 2015-2018

The growing season 2016-2017 is relatively warm with uneven rainfall (Figure 2). In May, when the growth is active, temperature values are in the norm 17.6°C and are accompanied by precipitation above those for the long term of 52.7 mm/m^2. The hot month of June combined with low rainfall causes stress in plants.

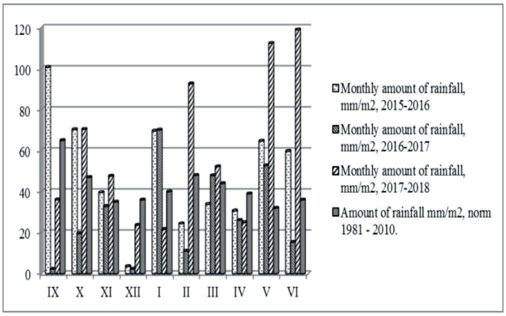

Figure 2. Rainfall sum (mm/m^2) during the study period 2015-2018

The 2017-2018 growing season is characterized as warm and moist. In May, heavy rainfall of 112.3 mm/m^2 was registered with long-term values of 32 mm/m^2.

2. Phenological development

Insufficient soil moisture and uneven distribution of rainfall during the growing season have the risk of loss of foliage and hence lower yields. In the absence of soil moisture, the plants form a small number of stems with small leaves. Yields decline sharply. In extreme rainfall, the plants become susceptible to fungal diseases. Irrigation was performed to reduce losses. During the study period, the beginning of the growing season begins in March (20-25 November). In the initial stages, especially in the first year after transplanting, the plants develop very slowly. Phenophase third - fourth leaf occurs in the period 7-10.IV. During this period, the plants grow slowly, and the weeds quickly, which carries the risk of strong weeding, and for this purpose weeding and digging were carried out. The studied leaf fertilizers were applied once at doses of 300 ml/da + 40 l water/da, in the beginning of May (03-05.V.), according to the scheme in three replications. Phenophase budding occurs in the period 09 -21.VI. During the study period, no differences were observed in the occurrence of the different phenological phases between the studied variants of foliar fertilization compared to the control.

3. Biometric indicators

3.1. Plant height on days 7 and 14 after application of organic fertilizers

In the first year of development, lemon balm forms a smaller number of stems up to 50 cm in height. Simultaneously with the formation of the stems, rhizome growth begins. Early in the spring of the second year, development progressed faster. The rhizomes have a greater branching ability, which allows for the formation of more stems and hence a larger number of leaves. When measuring the height 7 days after application of the fertilizers studied, no significant differences were observed in the treated plants relative to the control, but a percentage increase was more pronounced after Tryven treatment - (1.8% above the control - 44.73 cm) (Table 1).

Table 1. Plant height on days 7 and 14 after application of leaf fertilizers, averaged over the study period

Day / Variant	7th day		14th day	
	Height, cm	% compared to control	Height, cm	% compared to control
Control	43.95 a	100	51.19 a	100
Amalgerol	44.70 a	101.7	52.35 a	102,.3
Lithovit	44.66 a	101.6	52.42 a	102.4
Tryven	44.73 a	101.8	48.37 a	94.5

Duncan's Multiply Range Test ($P < 0.05$)

Increases in the value of the indicator were also reported when treated with Lithovit - 44.6 cm, and Amalgerol - 44.70 cm relative to the untreated control (43.95 cm), but the differences are not statistically proven. The application of leaf fertilizers complements plant nutrition and also affects the quality of production. 14 days after application of leaf fertilizers, higher percentages were observed compared to controls in the variants treated with Amalgerol and Lithovit, respectively, by 2.3% for Amalgerol and 2.4% for Lithovit. A value close to the control is reported in the variant treated with Tryven.

3.2. Number of stems per plant (rhizome)

According to the indicator the number of stems per plant (rhizome) - from the data in Table 2, it can be seen that in the variant treated with Tryven we have proven a greater number of formed plant stems - 11.6 compared to the control (9). Higher control values were also reported when treated with Lithovit and

Amalgerol, but these were not proven. In terms of the number of branches and number of leaves per stem, the treated variants with organic fertilizers show higher values than the control.

Table 2. Biometric indicators of a plant averaged over the study period

Variant	Number of stems	Number of branches per stem	Number of leaves	% leaves compared to control
Control	9.0 b	4.9 b	109.0 b	100
Amalgerol	9.7 b	7.1 a	179.0 a	164.2
Lithovit	9.3 b	5.3 b	119.0 b	109.2
Tryven	11.6 a	5.1 b	112.0 b	102.7

Duncan's Multiply Range Test (P < 0.05)

Amalgerol treatment has been shown to increase the number of branches and number of leaves relative to the untreated control. At a value of 7.1 branches per stem, 179 leaves were formed on average over the period. Amalgerol treated variants are followed by Lithovit treated versions with 5.3 branches and 119 leaves and Tryven - 5.1 branches and 112 leaves. Here, too, the values of the indicator are higher than the control but are not statistically proven (Table 2).

It can be said that the number of stems per plant increases only when treated with Tryven. According to the indicator the number of branches per stem and the number of leaves per plant, the application of the studied preparations leads to values greater than those of the control. Amalgerol has been shown to increase the values of both indicators relative to controls. The fertilizers studied led to an increase in the values of indicators - fresh mass (leaves + stems), fresh leaf mass and dry leaf mass (herb), compared to those of the untreated control (Table 3).

Table 3. Weight of fresh plant mass, fresh leaves mass and dry leaves mass of plant by variants averaged over the study period, g

Variant	Fresh biomass weight	%	Fresh leaves weight	%	Dry leaves weight	%	% of dry leaves of fresh leaves mass
Control	143.21b	100	69.62b	100	22.73c	100	32.65
Amalgerol	239.66ab	167.3	122.82a	176.4	31.07b	136.7	25.29
Lithovit	198.54b	138.6	117.08a	168.2	31.83ab	140.0	27.19
Tryven	250.05a	174.6	107.89a	154.9	37.72a	165.9	34.96

Duncan's Multiply Range Test (P<0.05)

In the indicator weight fresh mass of the whole plant, the highest value was shown in the variant treated with Tryven - 250.05 g, which is 74.6% more than the control (143.21 g). Amalgerol treated variants with 67.3% and Lithovit 38.6% also have higher values, but these differences are not proven.

The weight of the fresh leaf mass is strongly influenced by the applied leaf fertilizers and is proven to be higher than the controls in all three types of fertilizers. Amalgerol-treated plants have a fresh leaf mass of 122.82 g per plant weight, which is 76.4% above control. The implementation of Litovit increases the values of the indicator by 68.2% and Triven by 54.9% above the control. When measuring the weight of the dry leaf mass, the variants treated with Amalgerol - 31.07 g and Lithovit - 31.83 g were approximately the same values. They are proven to exceed the control variant - 22.73 g. The strongest influence on the weight of the dry leaf mass was observed in the variant treated with Tryven - 37.72 g, which is 65.9% above the control. It is statistically proven that the fertilizers studied lead to an increase in the values of the indicators compared to the untreated control. The data in Table 4 shows that, on average, for the study period, Triven and Amalgerol have been shown to increase yields of fresh mass (leaves + stems) per decare to a different degree than controls. The highest yield of fresh mass was obtained with the Tryven treated version - 989.2 kg/da, which was 7.5% above the yield of the control variant. Amalgerol, increased fresh weight yield by 6.5% (980 kg/da) above control (920 kg/da). The values for treatment with Lithovit are close to the control variant. In terms of fresh leaf yield, Lithovit has proven to increase the weight of fresh leaves by 21.1% over control - 541.6 kg/da.

An increase in the yield of fresh leaves was also reported when treated with Amalgerol - 502.2 kg/da (12.3% above control).

Table 4. Average yield of lemon balm kg/da by fertilizer variants

Variant	Yield of fresh biomass (leaves + stems)		Yield of fresh leaves		Yield of dry leaves mass	
	kg/da	%	kg/da	%	kg/da	%
Control	920.00c	100.0	447.23 c	100.0	146.14 b	100.0
Amalgerol	980.00 b	106.5	502.23 b	112.3	127.00 c	86.9
Lithovit	918.46 c	99.8	541.60 a	121.1	147.25 b	100.8
Tryven	989.23 a	107.5	426.80 d	95.4	149.21 a	102.1

Duncan's Multiply Range Test (P<0.05)

In the Tryven treated variant, despite the higher yield of fresh mass, no increase in the yield of fresh leaves was observed relative to the control. These differences between the fertilizer variants are due to the different composition of the fertilizers being studied, which also gives us a more accurate idea of their influence on lemon balm. After drying, the yield of dry leaves in the treated variants differs from the control.

Proven higher yields of dry leaves were reported with the Tryven treatment variant - 149.21 kg/da, which is 2.1% above control. A slight percentage increase in yield was also reported for the variant treated with Lithovit - 147.25 kg/da, but this difference from the control is not proven.Treatment with Tryven and Amalgerol increases the yield of fresh plant mass, but the yield of fresh leaves per decare is smaller than the control when treated with Tryven. This is due to the composition of the different fertilizers and the directing of nutrients to different organs of the plant. A similar variation of values is also observed in the dry leaf mass (herb).

When treated with Amalgerol, the yield of fresh leaves mass is increased, but after drying, the yield of dry leaves mass is less than that of the control.

The possibility of forming more leaves, but smaller in size, and the amount of dry matter in them, is yet to be explored. The data can be used when choosing a leaf fertilizer, depending on which part of the plant we want to influence according to the use direction - to increase the yield of fresh plant mass, for fresh leaves or for dry leaf mass (herb).

CONCLUSIONS

No differences in the occurrence of the phenological phases of the crop as a result of treatment with the leaf fertilizers compared to the control are taken into account.

A percentage increase in the values of the plant height indicator on the 7th and 14th days after treatment, more pronounced on the 14th day, was reported, but differences in control were not proved.

Treatment with Tryven leads to the formation of more stems per plant - 11.6 pieces compared to the control (9 pieces). This effect has not been demonstrated when treated with Lithovit and Amalgerol. Amalgerol treatment has been shown to increase the number of branches and number of leaves (7.1 number of branches per stem - 179 pieces) compared to the control (4.9 number of branches and 109 leaves). An increase in the values of the indicator was reported after the application of Lithovit and Tryven, but the difference from the control is not proved.

Tryven increased the weight of fresh mass of plant - 250.05 g, which is 74.6% above the control (143.21 g). The weight of the fresh leaf mass was strongly influenced by the applied products and was proven to be higher with Amalgerol -122.82 g (76.4% above control), Lithovit - with 68.2% and Tryven with 54.9% above control. Given the weight of the dry leaf mass, the variants treated with Amalgerol - 31.07 g and Lithovit - 31.83 g were approximately the same values. They are proven to exceed the control untreated variant (22.73 g). The weight of the dry leaf mass after Tryven administration increased also - 37.72 g, (65.9% above control), followed by the variants treated with Amalgerol - 31.07 g and Lithovit - 31.83 g.

Highest yield of fresh mass/da obtained after Tryven treatment - 989.2 kg/da and Amalgerol – 980 kg/da, from fresh leaves after treatment with Lithovit (541.6 kg/da) and Amalgerol (502.2 kg/da) and from dry leaves (herb) after Tryven treatment - 149.21 kg/da. Values exceed those of control and are statistically proven.

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ASPECTS OF THE INVOLVEMENT OF BIOTECHNOLOGY IN FUNCTIONAL FOOD AND NUTRACEUTICALS

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Abstract

The purpose of this paper is to briefly present the most important benefits of consuming of functional foods and nutraceuticals as well as the involvement of biotechnology in obtaining of these new foods. The functional food is a typical food that has specific nutrients added to it, like vitamins or minerals, fibres, probiotics or prebiotics. In general, this includes anything added for a specific functional purpose. Food science enables us to make some functional foods that wouldn't ordinarily be available. Knowing the science and chemistry of food and how ingredients interact helps us make some nutrients more readily available. The designation of functional foods and the attribution of health benefits of any kind are based on precise scientific criteria and rigorous safety and efficacy studies. The involvement of biotechnology in obtaining of functional foods and nutraceuticals is maximized and reproduced through: fermentative processes capable of generating functional compounds; correlating the structure of food macromolecules with their physiological function; modern non-thermal processing techniques, which provide nutrients in food but also ensure their stability and safety, etc. The beneficial effects of functional foods on health are due to biologically active compounds with physiological roles in the body. But these foods are not panacea for the wrong eating habits but they are adjuvants within a balanced diet. Diet is just one aspect of a complex approach to improving consumer's health. In fact, the functional foods are not tablets or capsules, but a healthy way of eating.

***Key words**: biotechnology, functional food, nutraceuticals, trends.*

INTRODUCTION

Alteration of the health status of humans due to the diet is closely monitored by researchers and nutritionists, which has led to the emergence of a new concept, that of "functional food".

The U.S. is the largest consumer of functional foods, it was a 44 billion dollar market in 2012 and it's increasing with at least 60% of people consuming functional foods, occasionally. By definition, a functional food is a typical food that has specific nutrients added to it like vitamins and minerals, to serve a specific purpose (Mulry, 2008).

A functional food can be a natural product, which contains biologically useful components or a food obtained through a technological intervention that increases its level of biologically active compounds. Biologically active compounds are components of foods that act positively on key functions of the body, relevant to health. They reduce the risk of diseases such as atherosclerosis, high blood pressure, myocardial infarction, diabetes, etc. Without a doubt, the richest sources of compounds with beneficial health effects are vegetables. Fresh fruits and vegetables, tea (especially the green one) are, due to their richness in polyphenols, a very efficient source of antioxidants necessary to prevent the excess accumulation of free radicals in the body.

Functional foods and beverages are not really new. The first functional beverages were wine and beer because they were consumed for their functional effects rather than just hydration. Tea is the most common functional beverage used worldwide today. Gatorade was one of the first modern functional products; it was specifically designed to replace sugars, calories and minerals lost during exercise (Mulry, 2008). Now, the functional foods trend is capitalizing on consumers' knowledge that diet has a major impact on health, both positive and negative.

Recent scientific research supports the health benefits of many common foods, and consumers increasingly recognize that incorporating more orange juice, carrots, broccoli, fish, garlic and green leafy vegetables into their diets can make a difference. Consumers are making the connection between a good diet and optimal health or disease prevention, but they do not really understand the term "functional foods". The International Food and Information Council reported "low to moderate" consumer awareness for functional foods, although awareness of foods that reduce cancer or heart disease risk was "fairly high" (Mulry, 2008).

MATERIALS AND METHODS

This is a documentary study. The main sources of the study consisted of various results published in scientific literature, some journals and scientific communications.

RESULTS AND DISCUSSIONS

Demographic trends of the population and socio-economic changes indicate the need for foods with higher health benefits (Roşculete and Roşculete, 2018). An increase in life expectancy and a desire for a better quality of life, as well as increased health care costs, have stimulated governments, physicians, researchers, agriculture and the food industry to find how to manage these changes more effectively.

The intersections of agriculture, biotechnology, and wellness are transforming global food industry (Bonciu and Sarac, 2016). Innovators are harnessing emerging technologies or applying existing technologies in new ways to design new ways to eat, responding to both to consumer trends and the imperative to improve the sustainability of the planet and human health. The resource management is a complex process involving an interdisciplinary approach because the management of natural resources has become a challenge at all levels (Butnariu, 2012; Butnariu and Caunii, 2013; Pandia et al., 2018, 2019; Rosculete et al., 2018, 2019).

Biotechnology presents an extremely rapid development and responds in the most natural way to major, fundamental human needs. It has a pronounced impact on society and environment. One of the most important implications of biotechnology in food science is that of ensuring nutritional value and amplifying the biological effects of food, which is actually the functional role of biotechnology. From this point of view, some of the strategies proposed by modern biotechnology are: use of lactic bacteria, true "cell factories" of functional compounds with altered metabolism, and with considerable yields of biosynthesis functional biocompounds, such as vitamins, for example; exploring the potential of lactic bacteria to produce exopolysaccharides; improvement of molecular tools to monitor the gene activity of probiotic species in the human intestine and to explain their functional activity; development of "vaccine foods", which contain strains of lactic bacteria that express epitopes of pathogenic species, for the purpose of immunizing the host, etc. (Abdel-Rahman and Sonomoto, 2016; Bosma et al., 2017).

Flavones contained in many functional foods can help lower mortality due to cardiovascular disease, reducing the risk of malignant disease. Soybean plays an important role in the prevention of cardiovascular disease, cancer and osteoporosis. Recent research suggests that isoflavones, compounds present in soy beans, prevent atherosclerosis (Pabich and Materska, 2019).

Oats are an important source of compounds (beta glucans) that reduce blood cholesterol levels and thus the risk of cardiovascular disease (Grundy et al., 2018).

Cold water fats (salmon, sardines, cod) and oilseeds (in pumpkin, nuts, walnuts) contain omega-3 polyunsaturated fats with anti-inflammatory effect, which limits the development of diseases such as rheumatoid arthritis, Alzheimer's, atherosclerosis, and with stimulating effect of the immune system (Bibus and Lands, 2015).

Dairy products are, without any reservation, excellent health-beneficial foods, especially fermented foods known as probiotics, foods that contain living microorganisms with complex effects on the host organism. Membranes of ingested bacteria can stimulate interferon formation, and the administration of yogurt containing live bacteria alters the immune response. Also, there is a decrease in cholesterol and an improvement in the associated cardiovascular disorders.

A food can become functional by the following biotechnological methods:

- Increasing the bioavailability or stability of a component recognized for its functional effects or for reducing the potential risk of disease;
- Increasing the concentration of a component present in the food to a point where it can

induce beneficial effects. For example, bio-fortification with a micronutrient to increase the daily intake;

- Replacing a component, usually macronutrient that is excessive, with a component with beneficial effects;
- Elimination of allergenic through biotechnology.

An item that is a functional food would include a note about added nutrients on its ingredient statement; the Nutrition Fact Panel would also identify additional nutrients and their levels, as well as nutrient content claims like "good source of," or "excellent source of," a particular nutrient. A functional food may also have structure/function claims like if orange juice has added calcium the package may say "calcium builds strong bones" (Mulry, 2008).

Nutraceuticals, a term often used interchangeably with functional foods, is, more accurately, parts of a food or a whole food that have a medical or health benefit, including prevention or treatment of a disease. Thus, the nutraceuticals definition is broader than that of functional foods. Included are dietary supplements or medical foods and functional foods (Mulry, 2008).

Functional genomics in food biotechnology involves: estimating the side effects of genetic changes in correlation with the behaviour of microorganisms in the product or the human gastrointestinal tract; estimating and anticipating the response of cells to stress; designing new antimicrobial systems; developing new metabolic engineering tools; implementation of specific and rapid methods of identifying the microorganisms of alteration and of the pathogens.

Microorganisms with incidence in food microbiology and biotechnology have relatively small genomes. An average bacterial genome can be sequenced in just a few days, becoming even a tendency for any study involving an industrial microbial strain to begin sequencing the genome.

In Figure 1 are indicated numerous biochemical nutraceutical categories including alkaloids, lipids, organic acids and polysaccharides, organosulphurs, phenols, phytic acids, phytosterols, and terpenes (Nwanodi, 2017).

Nutraceuticals represent an evolving new field of research that may generate a deeper knowledge of the mechanisms of action and the benefits that may derive from the use of evidence - based dietary patterns (Minuz et al., 2017). It is worth to mentioning the important role of some foods (such as fruits, vegetables and whole grains) or of their compounds (antioxidants, vitamins, prebiotics, etc.) in the prevention of diseases, which has led to the development of the functional food market in the context of profiling a new concept, that of optimized nutrition. Some vegetables are true natural nutraceuticals, which helps in the treatment of many diseases such as cancer for example. From this point of view, the species of the *Allium* genus namely garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and Chinese chive (*Allium tuberosum)* are representative (Zeng et al., 2017; Bonciu et al., 2018). Garlic is a very important source of dietary for antioxidant properties, including sulfur compounds, polyphenols, and carotenoids (Farooqui, 2013). Some effects of the garlic constituents to health's consumers is due by anticancer, antimicrobial, anti-inflammatory effect, etc. (Figure 2). Onion is an important source of dietary phytochemicals with proven antioxidant properties, such as organosulfur compounds, phenolic acids, flavonoids, thiosulfinates, and anthocyanins (Zhu et al., 2017). From this point of view, in Figure 3 is presented the physicochemical and functional properties of polysaccharides sequentially extracted from *Allium cepa*. Chinese chive is an important source of dietary phytochemicals with proven antioxidant properties, such as organosulfur compounds, flavonoids, and saponins. Major mechanisms of *Allium´s* organopolysulfides for preventive chronic disease include anticancer, preventive cardiovascular and heart diseases, anti-inflammation, antiobesity, antidiabetes, antimicrobial activities, and neuroprotective and immunological effects (Zeng et al., 2017).

Another concrete example of nutraceuticals is the willow bark derived analgesic aspirin, Aspergillus terreus, derived cholesterol-lowering lovastatin (George et al., 2016; Yuan et al., 2016; Nwanodi, 2017).

Communicating the health benefits of consumers is of great importance, so that they have the information they need to make correct choices about the preferred foods.

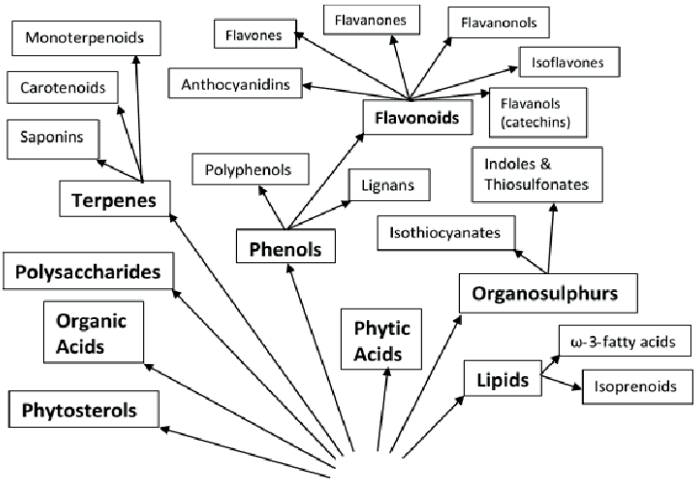

Figure 1. Nutraceuticals biochemical classification.
Source: Nwanodi, 2017

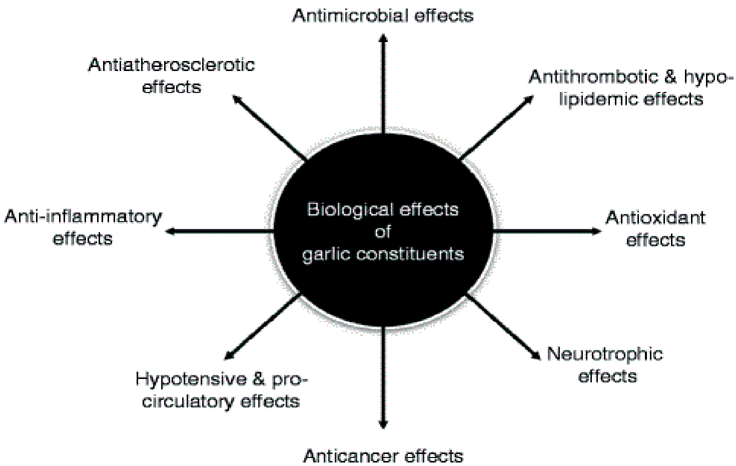

Figure 2. Some effects of the garlic constituents to health's consumers
Source: Farooqui, 2013

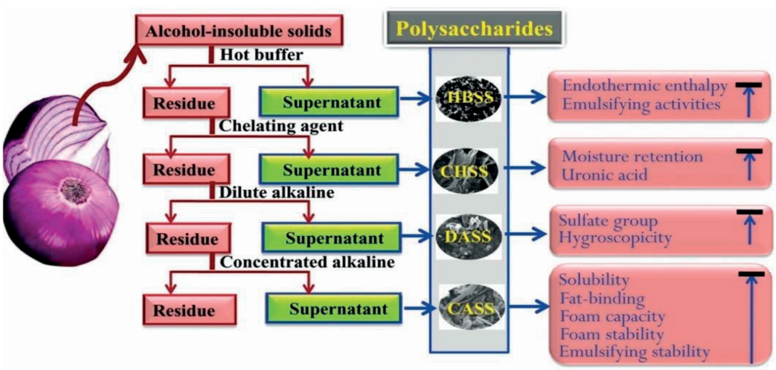

Figure 3. Physicochemical and functional properties of polysaccharides sequentially extracted from *Allium cepa*.
Source: Zhu et al., 2017

CONCLUSIONS

A functional food may be a whole natural food, a food to which it has been added or on the contrary, eliminated, a component by technological or biotechnological means, a food whose bioavailability has been modified or any combination of these variants.

Functional foods are products that contain various biologically active compounds and which consumed in a balanced diet, contribute to maintaining the optimum health of consumers; their role is rather to reduce the risk of some health problems.

The research opportunities in obtaining functional and nutraceutical foods as well as explaining the relationship between their consumption and improving the health of humans are the biggest challenge for scientists in the near future.

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IMPROVING SEWAGE SLUDGE COMPOST QUALITY BY VERMICOMPOSTING

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Abstract

Treatment and disposal of sludge produced during the waste water treatment is one of the most critical environmental issues of today. Therefore, different techniques are used for its disposal and recycling. Vermicomposting is one of the most ecologically and economically sound technologies for handling sewage sludge in order to convert it into a useful recyclable product. The aim of this study was to assess the effectiveness of vermicomposting process applied on three types of dehydrated sewage sludge coming from two different wastewater treatment plants using two earthworm species (Eisenia fetida and Eisenia andrei), as well as the compost quality. Biotic and abiotic parameters were used to assess the vermicomposting process and the quality of the obtained compost. The effect of heavy metal on earthworms was monitored durring the first stages of vermicomposting and at the end of the process. The compost quality was evaluated from an agronomic and ecological point of view through a germination seed test with cress seeds (Lepidium sativum).

***Key words**: earthworms, germination test, phytotoxicity, vermicompost quality, sewage sludge.*

INTRODUCTION

Large scale urbanization is leading to growth of sewage sludge production resulting from wastewater treatment plant in Romania in the last decades, raising serious environmental problems for their disposal. Treatment and disposal of sewage sludge produced during wastewater treatment represent one of the most critical environmental issues of today. The sludge produced is large in volume and hazardous. Numerous studies related to its safe handling, disposal and recycling techniques (Khwairakpam and Bhargava, 2009) have been conducted worldwide.

Sewage sludge can be recycled on agricultural soils because is rich in organic matter and nutrients, but its heavy metal content is a constraint (Khwairakpam and Bhrgawa, 2009; Wu et al., 2018) in the widespread adoption of this disposal method. Sewage sludge is a major contributor of toxic heavy metals such as cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), chromium (Cr) etc. to soils (Khwairakpam and Bhrgawa, 2009; Lv et al., 2016) due to its direct application as soil amendment. These metals may enter the human and animal body through consumption of the crops. Due to its high organic load, sewage sludge is a suitable waste for treatment through biological techniques such as composting and vermicomposting aimed at obtaining a stable product with a high agronomic value (Villar et al., 2016).

Recent studies have shown that vermicomposting is an efficient and alternative technique for the sewage sludge treatment (Yang et al., 2014; Lv et al., 2016; Malińska et al., 2016). Vermicomposting is a bio-oxidation and stabilization process of organic matter as a result of the interaction between microorganisms and earthworms (Khwairakpam and Bhrgawa, 2009; Villar et al., 2016), and it is a low cost (Nigussie et al., 2016), viable and sustainable option for sewage sludge management. Also, it is easy to operate and can

be conducted in contained space to produce a good quality fertilizer (Khwairakpam and Bhrgawa, 2009) for soil. Vermicomposting of sewage sludge has been widely used to stabilize heavy metals in SS (Benitez et al., 1999; Gupta and Garg, 2008; Lv et al., 2016; Suleiman et al., 2017; Wu et al., 2018). Vermicomposting mineralize most of the organic material and transform the residuals into stabilized humic-like materials (Benitez et al., 1999). It is well known that the addition of earthworms can accelerate the bio-stabilization process and the earthworms are considered as the crucial drivers of the vermicomposting process (Yang et al., 2014).
The aim of this study was to assess the effectiveness of vermicomposting process applied on three types of dehydrated sewage sludge, the impact of sewage sludge on earthworm species (*Eisenia fetida* and *Eisenia andrei*), as well as the vermicompost quality.

MATERIALS AND METHODS

These studies used dehydrated sewage sludges from two treatment plants labelled with the P and M initials. Thus, one of the sludges (SS_P) comes from P treatment plant, which serves about 160,000 inhabitants, and two sewage sludges come from M treatment plant (SS_1M; SS_2M), which serves about 32,000 inhabitants.
Physico-chemical analyses of compost were conducted within the soil pollution control laboratory of the Research Institute for Pedology and Agrochemistry in Bucharest.
The initial physico-chemical characteristics of the three types of sewage sludge are presented in Table 1.

Table 1. Initial physico-chemical characteristics of sewage sludge samples

	SS_P	SS_1M	SS_2M
pH	6.52	6.47	6.25
Moisture (%)	67.10	67.69	68.47
C/N	6.59	8.61	8.60
TOC (%)	24.37	22.39	22.70
N (%)	3.70	2.60	2.64
P (%)	1.74	1.73	1.74
K (%)	0.47	0.57	0.61

The contents in heavy metals i.e. cadmium, copper, chromium, nickel, lead, mercury and zinc (Cd, Cu, Cr, Ni, Pb, Hg, Zn) which are presented in Table 2 were also analysed.
The vermicomposting time was 45 days at 25°C. The moisture was maintained in each box at 70-80% (Wu et al., 2018).
After 45 days a product resulted, namely the vermicompost, which was structured, different from the sewage sludge, with a pleasant earthy smell and a dark brown colour.
After this stage, earthworms were separated from vermicompost and then weighed (being left previously for 24 hours in distilled water to empty the digestive tract). Subsequently, the material resulting from earthworms drying was prepared and subject to chemical analyses (Picture 2).

Table 2. Heavy metal contents of sewage sludge samples

	SS_P	SS_1M	SS_2M	WRAP-2011
Cd (mg/kg)	0.340	0.253	0.360	1.5
Cu (mg/kg)	168	167	168	200
Cr (mg/kg)	57	43	50	100
Ni (mg/kg)	41.00	37.00	39.13	50
Pb (mg/kg)	33.00	38.00	34.33	200
Hg (mg/kg)	0.29	0.22	0.24	1
Zn (mg/kg)	751.00	754.00	753.33	400

The earthworms belonging to genus *Eisenia* (*Eisenia fetida* and *Eisenia andrei*), were collected from a pile of plant debris resulting from the green spaces within the University of Agronomic Sciences and Veterinary Medicine of Bucharest campus.
The earthworms with an average length of 5.0 ± 0.2 cm and with a total mass of 100.0 ± 0.42 g were introduced into each box. After balancing, the earthworms were released in each box (Picture 1). No bulking materials have been used.

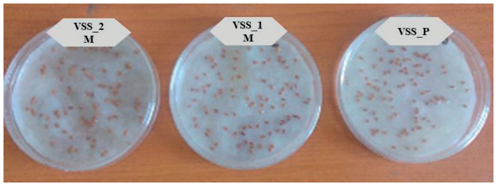
Picture 1. The three boxes with sewage sludge

Picture 2. The three boxes with vermicompost and the resulted earthworms

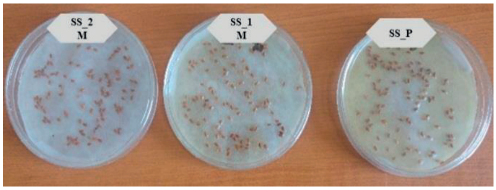
For the germination test, an aqueous extract was produced from both the vermicompost and the initial material (dehydrated sewage sludge). For this, 100 g samples from each of the 3 types of vermicompost as well as 100 g samples from each of the 3 types of dehydrated sludge were taken and mixed with 110 ml distilled water (Picture 3). The extracts obtained were well stirred and left to rest for 24 hours. Subsequently, the extracts were passed through filter paper, thus obtaining the supernatant.

Picture 3. Vermicompost and dehydrated sewage sludge extracts

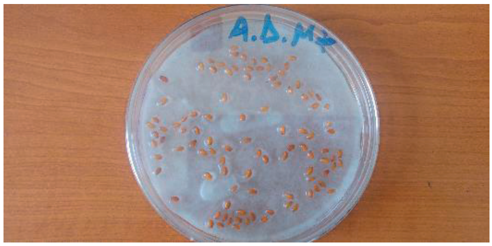
In order to evaluate the quality of the vermicomposts (VSS_P, VSS_1M and VSS_2M), a germination test was performed in Petri dishes (Picture 4) where those vermicomposts were compared to the three types of dehydrated sludge, SS_1P, SS_1M and SS_2M (Picture 5). A control variant with distilled water (ADM) was also performed (Picture 6). A filter paper was inserted in each Petri dish and 100 cress (*Lepidium sativum*) seeds were placed on it. Then, 5 ml of supernatant was added over the seeds and in the control variants, only distilled water was added. The Petri dishes were inserted in the incubator at 25ºC for 48 hours and then the germinated plants were counted for each variant and the evolution of the seedlings was observed. The seeds were sprayed daily with vermicompost extract, compost extract and distilled water, respectively. The initial number of germs was recorded, and their evolution was monitored. All tests were made in triplicates.



Picture 4. Germination test with vermicompost extract after sowing



Picture 5. Germination test with dehydrated sewage sludge extract after sowing



Picture 6. Germination test with distilled water (control) after sowing

RESULTS AND DISCUSSIONS

The physico-chemical characteristics of the vermicompost are presented in Table 3. In the initial stage, the moisture of the 3 types of dehydrated sewage sludge had very close values, 67.10% (SS_P), 67.69% (SS_1M) and 68.47% (SS_2M), and after vermicomposting it decreased to approx. 50%. The lowest moisture was registered at VSS_2M (48.45%), and the highest, at VSS_P (54.86%). Thus, compared to the initial status, the moisture differences were of 12.24% (VSS_P), 16.23% (VSS_1M) and 20.02% (VSS_2M).

Table 3. Physico-chemical characteristics of sewage sludge vermicompost

	VSS_P	VSS_1M	VSS_2M
Moisture (%)	54.86	51.46	48.45
PC (%)	63.81	66.47	68.37
OM (%)	35.54	36.91	28.01
pH	5.52	6.25	8.34
TOC (%)	17.38	18.10	13.93
N (%)	2.06	1.70	0.91
C/N	8.43	10.65	15.31
P (%)	1.03	0.77	0.21
K (%)	1.44	1.62	1.48
Ca (%)	3.17	3.34	2.38
Mg (%)	1.08	1.10	1.05

The vermicompost had an organic matter content (OM) between 28.01% (VSS_2M) and 36.91% (VSS_1M), and the total organic carbon content (TOC) had values between 13.93% (VSS_2M) and 18.10% (VSS_1M), generally lower than the initial content (24.37% at SS_P, and the other two types of sludge, SS_1M and SS_2M, had values just over 22%).
The decrease of TOC due to vermicomposting was also reported by other authors (Hait and Tare, 2011) and could be caused by carbon loss in the form of CO_2 as a result of the respiration of microorganisms (Nigussie et al., 2016; Suleiman et al., 2017).
At the same time, total N content also decreased and had values between 0.91 and 2.06% compared to the status before vermicomposting.
Other studies have reported an increase in total N content due to vermicomposting associated with a decrease in the amount of dry matter that could be due to the consumption of the substrate by earthworms and microorganisms and their metabolic activity (Suleiman et al., 2017).
The decrease in N content can be associated with the absence of food resources given the low values of C/N ratio in the initial stage (Wu et al., 2018). The C/N ratio at the end of vermicomposting had higher values, the highest value being registered at VSS_2M (15.31). The total P content decreased compared to the content of the initial material.
Regarding the pH values, they were decreasing by a pH unit at VSS_P compared to the initial value, and at VS_2M, there was an increase by two units compared to the initial value.
The pH value decrease in case of sewage sludge vermicomposting was also reported in other scientific papers (Yang et al., 2014; Lv et al., 2016), and it could be due to nitrogen and phosphorus mineralization (Suleiman et al., 2017).

Table 4. Heavy metal content of sewage sludge vermicompost

	VSS_P	VSS_1M	VSS_2M	Upper limits (WRAP, - 2011)
Cd (mg/kg)	1.187	1.091	0.881	1.5
Cu (mg/kg)	197	79	49	200
Cr (mg/kg)	65.87	41.41	28.8	100
Ni (mg/kg)	83.0	28.8	29.5	50
Pb (mg/kg)	67.08	18.96	22.29	200
Zn (mg/kg)	1232	627	233	400

The heavy metal contents of vermicomposts (Table 4) were compared with the initial values of the dehydrated sewage sludge content and with the upper limits for minimum compost quality for general use in United Kingdom (WRAP, 2011) in the absence of some recommended values for Romania. Increases of contents in all heavy metals analysed (Cd, Cu, Cr, Ni, Pb, Zn) were registered, in general, at VSS_P. However, regarding Cd, it registered increases of the content in all three vermicomposts compared with the initial values of dehydrated sewage sludge. The highest content was registered at VSS_P, and the lowest at VSS_2M. However, the Cd content did not exceed the limit value of 1.5 mg/kg (WRAP, 2011). The Zn content of VSS_P registered a large increase amounting to 1232 mg/kg compared to 751 mg/kg in the initial stage. The Zn content of the other two types of vermicompost decreased slightly but remained above the upper limit recommended by WRAP (2011). High values of Zn content have also been reported in previous studies (Suleiman et al., 2017).
The chemical analyses performed at the end of vermicomposting on earthworm samples revealed a high Cd content of those in case of VSS_P and VSS_1M. In case of VSS_P, a high Cu and Zn content was also highlighted. Previous studies have shown that earthworms are heavy metals accumulators and *Eisenia andrei* species has the highest accumulation capacity. Therefore, attention must be paid on the risk of introducing heavy metals into the food chain (Suleiman et al., 2017).

Table 5. Heavy metal content of earthworms at the end of vermicomposting

	VSS_P	VSS_1M	VSS_2M
Cd (mg/kg)	4.4	5.2	1.8
Cu (mg/kg)	55	23	34
Ni (mg/kg)	23.6	6.98	19.5
Pb (mg/kg)	16.2	5.3	10.8
Zn (mg/kg)	329	194	214

For the vermicompost quality evaluation, a germination test with cress (*Lepidium sativum* L.) was performed in three replicates. The seeds were germinated in an incubator, and after 48 hours from introduction into the incubator, the number of cress germs was counted. At this stage, the highest number of germs (the average of the three replicates) was registered in VSS_2M (92) vermicompost variant and the lowest number in SS_P (69) variant, compared to the control variant with distilled water, which had an average number of 96 germs (Figure 1, Picture 7). At 5 days after introduction into the incubator, the maximum number of seedlings (100) was registered in all variants with vermicompost, as well as in those with dehydrated sewage sludge, except for SS_2M.

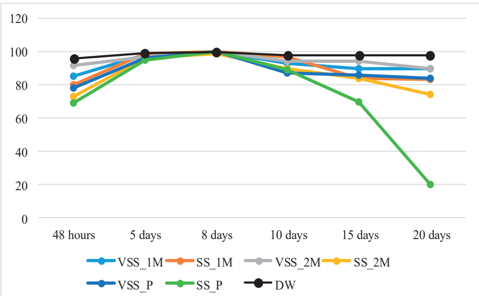
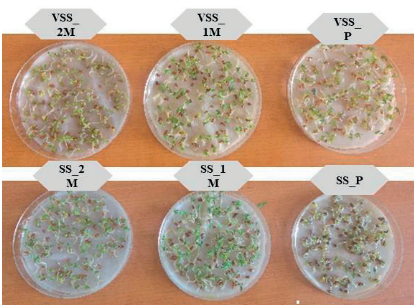
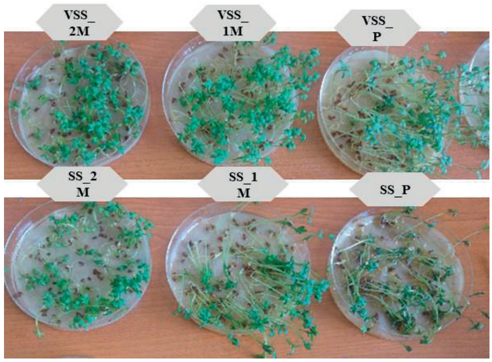


Figure 1. The evolution of the cress plants number under the influence of vermicompost extracts



Picture 7. Cress seed germination in vermicompost and dehydrated sewage sludge extract after 5 days

The evolution of seedlings and plants was monitored for 20 days. 10 days after the experiment establishment it was observed that the seedlings began to die, the most affected being those from the VSS_P and SS_P variants where the highest number of dead seedlings was registered, i.e 13 and 11 (Picture 8, Picture 9).



Picture 8. Cress seed germination in vermicompost and dehydrated sewage sludge extract after 10 days

Picture 9. Cress seed germination in distilled water after 5 and 10 days

After 20 days, the highest number of dead plants was registered in SS_P (80) variant, and in the vermicompost variants, most of the dead plants being recorded in VSS_P (16). In the vermicompost variants, on the 20th day, the highest number of viable plants was in average of 90 in VSS_1M and in VSS_2M followed by VSS_P (86). The plants may have been affected by the presence of heavy metals in the dehydrated sewage sludge variants. Previous studies have shown that sewage sludge cannot be independently used in vermicomposting process (Lim et al., 2016), and bulking materials is needed for enrichment of the diversity of microbial population and enzymatic activity (Hait and tare, 2011), and also to alleviate heavy metals phytotoxicity (Wu et al., 2018).

CONCLUSIONS

Vermicomposting is emerging as a most appropriate alternative to conventional aerobic composting (Hait and Tare, 2011), and vermicompost is a more advanced stage of raw materials decomposition than thermophilic compost (Nigussie et al., 2016). But, in order to ensure successful vermicomposting of sewage sludge, several suitable external materials should be applied to mitigate the toxicity of sewage sludge (Wu et al., 2018). The feed quality is of primary importance for the growth and reproduction of worms (Molina et al., 2013). However, further studies regarding heavy metal phytotoxicity, and their bioaccumulation by the earthworms are required.

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THE INFLUENCE OF THE TREATMENT AGAINST THE ATTACK OF THE MICROMYCETES *Polystigma rubrum* and *Stigmina carpophila* ON THE PLUM, SOIMARI LOCATION, PRAHOVA COUNTY

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Abstract

The purpose of the paper was to monitor the attack of pathogens Polystigma rubrum and Stigmina carpophila and making observations regarding the phytosanitary intervention on their attack on the plum in the research area in 2019. The biological material it was represented of cultivars: Stanley, Anna Spath and Romanian Gras. To the cultivar Romanian Gras it was noted that the attack of the micromicetes has diminished in the treated variants at 6.2% the attack of Polystigma rubrum and 8.2% of the attack of Stigmina carpophila fungus. Highest efficiency value has been registered at Romanian Gras cultivar with E = 69.75% in protection against Polistigma rubrum, and 71,1% against Stigmina carpophila followed by Stanley cultivar with effectivenes around 69% against the attack of micromicetes monitored.

Key words: *plum, micromicetes, cultivar, degree of attack, effectiveness.*

INTRODUCTION

The plum culture is recognized for the Prahova county area ensuring the Romanian market with fresh fruits and for industrialization (Alexandru et al., 2018). The health of the plum orchards is a continuous concern of the cultivators of this species (Alexandru et al., 2019; Popa et al., 2012; Vacaroiu et al., 2009). Plum-specific pathogens such as *Polystigma rubrum* and *Stigmina carpophila* are common in natural infection conditions in plum orchards in northwestern Romania (Miter et al., 2005), but also from the research area (Alexandru et al., 2019). *Polystigma rubrum* is the specific micromycete and frequency on *Prunus* species (Cannon, 1996; Roberts et al., 2918; Douglas, 2018), which causes plum-leaf blister considered a common disease in plum orchards (Iliev and Stoev, 2011). The pathogen *Stigmina carpophila* is known for its annual incidence on plum leaf (Alexandru et al., 2019; Gheorghieş and Geamăn, 2003) and causes significant losses in plum production (Yousefi and Shahri, 2014). Shot-hole disease of plum remains one of the most important foliar diseases of the Prunus species, and in particular shot-hole fungal disease produced by *Wilsonomyces carpophilus* (syn. Stigmina carpophila) (Adaskaveg et al., 1990).

MATERIALS AND METHODS

The research followed the influence of the treatment applied in the vegetation on the attack of plum-leaf blister caused by micromicet *Polystigma rubrum* and shot-hole desease of plum produced by the phatogen *Stigmina carpophila* in 2019, Soimari location, Prahova county.

Observations were made regarding the attack of the two pathogens before and after the treatments were applied.

The frequency and intensity of the attack were calculated according to the formulas: F = n x 100/N, in which n = the number of attacked plants/ organs, N = total plant/organ analysis, attack intensity was calculated using formula I = Σ (i x f) where: i = the percentage of the attack, f = the number of organs/plants with the respective attack percentage, n = the total number of attacked organs/plants analyzed. Based on these, the degree of attack was calculated according to the formula: = F x I/100, where: DA degree attack F = attack frequency, I = attack intensity.

The biological material was represented by the cultivars: Stanley, Anna Spath and Romanian Gras. The efficacy of the treatments was calculated according to Abbott's formula: E (%) = [(DAC-DAV)/DAC] x 100, where DAC =

attack degree control variant; DAV = attack degree in the treated variant.

RESULTS AND DISCUSSIONS

In 2019, a treatment scheme was applied in which recommended products were included in the control of the spectrum of plum pathogens, especially of the micromycetes *Polystigma rubrum* and *Stigmina carpophila* and of some specific pests of this culture. The data in table 1 indicates the products and concentrations used, the administration phenophase and the period of application of the tested products in the applied scheme. The application periods cover the vegetation period of the plum from rest to fruit development (Table 1).

Table 1. Treatment scheme applied to control *Polystigma rubrum* and *Stigmina carpophila* pathogens on plum in Şoimari location, Prahova County, 2019

The product	Concentration (%); dose (l,kg/ha)	Phenophase	Date of adminis-tration
			2019
Zeama bordeleza	2%	Vegetative retention	02.03
Confidor oil	1.5%	Vegetative retention	10.03
Topsin WDG (+ Calypso 480SC)	0.2% (+0.02%)	Green button	01.04
Luna experience 400 SC (+ Mospilan 20SG)	0.05% (+0.045%)	White button	14.04
Signum FG (+ Calypso 480SC)	0.5% (+0.02%)	Flowering corolla10-15%	29.04
Luna experience 400 SC (+ Novadim Progress EC)	0.05% (+0.075%)	Shake of the petals 10-15%	15.05
Signum FG (+ Mospilan 20SG)	0.5% (+0.045%)	Fruit development	07.06

In 2019, the micromycetes *Polystigma rubrum* and *Stigmina carpophila* registered a maximum frequency in all the cultivars studied, F = 100%. The intensity of the attack made the difference in the cultivars reaction to the attack of the two pathogens.

Regarding the intensity of the attack of the micromycete *Polystigma rubrum,* the small value was registered at the Romanian Gras cultivar with I = 20.5% followed by the Anna Spath cultivar with I = 25. 2%. The highest value of the degree of attack of the pathogen *Polystigma rubrum* was calculated in the Stanley cultivar where GA = 30%. The attack of shot hole disease was higher recording a value of attack rate 37% in Stanley cultivar. The lowest value of the attack degree was calculated at the Romanian Gras cultivar with a value of 30.1% (Table 2).

Table 2. Fungus attack *Polystigma rubrum* and *Stigmina carpophila* on plum in Şoimari location, Prahova County, 2019

Cultivar	Pathogen / disease					
	Polystigma rubrum/ plum-leaf blister			*Stigmina carpophila*/ shot-hole disease of plum		
	F (%)	I (%)	DA (%)	F (%)	I (%)	DA (%)
Stanley	100	30	30	100	37	37
Anna Spath	100	25.2	25.2	100	32.7	32.7
Romanian Gras	100	20.5	20.5	100	30.1	30.1

Following the application of the treatments presented in table 1 it is observed that the value of the attack fell after the significant decrease of the intensity of the attack, the symptoms of the diseases being present in the trees analyzed (F = 100%). Thus, in the case of the observations regarding the attack of the micromycete *Polystigma rubrum* in the Stanley cultivar, the degree of attack was reduced to 9.2%. In the Anna Spath cultivar, the degree of attack was reduced to 8%, compared to the untreated variant at which GA = 25.2%. The Gras Romanian cultivar registered the lowest attack of *Polystigma rubrum* in following the application of the treatments having about 6%. And in the case of the attack of the pathogen *Stigmina carpophila* the intensity of the attack decreased considerably, reaching 8.7% at the Romanian Gras cultivar. Stanley and Anna Spath cultivar recorded an attack rate of 11.5% and 10.2% respectively. In the case of Stanley cultivar a more pronounced reduction of the attack was observed in the treated variant as compared to the control variant (Table 3).

Table 3. The influence of the *Polystigma rubrum* and *Stigmina carpophila* on plum in Şoimari location, Prahova County, 2019

Culti var	Variant Untreated (control)/ treatment	Pathogen/disease					
		Polystigma rubrum/plum-leaf blister			*Stigmina carpophila*/shot-hole desease of plum		
		F (%)	I (%)	DA (%)	F (%)	I (%)	DA (%)
Stanley	Control	100	30	30	100	37	37
	Treatment	100	9.2	9.2	100	11.5	11.5
Anna Spath	Control	100	25.2	25.2	100	32.7	32.7
	Treatment	100	8	8	100	10.2	10.2
Romani an Gras	Control	100	20.5	20.5	100	30.2	30.2
	Treatment	100	6.2	6.2	100	8.7	8.7

The efficacy of the treatments applied in combating the attack of the pathogens

Polystigma rubrum and *Stigmina carpophila* was also calculated and as shown from the data in table 4 the highest efficacy was registered in the Romanian Gras cultivar in combating the shot-hole disease of plum, at which E = 71.1%. Regarding the Stanley and Anna Spath cultivars, the value of the treatment effectiveness was 68.91% and 67.88%.

Regarding the efficacy of the treatments on the red spot attack of the plum-leaf blister, the highest value of the efficacy was calculated in the Romanian Gras cultivar with E = 69.75% followed by the Stanley cultivar with E = 69.33%. In the Anna Spath cultivar it had an efficiency of 67.88% (Table 4).

Table 4. Effectiveness of the treatments applied in the control of the pathogens *Polystigma rubrum* and *Stigmina carpophila* in Şoimari location, Prahova County, 2019

Cultivar	Pathogen / disease				
	Untreated variant (control)/ treatment	*Polystigma rubrum*/plum-leaf blister		*Stigmina carpophila*/shot-hole desease of plum	
		DA (%)	E (%)	DA (%)	E (%)
Stanley	Control	30	69.33	37	68.91
	Treatment	9.2		11.5	
Anna Spath	Control	25.2	68.25	32.7	67.88
	Treatment	8		10.5	
Romanian Gras	Control	20.5	69.75	30.1	71.10
	Treatment	6.2		8.7	

Research has shown that under the conditions of them in the area of Romanian Gras cultivar had a good response to the treatment scheme applied (Alexandru et al., 2019). The year 2019 presented in the research area in the spring a cold and humid weather that allowed the manifestation of the shot-hole disease of plum which is harmful in cold and humid spring although it may occur and cause significant losses at any time during wet and cold weather (Evans et al., 2008).

Research on the control of plum pathogens highlights the role of treatments in the integrated management of key diseases of this species (Popa et al., 2012; Cristea at al., 2017).

CONCLUSIONS

The frequency of the attack was maximum in the pathogens monitored in all experimental variants. The application of the treatments considerably reduced the attack on all the plum cultivar analyzed. Romanian Gras cultivar reacted best to applying the treatments recording the lowest values of the attack of the micromycetes *Polystigma rubrum* and *Stigmina carpophila*. The highest values of the efficacy of the treatments against the monitored pathogens were registered in the Romanian Gras cultivar with an efficiency around 70% followed by the Stanley cultivar with an efficiency of about 69%.

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MICROALGAE EFFECTS ON THE PHOTOSYNTHETIC PERFORMANCE AND GROWTH PARAMETERS OF BARLEY GROWN ON SOIL CONTAMINATED WITH PETROLEUM PRODUCTS

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Abstract

The aim of the present study was to evaluate the effect of microalgae suspension (MS) treatment on soil health and on the potential for phytoremediation. One of the strategies to alleviate soil toxicity, which has become increasingly popular in recent years, is bioremediation by inoculation with specific microorganisms. We tested the effect of a mixture of four microalgae strains (Scenedesmus incrassatulus, Trachydiscus minutus, Chlorella sp. and Phormidium sp.) on the specific physiological parameters of a model barley crop cultivated on petroleum-contaminated soil. Non-destructive methods of analysis were used to evaluate the state of the photosynthetic apparatus and the ability to carbon assimilation from the plants. The results clearly show that petroleum-contaminated soil adversely affects the growth and development of the model culture, while treating the soil with the microalgae suspension significantly mitigates the negative impact. This is supported by the better growth and photosynthesis observed in plants grown on microalgae-treated soil. Therefore, the application of microalgae is an environmentally friendly and environmentally oriented strategy for improving soil health in areas affected by petroleum pollution.

Key words: *microalgae, photosynthesis, soil contamination, soil remediation.*

INTRODUCTION

Environmental pollution has been gaining ground in recent times. The daily use of products from the chemical and petrochemical industries is one of the major environmental problems today. One reason is the fact that the oil-related components have been shown to act as carcinogens and neurotoxic organic pollutants (Das & Chadran, 2011; Hatami et al., 2018). The processes of natural purification of the affected soils are slow and take thousands of years. Soils contaminated with petroleum products pose a constant risk to human health as well as to the sustainable functioning of ecosystems. (Park & Park, 2011; Tang, 2019). Some soil microorganisms, which are of particular importance for the course of biogeochemical cycles and for the maintenance of soil fertility, are sensitive to the presence of oil-based contaminants. The latest have a significant impact on the distribution of the species in the microbial community and generally have a negative impact on its biodiversity (Sutton et al., 2013). All these problems provoke a serious scientific interest on the topic in the last years with the aim to develop technologies for safe and rational usage of such contaminated soils and their proper remediation (Chen et al., 2015). Classic methods to treat petroleum contaminated soil include excavating its upper layers and their removal for treatment by physical or chemical methods (Hans-Holgar & Alexander, 2000; Juck et al., 2000). However, these procedures, despite being relatively effective, are expensive and disturb the ecological balance at the treated sites (Liu et al., 2012). A sound alternative is offered by the bioremediation approach, which utilizes living organisms to remove the toxic agents in the polluted soil or to alleviate their negative effects (Das & Chandran, 2011). The main advantages of bioremediation are its non-invasiveness and the relatively low cost (April et al., 2000). It is well known that microalgae are important members of the microbial community in soil ecosystems, but there is little or no information available in the literature about their involvement in biodegradation of hydrocarbons. It is suggested that the presence of microalgae may favor the activity of oil-degrading bacterial strains. These microalgae/

bacteria consortia can clean different pollutants more efficiently than individual microorganisms (Subashchandrabose et al., 2011; Chen et al., 2015). For instance, inoculation with blue-green algae such as *Calothrix elenkinii* stimulated the phyllosphere and rhizosphere microbiomes of okra (Manjunath et al., 2016). A possible mechanism explaining the favorability of soil microbial communities in response to inoculation with blue-green algae is related to the production and excretion of exopolysaccharides from the latter. Exopolysaccharides secreted by many microalgae species provide organic carbon for the growth and development of beneficial microorganisms, leading to the formation of beneficial biofilms in the rhizosphere (Xiao and Zheng, 2016; Chiaiese et al., 2018; Xia et al., 2020). Their association with soil elements helps in the solubilization, mineralization, and bioavailability of macro and micronutrients, thus improving crop performance (Manjunath et al., 2016; Chiaiese et al., 2018). Soil contamination with petroleum is unfavorable for plant growth as well, due to the significant decrease in the available nutrients (Adam & Duncan, 1999) and the rise in the concentrations of certain elements such as iron and zinc to toxic levels (John et al., 2011). Germination and seedling establishment are especially vulnerable stages in the plant life cycle (Vange et al., 2004). The usual symptoms observed in plants cultivated on petroleum contaminated soils include erosion of the epicuticular wax, degradation of chlorophyll, general reduction of the photosynthetic activity and respiration, accumulation of toxic substances, size and biomass decrease, which in the case of crops leads to the consecutive loss of yield (Bona et al., 2011). The evaluation of the phytotoxicity is most often based on indirect methods like the assessment of the total yield loss in comparison to neighboring non polluted regions. Other readily accessible indicators of phytotoxicity are the data for seed germination, dry weight or similar biometric characteristics (Baud-Grasset et al., 1993). However, more accurate approaches utilize functional physiological and biochemical indicators as well, since germination efficiency and growth parameters by themselves do not provide sufficiently objective information. Such functional parameters include activities of the main antioxidant enzymes like peroxidases, catalase and superoxide dismutase, membrane integrity, changes in the photosynthetic parameters, etc. (Cartmill et al., 2014; Wyszkowska et al., 2015). Photosynthetic performance and chlorophyll fluorescence are integral processes characterized the ability of the plant to cope with the stress factors (Gao et al., 2019; Tomar & Jajoo, 2019). By measuring these indicators, the degree of soil pollution and the effectiveness of the remediation techniques used can be estimated.

In the light of these actual problems the major goal of this study was to perform a plant assay for barley (*Hordeum vulgare* L.), which combines growth and functional parameters, in order to better evaluate the toxicity of petroleum product polluted soils as well as to assess the possibility for potential soil recovery by treatment with nonsterile microalgae cultures.

MATERIALS AND METHODS

Plant cultivation

The experiments were carried out with barley (*Hordeum vulgare* L.) as a model culture, variety Veslets. Plants were cultivated on soil in four different variants: Soil 4.5% + MS (polluted with 4.5% content of petroleum products, supplemented with microalgae suspension); Soil 4.5% (polluted with 4.5% content of petroleum products, no supplementation with microalgae suspension); Control + MS (non-polluted, supplemented with microalgae suspension); Control (non-polluted, no supplementation with microalgae suspension).

Each of these variants was grown in three replicates five plants per replicate in the following controlled conditions: photoperiod 16/8 h (light/dark), 250 μmol/m/s photosynthetic photon flux density (PPFD), 26/22ºC day/night temperature and 60-65% relative air humidity. After 21 days of cultivation, the plants were subjected to analyses for determination of various physiological parameters.

For the variants with microalgae supplementation, before sowing the soil was irrigated daily with an inoculation mixture of 4 microalgae strains (*Scenedesmus incrassatulus*, *Trachydiscus minutus*, *Chlorella* sp. and *Phormidium* sp.) with a final concentration of 0.5 mg/ml for each of the species for a period of 5 days. Cultures of these four strains were previously isolated from an oil-spill contaminated site near Sofia, Bulgaria, and were therefore considered a suitable candidate for the evaluation of microalgae-assisted bioremediation of oil-contaminated soil. These strains were kindly provided by colleagues from the Bulgarian Academy of Sciences, the Institute of Algology. For the non-supplemented varieties the irrigation was carried out with water. Within the period of their cultivation the plants were subjected to the following watering regimes: the variants supplemented with microalgae suspension were given 50 ml daily per pot, with alternation of microalgae suspension and water. For the other variants was used only water with the same quantity. Both groups were additionally supplemented twice with 50 ml ½ strength of modified nutrient solution: 0.505 mM KNO_3, 0.15 mM $Ca(NO_3)_2{\times}4H_2O$, 0.1 mM $NH_4H_2PO_4$, 0.1 mM $MgSO_4{\times}7H_2O$, 4.63 mM H_3BO_3, 0.91 mM $MnCl_2{\cdot}4H_2O$, 0.03 mM $CuSO_4{\cdot}5H_2O$, 0.06 mM $H_2MoO_4{\cdot}H_2O$, 0.16 mM $ZnSO_4{\cdot}7H_2O$, 1.64 mM $FeSO_4{\cdot}7H_2O$, and 0.81 mM Na_2-EDTA.

Determination of growth parameters

The assessed growth characteristics include fresh and dry weight of the plants, leaf area, shoot height and root length. The absolute dry weight of the entire plants and their individual organs was measured after fixation of the material at 105°C for 30 min and consecutive drying at 80°C for 48 h. Leaf area was quantified with an electric digital leaf area meter NEO-2 (TU-Bulgaria).

Assessment of photosynthetic parameters

Net photosynthetic rate (A) was measured on the second developed leaf of the plants with an open photosynthetic system LCA-4 (Analytical Development Company Ltd., Hoddesdon, England), equipped with a narrow chamber. Chlorophyll fluorescence analysis was performed using a Handy PEA fluorimeter (Handy Plant Efficiency Analyzer, Hansatech Instruments Ltd., King's Lynn, UK).

Measurement of photosynthetic pigments

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) were extracted in 80% acetone, determined spectrophotometrically and calculated according to the formulae of Lichtenthaler (1987). Data are presented as mg pigments/g fresh weight (mg/g FW).

Statistical analyses

One-way ANOVA (for $P < 0.05$) was used for all experiments. Based on the ANOVA results, a Tukey's test for main comparison at a 95% confidential level was applied.

RESULTS AND DISCUSSIONS

To assess the overall influence of petroleum contamination on barley plants, initially biometric experiments to measure fresh weight, dry weight, root and leaf length, leaf area and total number of leaves were conducted. The results are presented in Table 1. It is clearly seen that soil pollution with oil products considerably reduced all growth parameters. The observed differences are all statistically significant with $P < 0.05$. This is in concordance with numerous previous studies from authors that report the negative effect of oil on crops (Anoliefo & Edegbai, 2000; Akaniwor et al., 2007). Interestingly, supplementation with microalgae suspension seems to improve the growth parameters both in normal and in stress conditions. In certain cases, like the root fresh weight and leaf area in clean soil (Table 1) the differences between the means of the samples are almost twice between the supplemented and non-supplemented variant. The variants subjected to petroleum contamination all parameters showed the lowest values compared to the control plants. The microalgae application alleviates this phenomenon as slightly improves the growth parameters. The results obtained from the growth analysis showed the inhibitory effect of petroleum contamination and are in accordance with the results of other authors (Odjegba & Sadiq, 2002).

Table 1. Biometric parameters of young *Hordeum vulgare* L. plants grown either on control non-polluted or polluted soil with petroleum products and treated or not with microalgae suspension (MS). FW - fresh weight; DW - dry weight; LA - leaf area

Variants	Roots			Leaves				
	cm	FW	DW	cm	FW	DW	LA cm^2	Leaf count
Soil 4.5% + MS	17.5c	0.52c	0.055b	29b	0.57bc	0.063c	30.45b	3
Soil 4.5%	14c	0.36c	0.041b	22.2c	0.26c	0.049c	29c	2.5
Control + MS	32.3a	1.35a	0.108a	41.5a	1.70a	0.179a	78.1a	4.5
Control	23.5b	0.72b	0.088a	32b	0.99b	0.129b	39.2b	3

The data in the columns followed by the same letter (a, b, c) are not statistically significant for $P < 0.05$.

To complement the data from the biometric experiments parameters indicative of the physiological status of the photosynthetic apparatus as well as the intensity of transpire-tion were determined (Table 2). These include photosynthetic activity (A), transpiration rate (E) and stomatal conductance (gs). The results unequivocally demonstrate that petroleum contamination significantly disturbs the photo-synthetic processes in those variants since all three parameters were reduced (photosynthesis - 7.66, transpiration - 3.27 and stomatal conductance - 0.127). In the control plants, differences between both supplemented and non-supplemented with microalgae variants were barely detectable. On the other hand, when grown in polluted soil the plants watered with microalgae suspension partially restored their photosynthetic activity (10.135), while the transpiration rate (4.38) and the stomatal conductance (0.205) reached the levels of the controls.

Table 2. Measurement of the photosynthetic activity (A), transpiration rate (E) and stomatal conductance (gs) in young *Hordeum vulgare* L. plants, grown either on control non-polluted or polluted soil with petroleum products and treated or not with microalgae suspension

Variants	A (µmol m^2/s)	E (mmol m^2/s)	Gs
Soil 4.5% + MS	10.135b	4.38a	0.205a
Soil 4.5%	7.66c	3.27b	0.127b
Control + MS	12.35a	4.61a	0.209a
Control	12.3a	4.59a	0.208a

The data in the columns followed by the same letter (a, b, c) are not statistically significant for $P < 0.05$.

Not surprisingly the subsequent quantification of the main photosynthetic pigments also demonstrated that their levels are much lower in the variants grown on contaminated soil (Table 3). However, the ratio of chlorophylls relative to carotenoids was slightly augmented in the plants cultivated on contaminated soil (4.52) in comparison to the controls (4.38). This means that in barley carotenoids are more sensitive to the presence of petroleum products than chlorophylls. Since the carotenoids are supported by the findings for the inhibited photosynthesis in contaminated variants (Table 2). Photosynthesis is a process dependent on the photosynthetic pigments' quantity and the ability of all involved molecules to absorb, transform and transport the energy. This can be specific molecules which acts as antioxidants and protect against oxidative damage, their low concentration in the contaminated variants (0.48; 0.46) suggest for oxidative stress due to a photo-oxidation or other reason related to the petroleum-contaminated soil. The supplementation with microalgae didn't show any considerable effects neither in normal nor in stressful conditions, with the exception of a small increase of chlorophyll *a* and chl *a*/chl *b* ratio. These results and speculations are assessed by measuring chlorophyll fluorescence, one of the main markers for photosynthetic integrity and function (Paunov et al., 2018; Gao et al., 2019).

Table 3. Measurement of the photosynthetic pigment quantity in young *Hordeum vulgare* L. plants, grown either on control non-polluted or polluted soil with petroleum products and treated or not with microalgae suspension

Variants	Chl *a* (mg/gFW)	Chl *b* (mg/gFW)	Car (mg/gFW)	Chl*a*/Chl*b*	Chl/Car
Soil 4.5% + MS	1.50b	0.67b	0.46b	2.24b	4.76a
Soil 4.5%	1.43b	0.74b	0.48b	1.92c	4.52a
Control + MS	2.30a	0.84a	0.69a	2.74a	4.55a
Control	2.20a	0.82a	0.69a	2.68a	4.38b

The data in the columns followed by the same letter (a, b, c) are not statistically significant for P < 0.05.

The most common and widely used chlorophyll fluorescence analyses were performed on dark- and light-adapted leaf samples and subsequently different parameters characterrizing the steady-state status of the photosynthetic apparatus were calculated.
As presented in Table 4, petroleum contamination disturbs the energy migration from the antenna complexes to the chlorophyll of the reaction centers which leads to an increased minimal chlorophyll fluorescence emission in dark-adapted objects (Fo = 760). The lowest fluorescence (Fo = 595), as well as the highest quantum yield (Y = 0.776), were detected in the control plants treated with microalgae suspension. Similarly, the electron transport rate (ETR) calculated in light-adapted plants had the biggest value in the control + MS sample. This demonstrates that supplementation with MS has a positive impact on PSII photochemistry even in normal conditions. On the other hand, the addition of MS to contaminated soil apparently induces a rescue effect on the Y parameter, which was restored to the levels of the controls (a transition from 0.698 to 0.764). An interesting result is that MS treatment actually leads to a decrease of ETR in barley plants on contaminated soil (ETR = 18.7). A possible explanation could be that the MS suspension stimulates the plants' protective mechanisms, including lowering of the ETR, in order to prevent additional stress.

Table 4. Measurement of chlorophyll fluorescence parameters in young *Hordeum vulgare* L. plants, grown either on control non-polluted or polluted with petroleum products soil and treated or not with microalgae suspension. Fo - minimal fluorescence in dark adapted plants, Fm - maximal fluorescence in dark adapted plants, Y - quantum yield, F' - minimal fluorescence in light adapted plants, Fm'- maximum fluorescence in the light adapted plants, ETR - electron transport rate

Variants	Dark adapted			Light adapted			
	Fo	Fm	Y	F'	Fm'	Y	ETR
Soil 4.5% + MS	611b	2577b	0.763a	296d	817c	0.64a	18.7c
Soil 4.5%	760a	2525c	0.698b	337c	835b	0.60b	27.1b
Control + MS	595c	2664a	0.776a	342b	841a	0.59b	31.6a
Control	635b	2514d	0.747a	372a	660d	0.44c	27.2b

The data in the columns followed by the same letter (a, b, c, d) are not statistically significant for P < 0.05.

Bioremediation applies microorganisms, especially bacteria and fungi to remove soil contaminants or break them down into harmless compounds via, for instance, mineralization during which contaminants are used to produce carbon and energy. Phytoremediation removes contaminants from the environment by using plants and their micro-symbionts (Tang, 2019). The rationale behind the current research is investigating the possibility for establishment of productive plants/microorganisms symbiotic interactions in problematic areas contaminated with petroleum products. This would in turn stimulate and accelerate the soil detoxification by biological means and lead to future enhancement of the crop yield. The plant species chosen as a model for the study was barley because of its good growth in controlled conditions and because of a report that barley could be a good marker for phytoremediation of contaminated areas (Asiabadi et al., 2014). Growth and physiological parameters were evaluated both when plants were cultivated by themselves and when a mixture of 4 microalgae species was added. Microalgae pose numerous advantages as remediation agents since they have relatively low nutrient requirements, grow fast and produce a lot of biomass due to their autotrophic metabolism, and rarely produce toxic byproducts (Kumar & Oommen, 2012). Moreover, these organisms have been already shown to be effective for other kinds of soil pollution, for example with heavy metals (Suresh & Ravishankar, 2004).

One of the interesting observations in the present study was that the improvement of the photosynthetic parameters in the contaminated samples supplemented with microalgae was not due to higher quantities of photosynthetic pigments since the contents of the latter remained unaffected (Tables 2 and 3). Therefore the influence of the presence of microalgae seems to be at the functional, not the structural level, indirectly leading to increased efficiency of photosynthesis, most probably due to alleviation of stress symptoms.

In conclusion, the supplementation with microalgae suspension had a positive effect on the growth and development of the barley plants cultivated on polluted soil. This is shown by the increase in the growth parameters and the overall boost of photosynthesis. Therefore, inoculation with nonsterile microalgae cultures appears to be a promising approach to complement and accelerate phytoremediation in areas affected by oil spills. Follow-up studies on the topic would focus on the development of suitable approaches to inoculate bacterial/microalgae cultures in affected soils, either on their own or by specific vectors, as well as comparison of the performance of barley and other plant species in order to select the most appropriate candidates to overcome the negative effect of petroleum contamination and to quicken soil recovery.

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MARKET RESEARCH ABOUT AGRISO MOBILE APPLICATION FOR FARMERS

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Abstract

Nowadays, the use of smartphones has multiplied the utility of the phone, making it an extremely useful tool for different purposes. From young to old, the mobile device became extinct of a hand full of information from school, family, social, online shops, analysis laboratories, etc. Starting from their own experience and needs to exploit mobile devices, AgriSo mobile app developers created an application structured to help farmers have access to land much easier, oversee remote work running on their land, fuel used equipment when work but even when stationary with the engine running. AgriSo also provides information on the ground, how much need to increase humidity or lower it because the plantation to have a better result, define type of soil and what can be cultivated on it. Based on this application we have done a survey whose results are presented in the paper.

***Key words**: AgriSo, market research, mobile devices.*

INTRODUCTION

Mobile applications are useful and can make life easier. Apps nowadays can help us to solve decision processes more easily and in the simplest way. Mobile applications can be used by anyone: pupils, students, or people from various fields, with different occupations. As examples of applications created for different domains, we have:

- Tripadvisor - tour guide;
- Weather - weather;
- Metro and Feroviar - for public transport;
- Google maps - orientation in space;
- Google Translate - for translations;
- Social media phone applications: Skype, Facebook, WhatsApp, Telegram, etc.

In Romania, we meet six types of applications used:

- A farm management application: AgriSo;
- A transport stock Exchange: Kropapp;
- Monitoring of real-time: crop Agricloud;
- A temperature control in the silo: Silometer;
- A detection of diseases by drones: Serafim Drones;
- Satellite culture surveillance: Geosys.

In this paper, we focused on the application "AgriSo", created by two young people, intending to easily track agricultural land and monitoring problems to quickly make decisions regarding the good functioning of crops.

AgriSo - agriculture from the land to satellite

The application has been designed precisely to help farmers gain access to land more easily, to remotely monitor work on their land, the fuel used by the machines at work, and even when stationary with the engine running. "AgriSo" also provides information about the field, how much moisture needs to be raised or it needs to be low for the planting to produce the best possible result, tells us the type of soil and what we can cultivate on it.

The "AgriSo" application is tested by several farmers in Romania, Ireland, Ukraine and South Africa.

The utility of the AgriSo application:

- can be done satellite or drone scans where we can see problem areas and improve them;

- this is the application that monitors fuel consumption using satellite technology. This can help a farmer reduce costs by 25%;
- this is a digital farm management application to help improve the production processes of the crop sector for both small and large farms,
- we can also track production flow;
- we can make a check on the agricultural land,
- the fuel consumption can be checked at each technological stage.

For instance, in Figure 1, we show how we can be observed plantations according to what we grow on them.

Yellow plantations are those on which wheat has been grown and green plantations on which rape has been grown.

The intensity of the color shows the evolution of the plantation thus, we can see how it has developed and we can tell when we can harvest it.

Figure 1. Screenshot-Satellite view Agricultural Land

In our study, we want to gain information about the level of knowledge regarding the AgriSo application and the wish to use this application by the young population of farmers and future farmers.

MATERIALS AND METHODS

As we wanted to carry out market research on knowledge and use of the AgriSo application developed for farmers, we conducted our study through the basic survey tool (questionnaire).

The usefulness of the questionnaires is provided by different categories of information: the prioritization of needs, preferences, attitudes, consumption or purchasing habits, consumer motivations, and so on.

Also related to utility, reference is made to the persons interviewed to whom the categories of information mentioned may be associated. Thus, information on certain market phenomena or processes is circumscribed to the specific characteristics of the reference authorities.

For example, for the population, it is possible to collect information at the level of the various segments depending on sex, age, education, occupation, geographical area, residence environment, income achieved, structure and size of the household.

This data can be obtained more easily via the Internet.

The information obtained from the questionnaires is quantitative and qualitative and can be detailed according to certain criteria to allow a thorough knowledge of market aspects that cannot be addressed based on data from secondary sources.

In the case of our questionnaire, its administration was mostly done online through the iSondje.ro service.

The questions to which our questionnaire was intended to be answered were those who own agricultural businesses or who will take over the family business being in this area because the application studied is strictly logged into the location of the business in the field and provides all the data related to the state of the crop in that area.

The method used is a quantitative method related directly to an online survey.

RESULTS AND DISCUSSIONS

The size of the sample to be researched shall be determined by the degree of homogeneity of the Community, the extent of the probability error and the likelihood by which the results obtained from the time involved in the data collection are guaranteed and by the funds involved in the research.

If we consider the probability used to guarantee the results to be 95% ($z=1.06$), then for error = $\pm 5\%$, the volume of the sample will be:

$$n = \frac{z^2 \times p(1-p)}{\Delta_w^2} = \frac{1{,}06^2 x 0{,}5(1-0{,}5)}{0{,}05^2} = 112\ persons$$

Statistics of respondents

Our questionnaire started with 112 people, but 16 people did not want to mark any of the answers to question 3, so our questionnaire continued with 96 interviewees.

Criteria	Characteristics of the respondents
Male	68
Female	28
Total respondents	96
Average age	25.8 years

The first question was about the nature of the occupation because we want to know what are doing the respondents on our study, so we find out that most of the responders were students, followed by the employee, as it can see in Figure 2.

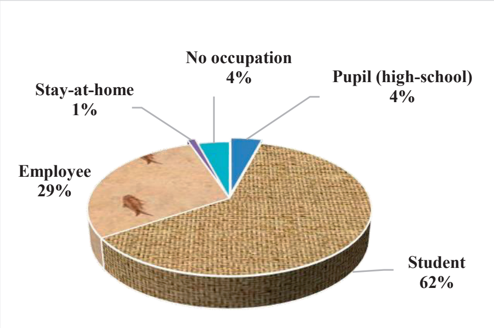

Figure 2. Respondents' nature of occupation distribution

The second question regards the education level, to understand if our respondents (not those who are students in the interview time) have a high-level education in the background, or plenty of work and life experience, or a mixture of those elements (Figure 3).

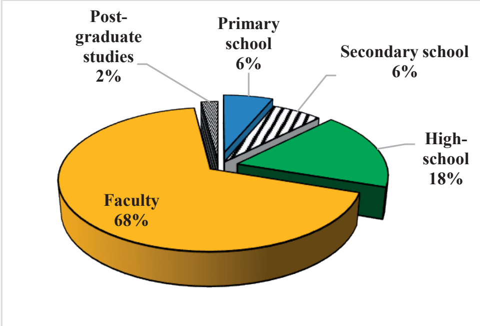

Figure 3. Respondents' education level distribution

When our respondents came on question number 3, from various motives, some of them do not want to answer. So, we adjust our questionnaire from 108 to 96 interviewees.

The sensitive question was about monthly incomes, and like we have shown the answers in Figure 4 most of them, over 70% have incomes under 2,500 lei.

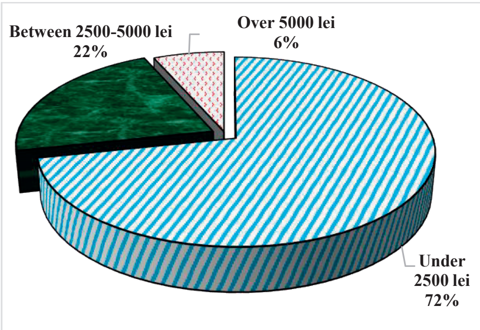

Figure 4. Respondents' education level distribution

The last demographic question is bounded to the area of living. So we divided into three areas such as urban, suburban and rural (Figure 5). We introduce the sub-urban element to gain data about the influence of the urban environment on the rural environment.

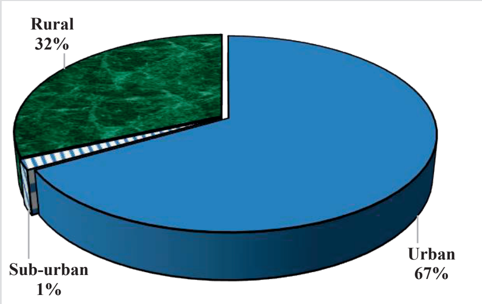

Figure 5. Respondents' area of living distribution

With the fifth question, we reach one of our items, to gain information about the level of knowledge regarding the AgriSo application, as a useful instrument in farm management. So, at the question "Do you know the AgriSo application" we have to determined answers Yes or No. The distribution of those answers is shown in Figure 6.

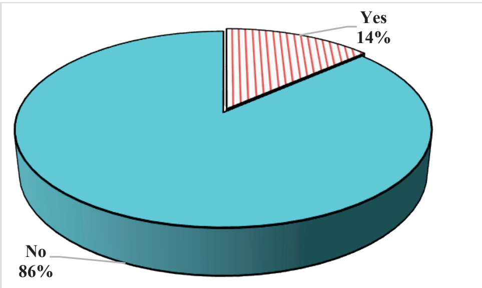

Figure 6. Respondents' Yes or No knowledge about AgriSo app distribution

We go on the next level and ask further, what respondents believe: AgriSo can be (or is) a useful application for agriculture or not (Figure 7).

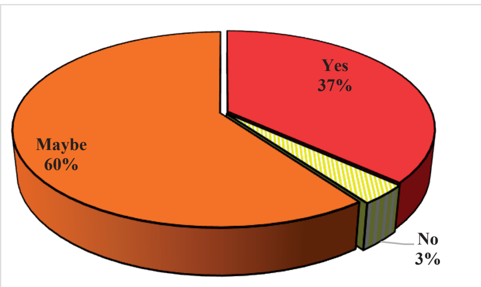

Figure 7. Respondents' answers for AgriSo - a useful app for agriculture distribution

This time we reach the second goal. Did the young farmers and future farmers wish to use the AgriSo application? The data gained is presented below in Figure 8.

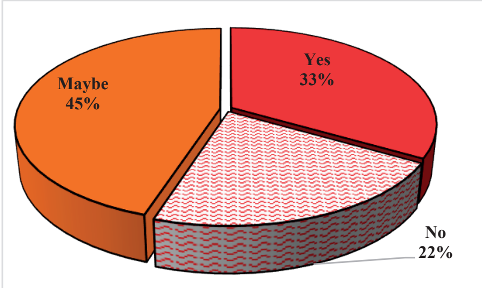

Figure 8. Respondents' wish to use the AgriSo app distribution

The next question is designated to verify if some of our respondents already use this application as an instrument in their farm management (Figure 9).

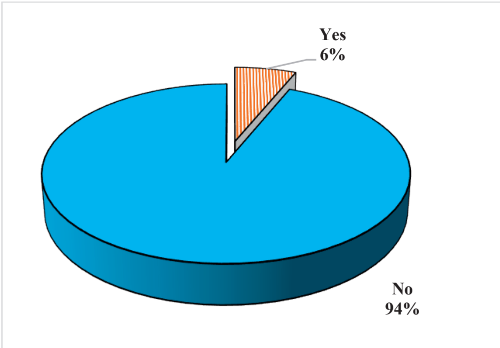

Figure 9. Respondents' that already use the AgriSo application

Of course, we want to know the opinion about the difficulty level in using this application. In Figure 10 we present the distribution of answers between simple, easy to use and complicated, difficult to use.

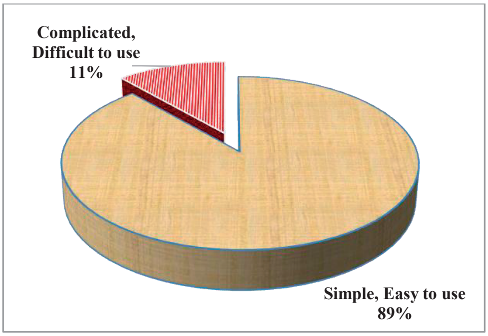

Figure 10. Respondents' opinion about the difficulty level of the AgriSo application

The last question ends in just ten ones in our interview. Most of the respondents consider that the AgriSo application can help them to grow more and more in their business (Figure 11).

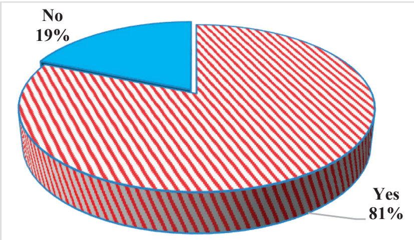

Figure 11. Respondents' opinion about AgriSo application as a useful instrument in farm management

CONCLUSIONS

We are at a time of rapid change in all domains. Our study shows the increased interest of young people in the AgriSo application shows a possible desire of young people to make their own business in the agricultural sector to which to apply knowledge from faculty and the farm monitoring technology through the AgriSo application.

The processing of demographic data showed that 68% were university-level respondents, while the lowest percentage was registered for post-graduate, secondary or primary school persons, 72% were those with monthly income below 2500 lei, only 6% of the respondents are over the income of 5000 lei. In urban areas, the majority of respondents live, 32% are rural residents and the difference of up to 100% is represented by people living in satellite locations near urban centers.

Only 14% of respondents know the AgriSo application, so this questionnaire also allowed those who did not know it to have basic information about this application.

37% believe that the AgriSo application is useful for agriculture, while 60% are undecided individuals who are expected to look further into this application and give a decisive response later. The high percentage of undecided people give a similar answer to the next question, about 45% may use the AgriSo application, while about 33% will certainly use it.

Regarding the application interface, 89% thought the application is simple and easy to use. 81% of respondents consider that the AgriSo application is a real aid to the activity (business) they have or intend to do, this shows the favorable opinion the interviewees have on the application being studied. The interviewees also emphasized that the application can be a future application, help us in the field of agriculture and help us to grow more and more even in a very easy and possibly less expensive way.

At the moment some of the people who responded to our study do not benefit from this application, but possibly after completing their studies those who want an agricultural activity will access the application that will certainly thank them, as we can see in the questionnaire, they marked in a number quite large that they like the application.

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THE DISTRIBUTION AND STRUCTURE OF THE PLANT COMMUNITIES FOUND IN THE DOBRICENI AND JGHEABURI FORESTS OF THE GOVORA RIVER BASIN, ROMANIA

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Abstract

The thematic area provided in this paper is situated in the Govora river basin, Vâlcea County. Within this study had been aimed the identification, description, diversity, ecological analysis and monitoring of the herbaceos and wooden plant communities, which belong to the Natura 2000 habitats and implicitly of the rare plant species, vulnerable, endemic whitin Dobriceni and Jgheaburi Forests from the Govora river basin. In this area we identified the following plant communities: Hieracio rotundati-Fagetum (Vida 1963) Täuber 1987 (syn.: Deschampsio flexuosae-Fagetum Soó 1962); Stellario nemorum-Alnetum glutinosae (Kästner 1938) Lohmeyer 1957; Quercetum petraeae-cerris Sóo (1957) 1969 and Carpino-Fagetum sylvaticae (Paucă 1941). According to the targets of this research, a very important place we gave to the complex study of the habitats: 91M0 Pannonian-Balkanic turkey oak sessile oak forests (CLAS. PAL.: 41.76); 9110 Luzulo-Fagetum beech forests (CLAS. PAL.: 41.11.); 91E0 Alluvial forests with Alnus glutinosa and Fraxinus excelsior (Alno-Padion, Alnion incanae, Salicion albae) (CLAS. PAL.: 44.3, 44.2 şi 44.13.) and 9130 Asperulo-Fagetum beech forests (CLAS. PAL.: 41.13). Considering the place where the study had been located to, the eco-pedo-climatic conditions and the anthropic term exerted I have considered that is necessary to develop some ecological studies (and using statistical methods (UPGMA si WPGMA, STYN-TAX 2000) for the most important plant community - Hieracio rotundati-Fagetum (Vida 1963) Täuber 1987 from this area.*

Key words*: Govora river, plant communities, forest, diversity, habitats.*

INTRODUCTION

The flora and the vegetation of a territory, besides its scientific importance, also presents a significant bioeconomic value, if we take into consideration its use in the economic-household activities (Niculescu, 2009). The vegetal carpet for this area of Oltenia in is a reflection of the very diverse stationary conditions, adding to a certain extent to the influence of the anthropo-zoogenic factors. The forests grow on large areas in Oltenia. They have a great importance in terms of biodiversity, but they also have a eco-pedo-genetic and economics role (Niculescu and Nuță, 2018).

MATERIALS AND METHODS

Study area

The territory under research is located in the Govora river basin, part of the SubCarpathian Area of Oltenia, in the Dobriceni Forest and Jgheaburi Forest, Valcea County.

The forest plant communities from this area have been analyzed and characterized from the chorological, ecological point of views. We also examined the floristic composition and physiognomy of the plant communities found in the phytocoenoses structure.

For the study of the plant communities we have used methods of phyto-sociologic research characteristic to the Central European phyto-sociologic School, which was based on the principles and methods elaborated by J. Braun-Blanquet (1926). Regarding the classification of plant communities it will be used the synthetic work written by J. S. Rodwell et al. (2002) and Applied Vegetation Science, Vol. no. 19 by Mucina et al. (2016). The quantitative assessment of the participation of every species to the vegetal association was performed with the help of the abundance-dominance index, according to the Braun-Blanquet scale. We gave a special attention to the calculation of the quantitative index *Bray-Curtis* (1957) and to performing the dendograms, by using the Group-Average method (UPGMA) in the program SYN-TAX 2000 (for the plant communities with minimum 10 relevées).

So, to identify the habitats, we looked into: *Natura 2000 in Romania Habitat fact sheets* (2008); *Habitats from Romania* by Doniţă N. et al. (2005); *Manual interpretation of Natura 2000 Habitats in Romania* by D. Gafta & O. Mountford - coord. (2008); and *Interpretation Manual of European Union Habitats* - EUR27 (European Commission. DG Environment. Nature and biodiversity, 2007) (Niculescu, 2016).

RESULTS AND DISCUSSIONS

In this area we identified the following plant communities: *Hieracio rotundati-Fagetum* (Vida 1963) Täuber 1987 (syn.: *Deschampsio flexuosae-Fagetum* Soó 1962; S*tellario nemorum-Alnetum glutinosae* (Kästner 1938) Lohmeyer 1957; *Quercetum petraeae-cerris* Sóo (1957) 1969 and *Carpino-Fagetum sylvaticae* Paucă 1941. According to the targets of this research, a very important place we gave to the complex study of the habitats: 91M0 *Pannonian-Balkanic turkey oak sessile oak forests* (CLAS. PAL.: 41.76); 9110 *Luzulo-Fagetum beech forests* (CLAS. PAL.: 41.11.); 91E0* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*) (CLAS. PAL.: 44.3, 44.2 şi 44.13.) and 9130 *Asperulo-Fagetum beech forests* (CLAS. PAL.: 41.13).

1. Ass. *Hieracio rotundati-Fagetum* (Vida 1963) Täuber 1987 (syn.: *Deschampsio flexuosae-Fagetum* Soó 1962) (Figure 1).

Distribution: The phytocoenoses of this plant community are situated in the Jgheaburi and Dobriceni Forests developed on poorly inclined to strongly inclined lands on acid brown soil. In the Jgheaburi forest the phytocoenoses it grows on very steep slopes.

Physiognomy and floristic composition. In the phytocoenotic composition of this plant community, apart from species *Fagus sylvatica and Hiercacium rotundatum*, there are also constant many species that belong to the coenotaxa: QUERCO-FAGETEA and FAGETALIA: *Carpinus hetulus, Poa nemoralis, Galium odoratum, Anemone ranunculoides, Helleborus purpurascens, Galium odoratum, Prenanthes purpurea, Actaea spicata, Dentaria bulbifera, Sanicula europaea,Brachypodium sylvaticum, Euonymus europaeus, Circea lutetiana, Lilium martagon, Galeopsis speciosa.*

The coverage of trees is good 75-85%. The herbaceous and bush cover layer are developed.

Figure 1. *Hieracio rotundati-Fagetum* (Vida 1963) Täuber 1987 plant coommunity

The winds, which are mainly influenced by the relief, can lead to destructions on large areas in this region. Such destructions were made in the last years in the Jgheaburi Forest (Figure 2).

Due to the difficult access in the area, these damages could not be totally cleared, and the collecting of the fallen trees was made through non-ecological procedures, thus considering them the consequence of anthropic impacts.

Figure 2. Anthropic impact in the Jgheaburi Forest

This plant community is part of the important Natura 2000 habiatat - 9110 *Luzulo-Fagetum beech forests* (CLAS. PAL.: 41.11.).

We paid much attention to the determination of the Bray-Curtis quantitative index, Euclidian index using the UPGMA method, and Jaccard index, using the WPGMA method and the achievement of dendrograms, using the program SYN-TAX 2000 (for the plant community with minimum 10 relevées).

In the UPGMA dendrogram, using the Bray Curtis index for this forest plant community, there are pointed out also 2 distinct clusters. In the first sub-cluster there are grouped relevées 1, 2, 8, 7, 4, 5, 10 and 9, especially due to *floristic*.

The latter clusters' relevées are grouped the relevées 3 and 6 - on high dominant values, due to the abundance of *Sambucus nigra* (abundance-dominance (AD) 2).

The values of the Bray-Curtis quantitative index reflecting the heterogeneity of the floristic structure of the phytocoenoses of the plant community with *Fagus sylvatica* from the Jgheaburi Forest (Figure 3).

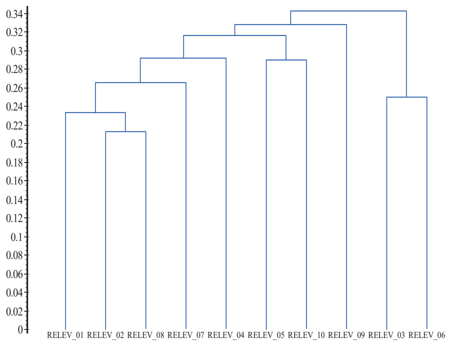

Figure 3. The dendrograma (UPGMA method, Bray-Curtis index) of *Hieracio rotundati-Fagetum* (Vida 1963) Täuber 1987 plant community

The dendrogram made using the Group-Average method (UPGMA) and the Euclidian index, highlights the separation of two distinct clusters, which, as it can be observed quantitative index values are very close.

In the first cluster we can notice the separation of the relevé 6 based on the present of the species *Lilium martagon*, absent in all the other relevées.

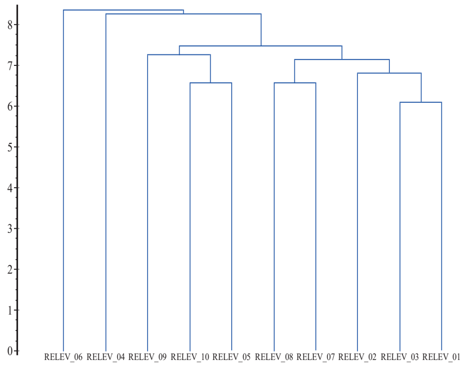

Figure 4. The dendrograma (UPGMA method, Euclidian index) of *Hieracio rotundati-Fagetum* (Vida 1963) Täuber 1987 plant community

The WPGMA method and Jaccard index, there can be noticed the grouped the relevées 8 and 2 relevées, from the rest of the relevées, based on the similarity of the phytocoenoses (Figure 5).

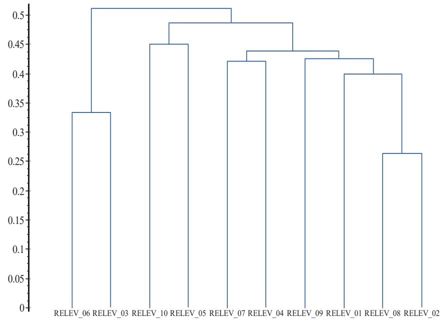

Figure 5. The dendrograma (WPGMA method, Jaccard index) of *Hieracio rotundati-Fagetum* (Vida 1963) Täuber 1987 plant community

2. Ass. S*tellario nemorum-Alnetum glutinosae* (Kästner 1938) Lohmeyer 1957

This plant community have been analyzed and characterized from the chorological, ecological, phytosociological point of views.

Distribution: The phytocoenoses of this plant community are situated in the Govora river basin, in the Dobriceni Valley, Pietroasa Valley, Cacova Valley in Stoenești, Gruiu, Neghinești and Dobriceni settlements.

From an ecological point of view, in this phytocoenoses prevail the mesophytes, meso-hygrophytes, micro-mesothermic and poorly acido-neutrophyle elements.

Physiognomy and floristic composition. In the phytocoenotic composition of this plant community, apart from species *Alnus glutinosa* and *Stellaria nemorum* there are also constant many species that belong to the: Alnetalia, Alno-Ulmion and Querco-Fagetea.
The coverage of trees is 70-75%. The herbaceous and bush cover layer are developed. It should be noted that these phytocenoses are strongly ruderalized and degraded due to zoo-anthropogenic factors.
3. Ass. *Quercetum petraeae-cerris* Sóo (1957) 1969
This plant community it is found on the small areas, sometimes island surfaces, in Dobriceni Forest. The phytocoenoses develops on compact forest brown soils, strongly podzolite, with a high percentage of carbonates.
Physiognomy and floristic composition. The phytocoeonoses of this plant community have a special composition, being characterised by the dominance of the species: *Asparagus tenuifolius*, *Geum urbanum*, *Poa nemoralis*, *Glechoma hirsuta*, *Melica uniflora*, *Polygonatum odoratum, P. latifolium*, *Galium scultesii, Lathyrus niger*, *Potentilla micrantha*, *Lychnis coronaria*, *Carex michelii*, *Lithosperumum purpureocaeruleum*, *Carex tomentosa*, *Festuca valesiaca*, *Euphorbia cyparissias*, *Vincetoxicum hirundinaria*, *Carex caryophyllea*, *Stachys officinalis.*
4. Ass. *Carpino-Fagetum sylvaticae* Paucă 1941.
Distribution: This type of forest is more common in the Stoienești area, on the hilly floor. The phytocoenoses develops on slopes (generally shaded) slightly-inclined medium, with different exposures, peaks and plateaus on the eutricombosoil.
Physiognomy and floristic composition.
The important and characteristic species for the pthycoenosis of tis plant community are: *Fagus sylvatica* ssp. *moesiaca, Carpinus betulus, Corylus avellana, Crataegus monogyna, Euonymus europaea, Galium odoratum, G. schultesii, Dentaria bulbifera, D. glandulosa Lathyrus venetus, Carex pilosa, C. brevicollis, C. sylvatica, Corydalis cava* ssp. *marschaliana, Brachypodium sylvaticum, Mercurialis perennis, Asarum europaeum, Anemone nemorosa, A. ranunculoides, Alllium ursinum, Lamium galeobdolon, Melica uniflora, Milium effusum, Aposeris foetida, Erythronium dens-canis* ssp.*niveum.*
Important species*: Neottia nidus-avis, Platanthera bifolia, Dentaria quinquefolia, Erythronium dens-canis* ssp. *niveum.*
We can mention it here some undesirable species which disturb the good development of the phycoenoses of this plant community: *Phellinus igniarius* Quel.), *Nectria galligena* Bres., *Xyleborus saxeseni, Trypodendron domesticum, Hylecoetus dermestoides, Taphrorynchus bicolor, Xyleborus monographus, Lymantria monacha*.

CONCLUSIONS

Following the botanical research, carried out in the upper basin of the Govora River, in the Dobriceni and Jgheaburi Forest between, there were identified for forest plant communities: *Hieracio rotundati-Fagetum* (Vida 1963) Täuber 1987 (syn.: *Deschampsio flexuosae-Fagetum* Soó 1962); *Stellario nemorum-Alnetum glutinosae* (Kästner 1938) Lohmeyer 1957; *Quercetum petraeae-cerris* Sóo (1957) 1969 *and Carpino-Fagetum sylvaticae Paucă 1941*.
The forest plant communities found in this area develops at the hilly level and the lower mountainous sub-level.
Regarding the biodiversity found in the forest phytocoenoses of this area, we can say that it is of particular interest in potential, variety, in the presence of many rare endangered and endemic species or Nature 2000. The forest plant communities from tus from Dobriceni and Jgheaburi Forest are part from important Nature 2000 habitats and are of the most diverse ones. Given the scientific, landscape, economic and social importance of this area we think that study is a must, therefore establishing for the future preservation measures.

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IMPORTANCE OF LONG-EARED OWL (*Asio otus* L.) IN RODENT REGULATION NUMBER IN URBAN AREAS

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Abstract

The winter trophic spectrum of two Asio otus colonies from Chisinau city was studied. There were collected and analysed 1432 pellets form the first colony (Ciocana district, 60 individuals) and 528 pellets from the second colony (Botanica district, 22 individuals). After the quantitative and qualitative analysis of the pellets 3567 individuals in Ciocana pellets and 1584 individuals in Botanica pellets were identified. The long-eared owl diet in both sites consists mostly of rodents - more than 90% of all identified animals, the birds constituted 4.93% and 3.35%, the shrews and the bats constituted less than 2%. The main prey of A. otus were the Microtus species, which constituted 61.9% in Ciocana pellets and 63.38% in Botanica pellets, followed by Apodemus sylvaticus (14.02% and 17.99%) and by the Mus species with 12.42% and 14.21%, respectively. Other rodent species registered less than 5%. Thus, the trophic spectrum of long-eared owl in winter period in urban area consists mostly of field vole. The proportion of synanthropic pest species Mus is rather high, which prove the huge importance of long-eared owl trophic activity in urban ecosystems.

Key words: *long-eared owl, urban ecosystems, trophic spectrum, rodents, biological control.*

INTRODUCTION

The long-eared owl (*Asio otus* L., Figure 1) is a sedentary bird and one of the most widespread nocturnal predators in Europe (Birrer, 2009). In cold period of the year the density of species increases due to the migrant individuals from the northern regions and they form rather large colonies of several tens of individuals. The hunting sectors of the long-eared owl are open type biotopes, including agricultural lands, where they hunt mainly rodents and occasionally birds, shrews and bats. After the digestion process the owls regurgitate as pellets the indigestible remains of consumed animals (bones, hair, feathers, fur). The analysis of pellets can provide data on the diet of the prey bird, the fauna of small mammals in a certain area, their density, their dynamics, etc. The long-eared owl is well adapted to anthropic environment and its wintering colonies are frequently registered in urban localities (Zubcov, 1986; Romanowski, 1988; Mori & Bertolino, 2015).

Many rodent species are important pests of agricultural crops, including in urban areas. Taking into consideration the huge importance of long-eared owl trophic activity in biological control of rodent pest species, especially in winter period, its diet was rather well studied in many regions of Europe (Goszczynski, 1977; Nilsson, 1981; Wijnandts, 1984; Tome, 1994; Galeotti & Canova, 1994; Romanowski & Zmihorscki, 2008; Birrer, 2009 etc.), as well as in the Republic of Moldova (Averin & Ganea, 1966; Anisimov, 1969; Zubcov, 1981; Nistreanu et al., 2015a; 2017). There are several studies concerning the long-eared owl's diet in urban areas (Barbu & Barbu, 1972; Barbu & Korodi Gal, 1972; Murariu et al., 1991; Laiu & Murariu, 1998; Banaru & Coroiu, 1997; Riegert et al., 2009, Sharikov et al., 2009; Sandor & Kiss, 2004; Kiat et al., 2008) and only few studies in the Republic of Moldova (Anisimov, 1969; Zubcov, 1986; Nistreanu et al., 2015b).

Figure 1. Long-eared owl (*Asio otus*) individuals from Ciocana site

The long-eared owl usually shows strong preference for *Microtus* voles, but in urban areas it hunts in open type biotopes within and outside the city, using also alternative prey (Pirovano et al, 2000; Kiat et al., 2008; Sandor & Kiss, 2008; Riegert et al., 2009).
The aim of the paper is the analysis of winter diet of two long-eared owl colonies located in opposite zones of Chisinau city and highlight the importance of trophic activity of the species in urban area.

MATERIALS AND METHODS

The studies were performed in winter periods of 2011-2012 in Chisinau city. Chisinau is the largest city of the Republic of Moldova, with the surface of 123 km^2, situated at the altitude of 82 m, coordinates 47^{0}01' N 28^{0}52' E. The climate is humid continental with warm summers and cool, windy winters. The winter period lasts 78-80 days. The average temperature in winter is -2.3°C and the minimum temperature during January and February can drop below -20°C.
One colony of long-eared owl (*Asio otus* L.) of 60 individuals was located in a courtyard of a school from Ciocana district (47.041N, 28.883E) with several dozens of tall coniferous and deciduous trees (*Picea abies, Populus nigra, P. tremula, Salix alba*) suitable for long-eared owl individuals. The second colony counting 22 individuals was located in Botanica district (46.989N, 28.866E) with tree vegetation of *Thuja orientalis, Picea abies, Populus nigra*. Both locations are situated within the city limits in heavily urbanized areas.
There were collected and analysed 1268 pellets form the first colony and 528 pellets from the second colony. Each pellet was measured, weighed and unfolded. The bone fragments were cleaned and sorted into categories. Small mammal species were determined according to cranial bones and dentition (Pucek, 1981; Popescu & Murariu, 2001). The sibling species *Microtus arvalis* and *M. rossiaemeridionalis*, *Mus musculus* and *M. spicilegus* that can't be differentiated morphologically were considered as genus *Microtus* and *Mus*, respectively.
The ecological analysis of the prey species was performed using the indexes of abundance (A = no*100/N, where no - number of individuals of a species, N - total number of individuals); frequency (F = nop*100/N, where nop - number of pellets with certain species, N - number of pellets); total biomass of consumed prey (B = no*G, where no - number of individuals of a species, G - mean weight of one individual). The mean weight of prey individual was calculated from our own data gathered during many year studies of mammals and birds. The trophic niche breadth was estimated using the *B* Levins' index: $B = 1/\Sigma p^2$, in its standardized version *Bs*: Bs = (B-1)/(n-1), where p is the fraction of items in the diet, and n is the number of possible food categories (Levins, 1968; Hulbert, 1978). Bs ranges from 0 (100% utilization of a single food category) to 1 (equal use of all categories).
During the study no animal was injured or sacrificed.

RESULTS AND DISCUSSIONS

In the first colony the pellet length varied from 1.55 cm to 8.34 cm, mean length of 4.03 cm. The pellet weight varied between 0.9 g and 7.2 g, mean - 2.71 g. The number of individuals per pellet was from 1 to 7, mean - 2.56 individuals. In the second colony the pellet length varied from 1.32 cm to 6.89 cm, mean length of 3.93 cm. The pellet weight varied between 0.9 g and 6.7 g, mean - 2.54 g. After the quantitative and qualitative analysis of the pellets 3567 individuals in Ciocana pellets and 1584 individuals in Botanica pellets were identified.

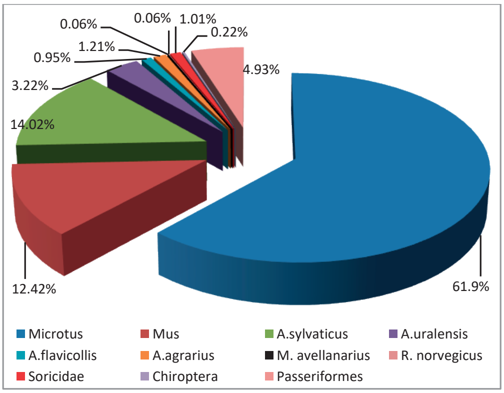

Figure 2. Trophic spectrum of long-eared owl from the first colony (Ciocana)

The trophic spectrum of long-eared owl in the first colony consisted of mammals from 3 orders (Soricomorpha, Rodentia, Chiroptera) and birds represented by Passeriformes, while in the second colony the bats have not been registered (Figures 2, 3).

In both sites *Microtus* species dominated with 61.9% and 63.38%, respectively. The wood mouse is the second species (14.02% and 17.99%), followed by the house mouse with 12.42% and 14.21%, respectively. Other *Apodemus* species constituted about 5% in the first colony and less than 1% in the second one. Other two rodent species have been registered in the first colony - the arboreal rodent *Muscardinus avellanarius* and the synanthropic species *Rattus norvegicus* with very low ratio (less than 0.1%), while in the second colony the forest species *Clethrionomys glareolus* (0.06%) and *R. norvegicus* (0.25%) were found. In both sites there were identified shrews with 1% and 0.44%, while bat species were found in the diet of the first colony only (0.2%). The birds constituted 4.93% and in 3.35%, respectively (Figures 2, 3). The diet of the first colony was more diverse, probably due to much higher number of individuals that hunted in a larger variety of ecosystems.

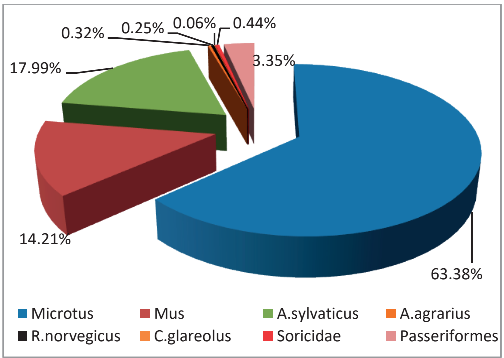

Figure 3. Trophic spectrum of long-eared owl from the second colony (Botanica)

The highest frequency in pellets from both urban areas belongs to *Microtus* species that was found in most of the pellets. The second species according to frequency was *Mus*, although it was the third after its abundance, but it was found in more pellets than *A. sylvaticus*, which also, had a rather high frequency (Table 1). The passerine birds had a frequency of 10.75% and 7.77%, respectively, they were found in many pellets. Other *Apodemus* species had a rather high frequency in pellets from Ciocana and very low frequency in Botanica pellets. The shrews had the frequency of 2-3% in both sites, while other species had a very low frequency.

Table 1. Frequency of animal species in *Asio otus* pellets

	Site	Ciocana		Botanica	
No.	Genus/species	No pellets	F, %	No pellets	F, %
1.	*Microtus*	917	64.04	402	76.14
2.	*Mus*	395	27.58	187	35.42
3.	*A. sylvaticus*	382	26.68	155	29.35
4.	*A. uralensis*	81	5.66	-	-
5.	*A. flavicollis*	102	7.12	-	-
6.	*A. agrarius*	41	2.86	5	0.95
7.	*M. avellanarius*	2	0.14	-	-
8.	*R. norvegicus*	2	0.14	4	0.76
9.	*C. glareolus*	-	-	1	0.19
10.	Soricomorpha	28	1.96	18	3.41
11.	Chiroptera	5	0.35	-	-
12.	Passeriformes	154	10.75	41	7.77

The total biomass of prey items constituted 139,175 g in both sites, with 93,774 g in Ciocana site and 45,401 g in Botanica site (Table 2). The highest biomass belongs to *Microtus* species, with 66,240 g and 30,120 g, respectively. In both colonies a rather high biomass was registered in *A. sylvaticus*, *Mus* species and birds (Table 2).

The trophic niche index varied between 2.19 to 2.37, the total index was 1.14. The total standardized index was 0.01 and varied from 0.137 to 0.171 and indicate that in Botanica site the prey categories had more even distribution in the diet (Table 2).

In both sites of Chisinau city the *Microtus* voles are the most important prey item in the winter diet of Long-eared owl, which was mentioned for many urban areas across Europe (Murariu et al., 1991; Laiu & Murariu, 1998; Banaru & Coroiu, 1997; Sharikov et al., 2009; Benedek & Sîrbu, 2010; Dzemian et al., 2012; Martelli & Fastelli, 2013 etc.). *Apodemus* species constitute an important trophic source for long-eared owl in urban area. Their share can vary between 3% and 66% (Romanowski, 1988; Laiu et al., 2002; Sharikov, 2006; Sharikov et al., 2009) depending on location, climatic conditions, hunting sectors and prey availability. In some urban areas the *Apodemus* species even were the most abundant prey during winter period (Pirovano et al., 2000; Dzemian et al., 2012).

Table 2. Individual number and biomass (g) of prey species in studied sites

Species	Site	Ciocana	Botanica	Total
Microtus	No	2208	1004	3212
	BM	66240	30120	96360
Mus	No	443	225	688
	BM	6645	3375	10020
A. sylvaticus	No	500	285	785
	BM	12500	7125	19625
A. uralensis	No	115	-	115
	BM	2300	-	2300
A. flavicollis	No	34	-	34
	BM	1190	-	1190
A. agrarius	No	43	5	48
	BM	1075	125	1200
R. norvegicus	No	2	4	6
	BM	200	600	800
M. avellanarius	No	2	-	2
	BM	40	-	40
C. glareolus	No	-	1	1
	BM	-	25	25
Soricidae	No	36	7	43
	BM	288	56	344
Chiroptera	No	8	-	8
	BM	96	-	96
Passeriformes	No	176	53	229
	BM	3200	3975	7175
Total ind.	**No**	**3567**	**1584**	**5151**
Total biomass, g	**BM**	**93774**	**45401**	**139175**
BTN		**2.369**	**2.198**	**1.139**
BTNs		**0.137**	**0.171**	**0.01**

No - number of individuals
BM - biomass
BTN - breadth of trophic niche
BTNs - breadth of trophic niche standardized

The abundance and biomass of *Mus* species is very high, which can be explained by the surface and structure of Chisinau city - there are many tall buildings and a massive production of waste. Data on high ratio of *Mus* species (up to 10%) in winter diet of the long-eared owl was noted for other large cities (Murariu et al., 1991; Bencova et al., 2006; Escala et al., 2009; Sharikov et al., 2009; Martelli & Fastelli, 2013).

Among other rodent species the brown rat was found in pellets with very low ratio, similar to other studies, where *R. norvegicus* registered up to 5% (Murariu et al., 1991; Laiu & Murariu, 1998; Romanowski, 1988; Sandor & Kiss, 2004), but in some large cities the species is one of the main prey, reaching about 20% and 60-70% biomass (Pirovano et al., 2000).

The representatives of shrews and bats have been registered in very low percent. The shrews represent an alternative prey type for *A. otus* and are usually hunted when the abundance of *Microtus* species is low (Korpimaki & Norrdahl, 1989). The presence of bats in *A. otus* pellets is usually accidental and constitutes less than 0.5% (Obuch, 1998).

The passerine birds constituted about 3-5% in long-eared owl diet, as well as in other urban studies, where their share varied between 1% and 10% (Romanowski, 1988; Bencova et al., 2006; Sharikov et al., 2009; Dzemian et al., 2012; Tulis et al., 2019).

The long-eared owl is a feeding specialist predator and hunt individuals that weight between 15 g and 50 g, therefore, the ratio of preferred prey - *Microtus* voles remain high in spite of the availability of other prey types (Goszczynski, 1977; Birrer, 2009). As adaptations to urban environment, may be considered the hunting of synanthropic rodent species (*Mus musculus*, *Rattus norvegicus*), the highest ratio of bird species as well as the use of many prey types (Wijnandts, 1984; Banaru & Coroiu, 1997; Pirovano et al., 2000; Sandor & Kiss, 2008; Kiat et al., 2008; Mori & Bertolino, 2015).

The low values of trophic niche breadth prove the high hunting specialization of the long-eared owl for *Microtus* voles, which are one of the most important rodent pests, and highlight its importance in rodent regulation in urban areas and surroundings.

CONCLUSIONS

The trophic spectrum of long-eared owl in Chisinau city is rather diverse and consists of mammals and birds, the rodents being the dominant trophic source with more than 90%. The *Microtus* species were the main prey in both sites and constituted more than 60%.

The proportion of synanthropic pest species from genus *Mus* was rather high, which prove the huge importance of long-eared owl trophic activity in urban ecosystems.

The total biomass of prey items constituted 139,175 g, among which the highest belongs to *Microtus* species with 93,360 g.

The low total value of trophic niche breadth (BTNs = 0.01) prove the high hunting specialization of the long-eared owl for *Microtus* voles, and highlight its importance in rodent regulation in urban areas and surroundings.

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BIOASSAYS RESISTANCE OF POLLEN BEETLE (*Meligethes aeneus* F.) TO SYNTHETIC PYRETHROID CYPERMETHRIN

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Abstract

Pollen beetle (Meligethes aeneus F.) as a key rape pest is controlled by use of a wide range of insecticides, to maintain low density. The downside of this method is destruction of the beneficial entomofauna, pollinators the possibility of developing resistance. Massive use in the synthetic pyrethroids to control the pollen beetle led to reduction their efficacy. Therefore, it is determined insecticidal resistance of pollen beetle (Meligethes aeneus F.) to synthetic pyrethroids. Resistance is refining by Bioassays, which includes a study of the reaction of an organism to a given substance, taking into account the concentration changing influences. To this end, they gathered pollen beetles for analysis. The results of the research proved presence of 10% resistance of pollen beetle (M. aeneus) to cypermethrin after 24 hours of action at the highest concentration of 0.250 ppm/cm^2.It was found, that the concentration and duration of cypermethrin action are inversely proportional of the resistance of the elderly.

***Key words**: pollen beetle, resistance, synthetic pyrethroids.*

INTRODUCTION

The pollen beetle (*Meligethes aeneus* F.) is the key pest of oilseed rape across Europe (Alford et al., 2003, Williams, 2004, 2010). In separate years the loss of it can reach 60-100% (Hansen, 1996; Coll et al., 1998; Johnen, 2000; Krostitz, 2000; Cook et al., 2004; Kazachkova, 2007; Milovanović, 2007; Ahmanl et al., 2009; Breitenmoser, 2012).

Receiving high yields depends exclusively on the successful fight against the pest. Most often the fight with the control of pollen beetle in practice is taking place using synthetic pyrethroids and neonicotinoids (Wegorek and Zamojaska, 2006; Slater et al., 2011). In the past years is observed reducing efficacy of synthetic pyrethroids, as a result of which this pest is has developed very fast resistance to most active substances. Speed of development of resistance depends of several risky ones factors and they can be divided into two groups. The first group includes agronomic risk: the number, the rate and time of applications of plant protection products for one generation, as well as the specificity of the plant protection product. The second group is risk, which includes migration circles of plants - Hosts of the enemy, its reproductive potential and speed presence in proximity of sensitive populations (Russell, 2001; IRAC, 2013).

The genetic background of resistance to insecticides can be from monogenic or polygeneous character (Groeters and Tabashnik, 2000), but most cases are caused by a single allele of resistance (McKenzie, 2000; Hollingworth and Dong, 2008). Changes at the genetic level are varied include: single nucleotide polymorphisms (SNPs) (Williamson et al., 1996), Genetic Amplification (Bass and Field, 2011), DNA methylation (Field, 2000), microsatellites/tandem repeats STR (Bass et al., 2013), alternative and or improper bindings (Sonoda et al., 2006) and RNA editing (Xu et al., 2006). Changes in DNA/RNA lead to complicated by chain physiological changes in arthropods and they can be extra classified in four main ones the mechanism of resistance. The reason for the development of the resistance of beetles to synthetic pyrethroids is frequent their use in recent years.

If only one is used and also active substance with the same mechanism of action, pests, which are less receptive, survive. As the percentage of insensitive individuals in the population, the efficacy of the active substances decrease

(Hoffmann et al., 1994). In the whole Europe pyrethroid insecticides are with long history in control of pollen beetle (Slater et al., 2011).

The earliest report on *M. aeneus* resistance to pyretroids in France and Scandinavia (Decoin, 2002; Hansen 2003, 2004, 2008; Milanović et al., 2019), its spreading in recent years across Europe has caused some majour pest control problem of oil seed raps in many countries in Europe - France (Ballanger et al., 2007), UK (Richardson, 2008), Denmark (Hansen, 2003, 2004, 2008; Kaiser et al., 2018), Poland (Wegorek et al., 2009; Philippou et al., 2011), Germany (Heimbach et al., 2006; Thieme et al., 2008; Heimbach & Müller, 2013), Swizerland (Philippou et al., 2011), Austria (Slater et al. 2011), Sweden (Kazachkova, 2007) and Czech Rebublic (Stara & Kocourek, 2017).

In many countries common practice is the execution of more than one vegetative treatment with insecticides against the pollen beetle (Richardson, 2008a, 2008b). Hansen (2003) studies resistance the pollen beetle (*M. aeneus*) agains active substances: tauflumivalate, lambda-cyhalothrin, esfenvalerate and dimethoate. Later in a row countries from West and West Central Europa (Heimbach et al., 2006), Finland (Tiilikainen and Hokkanen, 2008), Poland (Wegorek and Zamoyska, 2006), Czech Republic and Slovakia (Seidenglanz et al., 2015), United Kingdom (Richardson, 2008) there is established resistance the pollen beetle (*M. aeneus*) to synthetic pyrethroids. The results of conducted surveys show, that the beetles survive 99% standard doses of synthetic pyroethroids and up to 36% of dimethoate. The first case of show of resistance of rapeseed flower (*M. aeneus*) to the synthetic ones pyrethroids was announced in 1999 in the Champagne region in the Northeast France.

Resistance to pyrethroids the pollen beetle (*M. aeneus*) is not limited only to the individual compounds, but affects the entire chemical group of synthetic pyrethroid, though some of show them higher efficacy at recommended doses of administration, than others (Nauen, 2007). In 2007, in Europe the first strategy was adopted to manage resistance of pollen beetle (*M. aeneus*) in winter oilseed rape and is mainly based of treatment with thiacloprid, belonging to neonicotinoids, directed to nicotinic acetylcholine receptors of insects (Jeschke and Nauen, 2008). Thiacloprid is registered for control of pollen beetle, but since it was introduced tested insecticides, with different modes of action, their effect on pollen beetle, for improvement management of resistance (Longhurst et al., 2007; Schröder et al., 2009).

The limited set of insecticides to control pollen beetle make it necessary to look for new tools to fight a different mechanism of action, which means to provide long-lasting protection of rapeseed from enemies, conservation of useful entomofauna and crop pollinators.

The purpose of this study was to obtain information by testing the pyrethroid resistance of cypermethrin to answer the problems with the effectiveness of the insecticides used in the region of Northeastern Bulgaria and to obtain basic data that can be used in future monitoring actions to detect early changes in the susceptibility of pollen beetle to insecticides.

MATERIALS AND METHODS

Resistance was determined by the quantitative test BIOASSAY; this means the study of the reaction of a living being to a particular substance, taking into account the concentration and changing influences.

For the purpose of laboratory testing, the pollen beetles were collected from winter oilseed rape fields near Shoumen (43°23'25.8"N 26°48'13.8"E). The adult individuals were collected in sunny weather during phenological growth stages BBCH 52-55. The collection was performed according to the linear assessment method, which ensures that the beetles were collected from as many different points in the field (Kupfer and Schröder, 2015), which ensures that the beetles are collected from as many different points in the field as possible. The area was divided into 5 control points and, if necessary, the number of consecutive control point plants increased from 10 to 15 to capture the required number of beetles (520 in total). The beetles ware removed by shaking the plants and collected in plastic bags containing absorbent paper to absorb moisture. There were also 4 flowers for the food. The predatory insects caught in the collection of pollen beetles were removed.

In the laboratory, the beetles were counted in groups of 10 animals and placed in small plastic containers. The test was carried out by three test kits, each with four concentration levels. The concentrations went from 0.002 ppm to 0.250 ppm. Each group of 10 beetles was placed in each test tube and capped. This was done under a room temperature of 21°C.

RESULTS AND DISCUSSIONS

Synthetic pyrethroids are contact insecticides, which block addicts from pressure sodium channels in nerve membranes, as a result of which they follow opening cannot be closed again. Synthetic pyrethroids have a rapid initial effect against almost all insects. The cypermethrin enter the insects through the body cover, after which they spread throughout the body.
Cypermethrin is a neurotoxin, wherein the Na^+ -channels of nerve cells stop closing, so Na + ions flowing unhindered in the interior of the cage.
As a result, they appear uncontrollable nerve impulses, which lead first to agitation convulsions, then to violations of its coordination finally to paralysis.
Insect immobilized in within a few minutes, the so-called "knockdown" effect is %.

Survival of beetles reported in each tube at 1, 5 and 24 hours after application of test concentration. The insects they were not observed coordinated movements within 30 s, were classified as dead.
At a test concentration of 0.002 ppm/cm^2, after contact action in 24 hours, 97% survival in which after 1 and 5 hours they were not observed signs of poisoning in the pollen beetle.
Taking into account the levels of a concentration of 0.010 and 0.050 ppm/cm^2, after 1 and 5 hours, 3% or 7% of the beetles with signs of poisoning. After contact action in for 24 hours the number of beetles, with signs of poisoning was rising both levels of concentration reached 13%, which means survival rate of 87%. The highest concentration in this one experimental group was 0.250 ppm/cm^2. After contact time of 1 hour, of said concentration, 37% of beetles showed signs poisoning, after 5 hours the degree of disability increased to 43%. At the latter reporting after 24 hours survival was 10%. This means that at the highest concentration, used in biological research, resilience of Pollen beetle was 10%. From everyone results it can be said, that its concentration duration of action of cypermethrin are inversely proportional the resistance of the beetles (Table 1).

Table 1. Bioassays of cypermethrin against pollen beetle

Test concentration, μg/cm^3	**Mean number of vital beetles**	**Mean number of beetles with intoxication symptoms**	**Survival rate [%]**	**Efficiency [%]**
	after 1 hours			
untreated	10.0	0.0	100.0	0.0
0.002	10.0	0.0	100.0	0.0
0.010	9.7	1.3	97.0	3.0
0.050	9.3	1.7	93.0	7.0
0.250	6.3	3.7	63.0	37.0
	after 5 hours			
untreated	10.0	10.0	100.0	0.0
0.002	10.0	10.0	100.0	0.0
0.010	9.7	1.3	97.0	3.0
0.050	9.3	0.7	83.0	7.0
0.250	5.7	4.3	57.0	43.0
	after 24 hours			
untreated	10.0	10.0	10.0	0.0
0.002	9.7	0.3	97.0	3.0
0.010	9.0	1.0	90.0	10.0
0.050	8.7	1.3	87.0	13.0
0.250	0.1	9.0	10.0	90.0

The pollen beetle is known to be a species in which, in addition to its natural resistance, there is a strong resistance to some synthetic active substances (Tillikainen and Hokkanen 2008; Węgorek et al. 2009; Nauen et al, 2012; Seidenglanz et al, 2015). The results of our studies have shown that the pest exhibits high-level resistance to the test concentration of 0.002 ppm/cm^2. Changes in the level of sensitivity were observed at concentrations of 0.010 and 0.050 ppm/cm^2, but they were not significant. The reported high survival rate of individuals 8 of 0.250 ppm/cm^2 suggests mechanisms of detoxification in the test population that also determine the low efficacy of cypermethrin against pollen beetle. The pollen beetle resistance to pyrethroids has been reported to be due to a mechanism of physiological resistance based on oxidative enzymes (Slater and Nauen, 2007; Węgorek et al., 2011).

The experiments presented were carried out in connection with many signals from farmers for the low efficiency of chemical control of pollen beetle, especially when using pyrethroids. The results showed low toxicity of cypermethrin to pollen beetle. With this study, we report for the first time the existence of a resistant population of pollen beetle on oilseed rape in the area near the town of Shumen, Northeastern Bulgaria, but this claim requires lengthy observations and investigations. In view of the results obtained from our studies, literature and experience we can strongly recommend that alternative control of pollen beetle be sought. Strict adherence to the Sustainability Management Guidelines issued by local experts or published annually by the Insecticide Resistance Action Committee (IRAC) will exclude unnecessary combinations of insecticides and treatments and the emergence of new resistant individuals or populations.

CONCLUSIONS

As a result of research can draw the following conclusions:

-Established is 10% resistance of the pollen beetle to cypermethrin after 24 hours impact on the highest a concentration of 0.250 g/cm^2.

-The results have proven, that concentration and duration of action of cypermethrin are inversely proportional the resistance of the elderly individuals.

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URBAN AGRICULTURE OF BUCHAREST

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Abstract

The zone of influence of Bucharest is over five counties, with predominantly rural combinations mixed with urban functionalities. The city capitalize dynamically from an economic point of view, there are unique opportunities for dissemination and application of technological, social and cultural ideas. Why agriculture in the city, because it encourages innovation, niche opportunities, but in the long run these activities can return to (have a high chance of returning to) the countryside. Urban trade domestic agricultural and food depends on municipal support. Regulations on land use, facilities, access to various resources and the "garbage" generated by the community through regulations and the security of urban spaces in the broadest sense are essential for harmonious development. If the current population remains indifferent to the realization of the current procedures, transformations and environmental conditions that will had an impact on the future daily life, of culture, art and the city identity. The national program for support and applicability in urban agriculture in Romania is the "Minimum Program" for tomatoes and garlic, the most visible. The national action plan on climate change provides for the sustainability of agriculture and the greening of as large a percentage as possible, by creating of groups, the practice of works for friendly agriculture, but also the creation of local parts under shared responsibility. Urban agriculture is a hidden field in Bucharest, for these activities, but also from gardening, the analysis associated with consumption, reduction of fossil fuel and water resources is necessary, with an increased attention on the practices and policies regarding urban food strategies in the context of intelligent development of common living spaces with social, cultural and ecological impact. Satellite images and multi-criteria analysis of spatial land use conflicts in the Bucharest area, allows the observation of major changes, for example the central area through the communist project "Civic Centre" is a major change in the dynamics of the locality. These methods are crucial in achieving current and future urban regulations. Green infrastructure in cities should also to include urban agronomic activities with unique challenges other than conventional agriculture.

***Key words**: Bucharest, urban agriculture, Romania.*

INTRODUCTION

In Figure 1 below, it's the town planning of Bucharest, officially regulated by the authorities.

GENERAL URBAN PLAN OF THE MUNICIPALITY OF BUCHAREST, 2000, "ION MINCU" UNIVERSITY OF ARCHITECTURE AND URBANISM; RESEARCH, DESIGN, EXPERTISE AND CONSULTING CENTER (2000)

Figure 1. P.U.G. Map of Bucharest

The city is economically dynamic, applying ideas technological, social and cultural as seen in the maps above at the introductory level (Figure 2).

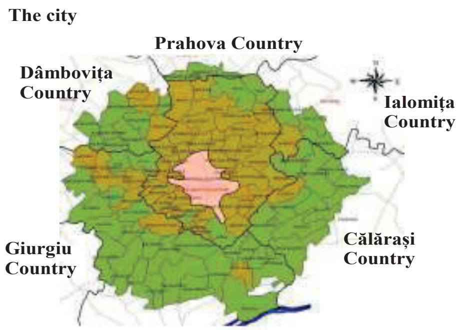

Figure 2. The influence of Bucharest (Source https://ilfov.insse.ro/)

The area of influence of Bucharest is over five counties, with predominantly rural combinations mixed with urban functionalities. The city capitalize dynamically from an economic point of view, there are unique opportunities for dissemination and application of technological, social and cultural ideas (Figure 3).

Figure 3. Group of maps on different topics
(Source www.google.ro/maps & https://ilfov.insse.ro/)

Why agriculture in the city, at the educational level concerns are for the daily life, the contributions of USAMV of Bucharest in agriculture and products (food) through analyses, publications, researches that have implications in the urban environment as well.

This relationship of the city's inhabitants with the urban anthropic nature green, regenerates the human spirit through the participation of the plant microlocat in the heart of the Romanian capital, the urban green space must stay and grow to support the physical, mental, spiritual and cultural health of citizens (Tronac et al., 2013).

MATERIALS AND METHODS

The method used was by analyzing the reports and open data from various public entities, together with the identification of the most relevant scientific articles, which are related to urban agriculture in Bucharest.

RESULTS AND DISCUSSIONS

Through the studies made in the periurban agricultural area of Bucharest mentioned below, there is a fairly high interest in plant adaptations and diversification.

The potato (*Solanum tuberosum* L.) is an important plant in the world, but also in Romania. The researchers from the didactic farm in 2013, checked three varieties of potatoes for the conditions in the area, cultivated non-irrigated and irrigated. The *Bellarosa* variety ranks first in terms of yield. “The specific cultivation conditions for Moara Domneasca ensure a high yield of tubers and a commercial quality of the potato, which offers the possibility to cultivate the potato in the periurban area of Bucharest.” (Marin et al., 2014).

Certain varieties of *Ribes* sp., *Prunus* sp., and other recommended by results of cultivation in urban and periurban gardens in Bucharest, agroecosystem (Bălan et al., 2014).

Based on the results observed by this team, they recommended certain varieties of Ribes and Prunus with the possibility of cultivation in urban and periurban gardens in Bucharest, providing details about the qualities of varieties and biotypes analyzed in different ecological, biochemical environments in an agroecosystem (Marin et al., 2014).

Also, the University of Bucharest, the Faculty of Biology through the Botanical Garden has a section of the Grandmother's Garden aims at a symbolic model of the traditional Romanian gardens in which vegetables, flowers, medicinal and aromatic plants, shrubs and fruit trees are well highlighted and sustainable for the botanical species and varieties consumed in the history of European food, gradually abandoned with the spread of plants brought from the New World. These sections of the Botanical Gardens have an educational purpose for visitors in terms of the diversity of food plants. These areas can be included as urban agriculture with a strong educational and biological character for the visiting public. It is the most visible UAB place in the Romanian Capital.

The need to identify climate-resistant roses in the SE area of Romania using the PCR-ISSR technique, 11 species and their variations were selected for their ability to produce informative patterns with genetic diversity. The ISSR method proved to be adequate and allowed the precise DNA imprinting for the exact identification and tracking of the varieties of roses (Duță-Cornescu et al., 2017).

The Botanical Garden has, among other sections, two symbolic models for botanical species and varieties for consumption. Visitors can observe the diversity of food possibilities, these places can be included as urban agriculture with a strong informative and biological character

Presented projects, but not only, it can be included in a percentage in urban agriculture, but they have a pronounced pedagogical character (didactic) with the final purpose of offering on the Romanian and European labor market specialties' in the agronomic field.
Urban trade domestic agricultural and food depends on municipal support (FAO, 2019).
The organizations based in Bucharest, which have the field of activity of agriculture or associated with it are very many and it was very difficult to disseminate the entities that actually carry out agriculture in Bucharest. There are only a few of these organizations and associations that have dealt with urban agriculture and such kinds projects.
A detailed analysis of how vegetables that play a key role are marketed and capitalized, which are sold with the attention to health, under possible and existing conditions, without damaging at markets in shops, supermarkets and traditional agrarian-food areas in Bucharest. The conclusion of the study is that all categories of stores are important for fresh fruits and vegetables, also relevant is the fact that the retail chain is important for local products, but with a number of deficiencies in product information, in large fresh product chains they are predominantly imported, as the required requirements cannot be met by many local producers (Ionescu et al., 2014).
The agricultural activity in Bucharest as UAB, are about 4-5 companies, but to determine how much of their producers is local, according to the unique agricultural register from DAMB is very difficult due to lack of data.
It is interesting that in the locality there are plant species keepers, which can be included in urban agriculture with planting material from the urban area.
On the other hand, in the agricultural register Bucharest I did not find an agricultural association or a cooperative that includes producers from the municipality, but there are numerous associations and agronomic organizations based fiscal, with members in different association systems, but which carry out the production in other places.
However, there is an active species that contains the name of the city, for the vine = "Muscat timpuriu de București", holder code 1059, from 1970.
Horizon 2020 - Call - Food and Natural Resources (H2020-FNR-2020) a vision for urban agriculture is the development and conflicting landscapes with different land uses and economic activities. The benefits are biodiversity, ecological infrastructure, climate and improved urban-rural connections. Through new business models based on urban agriculture will improving food security. But threats like aquifers from Montes Torozos (Spain) with high vulnerability, taken into account and identification of their source (Martinez-Allegri et al., 2014).
Presented results for 2007-2014 at USAMV Bucharest campus for eco-economic sustainability and biopomiculture by growing trees and shrubs of different species and their varieties, monitoring the adaptations to the urban area, which concludes that it is an UAB with developmental and pedagogical characteristics (Bălan et al., 2015).
The national program for support and applicability in urban agriculture in Romania is the "Minimum Program" for tomatoes and garlic, the most visible. The national action plan on climate change provides for the sustainability of agriculture and the greening of as large a percentage as possible, by creating of groups, the practice of works for friendly agriculture, but also the creation of local parts under shared responsibility.
In Bucharest, one applied for this funding program, according to the DAMB reports.
If the current population remains indifferent to the realization of the current procedures, transformations and environmental conditions that will had an impact on the future daily life, of culture, art and the city identity.
Regulations on land use, facilities, access to various resources and the rubbish generated by the community, through regulations and the security of urban spaces in the broadest sense are essential for harmonious development.
The characteristics of Bucharest compared to the surrounding area.
From these data we notice very large discrepancies between Bucharest, which has a population 4 times larger and 7 times less land than Ilfov (Table 1).

Table 1. Comparative densities between Bucharest and Ilfov County (According to https://ilfov.insse.ro)

	Bucharest	Ilfov	Share	
Population	1,940,000	451,839	4.294	0.233
Surface (ha)	23,787	158,300	0.150	6.655
Density (pop./ha)	81,557	2,854		

Also on the map (Figure 4) you can see local densities around the metropolis.

Figure 4. Density map of population by locality, Ilfov County (Source https://ilfov.insse.ro/)

Part of the capital's population almost certainly works in the "frontier" area of Bucharest.
The dominant agricultural character of Ilfov County according to the 2018 report DJS - ILFOV (Table 2) graphically expressed in Figure 5.

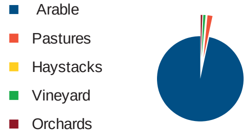

Figure 5. The proportion by categories of land existing in Ilfov County (According to INSSE - DJS - ILFOV

The dominant urban character of Bucharest Municipality according to the 2019 report DRSMB (Table 3) graphically expressed in Figure 6, the city has approximately 12% agricultural land, ~ 52% of arable and 28% cultivated of it.
Urban agriculture is a hidden field in Bucharest, for these activities, but also from gardening, the analysis associated with consumption, reduction of fossil fuel and water resources is necessary, with an increased attention on the practices and policies regarding urban food strategies in the context of intelligent development of common living spaces with social, cultural and ecological impact.

Table 2. Existing categories and areas of land in Ilfov County and the level of use of arable land (According to INSSE - DJS - ILFOV)

Lands Ilfov County (ha)	**Base 2014**			
Total	158,328	Used agricultural landscapes		
Agricultural	101,453	2016	2018	%
Arable	98,080	64,454	**66,769**	68.08
Pastures	1,875			
Haystacks	58			
Vineyard	812			
Orchards	628			
Degraded	1,162			

Table 3. Existing categories and areas of land in Bucharest and the level of use of arable land (Date according to INSSE – DRSMB 2019)

Lands of Bucharest (ha)	**Base 2013**	Arable land			
Total	23,787	2017	2018	2019	%
Agricultural	3,052				12.83%
Arable	**2,566**	1,461	1,609	1,608	52.69%
	Arable seeded	581	481	459	28.54%
Forests	611				
Waters	908				
Other	19,216				

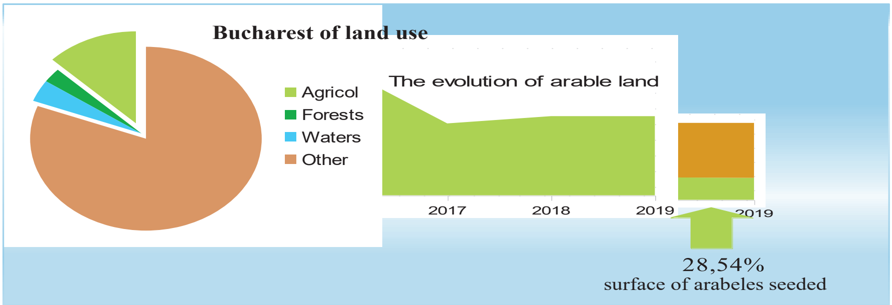

Figure 6. The proportion by categories of land existing in Bucharest
(Date from INSSE - DRSMB 2019)

Urban NGOs, especially in Bucharest, are very numerous, with many activities and numerous partnerships. Of these, there are very few projects on UAB carried out and in continuous expansion, which are laudable, some more visible, others less, but some projects initiated a few years ago have been started and abandoned. In Bucharest there are 5-6 organizations that deal directly with the promotion and implementation of urban agriculture projects. There are also (2-3) individual UAB initiatives promoted on Facebook, but I am convinced that in the suburbs and houses in Bucharest, where there is a green space, there are residents who practice gardening for their own consumption, but are not associated or do not have a legal form.
The multi-criteria spatial analysis (Figure 7) of the land use allows the observation of the locality changes, for the future and present urban regulations. (Loghin and Murătoreanu, 2011), (Ioja et al., 2013)

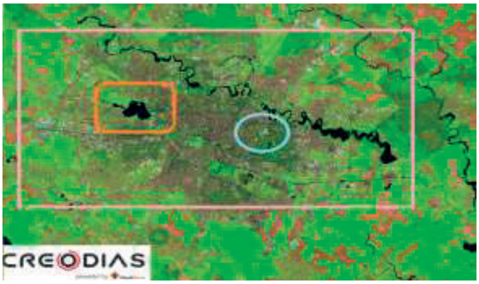

Figure 7. A multi-criteria spatial analysis model
(Source: https://creodias.eu/)

Green in cities should also to include urban agronomic activities (Bohn K. and Viljoen A., 2015)

CONCLUSIONS

Urban Agriculture of Bucharest (UAB) can invents, learn, cure and gardens, with a vision for biodiversity, sustainability and regulation of urban spaces, which are essential, increases the impact of food and practice gardening, but the indifference, standards not achieved by locals, and the Bucharest support just few urban farmers, can't reach to be a Green-city, without a variety of land use, including agronomic activities among others.

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THE INFLUENCE OF THE COMPOSTING METHOD AND THE PLANT BIOMASS ADDITION ON COMPOST QUALITY

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Abstract

Home composting could be proposed in order to improve the management of organic fraction of municipal solid waste (OFMSW) or biowaste especially in rural areas. In addition, different wild plants biomass with some ecological properties may be added as raw material, bulking agent and compost quality improver during composting. In order to assess the compost quality obtained in such conditions, a study in four small boxes (1.2 m x 0.8 m x 1.0 m.), made from local materials (wood and mesh from raffia), was carried out for a period of four months. A quantity of 550 ± 5 kilo of different precomposted OFMSW was introduced in each box. The compost was manually aerated with a fork two times per month. The compost temperature was registered daily by soil thermometers. In the 2nd month of the composting period, 10 kilos of fresh nettle (Urtica dioica L.) was added in one of the boxes. For compost quality assessment there were made chemical and physical analysis, maturity tests and microbiological analysis in order to identify the presence of pathogens.

Key words: *biodegradable waste, compost, home composting, maturity, germination index.*

INTRODUCTION

According to the framework waste legislation (Directive 2008/98/EC), since 2018, all urban and rural Romanian communities have been connected to centralized solid waste collection and management system services that are based on an integrated, hierarchical system, with waste prevention as the highest priority. Large amounts of municipal solid waste are collected daily.

One of the objectives of the Romanian National Waste Management Plan (NWMP, 2017) is to reduce the municipal biodegradable waste disposal, with a target for 2020 of 35% of the total amount produced in 1995. Also, separate waste collection is a permanent goal, and the agricultural use of products resulting from the treatment of biowaste by composting and/or anaerobic digestion is strongly encouraged (NWMP, 2017). In this regard, there is a growing interest of the Integrated Waste Management Centers (IWMC) in composting the organic fraction of municipal solid waste (OFMSW). But the large quantities of Organic Household Waste (OHW) that are source-separated at home and collected weekly in many rural areas could be composted at home and not transported away for treatment. The combined option of source-separated biodegradable waste and home composting may be seen as a valuable prevention action that contributes to reducing the generation of household waste (Puyuelo et al., 2013; Tatàno et al., 2015) and promoting the active participation of citizens in waste management and in the development of a self-management of organic waste (Ballardo et al., 2020) with low environmental impacts regarding emissions of greenhouse gases (Andersen et al., 2012; Ermolaev et al., 2014) that may reduce the operational waste management cost (Puyuelo et al., 2013) of rural communities. In addition, in the long run, the construction of landfills will affect smaller areas, and the sustainability of that process, as well as the environment resilience, will increase.

Composting the organic fraction of municipal solid waste (OFMSW) or biowaste is one of the most environmentally friendly technologies

(Barrena et al., 2014). It gained an important role in municipal solid waste management worldwide, and the compost may be used as soil conditioner as well as fertilizer. In order to provide crops with both nutrients and organic matter, it must be of high quality. But industrial composting requires high costs for building platforms, providing equipment, ensuring selective collection of waste and guaranteeing environment protection conditions. Therefore, especially in rural areas, home composting presents some potential benefits such as the avoidance of collection and transportation of biowaste (Barrena et al., 2014; Faverial and Sierra, 2014; Vázquez and Soto, 2017).

Home composting has been traditionally used in Romania, especially for manure and garden waste co-composting in the small family farming or in horticulture. But composting of organic household waste is less common. For villages, it can be much more attractive and sustainable to adopt home composting, especially where people have large gardens and can use compost for landscaping or for the production of fruits and vegetables for own consumption. Adopting composting of own organic waste can reduce the overall household waste management costs of communities, hold people responsible for waste production, and help increasing the sustainable use of resources.

The objective of this research was to evaluate the quality of the OFMSW compost produced in experimental conditions, and of the OHW compost produced in home composting natural conditions. For this objective, physico-chemical analyses were conducted, and a seed germination test was carried out.

MATERIALS AND METHODS

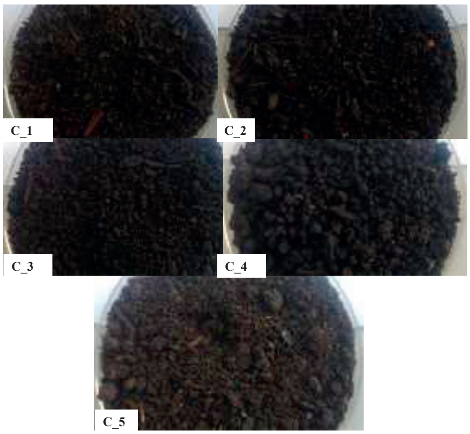
This section aimed to describe the composting facilities, the sampling methods and the analyses (Pictures 1 and 2).

Four compost were produced from organic fraction of municipal solid waste collected from an Integrated Waste Management Center. The OFMSW were in different stages of early composting at the collection time. During composting, the substrates were manually aerated with a fork, two times per month, the temperature was monitored daily using a thermometer and some bulking agent (dehydrated nettle plants) were added in box 2, one month after composting had started (the objective of this addition is not presented in this work).

1- Wood composting box lined with raffia mesh.
2- The four wood boxes loaded with the compost substrate.
3- Thermometers used for temeperature control.
4- Dehydrated nettle plants.

Picture 1. Facilities and compost production

One organic household waste compost was produced in rural natural aerated pile conditions for one year.



Picture 2. C_1, C2, C3 and C_4 - OFMSW compost, and C_5 - OHW compost used in the study

Physico-chemical analyses of compost were conducted within the soil pollution control laboratory of the Research Institute for Pedology and Agrochemistry in Bucharest. Moisture and dry matter were determined by gravimetric method. The pH was determined through the potentiometric method in 1/5 aqueous suspension. The total forms of mineral elements were determined by wet mineralization (H_2SO_4+H_2O_2) using the HACH Digesdahl method. The total nitrogen (N) was determined using the Kjeldahl method; the total phosphorus (P) was determined by spectrophotometry and the potassium content (K) was determined using the flam-photometric method; the total salt content and the electrical conductivity (EC) were determined using Romanian standard methods (STAS 7184/7-87, STAS 7184/12-88 chapter 2.1.6. and chapter 2.1.7; PTL 18); the organic matter content was determined through dry oxidation (LOI - loss on ignition), thus measuring the calcination losses (600°C, 2 h) and then the content in total organic C was calculated by multiplying the obtained result by 0.54. The heavy metals were determined through atomic absorption spectrometry in hydrochloric solution after the mineralization of the samples with strong acids. Laboratory methodology was used.

Samples of each compost were prepared for microbiological analyses. From each sample, 10 grams of compost were mixed with 50 ml of distilled water that was sterilized. The mix was stirred for 1 hour, and after that it was placed for 1-2 hours for sedimentation. Dilutions were made from the supernatant (- 2) and the Compact Dry discs were sown according to the manufacturer's instructions. Compact Dry discs were used to identify and quantify: *E. coli* and coliforms and *Salmonella*. The samples were incubated for 24 hours at the temperature specified by the manufacturer Compact Dry.

For phytotoxicity tests, a compost extract was prepared using 100 g of each compost and 150 ml of distilled water.

The experiment was set up on 2.03.2020 in 6 variants, and for each variant 4 replicates were performed. The germination test was made with cress (*Lepidium sativum*) seeds.

Picture 3. Compost extract

The compost extract was obtained by mixing a 100 g sample of compost with 110 ml of distilled water. The mixture was well stirred and left to rest overnight. The next day, the mixture was centrifuged for 15 minutes at 6000 rotations/minute. After centrifugation, the supernatant (Picture 3) was no longer passed through filter paper, but was pipetted and placed in the test tubes.

A filter paper with a 100 mm diameter was inserted in the Petri dishes and 25 cress seeds were placed on it. In each Petri dish, 5 ml of supernatant was added over the seeds. A control variant with distilled water was prepared. All variants were prepared in four replicates.

The Petri dishes (Picture 4) were inserted in the incubator at 25ºC for 48 hours. After 48 hours, the number of germinated plants for each variant and replicate was registered, the length of the radicles was measured and the germination index was calculated according to the formula proposed by Gariglio et al., 2002:

$$GI\ \% = G/G_0 \times L/L_0 \times 100, \qquad (1)$$

where:

GI % = germination index;

G = the number of plants that germinated in the compost extract;

G_0 = the number of plants that germinated in the distilled water;

L = the average length of the radicles in the compost extract;

L_0 = the average length of the radicles in the distilled water.

Picture 4. Germination test

RESULTS AND DISCUSSIONS

The physico-chemical characteristics and the key nutrient content analyzed in the compost samples are presented in Table 1. The pH values of compost ranged between 7.39 (C_1) and 8.17 (C_2). Mature compost should have pH values between 6 and 8 (Mustin, 1987; Zmora-Nahum et al., 2007; Papadopoulos et al., 2009; Barrena et al., 2014; Zhang et al., 2018; Cesaro et al., 2019). According to the Italian Regulation for compost classification (Legislative Decree n. 75/2010 cited by Cesaro et al., 2019), the threshold limits for pH values are 6 and 8.5. Considering the $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio values, two of our composts (C1 - OFMSW and C5-OHW) fall into the "very mature" category, and the other three (C2, C3 and C4 - OFMSW) in the "mature" category (Brinton, 2000).

All five composts were characterized by high contents of N, P, K. Thus, the total N content ranged between 1.04% (C_5) and 1.61% (C_3), the total P content ranged between 0.35% (C_1) and 1.14% (C_4), and the total K content ranged between 1.48% (C_5) and 2.89% (C_2). These values are related to the dry matter. According to Mustin (1987), the mature compost (140 days), obtained from urban household wastes, could have a total N content between 0.85% and 1.1% of the dry matter, a total P content between 0.2% and 0.35% of the dry matter and a total K content between 0.25% and 0.40% of the dry matter. Recent studies (Vázquez et al., 2015) reported total N content of 1.3% and 2.0% in composts that were obtained from organic wastes collected in a decentralized way. The same authors reported total P content between 0.49% and 0.53%, while the total K content ranged between 1.30% and 1.80%.

The five tested composts had Ca contents between 1.18% (C_1) and 3.39% (C_2) of the dry matter, which is according to the values reported by other authors (Mustin, 1987; Vázquez et al. 2015). Also, the total Mg content ranged between 0.53% (C_1) and 0.82% (C_2). According to Mustin (1987), the total Mg contents of the mature compost obtained from OFMSW should range between 0.25% and 0.40% of the dry matter. Relatively similar values were also reported by Vázquez et al. (2015).

The values of electrical conductivity (EC) and salt content of the composts are presented in Table 2. The EC values of the composts resulted from OFMSW ranged from 2090 μS/cm for C_4 to 4330 μS/cm for C_2. In fact, the value registered for C_2 is more than twice as high as the one registered for C_3 and C_4. The compost resulted from OHW (C_5) has an EC value of around 2660 μS/cm, which is relatively similar to C_3. Unlike other types of organic wastes, OFMSW tends to lead to a compost with high salt content, and the EC can exceed the threshold of 4000 μS/cm (Lasaridi et al., 2006). Zhang et al. (2018) point out to the EC increase of the compost resulted from co-composting of sewage sludge mixed with OFMSW. As OFMSW increases, the EC also increases, reaching 3200 μS/cm when the OFMSW share is of 85%. Barrena et al. (2014) reported an average EC value of 7200 μS/cm in the industrial compost obtained from OFMSW, unlike the home compost, which had an average value of 3900 μS/cm. The composts obtained from the biodegradable fraction of the municipal wastes, even if the wastes are source separated, can present high levels of inorganic salts compared with other substrates (Barrena et al., 2014). This may be due to a high degree of decomposition of the organic matter, especially rich in protein, which leads to the accumulation of various water-soluble salts (Barrena et al., 2014; Zhang et al., 2018). The plants are negatively affected by the excess of salts in the soil, and sodium (Na) can be harmful to the soil structure (Hargreaves et al., 2008). Thus, EC determination is necessary before establishing and applying some compost doses obtained from OFMSW in crop cultivation, because a high salt content, respectively an EC with high values, can lead to toxic effects when too high doses of compost are used.

Heavy metal concentrations of analyzed compost samples are presented in Table 3. The heavy metal content of the five studied compost samples was below the values established by the German Standards for this kind of compost, except for zinc (Zn) content. The compost produced under natural conditions from source separated organic household waste (C_5) had the lowest content of heavy metals, and cadmium (Cd) was not detected. One of the four OFMSW compost (C_2) had the highest content of Zn (705.0) mg kg^{-1}, compared with 500 mg kg^{-1}, which is the German standard (Brinton, 2000).

Table 1. Physico-chemical characteristics and key nutrient content analyzed in compost samples

Sample	pH	H_2O (%)	MO (%)	C_{org} (%)	N (%)	Ratio C/N	$N\text{-}NH_4^+$ (mg/kg)	$N\text{-}NO_3^-$ (mg/kg)	Ratio $N\text{-}NH_4^+/N\text{-}NO_3^-$	P (%)	K (%)	Ca (%)	Mg (%)
C_1	7.39	52.53	25.08	9.60	1.12	8.55	153	494	0.310	0.35	1.82	1.18	0.53
C_2	8.17	51.68	38.59	14.30	1.41	10.14	163	937	0.174	0.99	2.89	3.39	0.82
C_3	7.63	46.81	35.36	15.04	1.61	9.34	166	332	0.500	0.97	1.77	2.21	0.62
C_4	7.94	47.24	36.78	13.98	1.52	9.20	168	218	0.771	1.14	2.10	2.64	0.62
C_5	8.00	44.64	24.83	9.71	1.04	8.82	86	910	0.095	0.46	1.48	3.11	0.80

Table 2. Salt content of analyzed compost samples

Sample	CO_3^{2-} (mg)	HCO_3^- (mg)	SO_4^{2-} (mg)	Cl^- (mg)	Ca^{2+} (mg)	Mg^{2+} (mg)	Na^+ (mg)	K^+ (mg)	EC (µS/cm)	Cdt.* residue (mg)	Mineral residue (mg)
C_1	3.6	127	121	202	15	10	41	435	2690	1138	954
C_2	6.6	224	488	299	27	18	116	855	4330	2237	2033
C_3	4.5	113	133	151	17	8	47	343	2150	945	817
C_4	4.5	175	116	157	17	5	42	368	2090	925	884
C_5	3.0	144	215	135	27	15	30	529	2660	1414	1099

*conductometric residue

Table 3. Heavy metal concentration of analyzed compost samples (mg/kg dm)

Sample	Cd	Cu	Cr	Co	Ni	Pb	Zn
C_1	0.76	50.7	70.0	13.3	21.6	27.1	415.0
C_2	1.56	131.1	63.7	11.0	25.3	29.7	**705.0**
C_3	1.45	107.4	110.2	12.6	22.8	31.4	460.0
C_4	0.67	117.3	147.9	12.9	19.0	25.9	433.0
C_5	nd*	34.5	34.7	9.9	19.9	10.5	351.0
German standards[1]	3.0	150.0	150.0	-	50.0	150.0	500.0
Spanish[2] limits for: class A class B class C	0.7 2.0 3.0	70.0 300.0 400.0	70.0 250.0 300.0	na** na na	25.0 90.0 100.0	45.0 150.0 200.0	200.0 500.0 1000.0
European Commission 2006 (Eco-label)[3]	1	100	100	-	50	100	300

*not detected; ** not available

[1] Heavy Metal Content in MSW vs. Source-Separated Compost in Relation to Standards (Source: Kraus & Grammel., 1992 in Brinton, 2000)

[2] Limits for Classes A, B and C in Spanish legislation RD 506/2013 (in Vázquez et al., 2017).

[3] European Commission, 2006. Commission Decision of 15 December 2006 establishing revised ecological criteria and the related assessment and verification requirements for the award of the Community eco-label to growing media. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32007D0064 (in Vázquez and Soto, 2015).

Compared to the limit values of the heavy metals content in the compost, for the compost quality classes A, B and C mentioned in the Spanish legislation (Limits for Classes A, B and C in Spanish legislation RD 506/2013 cited by Vázquez et al., 2017), none of the studied composts can be included in class A because of the high levels of Zn. Even C_5 had a content of 351.0 mg kg^{-1} compared to 200, which is the limit for the Zn in the Spanish A class. But, the heavy metals contents registered in the analyzed samples from the compost obtained from household organic wastes (C_5) are below the levels indicated by the European Commission Decision of 15 December 2006 establishing revised ecological criteria and the related assessment and verification requirements for the award of the Community eco-label to growing media. In these conditions, it can be applied on agricultural soils in quantities of up to 30 t/ha per year of dry matter (Vázquez et al., 2015).

Salmonella spp. and *Escherichia coli* (*E. coli*) are commonly used indicator species for human and animal pathogens (WRAP/PAS 100:2011).

Table 4. Compost sample pathogen analysis results

	Compost sample	CFU/10 g
Salmonella	C1	Nd
	C2	Nd
	C3	Nd
	C4	Nd
	C5	Nd
Italian Regulation for compost classification, Legislative Decree n. 75/2010 (in Cesaro et al., 2019)		Absent/25 g
WRAP/PAS100:2011 (upper limit)		Absent/25 g
		CFU/10 g
E. coli and coliforms	C1	0,35x10^3
	C2	0,65x10^3
	C3	Nd
	C4	Nd
	C5	0,45x10^3
Italian Regulation for compost classification, Legislative Decree (in Cesaro et al., 2019)		1000-5000/g
WRAP/PAS 100:2011 (upper limit)		1000/g

The results of the microbiological analyses (Table 4) revealed that thermophilic temperatures were not reached during any of the composting methods applied to the studied composts, which in fact was also reported by other authors (Barrena et al. 2014). In these conditions, the microbiological analyses indicated the presence of *E. coli* and coliforms in three of the composts, but their level was below the maximum thresholds allowed by various regulations (Wrap/PAS 100:2011; Italian Regulation for compost classification, Legislative Decree n. 75/2010). *Salmonella* was not detected in any of the composts. However, taking into consideration the presence of *E. coli*, in order to ensure a good hygiene of the compost, the conditions for reaching the thermophilic temperature must be ensured during composting.

The germination index (GI) had values over 60, which is the minimum threshold allowed by the Italian legislation, i.e. the lowest value was of 67.87% at C_2 and the highest value was of 83.89% at C_4. C_5 had a germination index of 70.5% (Figure 1). Similar values were also reported by other authors (Cesaro et al., 2019).

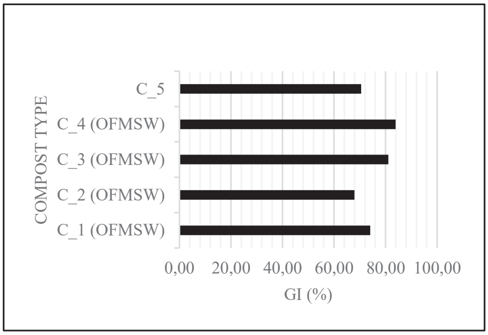


Figure 1. Germination index of five composts

CONCLUSIONS

According to the pH values, the $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio, the germination index and the microbiological indicators values, all composts used in this study reached the maturity. A more efficient separation of organic waste could prevent the presence of heavy metals, and for a good hygiene of the compost it should be possible to ensure thermophilic temperatures. In addition, home composting can be encouraged in rural areas where separation at source is more efficient.

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THE SOCIAL AND ECONOMIC CONTRIBUTION OF THE MAIN CATEGORIES OF NON-WOOD FOREST PRODUCTS FROM BUZAU COUNTY, ROMANIA

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Abstract

The paper work was aimed to explain the main categories of non-wood forest products from Buzau County, Romania. The truly value of non-wood forest were deeply studied especially because of their contribution on marketplace, enhancing in this way the economy. It is based on the statistical data provided by different software such as Expert Choice Desktop which brought the sensivity of each species described. The main categories of non-wood forest products analysed were: mushrooms, tree products, understory plants and animal origin. Every category contains a small number of non-wood forest products and all of these were taken through an analyze based on 19 well-established criteria. As a conclusion, NWFP have a huge contribution in human wellbeing and according to FAO, several million households world-wide depend heavily on NWFP for subsistence or income. The non-wood forest products exclude the potential raw materials, the main branches of constitution being the plants/plant products and also animal/animal products. An important contribution for the human population in Buzau county, especially in human needs and demands is represented by the different types of NWFP.

***Key words**: AHP, criteria, golden chanterelle NWFP, market potential, spruce seeds.*

INTRODUCTION

Non-wood forest products it's an important resource for humans, having an important social and economic contribution at global and national level and different applications in the new domain of bioeconomy.
The wood is the main element of the forest, but, beside this, the forest covers other resources, especially of vegetal nature, which are harvested and valorified of all districts. Distribution and range of NWFPs from Romania became rugged, in the most situations being dependent on environmental and ecological conditions of the forest sites. In essence, the harvesting potential is bigger in the counties situated in the mountain and hilly regions, where the forests are being well distributed than in the regions placed in the plain regions, where the difference between site conditions are not so semnificative.
The name of non-wood forest products contain itself the idea that as such a non-wood product not having in his constitution the wood as raw material (Ciuta, 1961).
In Romania, due to the fact that the variability of the environmental conditions has a high level, the potential in harvesting these is products become overestimated (Bragă and Dincă, 2019).
Since the presence of certain NWFPs is dependent on the presence of the forest stands and by taking into consideration that the distribution of the forests across the country in not uniform, there are counties with great potential in harvesting and marketing of several categories of NWFPs, such as edible mushrooms (Vasile et al., 2017). From the categories of non-wood forest products (NWFPs), the most common products spreaded worldwide are: edible mushrooms, forest berries and medicinal plants (Cântar, 2018). The aim of this paper work was to see the social and economic impacts of non-wood forest products in Buzău County from Romania and the actual contribution on the orientated-market and in human wellbeing.
The actual surface of Buzau Directorate is of 162.190 hectares, from which 159.498 hectares are represented by forests (Figure 1). The administrative surface of Buzau Directorate is done by the 7 districts, is of 84.880 hectares. Related to the surface which is occupied by

forests, this represents 25%, which the predominance of hardwood species (beech - 21.942 hectares) being followed by softwood stands (14.261 hectares) and oak stands (9.951 hectares).

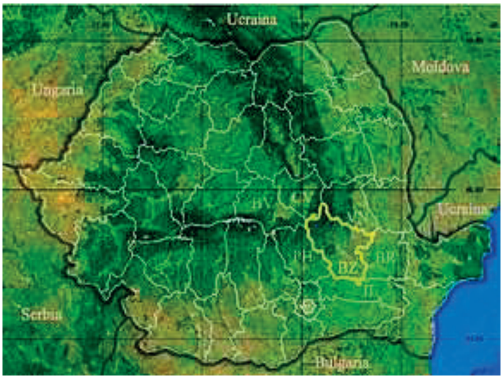

Figure 1. Location of the Buzau County, Romania

MATERIALS AND METHODS

As materials for this study it were used the projects, documents and reports which consists in activities elaborated by the Buzau Directorate. The main activities which are sustained by the directorate consisting in: hunting, fishing, game species and another services which rely on the actual regeneration of forest (forestry seedlings, ornamental seedlings and so on). The NWFPs were grouped in four categories, namely *Mushrooms*, *Understory plants*, *Tree products* and *Animal origin* and based on the above-mentioned data the most promising NWFPs were selected. These four categories were designed in the European project COST Action FP1203 and were taken also into consideration in similar studies recently conducted for Timis (Enescu et al., 2018), Bihor (Timiș-Gânsac et al., 2018), Arad (Pleșca et al., 2019), Vrancea (Tudor and Dincă, 2019) and Dolj (Cântar et al., 2018). As methods, for describing the all alternatives of all of these products, it was used the AHP-analytical hierarchy process, which was based on 19-well established criteria: 1) harvesting period, 2) harvested quantity/worker/8 hours, 3) harvesting cost, 4) knowledge for harvesting, 5) tools needed for harvesting, 6) complexity of the harvesting process, 7) development of the harvesting process, 8) knowledge for recognition, 9) distribution range, 10) biotic threats, 11) abiotic threats, 12) perishability, 13) market potential, 14) market demand, 15) "celebrity" of the product on market, 16) the price of the raw product, 17) the price of the derived product, 18) portfolio of derived products and 19) transport.

Every category was evaluated with absolute numbers situated in the interval (1…8). Using this scale, once the alternatives for making decisions were set, the numbers will be distributed more or less to the criteria of the products which are more reliable in the process of attributing. This evidence will be important for all users, making an hierarchy with all NWFPs that are having alternatives which suit best for the decision making process. Also, the Expert Choice Desktop (v. 11.5.1683) software package was used for the analyses.

RESULTS AND DISCUSSIONS

For each category were analyzed a series of the most important and requested non-wood forest products (NWFPs) for Buzau County (Figure 2).

For the mushrooms category, were selected 2 types of products: *Cantharellus cibarius* (chantarelle) *and Tuber* sp. (truffles). In the understory plants were included three types of non-wood forest products such as: *Corylus avellana* (hazelnut), *Alium ursinum* (wild garlic) and *Urtica dioica* (common nettle). The third category was represented by Tree products in which were analyzed only a single product derived from the genus *Picea*, being located in the spruce cones: spruce seeds. Also, there was some particularities related to the product of animal origin derived from the hunting activity, such as *Cervus elaphus* (red-deer) and *Meles meles* (badger). Beside the results obtained and mentioned in the Table 1, it was calculated the mean of alternatives for each species (Figure 3).

The first place is taken by the *Tuber* sp. (truffles), having a consistent number of alternatives (mean = 6.74) on potential market and demand and also is declared a "Celebrity" on the market.

Table 1. Alternatives ranking

Crite-rion	Mush-rooms		Tree products	Under-story plants			Ani-mal origin	
	Cantharellus cibarius	*Tuber* sp.	Spruce seeds	*Corylus avelanna*	*Alium ursinum*	*Urtica dioica*	*Cervus elaphus*	*Meles meles*
1	5	6	7	8	1	2	3	4
2	6	7	1	8	2	3	5	4
3	5	3	4	6	7	8	1	2
4	3	8	4	6	1	2	7	5
5	7	8	5	4	1	2	6	3
6	6	8	4	3	1	2	7	5
7	3	8	4	5	1	2	7	6
8	3	7	4	5	1	2	8	6
9	3	5	4	6	7	8	2	1
10	5	8	1	7	3	4	6	2
11	6	7	4	5	1	2	8	3
12	5	7	3	8	2	1	6	4
13	8	6	3	4	2	1	7	5
14	8	7	5	1	2	3	6	4
15	7	8	1	6	3	4	5	2
16	6	8	1	7	3	4	5	2
17	7	4	1	2	6	3	8	5
18	8	7	4	5	2	1	6	3
19	5	6	7	4	1	2	8	3

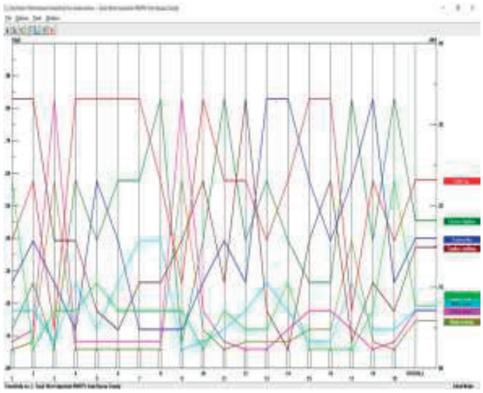

Figure 2. The diagram of sensivity of every species

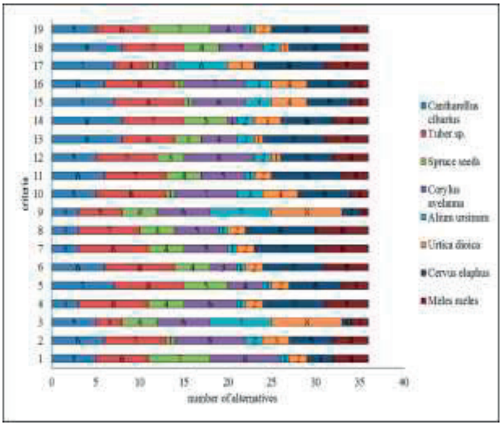

Figure 3. The alternatives of every species attributed to each criteria

The second place is occupied by the *Cervus elaphus* (red-deer) from the animal origin class, which reached the maximum number of alternatives (mean = 5.84) in 4 cases: knowledge of recognition, abiotic threats, the price of the derived products and the transport of the products. So, in this case, is essential to keep the products in safety, in special buildings, to monitor permanently the products with sensible character to avoid the possibility of installing the negative factors. Another product from the mushrooms class is *Cantharellus cibarius* (chanterelle) which scores the mean of 5.58, taking the third place. This mushroom is very appreciated in the marketplace, improving the potential and the huge demanding. Furthermore, there are a lot of derived products which can be used in many domains.

***Tuber* sp. (truffles)**

The origin of the term truffle comes for the Latin name Tuber, which meant tumor, excrescence or swelling (Simpson and Weiner, 1989). In the past, Balzac had a special passion for truffles, beside his prolific opera (Dincă and Dincă, 2012).

Truffles are micoritic mushrooms which are growing and developing underground, in the system formed by fine roots of trees (beech, hazel, oak, hornbeam and so on). In Romania, the only species of truffles which can be successfully cultivated is *Tuber aestivum*. In general, this species can be cultivated in the pastures, orchards and even in the vineyards, where the soil is still active and without the presence of another micoritic mushrooms which can consume the existing nutrients (Dincă and Dincă, 2014).

For instance, the initiation of the culture begin with the physico-chemical analyzes of the soil. The preference for this type of truffle is that the soils should be permeable, limestone, rich in organic substances and with moderate humidity (Dincă and Dincă, 2014). In order to discover and harvest them, it is needed to use a special category of trained dogs (Dincă and Dincă, 2015).

***Corylus avellana* (hazelnut)**

The experts from Poland found the important information about the composition of chemical components of hazelnuts shells, but being limited to the identification of phenolic compounds, such as flavonoid glycosides and aromatic acids (Ciemniewska-Żytkiewicz et al., 2015). At globally sense, hazelnut (*Corylus avellana* L.) is the one of the most cultivated and marketed nuts (Mencherini et al., 2017).

From the countries, the first country with the largest hazelnut production is Turkey (about 600,000 t/year) and the second is Italy (105,000 t/year) (www.fao.org).

In Romania, the hazelnut is meet starting from the plain zone reaching the high altitude of 1400 meters in the spruce stands. It has important esthetic value, being well cultivated in parks, gardens and orchards being a species which support the cuttings. Hazelnut is a resistant species at frost, but he cannot support the dryness. It prefers the fertile soils, rich in humus and in organic substances. His temperament is of light. The fruits are achenes, called "hazelnut", which have an soft pericarp in youth, becoming hard after the maturation. The hazelnuts are comestible and they are appreciating for the sweet taste.

Spruce seeds. Spruce is one of the most extended species from central and meridional yards, with a significant presence in Europe. In Romania the natural and cultural areal of spruce sum 1.43 million hectares, which means aproximatively 22% from the forest surface (Șofletea and Curtu, 2007). The particularity of the spruce seeds is that they have a high germinative power reaching 70-80% up to the 96% in good environmental conditions. Due to the fact that the seeds are winged it offers the possibility of dissemination on long distances, at 2-3 of tree height. Also, the seeds are not having resin in tegument being easy to carry them at long distances by wind. The maturation is annual, in the autumn.

There were a lot of studies regarding on the implementation of AHP for different categories of non-wood forest products in many counties in Romania. The studies in Cluj County, it was obtained a statistic with the highest rate in market potential for the mushrooms (chanterelle) and the lowest potential to the understory plants (Enescu et al., 2018). The results from Timis County were concretized by the abundance of Genus Boletus, which have a highest numbers of alternatives in market potential and a various scale of derived products (Enescu et al., 2018). In Prahova County, the rank of mushrooms was intermediate, in comparison with other counties such as Brasov or Maramures (Enescu et al., 2018). In Bacau County, based on the AHP results, the most important NWFPs were penny bun (*Boletus edulis*) and truffles (*Tuber* sp.) and less important ones was the category of understory plants (Blaga et al., 2019).

CONCLUSIONS

Non-wood forest products have a good economic and social contribution in Buzau county. The total surface covered by forest can be well managed in order to obtain good results in promoting and valorizing the NWFPs, not only the wood.

Using the AHP based on alternatives (1…8) attributed to 19-well established criteria, it was shows that the best results are achieved by the mushrooms category, such as *Tuber* sp. (truffles) and *Cantharellus cibarius* (chanterelle) both reaching maximum alternatives in the market potential and demand. Furthermore, these products have on consistent portofolio which offers a diversity of derived products. A high interest consist in the product of animal origin such as red-deer (*Cervus elaphus*) and badger (*Meles meles*), which need a good understanding in knowledge of managing well the sustainable production. Knowing the potential of Buzau County which is rich in NWFPs it is essential to implement a sustainable management for all of these resources.

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THE AGROECONOMIC VALUE OF REED CANARY GRASS, *Phalaris arundinacea* IN REPUBLIC OF MOLDOVA

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Abstract

Grasses are considered as one of the most important sources of feed for herbivorous animals and feedstock for renewable energy production. The aim of the current study was to evaluate the biomass quality (green mass, hay and haylage) from reed canary grass, Phalaris arundinacea, cv. Premier grown under the conditions of the Republic of Moldova. A rapid predictive method based on near-infrared spectroscopy (NIRS) was developed to measure crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL) and total soluble sugars (TSS). The results of the research revealed that the harvested reed canary grass (first cut) contained 25.1-25.6% dry matter. The biochemical composition and energy value of prepared hay and haylage: 109-127 g/kg CP, 92-95 g/kg ash, 417-443 g/kg ADF, 695-708 g/kg NDF, 28-44 g/kg ADL, 62-64 g/kg TSS, 54.4-56.4% digestible dry matter, 10.87-11.23 MJ/kg digestible energy, 8.92-9.22 MJ/kg metabolizable energy and 4.94-5.24 MJ/kg net energy for lactation. The biochemical methane potential of investigated Phalaris arundinacea biomass were 335-362 l/kg ODM. Reed canary grass, cv. Premier, produces high yields with optimal nutrient content, can be used as feed for ruminant animals, as well as substrate for the production of biomethane by anaerobic digestion.

Key words: *biochemical composition, biomethane potential, fodder value, green mass, hay, haylage, Phalaris arundinacea.*

INTRODUCTION

Grasslands serve critical habitat for many plant and animal species, have tremendous economic value ensuring humans food, forage for herbivores and ruminants animals, and feedstock for biorefinery, provides an important regulating ecosystem service such as pest control, carbon storage, protection of soil from erosion and nutrient leaching, contributing to a recreational values and cultural heritage. Recently, the interest in the efficient use and conservation of grassland has been restarted, efforts to conserve the remaining permanent grasslands. The creation of grasslands on polluted and degraded agricultural land are underway in many states and around the world.

Forage nutritive value affects forage utilization by herbivores. Higher nutritive value grasses have the potential to significantly increase milk or meat production and profitability of an expanding animal husbandry industry. Productive reconversion, considers agronomic criteria, such as the changes of current species by native species or alternative crops that are apt to survive and produce in areas susceptible to conversion.

Poaceae is clearly the most abundant and important family, accounting for about 24% of the Earth's vegetation, contained belong to 777 plant genera and includes 11461 accepted species names. *The Plant List* includes 110 scientific plant names of species rank for the genus *Phalaris* of these 19 are accepted species names. Reed canary grass, *Phalaris arundnacea* L. (syn. *Baldingera arundinacea* (L.) Dumort; *Phalaroides arundinacea* (L.) Rauschert; *Typhoides arundinacea* (L.) Moench) is a long-lived perennial grass, native to Europe, C_3 photosynthetic pathway. The stem is sturdy, hairless and hollow with some reddish coloring in the upper part, 60 to 200 cm tall. The leaf blades are flat with prominent ligules, usually green, flat, glabrous and taper gradually, 30-45 cm long and 0.8-1.2 cm wide. The leaves of the lower stem become light deprived as the plant grows and are replaced with new leaves higher up the stem. The inflorescence is branched panicles 7 to 40 cm long. Immature panicles are compact and resemble spikes, but open and become slightly

spreading at anthesis. Spikelets are lanceolate, 5 mm long and pale. The fruit is a caryopsis covered by coriaceous pallets, grain 1.5-4.0 mm long, 0.7-1.5 mm wide, containing a single grain, subovoid brown with faintly striate surface, the weight of 1000 seeds averages 0.9 g. *Phalaris arundinacea* develops an extensive, rhizomatous root system. New rhizomes originate almost entirely below the soil surface from buds at the nodes of other rhizomes. Roots and rhizomes may form an almost impenetrable sod.
A cool season grass, reed canary is one of the first grasses to sprout in spring, is adapted to poorly drained soils, it also is used for erosion control along streambanks, gullies, ponds, and lakes. It is very competitive once established and will frequently develop a solid monoculture. Reed canary grass has excellent frost tolerance and it also has good drought tolerance. It survives prolonged flooding by possessing anoxia tolerant rhizomes; once established, it can withstand continuous inundation for 60 to 70 days. Because of its importance as a pasture grass, most efforts to develop improved populations or cultivars have focused on solving problems related to agronomic performance or livestock utilization. New cultivars of reed canary grass have reduced gramine concentrations and no tryptamine or β-carboline alkaloids (Anderson et al., 2008). The productivity of *Phalaris arundinacea* reached 90 t/ha green mass, the digestible indices of nutrients: 72% crude protein, 55% crude fats, 65% crude cellulose and 72% nitrogen free extract (Medvedev & Smetannikova, 1981). This species has been an important component of permanent and temporary grassland, it has been the subject of much agricultural research (Alway, 1931; Chalupa et al., 1961; Tosi & Wittenberg, 1993; Ordakowski-Burk et al., 2006; Maruşca et al., 2011; Tokita et al., 2015), in the past decades has also been the subject of much research into biomass/energy crops which are suitable for biorefinery feedstocks (Anderson et al., 2008; Sepälä et al., 2009; Butkuté et al., 2014; Oleszek et al., 2019).
The objective of this research was to evaluate quality of the *Phalaris arundinacea,* and the possibility to use green mass, hay and haylage as feed for ruminant animals and feedstock for the production of biomethane.

MATERIALS AND METHODS

The cultivar *'Premier'* of reed canary grass *Phalaris arundinacea*, 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 green mass was harvested manually. The samples were collected in pre-anthesis period (1-st cut) in second and third growing season. The leaves/stems ratio was determined by separating the leaves from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). The prepared hay was dried directly in the field. The haylage was prepared from wilted green mass, shredded and compressed in well-sealed glass containers, after 45 days, the containers were opened and haylage were evaluated in accordance with standard laboratory procedures. Dry matter content was detected by drying samples up to constant weight at 105°C. Some assessments of the main biochemical parameters: protein, ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS) have been evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research-Development Institute for Grassland Brasov, Romania. The concentration of hemicelluloses (HC) and celluloses (Cel), digestible dry matter (DDM), dry matter intake (DMI), 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 obtained 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 protein (PB), acid detergent lignin (ADL) and hemicellulose (HC) values:

biogas $Yb = 670 + 0.44PB + 0.16HC - 3.02ADL$
biometan $Ym = 370 + 0.21PB + 0.05HC - 1.61ADL$

RESULTS AND DISCUSSIONS

We could mention that, the second growing season reed canary grass *Phalaris arundinacea* resumed growth and development in spring, in the middle March, when temperatures above 5-6 C were established, but in third growing season in the end March. The weather conditions in April-May 2019, with an optimal amount of rainfall, and lower air temperatures as compared with the previous year, helped the plants produce more shots and were favourable for their growth, development, and biomass production. The results regarding some agrobiological peculiarities of the *Phalaris arundinacea* 'Premier' and the structure of the harvested biomass are presented in Table 1. We would like to mention that the reed canary grass 'Premier', at the time of harvest, in the 2018 y. reached the height of 62.3 cm, but in 2019 y. the plants being the highest - 116.1 cm. In the harvested biomass the leaves content was 29.9-31.2%, the amount of dry matter - 25.1-25.6%. The green mass productivity from first cut in second growing season reached 35.9 t/ha, but in the third growing season 48.9 t/ha.

According to Maruşca et al. (2011) the potential yields of the *Phalaris arundinacea* 'Premier' in Romania were 65-80 t/ha green mass or 16-20 t/ha dry matter. Tokita et al. (2015) reported that the proportions of stem and leaf tissue in the harvested reed canary grass, were 62.0% and 29.0% at first cut, and 44.0% and 56.0%, respectively at second cut.

Table 1. Some agrobiological peculiarities and the structure of the green mass of the *Phalaris arundinacea* 'Premier'

Harvest period	Plant height, cm	Leaf, g		Stem, g		Productivity, t/ha	
		green mass	dry matter	green mass	dry matter	green mass	dry matter
Second growing season, 2018 y.	62.3	3.19	0.82	7.24	1.81	35.9	9.1
Third growing season, 2019 y.	116.1	3.92	1.03	9.79	2.42	48.9	12.5

Table 2. Biochemical composition and nutritive value of fodder from Phalaris *arundinacea* 'Premier'

Indices	second growing season, 2018 y.		third growing season, 2019 y.		
	green mass	hay	green mass	hay	haylage
Crude protein, g/kg	144	124	109	109	127
Minerals, g/kg	89	86	74	92	95
Acid detergent fibre, g/kg DM	375	387	411	443	417
Neutral detergent fibre, g/kg DM	615	623	685	708	695
Acid detergent lignin, g/kg DM	36	39	36	44	28
Total soluble sugars, g/kg DM	-	-	115	64	62
Digestible dry matter, %	59.7	58.8	56.9	54.4	56.4
Dry matter intake, % BW	1.95	1.93	1.75	1.69	1.73
Relative feed value	90	88	77	72	75
Digestible energy, MJ/ kg	11.82	11.65	11.32	10.87	11.23
Metabolizable energy, MJ/ kg	9.70	9.56	9.29	8.92	9.22
Net energy for lactation, MJ/ kg	5.72	5.58	5.31	4.94	5.24

Analyzing the results of the green mass quality of the *Phalaris arundinacea* 'Premier', Table 2, we found that dry matter of the harvested green mass contained 109-144 g/kg CP, 74-89 g/kg ash, 375-411 g/kg ADF, 615-685 g/kg NDF, 36 g/kg ADL, 115 g/kg TSS, 56.9-59.7% DDM. The natural fodder have RFV= 77-90, 11.32-11.82 MJ/kg DE, 9.29-9.70 MJ/kg ME and 5.31-5.72 MJ/kg NEl. The concentrations of crude protein was high in the natural fodder second growing season. The level of structural carbohydrates increased substantially in the fodder third growing season, which had a negative effect on digestibility, relative feed value and energy content. Some authors mentioned various findings about the green mass quality of the reed canary grass, *Phalaris arundinacea*. Jansone et al. (2012) mentioned that yields of studied reed canary grass varieties was 8.06-9.67 t/ha DM, crude protein contents 115.6-120.7 g/kg DM, digestible protein 37.8-45.4%. Tokita et al. (2015), reported that the nutrients contents of stems and leaves in harvested mass at the first cut were: 9.3% and 21.6% CP, 1.2 and 3.7% fat, 70.9 and 52.1% NDF, 42.0 and 24.4% ADF, 4.0 and 1.8% ADL, with digestibility 57.0 and 70.9 %, energy content 4119.9 and 4591.8 cal/g, respectively. Villalobos (2012), remarked that nutritional value of reed canary grass were 16.65-19.47% CP, 53.18-57.77% NDF, 34.60-36.86% ADF, 3.78-4.42% lignin, 18.79-20.91% hemicellulose, 31.04-32.34% cellulose, 63.38-70.55% IVDMD with estimated energy content 2.72-2.81 Mcal/kg DE, 2.10-2.18 Mcal/kg ME, 1.29-1.34 Mcal/kg NEl.

The period of winter feeding in our region lasts for about 200 days. Thus the production of preserved forages (hay, silage, haylage) is necessary, which enable the uniform and fully-valuable animal feeding during the whole year. Hay is a very popular and valuable feed for farm animals, a rich source of protein, vitamins and minerals, both in winter and throughout the year, especially for the young animals, pregnant females and breeding males. Feeding high quality hay can also reduce the level of grain supplementation needed during winter. The presence of bulky forages, including hay, in a feeding dose is essential, which determines the proper function of digestive track the fat content in milk. The prepared hay from *Phalaris arundinacea* 'Premier' contained 109-124 g/kg CP, 86-92 g/kg ash, 623-708 g/kg NDF, 387-443 g/kg ADF, 39-44 g/kg ADL, 54.4-58.8% DDM, 8.92-9.56 MJ/kg ME and 4.94-5.58 MJ/kg NEl. Thus, the preparation of the hay resulted in a decrease in the content of crude protein and an essential increase in the content of structural carbohydrates as compared with the freshly harvested mass, and fact had a negative impact on the net energy for lactation and relative feed values.

Several literature sources describe the nutritional performance of hay made from *Phalaris arundinacea*. Chalupa et al. (1961) found that hay contained 127.2-216.4 g/kg CP, 32.5-46.8 g/kg EE, 22.7-268.0 g/kg CF, 356.4-406.0 g/kg NFE, 62.2-70.2 g/kg ash, 536.3-604.2 g/kg total digestible nutrients and 2.407-2.694 Mcal/kg DE, the high-N fertilized applications rate increased the content of crude and digestible protein, fats, total digestible nutrients and energy, while crude fiber and nitrogen-free extract decreased. Archibald et al. (1962), reported that the composition of hay fed was: 21.3% CP, 2.3% EE, 29.1% CF, 37.2% NFE, 10.1% ash, 4.0% sugar, 20.3% cellulose, 5.6% lignin and 19.1% pentosans. Tosi & Wittenberg (1993), remarked that hay produced from reed canary grass contained 10.8-12.2% CP, 61.5-62.8% NDF, 36.7-38.0% ADF with 56.7-64.6% apparent dry matter digestibility, but timothy hay contained 14.4% CP, 59.2% NDF, 33.8% ADF with 60.9% apparent dry matter digestibility. According to Ordakowski-Burk et al. (2006) the nutrient composition of reed canary grass hay was 17.1% CP, 65.4% NDF, 33.5% ADF, 1.8% ether extract, 1.2% starch, 9.6% sugar and 2.3 Mcal/kg DE for horses, but timothy hay-14.4% CP, 62.6% NDF, 35.0% ADF, 2.5% ether extract, 1.6% starch, 11.5% sugar and 2.2 Mcal/kg DE for horses, respectively.

The preserved grass (silage and haylage) is the basis of most winter feeding systems and satisfactory animal performance is largely dependent on the adequate intake of good quality roughage fed. During the organoleptic assessment, it was found that the colour of the prepared haylage from *Phalaris arundinacea* 'Premier' was homogeneous olive, with pleasant smell, similar to pickled vegetables. The fermentation quality of haylage made from *Phalaris arundinacea* 'Premier' is illustrated in Table 3. The haylage material consolidated well and the fermentation was complete with pH values 4.65. It has been determined that the amounts of organic acids reached 34.8 g/kg DM, most organic acids in fixed form. The content of lactic acid reached 83.9 %, butyric acid was detected in fixed form- 1.4 %.

Table 3. The fermentation quality of the haylage from *Phalaris arundinacea* 'Premier'

Indices	*Phalaris arundinacea*
pH index	4.65
content of organic acids, g/kg	34.8
free acetic acid, g/kg	2.1
free butyric acid, g/kg	0.0
free lactic acid, g/kg	9.7
fixed acetic acid, g/kg	3.0
fixed butyric acid, g/kg	0.5
fixed lactic acid, g/kg	19.5
total acetic acid, g/kg	5.1
total butyric acid, g/kg	0.5
total lactic acid, g/kg	29.2
acetic acid, % of organic acids	14.7
butyric acid, % of organic acids	1.4
lactic acid, % of organic acids	83.9

The results of the investigations (Table 2) indicate that haylage from *Phalaris arundinacea* 'Premier', contained 127 g/kg CP, 95 g/kg ash, 695 g/kg NDF, 417 g/kg ADF, 28 g/kg ADL, with 56.4% DDM, 9.22 MJ/kg ME and 5.24 MJ/kg NEl. Thus, the preparation of the haylage resulted in a increase in the content of crude protein and an reduce level of structural carbohydrates as compared with the hay, and fact had a positive impact on the increase net energy for lactation. A similar relationship was noted in other study. Oleszek & Matyka (2017), remarked the chemical composition of ensiled reed canary grass were: 11.7% CP, 2.6% EE, 10.2% ash, 7.7% NFC, 32.2% cellulose, 26.6% hemicellulose, 8.6 % ADL. Utama et al. (2018) mentioned that prepared reed canary grass haylage contained: 547 g/kg DM, 11.1% CP, 2.6% fats, 7.3% ash, 38.5% CF, 66% NDF, 9.4 MJ/kg metabolizable energy.

Table 4. Biochemical composition and biomethane production potential of *Phalaris arundinacea* 'Premier' substrates

Indices	second growing season, 2018 y.		third growing season, 2019 y.		
	green mass	hay	green mass	Hay	haylage
Crude protein, g/kg DM	144	124	109	109	127
Minerals, g/kg DM	89	86	74	92	95
Nitrogen, g/kg DM	23	19.8	17.4	17.4	20.3
Carbon, g/kg DM	50.6	50.8	51.4	50.4	50.3
Ratio carbon/nitrogen	22	26	30	29	25
Cellulose, g/kg DM	339	348	375	399	389
Hemicellulose, g/kg DM	240	236	274	265	278
Acid detergent lignin, g/kg DM	36	39	36	44	28
Bio biogas potential, L/kg VS	663	645	653	627	686
Biomethane potential, L/kg VS	354	345	349	335	362

Biogas is one of the three most important biofuels in terms of raw material availability and costs of production. The technology of biomass conversion through anaerobic digestion and biomethane production represents the source of renewable energy with great potential, environmentally friendly and rapidly expanding in the latest years. The concentrations of organic constituents in the biomass and their availability, the carbon nitrogen ratio (C/N) plays a crucial role in the process of biomethane production (Vintilă & Neo, 2011; Vintilă et al. 2012; Dandikas et al. 2015).

The results regarding of the substrates quality and its biochemical methane potential are shown in Table 4. We found that investigated substrates from *Phalaris arundinacea* 'Premier', according to the C/N ratio, which constituted 22-30, met the established standards. The essential differences were observed between the content of cellulose, hemicellulose and lignin. The haylage substrate contained an acceptable amount of hemicellulose and a lower content of lignin as compared with the other substrates. The biochemical methane potential of investigated reed canary grass hay substrates was 335-345 l/kg ODM, green mass substrates 349-354 l/kg ODM, but haylage substrate reached 362 l/kg ODM. Krzystek et al. (2020), indicated the biogas potential of reed canary grass silage 490 l/kg and an annual methane productivity 2558 m^3/ha. Alvinge (2010) mentioned that the methane yield for *Phalaris arundinacea* was 323 l/kg, but *Typha latifolia*- 300 l/kg. Sepälä et al. (2009) obtained 296 l/kg from *Phalaris arundinacea.* Butkuté et al., 2014 reported that reed canary grass biomass contained: 37.2-42.2% cellulose, 19.0-22.9.0% hemicellulose, 3.17-7.66% ADL, with C/H=21.9-33.1 and its biomethane potential 316-426 l/kg VS.

CONCLUSIONS

The green mass productivity of *Phalaris arundinacea* 'Premier', at the first harvest, reached 35.9-48.9 t/ha, the dry matter contained 109-144 g/kg CP, 74-89 g/kg ash, 375-411 g/kg ADF, 615-685 g/kg NDF, 36 g/kg ADL, 115 g/kg TSS, 56.9-59.7% DDM, 11.32-11.82 MJ/kg DE, 9.29-9.70 MJ/kg ME and 5.31-5.72 MJ/kg NEl.

The biochemical composition and energy value of prepared hay and haylage can reach 109-127 g/kg CP, 92-95 g/kg ash, 417-443 g/kg ADF, 695-708 g/kg NDF, 28-44 g/kg ADL, 62-64 g/kg TSS, 54.4-56.4% digestible dry matter, 10.87-11.23 MJ/kg digestible energy, 8.92-9.22 MJ/kg metabolizable energy and 4.94-5.24 MJ/kg net energy for lactation.

The biochemical methane potential of the studied substrates was 335-362 l/kg ODM.

Under the conditions of the Republic of Moldova, the cultivar *'Premier'* of reed canary grass produces high yields with optimal nutrient content and can be used as feed for ruminant animals, as well as substrate for the production of renewable energy.

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BIOMASS QUALITY OF HEMP, *Cannabis sativa* L., AND PROSPECTS OF ITS USE FOR VARIOUS ENERGY PURPOSES

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Abstract

Plant biomass is an important renewable energy source in the world, and its importance has increased in regional and national energy strategies. The main objective of this research has been to evaluate the dry biomass quality of hemp, Cannabis sativa L., collected from the experimental field of the National Botanical Garden (Institute), Chişinău *as feedstock for the production cellulosic ethanol and solid bio fuel, namely briquettes. Corn stalks, Zea mays, were considered as control. The analysis of the chemical composition suggested that the dry matter of the whole hemp plants contained 556 g/kg cellulose, 309 g/kg hemicellulose and 105 g/kg acid detergent lignin, but corn stalks - 417 g/kg cellulose, 250 g/kg hemicelluloses and 82 g/kg acid detergent lignin. The theoretical ethanol yield from structural carbohydrates averaged 628 L/t in hemp substrates, as compared with 485 L/t in corn substrates. The bulk density of the milled chaffs of tested biomass, varied from 100 to132 kg/*m^3*, the gross calorific value - from 17.8 to 19.0 MJ/kg, the ash content - from 2.1 to 4.4%. The Cannabis sativa biomass was characterised by high gross calorific values and low ash content. The specific density of hemp briquettes reached 867 kg/*m^3*. Hemp, Cannabis sativa, may serve as multi-purpose feedstock for renewable energy production.*

Key words: biomass quality, briquettes, *Cannabis sativa*, *calorific values, theoretical ethanol yield.*

INTRODUCTION

The interest in alternative energy sources has increased in recent years due to the higher awareness of the negative impacts of climate change and the depletion of fossil fuel reserves. Hydro, bio, geothermal and solar energies are among these alternative energy sources. Biomass is the most developed renewable energy source with a large number of applications starting with direct heating for industrial or domestic purposes and ending with electricity generation or production of gaseous and liquid fuels. The flexibility of biomass as an energy carrier has led to the development of a wide range of biomass conversion technologies and growing biomass share within the global energy balance. According to Eurostat, bioenergy makes up over two thirds of the European Union's renewable energy production.
Since the time humans discovered fire, plant biomass has been a primary source of fuel. Plant biomass is an important renewable energy source in the world, and its importance has increased in regional and national energy strategies. Energy crops are a part of the modern biomass production and can be converted into various types of fuels or energy. The target of an intensified use of agricultural crops as renewable sources of energy thus represents an important question and challenge in the intersection between energy, environmental, agricultural, research and economic policy (Roman et al., 2016).
The combustion of solid biomass represents a direct conversion of biogenic primary energy sources. The densification of plant biomass into pellets and briquettes, contributes to improving its energy efficiency as a fuel, which makes it suitable for use by small households and by industrial consumers.
Ethanol is one of the most appealing fuels in the transportation sector, because it can be blended with petrol or used in its pure form in modified engines. Most of the bioethanol is

produced from cereals or sugar cane, but the share of cellulosic bioethanol is rapidly increasing. The advantage of the cellulosic bioethanol, compared to traditional grain/sugar ethanol, is the fact that it is possible to use entire above-ground biomass of a plant for bioethanol production, thus enabling better efficiency and land use. Using lignocellulosic agricultural residues and energy crops as raw materials for the production of ethanol fuel will also minimize the potential conflict between food and fuel production.

Hemp, *Cannabis sativa* L., family *Cannabaceae,* is one of the oldest crops in the world dating back to 8,000 B.C. It is an annual short day, C3 plant, stems growing up to 4 m tall, leaves are finely hairy with alternate leaves, palmately divided into 3-11 lanceolate toothed leaflets, male flowers are yellow-green, up to 5 mm in diameter, borne in small panicles, the female plants are a darker green, with denser foliage and tightly bunched panicles, the flowers are closely surrounded by tubular bracteoles. The female axillary leafy clusters do not form any compact strobilus. The fruiting body is greyish-brown in colour and about 4 mm long. The deep (1.0-1.5 m), tap root system of hemp contributes to a good aeration of the soil and optimum air/water conditions. It also loosens the soil and allows the plant to use water from deeper layers of the soil. Hemp is a crop producing big amounts of biomass, which, when ploughed down, contributes to the fast restoration of the biologically active soil layer on degraded land. Furthermore hemp requires little to no use of fertilizers and pesticides making it a very sustainable crop. Hemp has historically been used in fibre, food and medicinal production. The cultivation, possession and use of hemp were strictly forbidden in most countries of the world during the 20 century. Today hemp is a niche crop, cultivated on more than 33,000 ha in the European Union. Fibrous hemp is a plant that easily adapts to new conditions of vegetation and is characterized by a rich diversity of forms. Hemp stem, depending on the cultivars, consists of approximately 20-40% of fibre, which is outside of the stem, and 60-80% of wood (hurds). It is a phytosanitary plant which makes possible its introduction into a variety of crop rotations. In Lithuania, hemp produced 16.2-22.6 t/ha of dry biomass (Jankauskienė and Gruzdevienė, 2010).

Since ancient times, hemp has been grown in our region for fibre, oil and food production, animal bedding and fuel (Tabără, 2009). However, in recent years, there has been increasing interest with regards to using hemp crop to obtain raw material for biorefineries and generation of energy. Due to the crops' high biomass yield and oil content, it can be used as a feedstock for the production of solid biofuels, biogas, biodiesel and cellulosic bioethanol (Alaru et al., 2011; Kreuger et al., 2011; Prade et al., 2011; Tutt et al., 2013; Jasinskas et al., 2014; Kuglarz et al., 2014; Kraszkiewicz et al., 2019). The calorific value of hemp biomass varied from 17.9 to 19.8 MJ/kg depending on the part of the plant (Mankowski et al., 2014).

The main objective of this research was to evaluate the dry biomass quality of hemp, *Cannabis sativa*, as feedstock for the production cellulosic ethanol and solid bio fuel-briquettes.

MATERIALS AND METHODS

Industrial hemp, *Cannabis sativa* cultivar 'Bialobrzeskie' was cultivated at the experimental plot of the National Botanical Garden (Institute) "Alexandru Ciubotaru", Chişinău, latitude 46°58′25.7″N and longitude 28°52′57.8″E, served as subjects of the research, and corn stalks (*Zea mays*) were used as control.

Hemp seeds were sown in middle May, 200 viable seeds m^2. Hemp stalks were collected in the September-February period. Stems and leaves (including fine stems) were separated manually after drying and weighed.

The hemp stalks were chopped and disintegrated by knife mill with a sieve with the mesh size of 1 mm. The content of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 at the Research-Development Institute for Grassland Brasov, Romania. The cellulose is ADF minus ADL and hemicelluloses is NDF minus ADF. Theoretical Ethanol Potential (TEP) was calculated according to the equations of Goff et al. (2010) based on conversion of hexoses (H) and pentoses (P) sugars:

$$H = [\% \text{ Cel} + (\% \text{ HC} \times 0.07)] \times 172.82$$

$$P = [\% \text{ HC} \times 0.93] \times 176.87$$

$$TEP = [H + P] \times 4.17$$

For the production of solid biofuel, the harvested hemp stalks were chopped into chaff with the use of a stationary forage chopping unit. The chopped phytomass was milled in a beater mill equipped with a sieve with diameter of openings of 10 mm using the equipment SM 100. The physical and mechanical properties of dry biomass were determined according to the European Standards, at the State Agrarian University of Moldova: 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 550ºC in a muffle furnace HT40AL according to SM EN ISO 18122; 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 briquetting was carried out by hydraulic piston briquetting press BrikStar model 50-12 (Briklis). The mean compressed (specific) density of the briquettes was determined immediately after removal from the mould as a ratio of measured mass over calculated volume.

RESULTS AND DISCUSSIONS

We could mention that, under the climatic conditions of the Republic of Moldova, the hemp seed emerged in 4 days after sowing and reached growth stage of the first pair of true leaves in 8-9 days after sowing. During the first month after the emergence of seedlings, the root system developed more intensively. The daily growth of the main root during this period was from 1.5 to 1.8 cm, the lateral roots appeared 8-10 days after emergence, when the length of the main root was 11-13 cm. The taproot system of the hemp was strong, well developed, penetrating to a depth of up to 200 cm, developed 25-50 lateral roots, due to which the plants were resistant to flattening and unfavourable weather conditions (heavy rains, wind). They very effectively used the nutrients from the soil, especially below the arable layer. It has been established that at the beginning of June, hemp plants were already 12-14 cm in height, had 3-5 pairs of true leaves. The aboveground part of the plants developed more intensively at the end of June, in the middle of August, the height of hemp plants reached 360-385 cm. The buds emerged at the beginning of July and the first flowering hemp plants appeared only in the second half of July, the mass flowering period lasted 29-36 days. The beginning of maturity of seeds was noticed at the end of August and finished in October.

It is known that leaf and moisture contents in the harvested energy biomass influenced the costs of transport, storage, drying and processing. In the middle of August, some of the leaves on the lower part of the stem turned yellow and fell. The results of the assessment of moisture and leaf contents in the in harvested energy biomass of *Cannabis sativa* are shown in Table 1. After taking samples in the first days of September, it was found that the moisture and leaf contents of aboveground parts were 72.8% and 34.1%, respectively. In October-November, the defoliation and dehydration of the hemp stems accelerated, the leaf content in the harvested biomass decreased to 4.0%, and the moisture content lowered to 22.3%, respectively. In winter period, when temperatures below 0°C were recorded, the leaves were falling and the stems were already dry and could be harvested and chopped directly in the field. The yield of harvested hemp stems in February was 9.5 t/ha.

Table 1. Moisture and leaf contents in the harvested energy biomass of *Cannabis sativa*, %

Harvesting Period	*Cannabis sativa*	
	Moisture content	Leaf content
September	72.8	34.1
October	55.0	15.1
November	22.3	4.0
December	17.7	2.7
January	14.3	0.5
February	10.4	0.1

Mankowski and Kolodziej (2008), mentioned that, hemp generates about 10-15 t of biomass per hectare and it has been estimated that 1 ha of hemp absorbs about 2.5 tons CO_2, which results in a significant reduction of the greenhouse effect. The research conducted in the University of Bologna, Italy by Zatta and Venturi (2009), revealed that, depending on the harvest time, the hemp stem dry matter yield varied from 11.2 to 16.5 t/ha. Prade et al. (2011) demonstrated that hemp grown for energy could provide yields of 14.4 t/ha when harvested in autumn and 9.9 t/ha when harvested in spring. Kolarikova et al. (2013) stated that, in the Czech Republic, the hemp yield from autumn harvest was 22.1-25.5 t/ha or 8.6-9.65 t/ha dry matter.

The major components of lignocellulosic biomass are cellulose, hemicellulose and lignin. Cellulose is a crystalline and linear structure made up of units of glucose strongly linked together by β-1-4-glycosidic bonds. These linkages give cellulose very high crystalline structure making it resistant to degradation. It is the most abundant organic polymer on earth. Hemicellulose, on the other hand, consists of linear and highly branched mixture of pentoses (xylose and arabinose) and hexoses (glucose, galactose, and mannose). Lignin is a highly branched polyphenolic polymer, which gives stability to biomass structure. The contents of these components vary significantly depending on the plant species, type of biomass, harvesting period. The possibility of converting lignocellulosic biomass in bioethanol fuel is currently an area of great research interest around the world.

The bioethanol yields are influenced by several factors, including biomass yield and its tissue composition (ratios of cellulose, hemicellulose and lignin). Analyzing the cell wall composition of dehydrated stems, Table 2, we could mention that the concentrations of structural carbohydrates in hemp, *Cannabis sativa*, substrates are much higher in comparison with corn stalks, *Zea mays*, substrates. The analysis of the chemical composition suggested that the dry matter of the whole hemp plants contained 556 g/kg cellulose, 309 g/kg hemicellulose and 105 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 structural sugars in hemp stalks: 85.0 g/kg pentoses and 35.6 g/kg hexoses, but in corn stalks - 75 g/kg and 41 g/kg, respectively. The theoretical ethanol yield from structural carbohydrates averaged 628 L/t in hemp substrates, as compared to 485 L/t in corn substrates.

Table 2. The cell wall composition and theoretical ethanol potential of *Cannabis sativa* dry matter

Indices	*Cannabis sativa*	*Zea mays*
Acid detergent fibre, g/kg	661	499
Neutral detergent fibre, g/kg	970	749
Acid detergent lignin, g/kg	105	87
Cellulose, g/kg	556	417
Hemicellulose, g/kg	309	250
Hexose sugars, g/kg	99.8	75.1
Pentose sugars, g/kg	50.8	41.1
Theoretical ethanol potential,	628	485

Some authors mentioned various findings about the hemp biomass quality. According to Zatta and Venturi (2009), the chemical composition of stems of female hemp plants, harvested at full flowering stage, was: 58-63% cellulose, 13-17% hemicellulose and 9-10% lignin, but hemp stems harvested at beginning of seed maturity - 62-65% cellulose, 12-16% hemicellulose and 9-10% lignin, the calculated bioethanol production varied from 2799 up to 4500 litres/ha. Agbor et al., 2011, mentioned that purified hemp substrates contained 91.30% NDF, 86.36 ADF, 4.94% hemicellulose, 78.94% cellulose, 7.92% lignin with specific ethanol yield 1.99 mMol/g cellulose, but purified wood substrates 95.57% NDF, 93.40% ADF, 2.17% hemicellulose, 91.27% cellulose, 2.13% lignin, specific ethanol yield 1.79 mMol/g cellulose, respectively. Tutt and Olt (2011) stated that hemp contained 5.25% ash, 53.86% cellulose 10.6% hemicellulose, 8.76% lignin; the glucose yield was 312.7 g/kg or 58.06% from possible maximum values. Thygesen et al. (2007) reported the chemical composition of raw hemp fibre was as follows: 55-72% cellulose, 8-19% hemicellulose, 2-5% lignin, <1% wax and 4% minerals; hurds had higher content of lignin (19-21%) and hemicellulose (31-37%), but lower amount of cellulose (36-41%). Wawro et al. (2019) found that the chemical composition of hemp biomass before pre-treatment consisted of 50.82% cellulose, 27.79% hemicellulose and 14.68% lignin, but after alkaline treatment, the cellulose content in biomass increased to 62.70%, lignin to 15.12% and hemicellulose content decreased significantly to 20.16%. In comparison, for sorghum crop, the theoretical ethanol potential ranged from 560 to 610 L/t of dry biomass (Goff et. al., 2010).

Biomass is very difficult to handle, transport, store and utilize in its original form due to factors that can include high moisture content, irregular shape and sizes, and low bulk density. Densification can produce more compact products with uniform shape and sizes that can be more easily handled using existing handling and storage equipment and thereby reduce the costs associated with transportation, handling and storage. Baling, briquetting and pelleting are the most common biomass densification methods used for solid fuel applications. The quality and structural integrity of a briquette is affected by the size of particles, moisture, contents of lignin and cellulose, but also by the cellular structure of plant stems and leaves. It is known that low ash and moisture contents increase combustibility, and high bulk density fuel is convenient to be transported. Physical and mechanical properties of hemp biomass and prepared briquettes are presented in Table 3. The ash content in hemp biomass was lower (2.1%) than in corn stalks (4.4%), hemp has an excellent gross calorific value (19.0 MJ/kg), greater than corn stalks (17.8 MJ/kg), due to the fast defoliation and the stems with higher concentrations of cellulose, hemicellulose and lignin.

Table 3. Some physical and mechanical properties of biomass and solid biofuel

Indices	*Cannabis sativa*	*Zea mays*
Ash content of biomass, %	2.1	4.4
Gross calorific value of biomass, MJ/kg	19.0	17.8
Bulk density of chopped chaffs, kg/m^3	117	87
Bulk density of milled chaffs, kg/m^3	132	100
Specific density of briquettes, kg/m^3	867	923
Net calorific value of briquettes, MJ/kg	15.4	14.0

The bulk density of the hemp chopped chaffs reached 100 kg/m^3 and milled chaffs – 132 kg/m^3. The densified hemp solid fuel-briquettes have optimal specific density (867 kg/m^3), but still lower than corn briquettes (923 kg/m^3), perhaps because of the anatomical structure and the high content of fibre in hemp biomass. The net calorific value of hemp briquettes reached 15.4 MJ/kg and corn stalks 14.0 MJ/kg.

There are different results concerning the physical and mechanical properties of hemp reported in research studies conducted by other authors. Alaru et al. (2011) determined that the briquettes from dioecious hemp had better quality, because its male plants increased the mean content of lignin in the briquetting material, dioecious hemp had 5.3-6.3% ash, 77.0-79.0% volatiles, dry mass calorific value 16.6-16.7 MJ/kg, actual calorific value 15.3-15.4 MJ/kg. Prade et al. (2011) stated that the calorific value of hemp biomass increased from 17.5 MJ/kg in July to 18.4 MJ/kg during the period August-December, to an average of 19.1 MJ/kg during the period January-April. According to Poisa et al. (2016), in Latvia, for the hemp cultivar ‘Bialobrzeskie’ the calorific value of stem biomass was 18.68 MJ/kg and of shives - 18.16 MJ/kg, the ash content was 2.16% in shive biomass and 3.02% in stem biomass, the ash melting temperature for the hemp stems was 1393.17ºC and for shives - 1368.85ºC. Jasinskas et al. (2014) noted that bulk density of seeder hemp chaff was 28.2 kg/m^3 DM and hemp mill 101.0 kg/m^3 DM, the specific density of pellets was 877.8 kg/m^3 and the calorific value – 18.2 MJ/kg. Kakitis et al. (2011, 2014) mentioned that hemp had gross calorific value of 18.29 MJ/kg and net calorific value of 15.54 MJ/kg, the ash content was 2.97%, the density of briquettes reached 1135 kg/m^3, the splitting force for particles reached 115 N/mm. Kolarikova et al. (2013) stated that, in Czech Republic, the calorific gross value of hemp was 18.6-19.3 MJ/kg and the net calorific value was 15.8-17.3 MJ/kg. Kraszkiewicz et al. (2019) reported that the heat of combustion of hemp biomass was 18.089 MJ/kg and the calorific value 16.636 MJ/kg, the ash content 2.51%, the physical properties of briquettes: the density of briquettes 828 kg/m^3 and the mechanical durability - 98.17%.

CONCLUSIONS

The dry matter of the whole hemp plants contained 556 g/kg cellulose, 309 g/kg hemicellulose and the theoretical ethanol yield from structural carbohydrates averaged 628 L/t. The *Cannabis sativa* biomass was characterized by high gross calorific values (19.0 MJ/kg) and low ash content (2.1%).

The specific density of hemp briquettes reached 867 kg/m^3 and the net calorific value – 15.4 MJ/kg.

Hemp, *Cannabis sativa* may serve as multi-purpose feedstock for renewable energy production.

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THE IMPACT OF AGRICULTURAL MANAGEMENT PRACTICES ON THE SPECIES COMPOSITION OF BIRDS IN SOUTH BULGARIA

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Abstract

Agriculture is one of the most important areas of human activity and therefore has a crucial impact on the environment and the landscape. Its main objective is primarily produce high-quality raw materials for the food industry and animal husbandry. At the same time, agriculture is one of the main contributors to the loss of biodiversity worldwide. In recent decades, agricultural intensification has led to a serious decline in biodiversity including Farmland Birds. In order to analyze the impact of agricultural practices on the species composition of birds a study of two types of agricultural farming systems- organic farming and conventional was conducted. Birds were studied in four types of crops - rose, lavender, apple and pea on the territory of nine different villages in South Bulgaria. 48 bird species from nine orders have been identified. Direct impact of agricultural practices on the species composition of birds was not established.

Key words: *agriculture, conventional, farmland birds, organic farming.*

INTRODUCTION

Conservation of biodiversity and ecosystems is interconnected to the agricultural practices applied to the different types of crops. In the recent years in Bulgaria there has been an increasing interest in the cultivation of medicinal and essential oils plants, legumes and fruit crops. Areas with lavender and oilseed rose. Apple is one of the most profitable for Bulgarian agriculture. To maintain the ecological functions of agro-ecosystems it is essential to implement agricultural practices that provide greater sustainability to both agro-ecosystems themselves and their adjacent natural and semi-natural ecosystems. Birds play a key role in the economy of nature. They are very important for agriculture, forestry, green spaces etc. The various bird species - insectivorous and predatory feed on invertebrates, insects and rodents and thus destroy a number of pests on crops, fruit trees, forest and ornamental plants. Their contribution is highlighted in a number of references worldwide. Beneficial birds in agriculture are found everywhere in Bulgaria, but unfortunately their number in many places is relatively small.

Intensification of agriculture poses the greatest threat to biodiversity beyond climate change and the spread of invasive species (Pullin, 2002).

In recent decades, agricultural intensification has led to a serious decline in biodiversity, including Farmland Birds (Chamberlain et al., 2000; Donald et al., 2001). This decline is still ongoing (Gregory et al., 2005; Donald et al., 2006; Flade et al., 2008), although agri-environment schemes have been implemented as an important policy tool (Kleijn & Sutherland, 2003).

The main objectives of these schemes are the reduced use of pesticides to protect biodiversity. Several studies have shown the positive effects of agri-environment schemes on birds (Bengtsson et al., 2005), but a Pan-European study can't prove an overall positive effect (Kleijn et al., 2006).

MATERIALS AND METHODS

In order to study the impact of agricultural practices on priority crop systems and evaluate their impact on biodiversity and ecosystems, as well as the services they provide, the following field sites have been identified (Table 1).

Table 1. Study areas, crops and farming practices for study the species composition of birds

Region	Area	Crop	Type of farming
Stara Zagora	Skobelevo village	Rose	Conventional
Stara Zagora	Asen village	Rose	Organic
Stara Zagora	Koprinka Dam	Lavender	Conventional
Stara Zagora	Asen village	Lavender	Organic
Plovdiv	Brestnik village	Apple	Conventional
Plovdiv	Yagodovo village	Apple	Organic
Plovdiv	Sadovo town	Pea	Conventional
Plovdiv	Sadovo town	Pea	Organic
Stara Zagora	Chirpan town	Pea	Conventional
Stara Zagora	Chirpan town	Pea	Organic
Burgas	Karnobat town	Pea	Conventional
Burgas	Karnobat town	Pea	Organic

The survey was conducted between May and August 2019, this period covering the breeding season and autumn migration of the birds.

A specialized field form for suspected occurring bird species has been developed based on literary data before field work. It helps for faster and accurate monitoring of the birds in the study areas.

Personal geographic information from the study areas was imported used Google Earth Pro for easier viewing, such as:

- Geographic relief;
- Access roads;
- Surrounding landscape;
- Habitat types;
- Protected areas.

Adjacent protected areas have also been imported with specialized KML files available on the Natura 2000 Network Viewer online platform of the European Environment Agency. For determination the species composition of birds in different study areas and sites, field surveys were carried out using the methods of monitoring birds from stationary points, using predetermined linear car tours and determining by birds sounds. An innovative field research technique, which is an advantage of remote sensing with high efficiency and productivity, reliability of information and the ability to use in difficult conditions, has also been used:

- Binoculars Nikon MONARCH 5 8x42
- Field scope tube SWAROWSKI 80HD
- Camera Nikon D 71000
- Camera Lens Nikon AF-S Nikkor 200-500mm
- GPS Garmin Montana 610
- Drone 4K камера DJI Mavic Pro FLY MORE COMBO

RESULTS AND DISCUSSIONS

As a result of preliminary studies, two types of agricultural practices have been identified - conventional and organic. They were studied in four types of crops - rose, lavender, apple and pea on the territory of nine villages.

As a result of studies have been identified 48 bird species from 9 orders - order Galliformes, order Ciconiiformes, order Accipiterformes, order Falconiformes, order Columbiformes, order Strigiformes, order Coraciiformes, order Piciformes and order Passeriformes.

Results from field sites:

I.

Region: Stara Zagora;

Area: Skobelevo village;

Crop: Rose;

Type of farming: Conventional

24 bird species have been identified in 5 orders:

Order Accipiterformes: *Circus pygargus, Buteo buteo, Accipiter nisus.*

Order Falconiformes: *Falco tinnunculus.*

Order Columbiformes: *Columba palumbus, Streptopelia turtur, Cuculus canorus.*

Order Piciformes: *Dendrocopos major*

Order Passeriformes: *Alauda arvensis, Galerida cristata, Hirundo rustica, Delichon urbicum, Motacilla alba, Luscinia megarhynchos, Saxicola torquatus, Turdus philomelos, Turdus merula, Parus major, Pica pica, Garrulus glandarius, Sturnus vulgaris, Fringilla coelebs, Carduelis carduelis, Emberiza calandra.*

II.

Region: Stara Zagora

Area: Asen village

Crop: Rose

Type of farming: Organic

36 bird species have been identified in 8 orders:

Order Galliformes: *Perdix perdix.*

Order Ciconiiformes: *Ciconia ciconia, Ciconia nigra.*

Order Accipiterformes: *Aquila pomarine, Circaetus gallicus, Aquila pennata, Circus cyaneus, Circus pygargus, Buteo rufinus, Buteo buteo, Accipiter gentilis.*

Order Falconiformes: *Falco tinnunculus.*

Order Columbiformes: *Columba palumbus, Streptopelia turtur, Cuculus canorus.*

Order Coraciiformes: *Upupa epops, Merops apiaster, Coracias garrulous.*
Order Piciformes: *Dendrocopos major, Dendrocopos syriacus.*
Order Passeriformes.

III.
Region: Stara Zagora
Area: Koprinka Dam
Crop: Lavender
Type of farming: Conventional
7 bird species have been identified in 2 orders:
Order Ciconiiformes: *Ciconia nigra.*
Order Passeriformes: *Alauda arvensis, Hirundo rustica, Delichon urbicum, Pica pica, Sturnus vulgaris, Emberiza calandra.*

IV.
Region: Stara Zagora
Area: Asen village
Crop: Lavender
Type of farming: Organic
36 bird species have been identified in 8 orders:
Order Galliformes: *Perdix perdix.*
Order Ciconiiformes: *Ciconia ciconia, Ciconia nigra.*
Order Accipiterformes: *Aquila pomarine, Circaetus gallicus, Aquila pennata, Circus cyaneus, Circus pygargus, Buteo rufinus, Buteo buteo, Accipiter gentilis.*
Order Falconiformes: *Falco tinnunculus.*
Order Columbiformes: *Columba palumbus*, *Streptopelia turtur, Cuculus canorus.*
Order Coraciiformes: *Upupa epops, Merops apiaster, Coracias garrulous.*
Order Piciformes: *Dendrocopos major, Dendrocopos syriacus.*
Order Passeriformes.

V.
Region: Plovdiv
Area: Brestnik village
Crop: Apple
Type of farming: Conventional
15 bird species have been identified in 5 orders:
Order Galliformes: *Perdix perdix.*
Order Accipiterformes: *Buteo buteo.*
Order Columbiformes: *Columba palumbus, Streptopelia turtur, Cuculus canorus.*
Order Coraciiformes: *Upupa epops.*
Order Passeriformes: *Alauda arvensis, Galerida cristata, Luscinia megarhynchos, Turdus philomelos, Turdus merula, Parus major, Lanius excubitor, Sturnus vulgaris, Emberiza melanocephala.*

VI.
Region: Plovdiv
Area: Yagodovo village
Crop: Apple
Type of farming: Organic
8 bird species have been identified in 2 orders:
Order Piciformes: *Dendrocopos major, Dendrocopos syriacus.*
Order Passeriformes: *Galerida cristata, Hirundo rustica, Delichon urbicum, Luscinia megarhynchos, Turdus merula, Parus major.*

VII.
Region: Plovdiv
Area: Sadovo town
Crop: Pea
Type of farming: Conventional
11 bird species have been identified in 6 orders:
Order Ciconiiformes: *Ciconia ciconia.*
Order Columbiformes: *Cuculus canorus.*
Order Strigiformes: *Tyto alba, Athene noctua.*
Order Coraciiformes: *Upupa epops, Merops apiaster.*
Order Piciformes: *Dendrocopos major.*
Order Passeriformes: *Alauda arvensis, Galerida cristata, Hirundo rustica, Delichon urbicum.*

VIII.
Region: Plovdiv
Area: Sadovo town
Crop: Pea
Type of farming: Organic
11 bird species have been identified in 6 orders:
Order Ciconiiformes: *Ciconia ciconia.*
Order Columbiformes: *Cuculus canorus.*
Order Strigiformes: *Tyto alba, Athene noctua.*
Order Coraciiformes: *Upupa epops, Merops apiaster.*
Order Piciformes: *Dendrocopos major.*
Order Passeriformes: *Alauda arvensis, Galerida cristata, Hirundo rustica, Delichon urbicum.*

IX.
Region: Stara Zagora
Area: Chirpan town
Crop: Pea

Type of farming: Conventional
11 bird species have been identified in 5 orders:
Order Ciconiiformes: *Ciconia ciconia.*
Order Accipiterformes: *Circus pygargus, Buteo buteo.*
Order Falconiformes: *Falco tinnunculus.*
Order Coraciiformes: *Upupa epops, Merops apiaster.*
Order Passeriformes: *Galerida cristata, Hirundo rustica, Delichon urbicum, Motacilla alba, Fringilla coelebs.*

X.
Region: Stara Zagora
Area: Chirpan town
Crop: Pea
Type of farming: Organic
19 bird species have been identified in 8 orders:
Order Galliformes: *Perdix perdix.*
Order Ciconiiformes: *Ciconia Ciconia, Ciconia nigra.*
Order Accipiterformes: *Aquila pomarine, Circus pygargus, Buteo buteo, Accipiter gentilis.*
Order Falconiformes: *Falco tinnunculus.*
Order Columbiformes: *Columba palumbus, Streptopelia turtur, Cuculus canorus.*
Order Coraciiformes: *Upupa epops, Merops apiaster.*
Order Piciformes: *Dendrocopos major.*
Order Passeriformes: *Motacilla alba, Parus major, Oriolus oriolus, Fringilla coelebs, Emberiza calandra.*

XI.
Region: Burgas
Area: Karnobat town
Crop: Pea
Type of farming: Convetional
15 bird species have been identified in 5 orders:
Order Ciconiiformes: *Ciconia Ciconia.*
Order Accipiterformes: *Aquila pennata, Circus pygargus, Buteo rufinus, Buteo buteo.*
Order Falconiformes: *Falco tinnunculus.*
Order Coraciiformes: *Merops apiaster.*
Order Passeriformes: *Alauda arvensis, Galerida cristata, Motacilla alba, Parus major, Cyanistes caeruleus, Lanius excubitor, Lanius collurio, Pica pica, Emberiza calandra.*

XII.
Region: Burgas
Area: Karnobat town
Crop: Pea
Type of farming: Organic
20 bird species have been identified in 7 orders:
Order Ciconiiformes: *Ciconia Ciconia.*
Order Accipiterformes: *Buteo rufinus, Buteo buteo, Accipiter nisus.*
Order Falconiformes: *Falco tinnunculus.*
Order Columbiformes: *Columba palumbus, Streptopelia turtur, Cuculus canorus.*
Order Coraciiformes: *Merops apiaster.*
Order Piciformes: *Dendrocopos major.*
Order Passeriformes: *Alauda arvensis, Galerida cristata, Motacilla alba, Luscinia megarhynchos, Parus major, Cyanistes caeruleus, Lanius senator, Pica pica, Sturnus vulgari, Emberiza calandra.*

CONCLUSIONS

Due to the fact that the birds inhabit vast territories and cover large areas, and the fields studied are relatively small in size, no direct dependence on agricultural practices and crops on the species composition of the birds were established. In this case, the number of birds in the area is determined by the type of habitats in the surrounding area - the presence of arable and arable land, forest areas and anthropogenic conditions. Equally important is the presence of Natura 2000 Ecological Network Protected Areas, Protected Areas, National and Natural Parks and Reserves. The species composition of the studied fields is largely influenced by the composition in the Protected Areas and Territories located nearby.

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