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SUMMARY

SOIL SCIENCES

CROP SCIENCES

MISCELLANEOUS

SOIL
SCIENCES

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RESEARCH ON EARTHWORM COMMUNITY IN MAIZE CROP IN BORCEA, CĂLĂRAȘI

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Abstract

Earthworms play a variety of important roles in ecosystems. Their feeding and burrowing activities incorporate organic residues and amendments into the soil, enhancing decomposition, humus formation, nutrient cycling, and soil structural. Earthworms feed on plant debris (dead roots, leaves, grasses, manure) and soil. The purpose of this study was to assess the presence of earthworm species in maize crops in Borcea commune in Călărași Country over the years 2020-2022. The sampling consisted of 15 pits of 25 x 25 x 40 cm. Five earthworm species belonging to three genera Aporrectodea, Allolobophora, Eisenia fetida and Dendrobaena were identified. he most abundant species was Aporrectodea caliginosa. This study reports the first data on earthworm fauna for the Borcea, Călărași.

Key words: *earthworms, Lumbricidae, diversity of populations, agriculture, maize.*

INTRODUCTION

Earthworms play a significant role in maize cultivation, contributing to soil health and crop productivity in several important ways: aeration and porosity, aggregation, nutrient cycling and availability (I. earthworms consume organic matter and decompose it and release nutrients in forms that plants can readily absorb (Darwin, 1881) and II. Castings are rich in nutrients like nitrogen, phosphorus, potassium an micro nutrients and the castings act as natural fertilizer that improve soil fertility), enhanced microbial activity (microbial populations stimulates by earthworms gut, play a crucial role in nutrient cycling and organic matter decomposition, also enzymes and hormones (Aira et al., 2005) that are secreted by earthworms enhance microbial activity and soil health. Another important role of earthworms: soil pH and buffering capacity (earthworms castings can help buffer soil pH (Gong et al., 2019), making it more neutral and conducive for maize growth, also their activities can reduce soli acidity), disease and pest management, organic natter incorporation, root development (Earthworms can facilitate symbiotic relationships between maize roots and beneficial mycorrhizal fungi).

Earthworms contribute to soil aggregation mainly through the production of casts, although earthworm burrows can also contribute to aggregate stability, since they are often lined with oriented clays and humic materials (Jeanson, 1964) which can form a stable structure. Most workers agree that earthworm casts contain more water-stable aggregates than the surrounding soils.

The population of an earthworm species at any one time is made up of young immature, well-grown immature (adolescent), mature, and senescent individuals, the relative proportions depending on the time of year

Large amounts of insecticides, herbicides and fungicides are applied to soil to control pests. Some of these are general biocides and may also kill earthworms. For instance, it has been shown that copper fungicides are toxic to earthworms (Edwards, 1977)

Herbicides can affect earthworm populations either directly, or indirectly by killing the vegetation on which the worms feed. Not many herbicides decrease earthworm populations directly. Pollution from neonicotinoid insecticides and heavy metals has the potential to inhibit growth as well as damage DNA and cause oxidative stress (Yan et al., 2021).

Earthworm populations are generally lower in arable land comparative to undisturbed habitats (Chan, 2001). Direct mortality level depends on the severity and frequency of soil disturbance. Cuendet (1983) estimated that 5 to 10% of the earthworm biomass was brought to the surface by plowing, with about 25% of these earthworms mortally wounded. Rotary cultivation can reduce numbers by 60 to 70% (Boström, 1988).

Edwards et al. (1988) estimated that there were 1.6 million *L. terrestris* burrows per ha in a no-tillage maize field. These burrows are particularly important from the standpoint of water infiltration, because they open to the surface, are nearly vertical, and can as much as 2 m deep.

MATERIALS AND METHODS

Study area

The soil sampling was performed in maize crops in the experimental field from Borcea town, over the years 2020-2022. Borcea is a commune located in the Călărași County in Romania. Situated in the southeastern part of the country, Călărași County lies in the historical region of Muntenia. The commune of Borcea is positioned along the banks of the Borcea branch of the Danube River, which contributes to the area's agricultural suitability. The soil in Borcea, Călărași, Romania, is predominantly chernozem, also known as "black earth". Chernozem is a rich, fertile soil that is highly prized for agricultural activities. This type of soil is characterized by a high organic matter content and good structure, making it particularly suitable for growing crops like maize, wheat, and sunflowers. The soil in Borcea, Călărași, Romania, is predominantly chernozem, also known as "black earth". Chernozem is a rich, fertile soil that is highly prized for agricultural activities. This type of soil is characterized by a high organic matter content and good structure, making it particularly suitable for growing crops like maize, wheat, and sunflowers. Borcea receives an average annual precipitation of around 500-600 mm. Average temperature in Borcea is 23.9°C.

Earthworm sampling

Earthworms were collected in March-May and September 2020-2022. The soil was extracted using a spade and was put into a high sided tray in order to prevent earthworm escape. The extracted soil was hand-sorted for living earthworms. It was made it 15 pit soil and each soil pit had sides of 25 cm x 25 cm and 40 depth; the distance between pit soil was 10-30 cm on the crop row. The adult specimens were fixed in 70% ethanol, analysed under a stereomicroscope and identify to the species level. Juveniles are kept in the soil in the lab conditions to obtain adult stage.

Agricultural techniques

In 2020, the following technical works were carried out: preceding crop: alfalfa; sown corn directly; irrigated soil; fertilized with 163 kg nitrogen/ha.

And phytosanitary treatments: fighting weeds with the following active substances: glyphosate 4 l/ha (herbicide); 40 g/l nicosulfuron 1 l/ha; cypermetrim 150 ml/ha.

In 2021, the following technical works were carried out: preceding crop: corn; cultivator, combiner and sower; irrigated soil; fertilized with 163 kg nitrogen/ha. And phytosanitary treatments: fighting weeds with the following active substances: glyphosate 4 l/ha (herbicide); 40 g/l nicosulfuron 1 l/ha; cypermetrim 150 ml/ha.

In 2022, the following technical works were carried out: preceding crop: corn; cultivator, combiner and sower; irrigated soil; fertilized with 163 kg nitrogen/ha. And phytosanitary treatments: fighting weeds with the following active substances: glyphosate 4 l/ha (herbicide); 40 g/l nicosulfuron 1 l/ha; cypermetrim 150 ml/ha.

RESULTS AND DISCUSSIONS

Due to the fact that the land is worked in a no-till system, we can see a very big difference in terms of earthworm populations. The month of September and April offers the best conditions for their development for adults. The lowest results are recorded in May (Table 1).

Table 1. The number of adult and juvenile Lumbricidae

Year of sampling	**The number of adult and juvenile Lumbricidae**			
	March	April	May	September
2020	25	30	20	27
2021	22	21	17	30
2022	28	32	27	27
Total	75	83	64	84

In the Table 2 we notice that the year 2022 was the most suitable for the development of earthworms. In 2020, 48 adult individuals were collected and 54 juvenile earthworms, in 2021, 41 adults were collected and 49 juvenile earthworms and the last year of study in 2022, in all 4 months of the study, 51 adult individuals were collected an 63 juveniles.

Table 2. The number of adult

Year of sampling	The number of adult			
	March	April	May	September
2020	10	17	9	12
2021	11	10	7	13
2022	17	17	12	5
Total	38	44	28	30

The most abundant earthworm juveniles was recorded for all 3 years in September with a total of 54 individuals and the lowest is recorded in May with 36 individuals (Table 3).
It’s possible that the influence of sowing, the herbicide as well as the seed treatment to influence earthworm populations in may because the temperature is not so high to affect earthworms.

Table 3. The number of juveniles

Year of sampling	The number of juveniles			
	March	April	May	September
2020	15	13	11	15
2021	11	11	10	17
2022	11	15	15	22
Total	37	38	36	54

In all 3 years of research, the most abundant earthworm species is *Aporrectodea caliginosa*. The most individuals met in April and the fewest in September, the populations being mostly influenced by the drought (Tables 4-7 and Figures 1-6).

Figure 1. *Aporrectodea rosea*

Figure 2. *Allolobophora chlorotica*

Figure 3. *Aporrectodea caliginosa noctura*

Figure 4. Earthworm at the surface on plant residues

Table 4. Earthworms species in 2020

Species	March	April	May	September
Aporrectodea caliginosa	3	8	3	5
Aporrectodea longa	2	3	2	2
Dendrobaena sp.	1	2	1	1
Allolobophora chlorotica	1	1	1	-
Aporrectodea caliginosa nocturna	2	2	2	3
Eisenia fetida	1	1	-	1

Table 5. Earthworms species in 2021

Species	March	April	May	September
Aporrectodea caliginosa	4	3	3	5
Aporrectodea longa	2	2	2	2
Dendrobaena sp.	1	1	-	1
Allolobophora chlorotica	1	-	-	-
Aporrectodea caliginosa nocturna	2	3	2	4
Eisenia fetida	1	1	-	1

Table 6. Earthworms species in 2022

Species	March	April	May	September
Aporrectodea caliginosa	10	7	3	2
Aporrectodea longa	3	3	2	1
Dendrobaena sp.	1	1	-	-
Allolobophora chlorotica	1	1	-	-
Aporrectodea caliginosa nocturna	2	3	2	2
Eisenia fetida	-	2	1	-

Table 7. Total Earthworms species in 2020-2022

Species	March	April	May	September	Total 2020-2022
Aporrectodea caliginosa	17	18	12	12	59
Aporrectodea longa	7	8	7	5	27
Dendrobaena sp.	3	4	1	2	10
Allolobophora chlorotica	3	2	1	0	6
Aporrectodea caliginosa nocturna	6	8	6	9	29
Eisenia fetida	2	4	1	2	9

The least common adult earthworm individuals are *Allolobophora chlorotica* and *Eisenia fetida*, these are species of earthworms that feed on the remains of vegetation on the surface of the soil and with the high temperatures in this area and the intensive drought, the populations of these species are influenced by the climatic conditions and also the influence of herbicides, chemical fertilizers and seeds treatments with neonicotinoides.

In the first year of experience, 2020, the fewest individuals were registered and in 2022, the most individuals was colected. The influence of no-till technology has a beneficial influence on the soil structure as well as the earthworm populations.

Figure 5. Juvenile earthworm

Figure 6. *Aporrectodea longa*

CONCLUSIONS

Our data from 2020-2022 showed presence of six species of earthworms *Aporrectodea longa, Aporrectodea caliginosa, Aporrectodea caliginosa nocturna, Allolobophora chlorotica, Dendrobaena* sp.*, Eisenia fetida.*

The most earthworms (adults + juveniles) were found in maize crop especially in the 2022 in April and September. The most abundant species was *Aporrectodea caliginosa* with a total of 59 adult individuals found in the 3 years of study, in March 2022 it was found 10 individuals.

The next species with the most adults is *Aporrectodea caliginosa nocturna* with 29 adult individuals and the species with the fewest specimens being *Allolobophora chlorotica* with 6 adult individuals. In this three years of study, it appears that the April month is the most favorable for the development and activity of earthworms.

Under no-till conditions the earthworm populations are much more abundant than in those where plowing is applied. Analyzing the 3 years of studies, we can see the difference. From year to year, more individuals were collected due to the humidity conditions in the soil, the fact that there are more plant residues as food for earthworms and last but not least, let's not forget that the exclusion of plowing offers the development of earthworm populations.

In March, April and September on the surface of the soil are many plant residues and also the temperature of the soil and the humidity are suitable for earthworm populations.

And another aspect it is that has been scientifically proven that plowing reduces up to 70% of earthworm populations.

For alive and healthy soil, the technique of cultivating maize in no-till is beneficial. It can be seen from the data that every year more and more adult and juvenile individuals have been found that have a positive impact on soil fertility and structure.

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LAND CAPABILITY AND SUITABILITY ANALYSIS FOR VINEYARDS IN SĂLAJ COUNTY, ROMANIA

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Abstract

Land capability classification (LCC) for soils ranking is used to link the peculiarities of an area to the productions and the applied management. Terroir has a great influence on viticultural activities and wine quality. The aim of this study was to analyze and compare the components of Carastelec and Cămăr vineyards' terroirs and determine the influence of the soil and land characteristics on the suitability and capability of these lands for vine culture. Data on soil and climate were gathered. Soil samples were collected, analyzed, and modelled to establish their capability and suitability for vine use followed by land capability improvement approaches and grape varieties recommendation. The results reveal that the studied areas consist of different soil types and ecologically homogenous territories which ranks them into different capability and suitability classes suggesting that they have a moderate capability for table grapes production and a higher one for wine grapes production. By applying works to reduce or cancel the identified limitations, the ranking was higher for both vineyards and recommendations of the moist suitable grape varieties to be cultivated were made.

Key words*: land capability, soil, grapes, wine, classification.*

INTRODUCTION

Land capability classifies land based on the potential for different uses, e.g., agriculture, forestry, grazing, depending on the environmental and soil characteristics. Many kinds of land capability classification systems have been developed (FAO, 1976; Gizachew & Ndao, 2008; Hall, 2008; USDA, 1997; Klingebiel & Montgomery, 1976; Lynn et al., 2009; Rowe et al., 1981; Tesfay et al., 2017) using different principles and parameters. Land capability classification is a useful instrument to evaluate the terroir conditions.

Regarding vineyards and wine making, it is well known that all wine brands owe their specific characteristics and qualities to such terroirs (Brillante et al., 2020).

Developed in France and adopted globally, mostly in the wine industry, the concept became more precise: from a largely descriptive regional science to a technical research field with the main focus on the variation of biophysical characteristics of a vineyard site and their interaction with vine performance (Bramley, 2020). Concerning soil-vine interaction, most of the research studies involve precision agriculture methodologies used to better elucidate the contribution of both ecological and pedological factors (geology, lithology, climate, groundwater, vegetation, and fauna) to the formation of vineyard soils and their influence on different grape varieties and rootstocks. Currently, zoning, constitutes the first step in site selection and has a crucial role in the optimization of vineyard management. Similarly, they increase production efficiency and enhance the site-specific peculiarities of the product (Bramley, 2020; Vaudour et al., 2015).

Soil types also play an important role in vineyard performance and grapes composition (Echeverría et al., 2017). Interactions between vine and climate-soil system were studied by several authors. The results suggest that the effects of climate, soil and variety on vine behavior were highly significant. Furthermore, it has been reported that the anthocyanin concentration of the grapes was mostly influenced by the climate and the soil than by the vine variety (Jones, 2015; Van Leeuwen et al., 2004). Other studies suggest that soil temperature has a crucial role and a great effect on vine phenology and roots affecting their size and function (Lanyon et al., 2004; van Leeuwen et al., 2018).

Soil quality is another important parameter with an essential role in vine development and performance, exhibiting a strong linear association between soil and yield quality (Coipel et al., 2006).

Geological features of the terroir were investigated by various authors who consider that climate change has a sensitive impact on grapevine cultivation, affecting phenological stages in different terroirs increasing vulnerabilities in the future. They also suggest that more studies of both the terroir and the wine industry are needed to reduce this vulnerability (Bargmann, 2003; Bonnardot, 2002; Conradie et al., 2002; Hancock, 1999; Haynes, 1999; Holand & Smith, 2010; Maltman, 2008; Morlat, 2001).

In the capability system, soils are generally grouped in different levels, in accordance with the used system. Most of the systems consider climatic conditions, soil, and terrain conditions as being the most important, having different influences depending on the crop type, level of inputs and management.

The selection of attributes, data sets and indicators also need to be relevant for the standardization of soil quality attributes and their analyses (Mueller et al., 2010). Some indicator sets and thresholds have been developed for typical regions or countries (Barrios et al., 2006; Govaerts et al., 2006; Sparling et al., 2008; Teaci, 1980). Also, some systems take into consideration the improvement capabilities (Dalal-Clayton & Dent, 1993; Teaci, 1980). Land attributes can be related to either the direct use of the land or the possibilities of a major improvement of the land conditions if it's possible (Bennema, 1978). According to Vlad (2001) a land evaluation method is characterized by (1) the set of primary data used, (2) the set of evaluation criteria and land suitabilities used and (3) the evaluation models used for determining the evaluation criteria and land suitabilities.

Some research results reveal that Romanian vineyard terroirs could be classified by ecosystems: North, South and East Carpathian, Banatic, Dobrogea and Danubian. According to Toti & Ignat (2011), critical ecopedological factors that determine the architectonics of the vine root system are the edaphic factors which subserve or set back the development and functioning of the root system in terms of shape, length, thickness, and efficiency. In this context, the need for more specific studies to reveal the variables of other terroirs from Romania. Therefore, the main aim of this research was to i) identify the types of soils and analyze their properties ii) to assess land suitability and capability for wine and grapes in both natural and improved conditions, in two vineyards (Carastelec and Camăr) from Sălaj County, Romania.

MATERIALS AND METHODS

In order to assess LCC various land qualities and characteristics were analyzed in both Carastelec and Camăr vineyards. The most important land characteristics which should be included in any LCC are topography, soil, and climate. All of these, but especially topography and soil, are significant components in the determination of land units.

Location and site description

Carastelec and Camăr vineyards belong to the Wine Center of Sylvania which is situated in the North-Western part of Romania, in Sălaj County.

The plantations are located on low hills, between 220 and 320 m above sea level in Camăr, and between 200-230 m in Carastelec, on a moderately inclined slope (5-15%) in Camăr, and with 10-15% inclination in Carastelec, mostly with southern and south-western exposure.

In Camăr, the areas intended for the establishment of the vineyard are located on a slightly uneven slope, with favorable exposure and a soil with medium fertility. The plantations in Carastelec are located on a slightly uniform slope, with favorable exposure, highly fertile soil and unterraced surfaces. The predominance of southern and south-western exposure of the lands favors successful vine cultivation. Depending on the slope, it might not be necessary to carry out specific correction works in order to set up the

plantation. All these features guarantee obtaining high-quality grapes (Genoiu, 2015).
From geomorphological point of view, the territory of Carastelec commune is part of the Someș Plateau, whose external or north-western side includes the Sylvania Hills or the Salaj Platform, gradually leaning towards the Tisa Plain. The relief, rather than the microrelief, caused changes in the soil formation process, thus the soils evolved differently, according to the geomorphological unit to which they belong.
It is worth mentioning that the whole area comprises hills and elevated land, mostly with Northwest-facing slopes with hills ranging between 881 m (Plopiș) and 151 m (near the Barcău River). As a result of the geological evolution, the generated soil types by the parent rock found in this area are: carbonate clay minerals (typically light green or purplish), carbonate-cemented sandstones alternating with carbonate clay and colluvium. This area is currently serving as a wine processing center and a sparkling wine factory. Thus, Vinum Partium Winery was established in Carastelec commune and Fort Silvan 47 Winery in Camăr commune. The vineyards are surrounded by the forest in the vicinity.
Carastelec vineyard covers an area of 22.4 hectares. The land was split into 8 vine plots, each consisting of several sub-plots. The Camăr vineyard is slightly bigger, covering an area of 36.08 hectares, and is divided into 14 lots of grapevines, with their sub-plots.

Climate

Sălaj County is under the direct influence of western air masses. Atmospheric circulation, as well as relief, by its appearance and altitude, create climatic differences, on one hand between the West and East of the county, and on the other hand, between the main geomorphological units. The climate of Carastelec and Camăr region are characterized by moderate temperate-continental climate, with Sub-Mediterranean oceanic influences, with the characteristic climate of low-elevation regions. The annual average rainfall is 626 mm, although unevenly distributed, sufficient for most agricultural crops during years considered as normal. The highest average quantities of rainfall/month have been recorded in June (94.5 mm) and July (80.3 mm) while the lowest values have been registered in January (34.1 mm) and February (30.2 mm). Concerning the mean annual values of the climatic water balance, the studied land areas fall into the class of low precipitation surplus and are characterized by moderate erosion (code 0127) (according to data provided by the National Meteorological Administration, Regional Northern Transylvanian Meteorological Centre, 1982-2011).
The average annual temperature exceeds 9°C while the temperature amplitude ranges from 19.3 and 27.6°C. From a pedo-geoclimatic point of view, Carastelec and Camăr communes fall into a moderate cool-humid climate, with moderately rugged relief, and Luvisol as the predominant soil, identified with 78/13aIIID-BP in the climatic microzone class (Florea et al., 1999).

Soil description and characteristics

In order to identify soil types and describe their characteristics for both territories (Carastelec and Camăr) three soil profiles were opened for each territory to depict all the horizons. The soil profiles were opened at the minimum depth of 125 cm and soil layers were observed and described to classify and interpret the soil for various uses. Field observations were followed by the collection of 34 soil samples taken from each horizon which were then transferred to the laboratory to determine the physical and chemical characteristics of the soils. The soil samples were dried first at 30°C, then grinded and filtered to prepare them for further analyses.

Land suitability and capability evaluation

Land evaluation is a very complex process based on the land characteristics and its resources which are matched with a specific use of the land according to scientifically standardized techniques. In this regard, land suitability and capability could be assessed for present (current) land conditions without applying any input, or for future conditions which could be reached after land improvement (potential land suitability). To define current land suitability, soil characteristics and environmental data related to growth requirements of a specific crop need to be

evaluated. Potential land suitability refers to a future state, after land improvements have been applied and the productivity of the land was enhanced, thus creating more suitable conditions for the crop (Ritung et al., 2007). In Romania, the LCC system is called Databases of agricultural soil-land units in Romania and the support system of expertise on soil-land-BDUST, developed by The National Institute for Pedology and Agrochemistry (ICPA Bucureşti), which was used for the current study. Data processing and mapping of the soil units (US), the ecologically homogeneous territories (TEO) and favorability and suitability maps were performed using Global Mapper program (ICPA, 2016).
The main parameters scored and used in order to establish suitability and capability classes for the vineyards under study were: the average annual temperatures, the average annual rainfall, the degree of glazing and stagnation, texture, salinization/alkalization, slope, erosion hazard, total porosity, carbonate content, groundwater depth, soil pH, useful soil volume, and organic matter content. In order to obtain the suitability scores, parameters matching system between land quality and land characteristics with plant's requirements was used according to the evaluation flow presented in Figure 1.

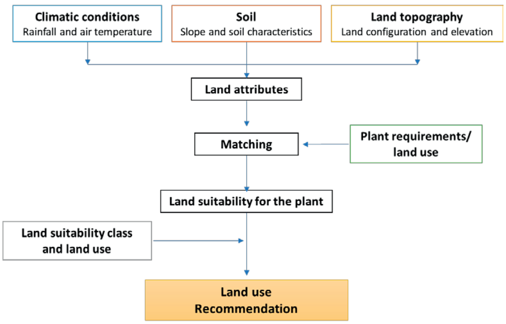

Figure 1. General framework for land evaluation

Florea et al. (1986) suggests that based on land evaluation scores, lands can be listed in five different classes: the first one with the highest scores being considered the most suitable: Class 1-81-100, followed by Class 2-61-81, Class 3-41-60, Class 4-21-40 and Class 5-0-20. Suitability classification according to the guidelines of FAO (1976) is divided into Order, Class, Sub Class, and Unit. The Order is the global land suitability group. Furthermore, Land suitability Order is divided into S (Suitable) and N (Not Suitable). Class is the land suitability group within the Order level which defines six soil quality classes based on the data obtained due to soil evaluation as follows:

- Class 1 - high-quality soils;
- Class 2 - good quality soils;
- Class 3 - medium quality soils;
- Class 4 - low-quality soils;
- Class 5 - very low-quality soils;
- Class 6 - unsuitable soils for the crop chosen.

In addition to the evaluation and soil quality classes, lands are also evaluated according to their capability. As described by the global Land Capability Classification (LCC) System, the capability of a land is determined by its potential to be suitable for certain uses and to assess if there are any risks of degradation. In this regard, it must be mentioned that some land restrictions could be ameliorable (by choice) and due to the improvement, they could fall into higher classes of suitability and capability, while other restrictions of an absolute nature could not be improved (climate, edaphic volume, etc.). The description of these classes are as follows: Class 1 - land with very good suitability for crops, without any restrictions; can be grown without applying measures to prevent degradation or improve the soil (ensures very good yields); Class 2 - land with good suitability, with low limitations, the risk of soil degradation or existing deficiencies can be improved by current practices or ameliorative measures (provides good yield); Class 3 - land with medium suitability, with moderate limitations, which limits the use of agricultural crops and requires improvement measures to prevent degradation (provides medium yield); Class 4 - land with low suitability, with severe limitations, which leads to significant decrease in crop yields and requires intensive planning and/or improvement measures in order to ensure yield safety; Class 5 - land with very severe limitations, unsuitable for agricultural crops, orchards or vineyards without special, complex and intensive land improvement measures. However, after improvement, these lands could be reconsidered and proposed for: arable land if the weather conditions are suitable for the prioritized agricultural crops (AL); orchards (OR) or vineyards (VY). Class 6-land with extremely severe limitations which cannot be used for agricultural crops nor for orchards or vineyards even after improvement measures.

For the establishment of a vineyard, besides the results of the soil analyses, it is very important to take into consideration the ecological factor as well, which can influence the growth and the production of the vine. Depending on the characteristics of the land associated with the eco-pedological factors (relief, hydrology, etc.), the suitability of the land can be established.

Every parameter analyzed in this study was associated with a coefficient from 0-1, where 1 represents maximum suitability and 0 means completely unsuitable. The final score was then calculated based on the coefficients and multiplied by 100 (Teaci, 1980).

While for the land suitability classes the evaluation scores are used. The grouping of land units in relation to capability for field crops and other uses is done in classes, subclasses, groups, and subgroups, in relation to the nature and the intensity of the limiting factors of production (relief, climate, soil). The subclass is marked with capital letters corresponding to the limiting factors, and Arabic numbers (2 to 6) added to the symbols of the limiting factors (Table 1) are used to mark the groups (Figure 2).

Table 1. The restrictions used for the establishment of the capability classes

Symbol	Restrictions
S	Salinization
A	Acidity
M	Reserve of humus
K	$CaCO_3$ content
N	Coarse texture
C	Fine texture
T	Compactness
V	Low edaphic volume
O	Reduced bearing capacity
P	Slope
E	Surface erosion
R	Deep erosion
F	Slides and falls
Z	Land cover with stones and rocks
U	Unevenness of the terrain
Q	Excess of water table
W	Excess of stagnant water
H	Flooding
L	Excess of moisture on slopes
G	Anthropical degradation
B	Low temperatures
Δ	Moisture deficit

The higher the value, the higher the intensity of the limiting factor. One and the same terrain can have restrictions of different intensities (Q4, W2, etc.) or several restrictions of the same intensity (Q4, W4, etc.). The placement in the class is done by considering the restriction or restrictions of the highest intensity.

Land capacity classes can change to higher classes if the existing limitations can be permanently removed or reduced to some extent by economically feasible rehabilitation measures.

These can happen by providing irrigation, ensuring adequate drainage, damming, or stabilizing the land, terracing. The application of these measures allows a new assessment of the land, namely potentiated regime, by using new coefficients for the new state of the corrected indicator/limitation.

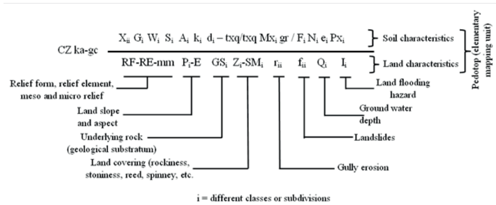

Figure 2. Capability formula/Soil-land unit, completing information on soil (upper part) with information on environmental (underlined part)

RESULTS AND DISCUSSIONS

Soil conditions

In contrast to other crops, vines are able to add value and perform successfully even on lands with low agricultural values. They only need sunshine, warmth, a little water, a low amount of soil volume and a low dose of nutrients in the soil to thrive. However, soil characteristics are essential for vine growing. In general, soil layers are destroyed and relocated due to terracing or deep plowing right before vine planting which poses a negative effect on both physical and chemical characteristics of the soil. Carastelec and Camăr communes are located in the north-western part of Sălaj County, north-western part of the country, overlapping almost entirely with the connection area between the Apuseni Mountains and the Eastern Carpathians, known as the Someşana Platform. From geomorphological point of view, the territory of Carastelec commune is part of the Someșan Plateau. During this research it has been observed that the relief had an important impact on the soil formation process in this area. It should be noted that the whole area is dominated by hills with their slopes oriented to the north-west. The maximum elevation recorded is 881 m (Plopiș), while the lowest is 151 m. This difference of 730 m elevation must be considered when distributing the production across the territory. The hills are fragmented and both surface and deep soil erosion were observed in this area. Soil analyses revealed that in Carastelec area, in the Vinum Partium Vineyard covering 22.4 hectares the only soil type identified was Anthrosol (Florea & Munteanu, 2012) according to RSST (Anthrosols in WRB), with three subtypes (US1, US2 and US3) as presented in Figure 3.

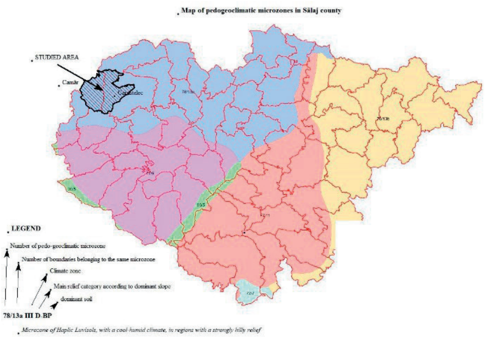

Figure 3. Map of pedogeoclimatic microzones in Sălaj county (adapted from Florea et al., 1999)

In this area, three soil profiles were opened as follows: Profile 1. Anthrosol aric preluvic-stagnic Ao1d-Ao2-ABd-Bt1w-Bt2w-Cn, deep, developed on mezobasics hard rocks; Profile 2. Antrosol aric preluvic Ao1d-Ao2d-ABd-Bt1-Bt2-Cn, developed ob mezobasics hard rocks and Profile 3. Antrosol aric cernic Am1d-Am2d-ABd-Bt1-Bt2-Cn, developed ob mezobasics hard rocks.

All analyzed profiles present a Bt horizon richer in clay (about 60 cm depth), which could cause compaction at the level of the roots correlated with a much slower percolation of water. But these conditions can be readily reversed through deep loosening, which was the main improvement measure for potentiated evaluation.

At Camăr, within the plots in Podgoria Fort Silvan 47 on 33.58 hectares the predominant soil type identified was Phaeozem, with the three subtypes as follow:

- Profile 1. Haplic Phaeozems Am1-Am2-AC-Ck, well developed on carbonatic clays (US1)
- Profile 2. Vertic Stagnic Phaeozems Am1y-Am2y-ABy-Bt1yw-Bt2yw-Ck., well developed on carbonatic clays (US2).
- Profile 3. Luvic Stagnic Phaeozems Am1-Am2-AB-Bt1w-Bt2w-Cn, well developed on clays (non-carbonatic) (US3).

The parent material represented by clays stimulates the accumulation of organic matter in larger quantities and ensures a larger reserve of humus, but the presence of swelling clays also determines the appearance of the vertical character (US2) which prevents the normal development of the roots. Also, lower porosity values than in the case of anthrosol also determine the appearance of stagnant character (US1 and US3). Grapevines are tolerant to waterlogging but only during the inactive phase, not during the growing period (Campbell-Clause & Moore, 1991). As a result, in addition to deep loosening, works to remove excess stagnant water are also recommended.

In both areas, the slope is slight to moderate which is desirable in vineyard sites since it accelerates the drainage of cold air from the vineyard. Cold air is denser than warm air and will drain downhill and therefore there is no need for protection during cold periods of the year (Bufu, 2014; Genoiu, 2015).

Climate

The studied lands fall into the class of low surplus annual average hydroclimatic balance and into the middle erosion class (code 0127) according to the National Meteorological Administration, Regional Meteorological Center Transilvania-Nord, 1982–2011.

From pedological and geoclimatic point of view, Carastelec and Camăr communes are characterized by a moderately cool-humid climate, with moderately rugged relief, with whitish Luvosol as the predominat soil, which allows this land to be classified by the climatic microzone with the symbol 78/13aIIID-BP (Florea et al., 1999) as presented in Figure 3.

From pedological and geoclimatic point of view, Carastelec and Camăr communes are characterized by a moderately cool-humid climate, with moderately rugged relief, with Albic Luvosol as the predominant soil, which allows this land to be classified by the climatic microzone with the symbol 78/13aIIID-BP (Florea et al., 1999) as presented in Figure 3.

The annual rainfall of 626 mm is evenly distributed, with a slight surplus in May–June and with the least precipitations in December–March. The significant decrease in rainfall in August, September and October has a positive impact on soil temperature and fruit ripening and provides good phytosanitary status for the grapes. The significant decrease of the average air temperature by 3-4°C, in September, helps the preservation of chemical components in fruits sensitive to higher temperatures-flavors, acids-in grapes (ONVPV, 2014). The number of foggy days with high humidity level is very low which enhances the helio-thermal availability for vineyards in this area and guarantees the production of high-quality. aromatic white and red wines. The De Martonne annual aridity index (IaDM) is 36.9 and the monthly index varies between 58.8 in January and 26.1 in September, values which indicate no month with moisture deficiency in the studied areas (Genoiu, 2014) (National Meteorological Administration, Regional Meteorological Center Transilvania-Nord, 1982-2011).

The natural shelter of the Meseș Mountains makes the extreme minimum temperature one of the least harsh in Romania. In the studied area, the frequency of the absolute minimum temperature in winter, unfavorable for vines, is low (Oșlobeanu et al., 2014). Due to the southern exposition of Carastelec and the Southwestern orientation of Camăr the slopes benefit from extra light and warmth.

Based on the climatic parameters recorded, it can be claimed that from climatic point of view the investigated sites, namely, Carastelec and Camăr are suitable for vineyard establishment (Genoiu, 2015).

Land suitability and capability

Land suitability and capability are often confused or even considered identical; however, they define two different and very important characteristics of a certain land. Thus, suitability is evaluated based on the positive features of the land which can facilitate successful production, whereas land capability is defined mostly focusing on the features (limitations or restrictions) which can prevent the land to be used for certain agricultural land categories, e.g.,vines, arable, orchards or pastures (Blaga et al., 2008).

Similar to this study a number of LCC researches have been used as a valid tool in helping land managers and land use planners to manage land considering soil proprieties and potentialities, identifying areas with physical constraints for a range of land uses (Girmay et al., 2018; Scopesi et al., 2020).

Following data analysis and processing, the evaluation scores were assessed, and then quality classes, suitability and land capability classes were defined for the wine-producing vineyard and for grapes production in both natural and improved conditions, for Carastelec (Table 2) and Camăr (Table 3) vineyard as well.

Ecologically Homogeneous Territories (TEO) represent “a distinct portion of land on which all natural factors, or in the case of improved and anthropogenic surfaces, manifest themselves uniformly, the portion of territory cannot be further divided according to any of the criteria used” (Teaci, 1980). Therefore, the same type of soil could be divided into multiple TEOs based on the changes of the land characteristics (different slope, different orientation, etc). In total, seven TEOs were separated, three in the Carastelec plantation and four in the Camăr plantation. The separation of TEOs was achieved by overlapping soil and the topographic maps based on the following data: orientation, slope, hydrographic basin, main relief, meso- and micro-relief form, risk of surface erosion, deep erosion and landslides, corrected annual average air temperature, corrected annual cumulative average precipitation, groundwater depth, reed cover, stands, mounds or ridges, degree of land cover with stones, degree of reed bed land cover, feasibility for land improvement and agropedoameliorative works, earthworks land terracing and anti-erosion works (Vlad, 2015).

a. Carastelec vineyard. The results suggest that, in natural conditions, regarding the wine producing sector, no differences have been recorded between US 1 and US 2, both sharing the same evaluation score (58) indicating medium suitability with medium quality soils with moderate limitations, which limits the use of agricultural crops and requires improvement measures to prevent degradation. US 3 was slightly different, falling into the 2nd quality class with low quality soils, but with medium suitability associated with moderate limitations. The final scores of land suitability and capability indicate that in Carastelec US 3 and TEO 3 had the highest evaluation scores defining good and medium quality soils but low land suitability and capability for both wine and grape production (Table 2).

These results show that the land is more suitable for cultivating wine grapes varieties than table grapes varieties. Similar results were obtained in Ankara province (Uyan et al., 2023), assuming a relative match between LCC systems used in the evaluation.

Table 2. Evaluation scores, suitability, and land capability classes for Carastelec vineyard

		Natural conditions											
		wine				grapes				average			
No. US	No. TEO	Evaluation score	Quality classes	Suitability classes	Land capability classes	Evaluation score	Quality classes	Suitability classes	Land capability classes	Evaluation score	Quality classes	Suitability classes	Land capability classes
1	1	58	III	V	III	52	III	V	III	55	III	V	III
2	2	58	III	V	III	46	III	VI	III	52	III	V	III
3	3	65	II	IV	III	58	III	VI	III	62	II	IV	III
		Potential conditions											
		wine				grapes				average			
No. US	No. TEO	Evaluation mark	Quality classes	Suitability classes	Land capability classes	Evaluation mark	Quality classes	Suitability classes	capability	Evaluation mark	Quality classes	Suitability classes	Land capability classes
1	1	71	II	III	III	63	II	IV	III	67	II	IV	III
2	2	87	I	II	III	84	I	II	III	86	I	II	III
3	3	79	II	III	III	70	II	IV	III	75	II	III	III

Regarding the potential suitability evaluation, the results show that after improvement, US 2 became the most suitable and capable area for wine and grapes production out of the three areas under study, followed by US 3.

Based on the eco-pedological data, maps were generated to represent the soil unit, together with the ecologically homogenous territorial units (TEOs) for Carastelec (Figure 4).

For Carastelec vineyard, TEO 1 and 2 fall into the 3rd quality class, and TEO 3 into 2nd quality class, according to the value of scores in natural conditions (Figure 5). The implementation of improvement measures changes the classification of quality classes of territorial-homogeneous units, up to one class, 2nd and 1st respectively (Figure 6). This new state of the land assures better conditions for vine growth and presumably more production.

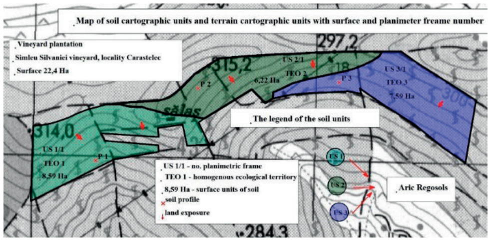

Figure 4. Mapping of soil units (US) and ecologically homogenous territorial units (TEO), plots and surfaces, Carastelec vineyard

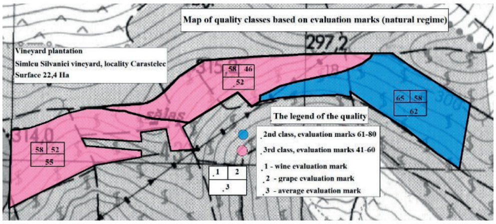

Figure 5. Map of quality classes per TEO units, Carastelec vineyard (natural conditions)

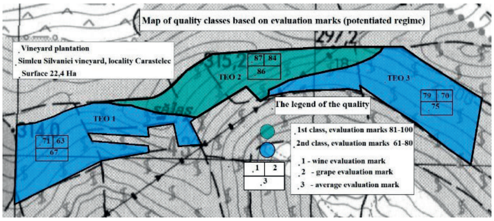

Figure 6. Map of quality classes per TEO units, Carastelec vineyard (improvement conditions)

For vine cultivation, the suitability maps for the Carastelec plantation showed an average suitability, the best degree of suitability being registered in the case of TEO-3, as part of the 4th class, with favorability grades between 65–58 points. TEO 1 and 2 fall into the 5th suitability class, with an average of 55 and 52 points, respectively (Figure 7). After improvements, the suitability degree increases (Figure 8), reaching the 2nd suitability class for TEO 2 and the 2nd suitability class for TEO 1 and 3, with 75 suitability points and 67 points, respectively, for grape vines.

Based on the identified limits (indicators with coefficients below 0.7), reduced porosity and compaction respectively, the recommended improvement work was deep loosening. The lands used for horticultural activities, in particular for the vine culture, fall into the 1st to 3rd capability classes (Campbell-Clause & Moore, 1991). As a result, the lands in the Carastelec vineyard fall into the optimal category both under natural and potentiated conditions (Figure 9).

b. Camăr vineyard evaluation marks in natural conditions (Figure 10) are lower, leading to classification into the 3rd quality class for TEO 1, 3 and 4 and into the 4th class for TEO 2 (Figure 11). In the potential regime (Figure 12), a higher classification is observed for TEO 2, with an average of 99 favorability points and is classified into the 1st quality class, while TEO 1, 3 and 4 reach the 2nd favorability class.

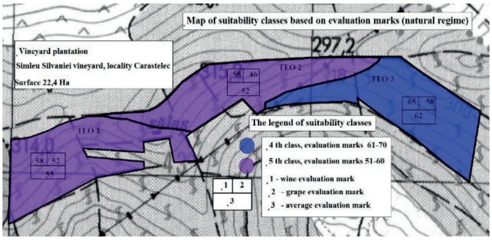

Figure 7. Map of suitability classes per TEO units, Carastelec vineyard (natural conditions)

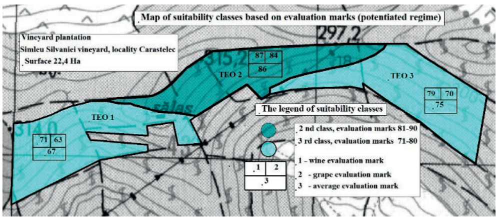

Figure 8. Map of suitability classes per TEO units, Carastelec vineyard (improvement conditions)

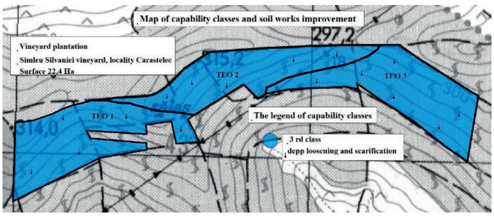

Figure 9. Map of capability classes per TEO units, Carastelec vineyard (improvement conditions)

The final scores of land suitability and capability indicate that in Camăr US 3 and TEO 4 had the highest evaluation scores defining medium and low-quality soils and also low land suitability and medium capability for wine production and, low land quality, suitability and capability for grapes production on the other hand (Table 3).

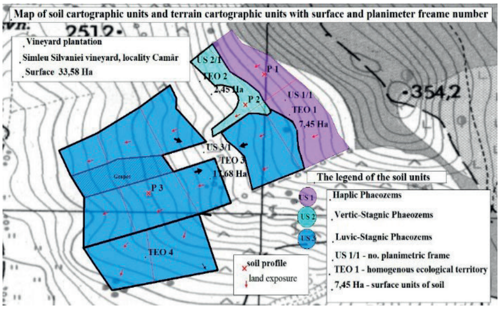

Figure 10. Map of soil units (US) and ecologically homogenous territorial units (TEO), plots and surfaces, Camăr vineyard

Table 3. Evaluation scores, suitability, and land capability classes for Camăr vineyard

Natural conditions													
		wine				grapes				Average			
No. US	No. TEO	Evaluation score	Quality classes	Suitability classes	Land capability classes	Evaluation score	Quality classes	Suitability classes	Land capability classes	Evaluation score	Quality classes	Suitability classes	Land capability classes
1	1	52	III	V	III	41	III	VI	III	47	III	VI	III
2	2	38	IV	VII	IV	26	IV	VIII	IV	32	IV	VII	IV
3	3	47	IV	VI	III	36	IV	VII	III	42	III	VI	III
	4	47	III	VI	III	36	IV	VII	III	42	III	VI	III
Potential conditions													
		wine				Grapes				Average			
No. US	No. TEO	Evaluation mark	Quality classes	Suitability classes	Land capability classes	Evaluation mark	Quality classes	Suitability classes	Land capability classes	Evaluation mark	Quality classes	Suitability classes	Land capability classes
1	1	78	II	III	III	76	II	III	III	77	II	III	III
2	2	105	I+	XI	IV	92	I	I	IV	99	I	I	IV
3	3	78	II	III	III	76	II	III	III	77	II	III	III
	4	78	II	III	III	76	II	III	III	77	II	III	III

In Camăr vineyard, the lower evaluation scores in natural conditions led to the classification into the 5th suitability class for TEO 1, 6th class for TEO 3 and 4, and 7th class for TEO 2 (Figure 13).

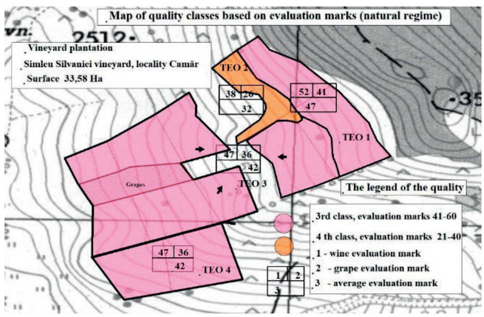

Figure 11. Map of quality classes per TEO units, Camăr vineyard (natural conditions)

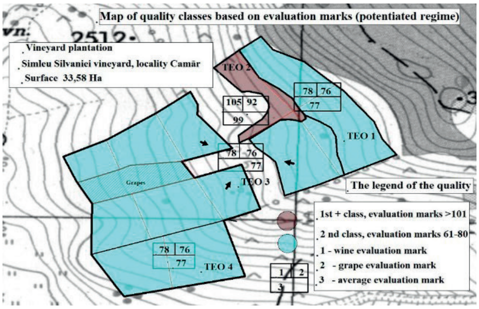

Figure 12. Map of quality classes per TEO units, Camăr vineyard (potential conditions)

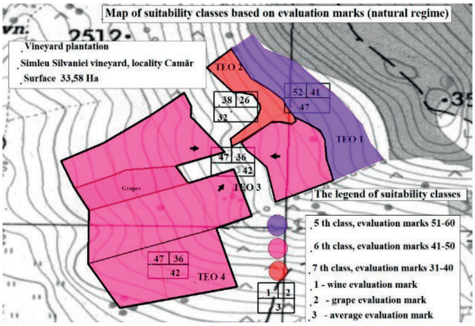

Figure 13. Map of suitability classes per TEO units, Camăr vineyard (natural conditions)

The enhanced favorability scores allow the classification into higher suitability classes; class 11 with over 100 favorability points for TEO 2 and the 3rd suitability class for the rest of TEOs with 77 favorability points (Figure 14).

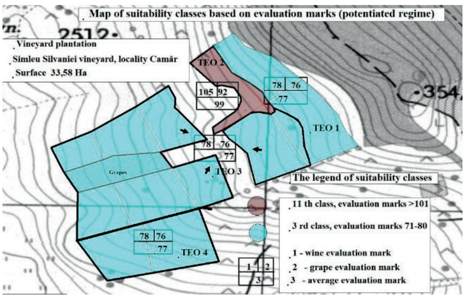

Figure 14. Map of suitability classes per TEO units, Camăr vineyard (potentiated regime)

The suitability for grape vine cultivation, as well as for the application of improvement works, shows a higher suitability of the Carastelec vineyard as compared to Camar vineyard, all TEOs being classified into the 3rd suitability class, while the recommended improvement work is deep soil loosening (Figure 15). Instead, the TEOs from the Camăr plantation fall into the 3rd and 4th suitability classes, and along with deep soil loosening, drainage works are also recommended to remove the excess of groundwater moisture (Figure 15).

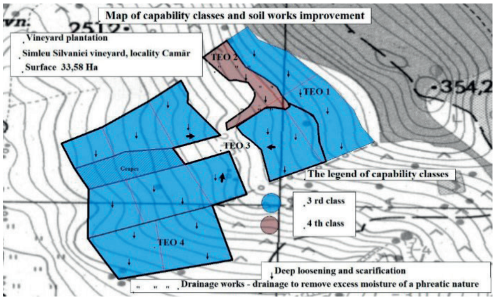

Figure 15. Map of capability classes per TEO units, Camăr vineyard

The lands in Camar vineyard, because of more limitations (stagnant water, compactness, low porosity) in natural conditions, fall into the 4th class and their suitability for vines is dependable on the improvements of limitations. LCC can be used to make recommendations about grapes varieties (Wanyama et al., 2014; Parker et al., 2020). The grape varieties indicated to be grown in the terroir conditions of Carastelec are those used to produce sparkling wine such as Royal Maiden, Italian Riesling, Pinot Noir, and Pinot Gris, and the recommended varieties for the Camăr vineyard are Traminer, Muscat Ottonel, Merlot, Black Maiden and Cadarca.

CONCLUSIONS

The application of LCC in the vineyards of Carastelec and Camăr allowed the identification of suitability and capability for vine culture, for both wine grape varieties and table grape varieties. By highlighting the suitability and capability categories in the natural regime, it was possible to pick out the improvement works specific to each restriction/limitation, so that through the evaluation in the potentiated regime, the assessment of favorability and suitability was increased. For each vineyard, depending on the climate, soil and terrain conditions, suitable wine grape varieties were identified for successful cultivation.

The identification of terroir elements gives the decision makers (from farmers to government agencies) a sense of prediction concerning the potential of the land, either for current or proposed uses.

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DYNAMICS OF SOIL BACTERIAL COMMUNITIES UNDER WINTER WHEAT IN THE NORTH-EAST PART OF ROMANIA

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Abstract

Agricultural soil microbial communities are influenced by a multitude of factors, including seasonality and local environmental conditions, management practices and their interaction, and cultivated species. Seasonality can result in variations in temperature, precipitation, soil moisture, and solar radiation, all of which drive rates of soil microbial metabolism and respiration, as well as taxonomic composition. Agricultural crop species can significantly impact the soil microbial population through the release of root exudates. Root exudates are a diverse array of organic compounds that are actively secreted by plant roots into the surrounding soil. These compounds include sugars, organic acids, amino acids, vitamins, enzymes, and other secondary metabolites. Root exudates serve various functions, including providing a carbon source for soil microorganisms, facilitating nutrient uptake by plants, and mediating plant-microbe interactions. This study investigates the dynamics in bacterial communities within the rhizosphere of winter wheat across different growth stages and geographical regions in north-eastern Romania. Soil samples were collected from two locations in Iasi County, one location in Botosani County, one location in Vaslui County, and one location in Galati County. Soil sampling occurred three times during the winter wheat growing season: in the fall of 2022, in April 2023, and in May 2023, covering three phenological stages (emergence, stem elongation, and flowering). The results obtained indicated differences between the wheat crop's phenophase and between locations; however, statistical analysis of the data demonstrated that these differences were not statistically significant.

Key words: *soil microbiome, soil microbial community, winter wheat.*

INTRODUCTION

In the year 2022, according to available statistical data, wheat was cultivated on approximately 219 million hectares of land worldwide, making it the most cultivated crop globally. Since 1961, the global wheat-growing area has fluctuated between 200 and 240 million hectares. The cultivated wheat area peaked around 1980 and has slowly declined to its current level (Shiferaw et al., 2013). Despite the relative stability of the wheat-growing area (including a modest decrease over the past half-century), the increase in global wheat production is attributed to consistent yield improvements. Yields have steadily increased from a global average of just over 1 tonne/ha in the early 1960s to 3.6 tons/ha currently (Roy et al., 2006). In Romania, the average wheat yield is over 4 tons/ha (Gafencu, 2019). Wheat is cultivated in over 120 countries across Africa, South, Central and North America, Asia, Europe, and Oceania, making it a vital crop for both emerging economies and developed countries.

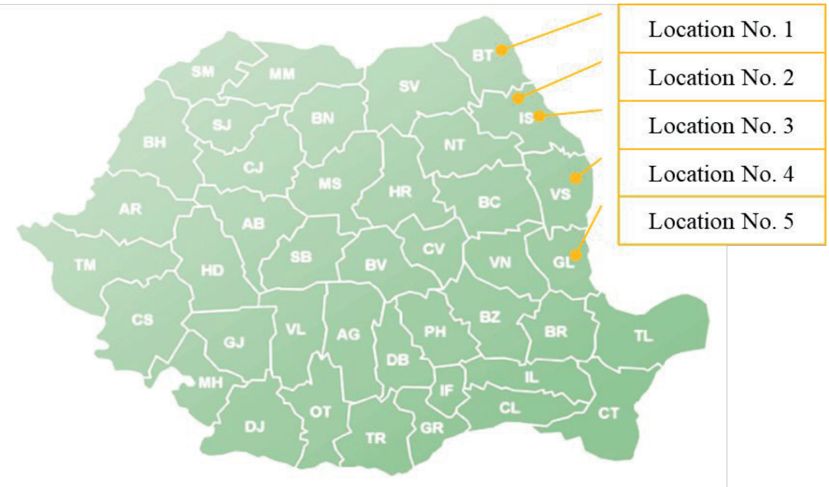
Global demand for wheat has been uninterrupted and continues to rise steadily since its cultivation began, projected to increase by 60% by 2050, as wheat serves as a staple food for approximately 3 billion people, over a third of whom are among the poorest, and provides about one-fifth of the calories and proteins in the human diet. Wheat production is essential for global food security and political stability, which is why wheat is the most cultivated plant in the world (Erenstein et al., 2022).

To ensure sustainable and efficient wheat production, it is essential to understand and manage the health of the soil in which this crop is grown. Soil microorganisms play a crucial role for soil and plant health, and understanding them is essential for optimizing agricultural production. Therefore, knowledge of the soil microorganisms in which wheat is cultivated is not only useful but also indispensable for ensuring sustainable, efficient, and climate-resilient agricultural production. Research and understanding of soil microbiota can significantly contribute to improving agricultural practices and promoting global food security.

The main purpose of this research was to investigate the abundance and diversity of bacterial communities in the rhizosphere of winter wheat cultivated in five locations in north-eastern Romania, as well as to examine the changes occurring in these microbial communities throughout the wheat growing season.

MATERIALS AND METHODS

For this study, soil was sampled from five different locations in the northeastern region of Romania (Figure 1). Soil samples were collected from two locations in Iași County, one location in Botoșani County, one location in Vaslui County, and one location in Galați County.



Legend: Location No. 1: Stefanesti, Botosani; Location No. 2: Andrieseni, Iasi; Location No. 3: Ezareni, Iasi; Location No. 4: Codaesti, Vaslui; Location No. 5: Slobozia-Conachi, Galati

Figure 1. The locations where soil samples were collected

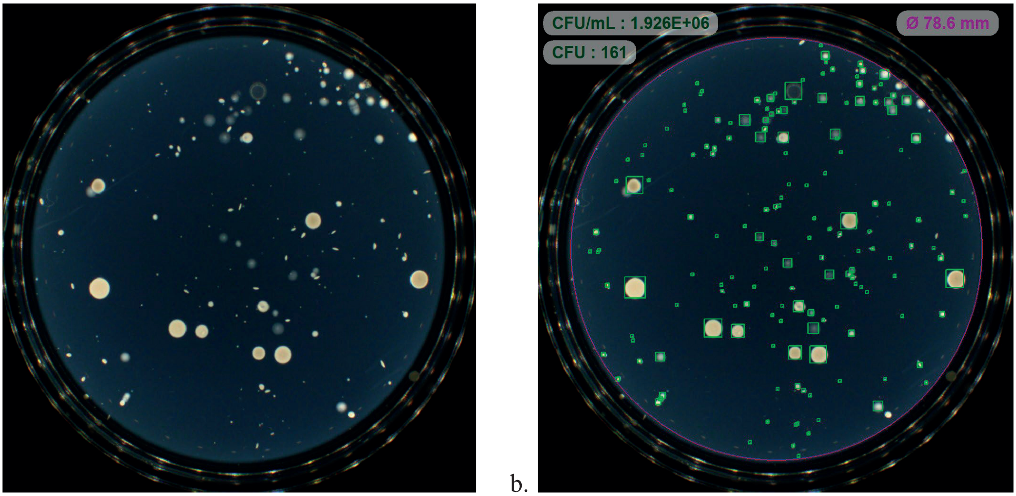
Soil sampling was conducted three times during the winter wheat growing season: [1] the first sample was taken in the fall of 2022, after crop sowing and emergence of wheat plants, [2] the second sample was taken in April 2023, and [3] the third sample was taken in May 2023, during wheat flowering.

Soil samples were collected from the rhizosphere of wheat plants, at a depth of 7-10 cm. From each wheat-cultivated plot in each of the 5 locations, an average sample was taken, consisting of 10 initial probes.

Soil sampling was conducted following all aseptic conditions to avoid soil contamination during collection. Soil samples were collected in paper bags and transported to the Microbiology Laboratory of the Faculty of Agriculture at Iasi University of Life Sciences for further investigation. Once in the microbiology laboratory, the soil samples were homogenized and ground. The organic matter present in the soil samples was removed using sterile tweezers.

For determining the total number of colony-forming units (CFUs) in the soil, the serial dilution method was used, and cultivation was performed on Potato Dextrose Agar (PDA) medium (Sarchlau, Spain, 39 g l^{-1}), in two different compositions: classic, and with Streptomycin. The antibiotic streptomycin (35 mg·L^{-1}) was used to control the reproduction of Gram-negative bacteria (Gafencu et al., 2021).

Serial dilutions were prepared in sterile water with a dilution factor of 10 (dilutions 10^{-1}, 10^{-2}, ..., 10^{-6}). This method generated a series of dilutions in which the number of microorganisms decreased. To prepare these dilutions, 9 mL of double-distilled sterilized water at 120°C for 30 minutes was distributed into sterile tubes with a capacity of 15 mL. Subsequently, 1 g of soil was weighed on a sterile watch glass and introduced into the first dilution tube. After vigorous shaking for five minutes, a 10^{-1} (1/10) dilution was obtained. Successive dilutions, such as 1/100 (10^{-2}), were then prepared by transferring 1 mL of suspension from the previous dilution to another test tube containing 9 mL of sterile water. This process was repeated to obtain further dilutions. 1 mL of suspension from each dilution was plated on Petri plates, and after an incubation period of 24 hours at 28°C, bacterial colonies (Figure 2) were counted using an automatic colony counter (Scan® 1200, Interscience, France). To determine the number of bacteria in one gram of soil, the number of colonies that developed on the Petri plate was multiplied by the inverse value of the dilution (Gafencu & Ulea, 2023).



a. b.

Figure 2. Bacterial Colonies on Potato Dextrose Agar media: using the Petri plate as a counting method (a - before counting, b - after counting with Scan® 1200, Interscience, France)

The counting result was correlated with the dilution used, and the final result was expressed in colony-forming units (CFUs) per 1 gram of soil (Gafencu et al., 2023).

Statistical analysis of the data obtained in the experiments was performed using the SPSS program (IBM SPSS Statistics 26) for Windows. For the statistical analysis of the data, a benchmark represented by the average value was utilized for comparison with the obtained values. Additionally, mean values were determined for each sampling moment, including the total number of bacteria, Gram-positive bacteria, and Gram-negative bacteria.

RESULTS AND DISCUSSIONS

In all five locations, the winter wheat crop was preceded by sunflower crop. In all location, the winter wheat cultivar Glosa was cultivated. Soil cultivation practices were similar across all locations, with land preparation occurring after sunflower harvesting. Plant residues were shredded, followed by scarification, and then preparation of the seedbed for the wheat crop. Chemical fertilizers were applied in moderate amounts at all locations. Additionally, pesticides were applied in all locations to control weeds, pests, and diseases.

The soil serves as a vital habitat for plants as well as for soil microorganisms. Plants influence the composition and dynamics of microbial communities present in the rhizosphere, and recent studies have evaluated the factors that influence the composition of these root-associated communities (Berg & Smalla, 2009). Such studies are important because the structure of rhizosphere microbial communities can significantly affect nutrient availability for plants, organic matter decomposition, phytopathogen activity, and so on (Chapparo et al., 2012; Howard et al., 2020). Studies on soil microbiota are essential for understanding how plants shape these communities and for evaluating the impact of these microorganisms on plant health and soil fertility (Lamb et al., 2011; Tkacz et al., 2020). For a detailed analysis of the variations in the total number of bacteria within the winter wheat rhizosphere, across different seasons and different locations, we can closely examine the dynamics and differences among the recorded values (Figure 3).

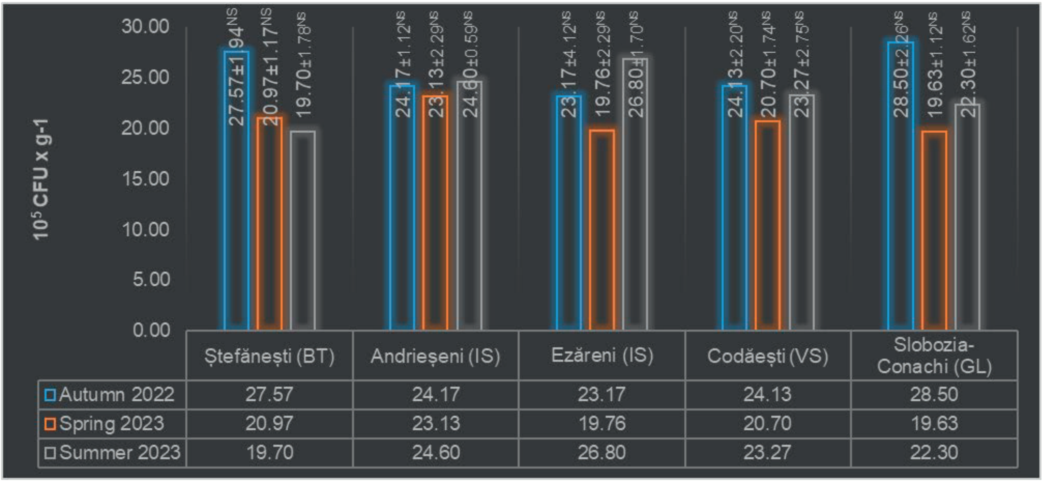


Figure 3. Comparison of total bacterial number colonizing the rhizosphere of winter wheat across locations and seasons

The results highlight that the highest values of the total number of bacteria were recorded in the autumn of 2022 in the rhizosphere of winter wheat cultivated in Slobozia-Conachi Galați and Ștefănești - Botoșani, with values of 28.50±2.26 and 27.57±1.94 CFU x 10^5 g^{-1} of dry soil, respectively. The lowest values were recorded in Ezăreni - Iași, with 23.17±4.12 CFU x 10^5 g^{-1} of dry soil. The data indicate that there are differences between locations regarding the total number of bacteria, especially between Ștefănești - Botoșani and Ezăreni - Iași. However, the statistical interpretation of the results suggests that no significant differences were identified between locations or between the monitored seasons regarding the total number of bacteria.

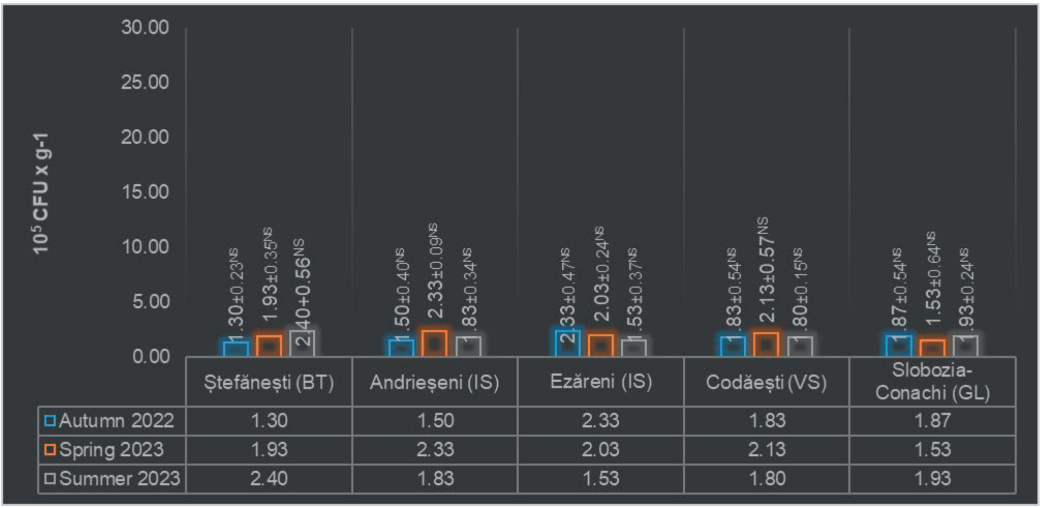


Figure 4. Comparison of Gram-positive bacteria colonizing rhizosphere of winter wheat across locations and seasons

The analysis of Gram-positive bacteria data (Figure 4) reveals important variations in their abundance across different locations (Ștefănești, Andrieșeni, Ezăreni, Codăești, and Slobozia-Conachi) over three seasons (Autumn 2022, Spring 2023 and Summer 2023).
The population size of Gram-positive bacteria exhibits the highest levels of abundance in winter wheat rhizosphere collected from Ezareni, especially in Autumn 2022 and Spring 2023. The abundance of Gram-positive bacteria from soil collected from other locations such as Slobozia-Conachi and Ștefănești show variations between seasons.
These fluctuations suggest that factors influencing the growth of Gram-positive bacteria may vary over time, even within the same geographical area. Despite observed variations in the abundance of Gram-positive bacteria across locations and seasons, the statistical tests do not provide sufficient evidence to support the significance of these differences.
The analysis of data regarding Gram-negative bacteria (Figure 5) indicates that no statistically significant differences have been identified between the values recorded in different locations or across the monitored seasons.
The highest values of the Gram-negative bacteria were recorded in the autumn of 2022 in the rhizosphere of winter wheat cultivated in Slobozia-Conachi Galați and Ștefănești - Botoșani, with values of 26.63±2.64 and 26.27±1.71 CFU x 10^5 g^{-1} of dry soil, respectively.
The lowest values of the Gram-negative bacteria were recorded in the summer of 2023 in the rhizosphere of winter wheat cultivated in in Ștefănești - Botoșani, with 17.30±1.30 CFU x 10^5 g^{-1} of dry soil.

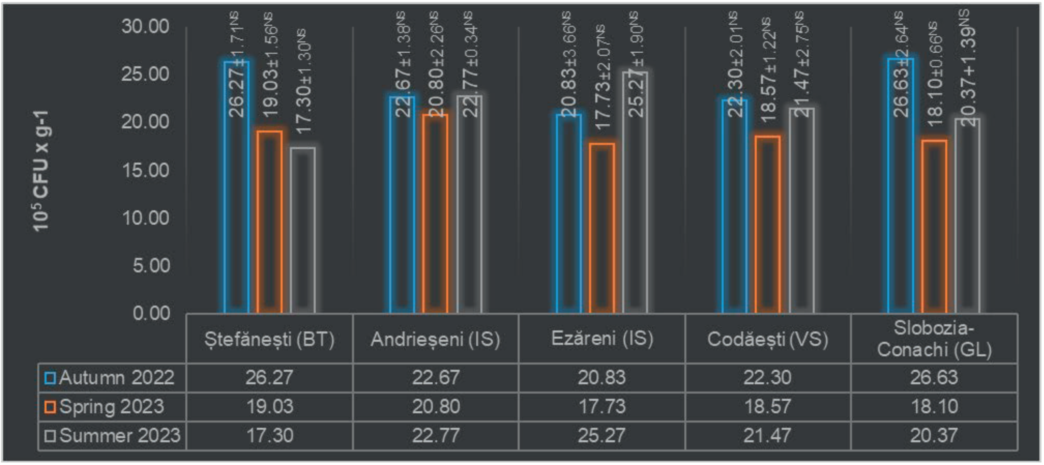


Figure 5. Comparison of Gram-negative bacteria colonizing rhizosphere of winter wheat across locations and seasons

Although there are variations in the abundance of these bacteria across different locations and seasons, the statistical analysis does not provide sufficient evidence to support the significance of these differences.

CONCLUSIONS

Taking into account all the data provided in this study regarding the total number of bacteria, Gram-positive bacteria, and Gram-negative bacteria across various locations and seasons, several conclusions can be drawn: Firstly, while significant variations in bacterial abundance were observed across different locations and seasons, statistically, these differences are not assured, suggesting that the factors influencing these variations may be consistent across the entire study area. Secondly, soil collected from Ezăreni (IS) exhibited the most variation in bacterial abundance, often ranking among the highest or lowest depending on the season. Thirdly, the season played a significant role in bacterial dynamics, with some locations showing significant increases or decreases between the monitored seasons.
Overall, these results underscore the complexity of bacterial dynamics in various environments

and highlight the importance of further research to better understand microbial interactions and their influences in different ecosystems.

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USING THE BULK DENSITY AND PARTICLE SIZE COMPOSITION OF SOIL AS SUSTAINABILITY INDICATORS TO CHARACTERIZE A SILVOARABLE ECOSYSTEM OF ROMANIA

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Abstract

The aim of the study was to identify the values of several soil parameters (particle size composition and bulk density) in a silvoarable ecosystem combining two types of plant cultures: a plantation of no-tilled hybrid poplar (Populus spp.*) trees and a conventional tilled rapeseed crop (Brassica napus). The studied parameters have been chosen because these are sustainability indicators used in the monitoring of the agricultural ecosystems in general, but in particular to characterize a rare type of agricultural ecosystems in Romania, the agroforestry ecosystems, and in this case a silvoarable ecosystem, because these are poorly studied and described in this country. The results have shown in the 0-10 cm topsoil of the poplar plantation negative Pearson's correlations between soil bulk density and respectively silt, colloidal clay and physical clay, and positive Pearson's correlations between soil bulk density and respectively coarse sand and fine sand, and inverse correlations between the same factors in the 20-30 cm poplar soil. The coarse sand and the fine sand have negatively correlated with other textural fractions of the soil (silt, colloidal clay, physical clay) in both plant types, excepting the depth 20-30 cm in the poplar soil. The silt fraction was positively correlated with the clay fractions (colloidal and physical, respectively) in both plant types, excepting the depth 20-30 cm in the poplar soil, and a positive correlation has been found between the colloidal clay and the physical clay fractions in both plant types and for all soil depths. In this study, for the topsoil (0-10 cm) of the two components (poplar plantation and rapeseed crop) there were not found statistical significant differences between the proportions of the fractions coarse sand, colloidal clay and physical clay, but there were found for fine sand and silt fractions.*

Key words*: agroforestry, particle size distribution, texture, Populus, poplar, rapeseed.*

INTRODUCTION

The particle size composition of the soil is an indicator of soil sustainability by influencing several processes in soil, among which the soil permeability has been widely studied, because the proportion of the soil fractions, their size and arrangement determine several parameters of soil which are water-related (water retention, water infiltration), such as the permeability coefficient, internal seepage (Zhao et al., 2024), water evaporation, pore size characteristics of soil (Li et al., 2024), or are very important in assessing the sustainability of agroecosystems, such as nutrient cycling, fertility (Wang et al., 2022a; Azaryan et al., 2022) soil aggregate stability (structural stability), soil leaching, soil erosion (Wang et al., 2022b). However, the soil particle size distribution is a very important parameter for understanding the soil mechanics and geotechnics, which influence its productivity, its pollutant retention or leaching (Bari, 2023) and, generally, its resilience on long term to accomplish its role in the agroecosystems and give humanity the expected sustainable economic benefits (mainly food production). Besides the influence on the physico-chemical properties of the soil, the particle size distribution of soil was found to influence several biological features of the pedosphere, like the microbial biomass and decomposition (Wang et al., 2022c; Duan et al., 2024) and the morphological traits of the roots (Azaryan et al., 2022).

The bulk density of soil is also an important physical indicator of soil sustainability because this influences many characteristics of the soils which describe its sustainability: the soil fertility by influencing the nutrient cycles, such as the stocks of soil organic carbon (Fenton et al., 2024) and the microbial activity (Gui et al., 2023), the soil compaction by estimating the packing density (Panagos et al., 2024), the water retention (Liu et al., 2024), the water hygroscopy (Zheng et al., 2021), the soil CO_2 emissions (Gui et al., 2023), or the soil temperature (Qiao et al., 2023) and pollutant contamination (Korchagina et al., 2014). The main driver of the bulk density variations was reported to be the land cover type and respectively the crop type when the soil use is agricultural (Panagos et al., 2024), but other studies (Larsbo et al., 2024) revealed the implications of soil pedofauna (earthworms) in changing the vertical profiles of the bulk density. The relationships between the textural fractions of the soil and its bulk density is a subject insufficiently approached in agroecosystems. In Romania, the agroforestry ecosystems are low studied, and the information about the relations existing between the physical factors of the soil in the silvoarable systems are lacking. This study aims to provide information about these relationships considering the importance of the approached soil parameters (particle size composition and bulk density) in characterising the soil sustainability.

MATERIALS AND METHODS

The studied ecosystem is a silvoarable ecosystem (FAO 2017) from Timiș County, Romania (45.45418°N, 20.90334°E) consisting of two components: a poplar plantation (Euro-American hybrid poplar trees: *Populus deltoides* × *Populus nigra*) which border an agricultural crop of rapeseed (*Brassica napus* L., the hybrid LG Architect).

The study aimed to characterize the soil (vertisol) of the two plant ecosystems (poplar and rapeseed) through physical parameters known as sustainability indicators of soil in agroecosystems: particle size composition (coarse sand (2.0-0.2 mm), fine sand (0.2-0.02 mm), silt (0.02-0.002 mm), colloidal clay (<0.002 mm), physical clay (<0.01 mm) and bulk density. The fractions of the particle size composition have been analyzed for three depths (0-10 cm, 10-20 cm, 20-30 cm) in the poplar soil and for one depth (0-10 cm) in the rapeseed soil. The argument for analyzing only one depth in rapeseed soil is represented by the different agricultural technologies applied for rapeseed soil, which consist of mechanical disturbance on a depth by 30 cm, as compared with the poplar plantation where the soil was not disturbed through mechanical workings for eight years. This is also the reason why the bulk density was not monitored in the rapeseed soil. The soil analyses have been performed according to the following methodology: the particle size composition has been established according to STAS 7184/10-79-PS-04 (Kaczynski's method for determination, the Atterberg scale for interpretation), and the bulk density according to ISO 11272:2017. The statistical interpretation was made using the software IBM SPSS 28.0.0.0.

RESULTS AND DISCUSSIONS

The fractions of the particle size composition of the studied soils (planted with rapeseed and respectively with poplar hybrids) analyzed for three depths (0-10 cm, 10-20 cm, 20-30 cm) in the poplar soil and for one depth (0-10 cm) in the rapeseed soil are shown in Table 1, respectively Figure 1.

Table 1. The proportions of the soil textural fractions (mean values) of the two components of the studied silvoarable ecosystem (*Populus* spp. plantation and *Brassica napus* crop)

Soil depth (cm)	Soil parameters in *Populus* spp. (hybrid poplar) plantation (mean values)					
	Coarse sand (2.0-0.2 mm)	Fine sand (0.2-0.02 mm)	Silt (0.02-0.002 mm)	Colloidal clay (<0.002 mm)	Physical clay (<0.1 mm)	Bulk density (g/cm^3)
0-10 cm	6.10±2.90	41.93±1.77	23.93±1.45	28.03±2.77	41.00±3.67	1.470±0.026
10-20 cm	6.36±2.59	42.10±1.70	23.33±1.92	28.20±2.26	37.06±9.42	-
20-30 cm	6.06±3.09	35.06±9.93	30.00±10.05	28.86±2.82	41.93±2.91	1.473±0.056
Soil depth (cm)	Soil parameters in *Brassica napus* (rapeseed) crop (mean values)					
	Coarse sand (2.0-0.2 mm)	Fine sand (0.2-0.02 mm)	Silt (0.02-0.002 mm)	Colloidal clay (<0.002 mm)	Physical clay (<0.1 mm)	
0-10 cm	4.20±2.51	40.63±5.70	25.10±2.98	30.06±5.20	43.46±6.67	

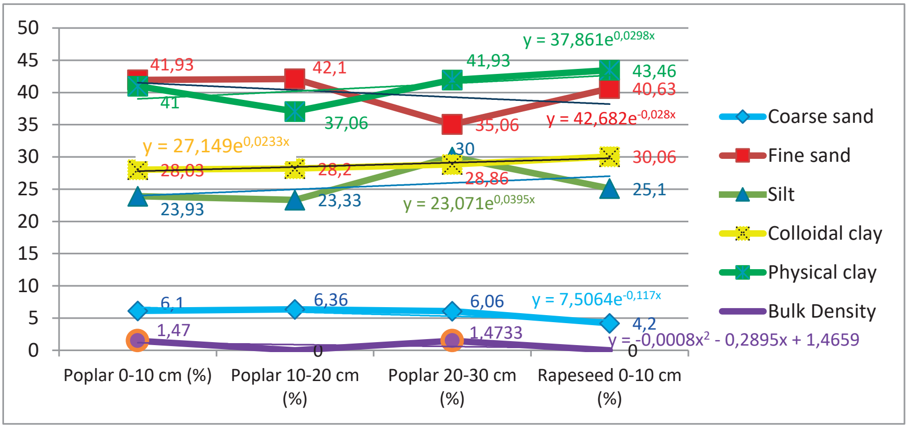

Figure 1. Particle size composition of the soil in the studied silvoarable ecosystem

In this study, for the two components (poplar plantation and rapeseed crop) of the researched silvoarable ecosystem, there were not found statistical significant differences between the proportions of the following textural (particle size composition) fractions of the topsoil (0-10 cm): coarse sand, colloidal clay, and physical clay. These findings show that, despite the different agricultural technology and land use, the particle size distribution of the rapeseed soil is not statistically different as coarse sand, colloidal clay and physical clay from that of the poplar soil. Considering that the poplar plantation has been established eight years earlier, a possible explication for these not significant differences could be the time necessary to produce changes in a vertisol by introducing a woody species. Another reason could be the higher stability of the clay due to its mineral-organic association with the organic matter (Chen et al., 2018) as compared with other particle fractions of the soil. The plant root biomass in the rapeseed topsoil and the litter input in the poplar topsoil could action as equivalent factors in maintaining the supply with organic matter of the soil and could explain the achieved result. The coarse sand is considered a non-erodible soil element (Lipiec et al., 2016) and a contributor to the internal spatial architecture of the soil which promote plant root penetration and thus its stability and low migration along soil profile.

Statistical significant differences (paired samples t-test, $p<0.05$) were however found for the finer fractions of the soil particles, i.e. for the fine sand, which in the poplar topsoil was with 3.11% higher than in the rapeseed topsoil, respectively for the silt, which in the rapeseed topsoil was with 4.88% higher than in the poplar topsoil, at 0-10 cm depth (Figure 2).

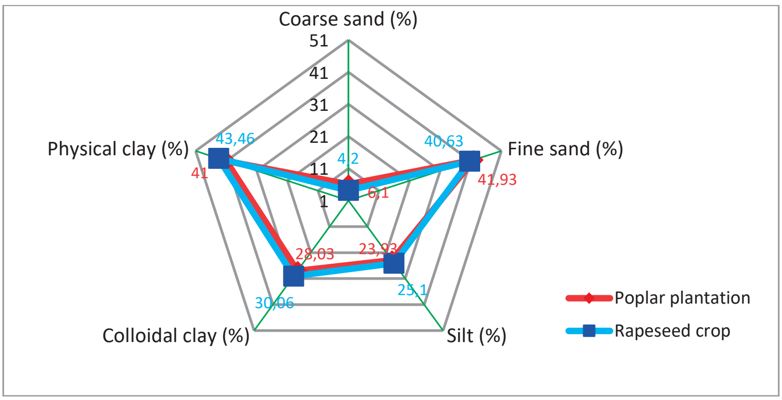

Figure 2. Particle size composition of the topsoil (0-10 cm) in the studied silvoarable ecosystem

Although these differences are small, these could have important impacts at soil level since other studies (Liang et al., 2020) showed that small variations (0-25%) of the clay fraction can considerably impact several soil properties such as the critical hydraulic gradient, the critical flow velocity and the internal erosion by influencing the behaviour of the find sand in the analyzed soil. Several researches showed that the finer particles of the soil texture are more susceptible to vary due to the particle breakdown during the mechanical agricultural workings and farming practices (Birhanu et al., 2016; Jacobs et al., 2024), or due to their susceptibility to contribute to soil losses through erosion (Ampontuah et al., 2006).

The Pearson's correlations ($p<0.05$) have been calculated to identify the relationships existing between all fractions of the particle size composition both for the soil planted with rapeseed and poplar (Table 2).

Table 2. Pearson`s correlations ($p<0.05$) between the textural (particle size composition) fractions of the soil in *Populus* spp. plantation and *Brassica napus* crop

Plant type/Soil depth (cm)	Soil textural fraction	Pearson's correlation coefficient (*p<0.05)					
		Coarse sand (2.0-0.2 mm)	Fine sand (0.2-0.02 mm)	Silt (0.02-0.002 mm)	Colloidal clay (<0.002 mm)	Physical clay (<0.1 mm)	Bulk density (g/cm^3)
Populus spp. (hybrid poplar) 0-10 cm	Coarse sand (2.0-0.2 mm)	-	0.599	-0.902*	-0.958*	-0.948*	0.923*
	Fine sand (0.2-0.02 mm)	-	-	-0.886*	-0.804*	-0.822*	0.860*
	Silt (0.02-0.002 mm)	-	-	-	0.988*	0.992*	-0.998*
	Colloidal clay (<0.002 mm)	-	-	-	-	0.999*	-0.994*
	Physical clay (<0.1 mm)	-	-	-	-	-	-0.997*
Populus spp. (hybrid poplar) 10-20 cm	Coarse sand (2.0-0.2 mm)	-	0.882*	-0.971*	-0.987*	-0.783	-
	Fine sand (0.2-0.02 mm)	-	-	-0.968*	-0.944*	-0.983*	-
	Silt (0.02-0.002 mm)	-	-	-	0.996*	0.907*	-
	Colloidal clay (<0.002 mm)		-	-	-	0.870*	-
Populus spp. (hybrid poplar) 20-30 cm	Coarse sand (2.0-0.2 mm)	-	-0.157	0.127	-0.998*	-0.994*	-0.994*
	Fine sand (0.2-0.02 mm)	-	-	-0.999*	0.215	0.257	-0.273
	Silt (0.02-0.002 mm)	-	-	-	-0.186	-0.229	0.301
	Colloidal clay (<0.002 mm)	-	-	-	-	0.999*	0.880*
	Physical clay (<0.1 mm)	-	-	-	-	-	0.858*
Brassica napus (rapeseed) 0-10 cm	Coarse sand (2.0-0.2 mm)	-	0.971*	-0.963*	-0.994*	-0.996*	-
	Fine sand (0.2-0.02 mm)	-	-	-0.999*	-0.991*	-0.988*	-
	Silt (0.02-0.002 mm)	-	-	-	0.986*	0.983*	-
	Colloidal clay (<0.002 mm)	-	-	-	-	0.999*	-

The proportions and associations of the differently sized physical fractions of the soil are important features because these determine the soil capacity to retain the water, its hygroscopy (Arthur et al., 2021), and therefore this study aimed to identify the possible relationships existing between the textural fractions of the researched soil and between these and soil bulk density. It was found a negative correlation between the factors coarse sand and silt both in poplar soil and in the rapeseed soil, excepting the soil depth 20-30 cm in poplar plantation, where the correlation was not statistically significant. Also, the factor coarse sand was negatively correlated with the clay content (colloidal clay and physical clay) for all soil depths in both plant types. The correlation between soil coarse sand and bulk density has been studied only in poplar plantation and there was found to be positive in the soil layer 0-10 cm and negative in the soil layer 20-30 cm. Negative correlations of the coarse sand with soil silt and clay have been previously found in an Entisol (Gubiani et al., 2021). The same study reported also a negative correlation between the fine sand and silt fractions of the soil. Negative correlations between the clay content and the coarse sand content of the soil have been previously reported by other researches (Xi et al., 2015; Valladares et al., 2006), which found that the clay content is associated, to some extent, with the content of organic carbon of soil (Xi et al., 2015), whereas the coarse sand content has been associated with a low content of organic carbon (Wang et al., 2019).

The soil textural fraction fine sand was negatively correlated with silt in all soil depth for both plant types. This finding is consistent with other researches regarding the particle size distribution of soil (Erktan et al., 2016) which have found that the fine sand and silt act as drivers upon the soil aggregate stability, the fine sand enhancing it whereas the silt content was associated with its decrease, emphasizing the importance of the factors fine sand and silt in determining the structural stability of soils especially in the early stages of the ecological succession of the disturbed sites, when the content of the organic matter of soil is low, resulting that the soil aggregate stability is relying also on physical and mechanical factors, such as soil particle size distribution or fine root network.

The fine sand fraction was also negatively correlated with the colloidal clay and with the physical clay in both plant types (excepting the soil depths 20-30 cm in poplar plantation, where the correlation was not statistically significant). Other studies (Liang et al., 2020) indicated that the relations of the fine sand with the clay in soils influence the internal erosion and the hydraulic conductivity of it. Also, the clay was found to be responsible for the increasing of the soil cohesion which depends on the ratio of cohesive (clay)/non-cohesive (sand) particles in the soil and on the fine particles of the soil rather than on the coarse particles of it (Wibisono et al., 2017). The fraction fine sand in the poplar soil showed a positive correlation with the bulk density only in the 0-10 cm topsoil. Similar relationship has been also found by Aşkin and Özdemir (2003) in a grassland soil and it indicated that the sand content was the soil fraction with the higher direct effects on soil bulk density than any other particle fraction and also with indirect effects through other soil properties on the bulk density of the analyzed soil.

The soil fraction silt has shown positive correlations with the clay (both colloidal and physical) in both plant types, excepting the soil depths 20-30 cm in poplar plantation, where the correlation was not statistically significant. The soil silt was negatively correlated with the bulk density only in the 0-10 cm poplar topsoil. Similar relationship between soil silt and soil bulk density has been also found by Aşkin and Özdemir (2003), which found in a grassland soil of hot Mediterranean climate the following order of direct influence of the soil particle fractions on the soil bulk density: sand, clay, silt, organic matter, fine sand.

In this study, positive correlations between the soil fractions coarse sand and fine sand were noticed only for the superior soil layers, respectively 10-20 cm in the poplar soil and 0-10 cm in the rapeseed soil. The main difference between the properties of the coarse and fine sand in soils consists on their influence on soil liquid permeability because of the porosity resulted from their arrangement along the soil profile (Wang et al., 2022a). In our study, in the poplar soil, the positive correlations between coarse and fine sand have been found in the soil layer with the largest amount of coarse sand and respectively of fine sand (10-20 cm).

The correlations between the soil textural fractions colloidal clay and physical clay were positive for both plant types and for all studied soil depths.

The relations of soil bulk density with the clay (both colloidal and physical) in the poplar soil were found to be negative in the soil layer 0-10 cm and positive in the soil layer 20-30 cm. A study regarding the bulk density of the European soils (Panagos et al., 2024) made on three depth intervals (0-10, 10-20, 20-30 cm) showed that, generally, the values of this parameter increase with the depth, with approximately 16% for the depth 20-30 cm as compared with the soil depth 0-10 cm. The same study revealed that the disturbed soils (arable lands) have the bulk density higher with 44-60% than the woodlands. In the present study, the difference between the same depth layers (0-10 and 20-30 cm) of the poplar soil was lower than 1% for bulk density. We consider this is a consequence of the no tillage technologies for eight years in the poplar plantation. Another direction to explain this low difference between the bulk densities of the soil layers was to investigate the earthworm presence in this soil, which revealed in the poplar soil the massive presence of the epigeic earthworms, which not contribute through their feeding behaviour to the variations of the bulk density, but this data is not published in this study. Positive correlations between the bulk density and the colloidal clay and the direct dependence of the bulk density on the colloidal clay content have been found previously by Suzuki et al. (2015) in the same range of soil depth. In our study, the bulk density has been measured only in the poplar soil and it has been positively correlated with the colloidal clay only for the depth 20-30 cm, because for the depth 0-10 cm the correlation has been negative. Other studies showed that the variation of the values of bulk density between the soil layers is associated with the various amount of organic matter, the biological activity or the different architecture of the plant roots.

CONCLUSIONS

In this study, for the topsoil (0-10 cm) of the two components (poplar plantation and rapeseed crop) of the researched silvoarable ecosystem there were not found statistical significant differences between the proportions of the fractions coarse sand, colloidal clay and physical clay, but there were found for fine sand and silt.

The results have shown in the 0-10 cm topsoil of the poplar plantation negative Pearson's correlations between soil bulk density and respectively silt, colloidal clay and physical clay, and positive Pearson's correlations between soil bulk density and respectively coarse sand and fine sand, and inverse correlations between the same factors in the 20-30 cm poplar soil.

The coarse sand and the fine sand have negatively correlated with other textural fractions of the soil (silt, colloidal clay, physical clay) in both plant types, excepting the depth 20-30 cm in the poplar soil.

The silt fraction was positively correlated with the clay fractions (colloidal and physical, respectively) in both plant types, excepting the depth 20-30 cm in the poplar soil.

A positive correlation has been found between the colloidal clay and the physical clay fractions in both plant types and for all analyzed soil depths.

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PEDOLOGICAL AND AGROCHEMICAL STUDY ON THE AREA IN PERIȘORU AREA, CĂLĂRAȘI COUNTY

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Abstract

The pedological mapping was carried out in order to identify the soil type and assess its fertility based on physico-chemical characteristics. At the same time, agrochemical samples were collected from the arable horizon (0-20 cm) in order to determine the nutrient content. The main physical characteristics were determined, based on the collection of soil samples in natural settlement (metal cylinders). Based on the nutrient content, the crop fertilization plan was developed for each species and the expected production. Based on the results obtained, the humus content of the soil shows low values (less than 4%) unsuitable for a typical chernozem formed in the Baraganului area. A bonitation study was conducted for arable use, the soil being classified at the lower limit of Class II with 66 bonitation points, due to the less favorable climatic conditions and the phreatic level located below 10 m depth.

Key words: *improvement, soil cover, fertilization plan, agricultural technologies.*

INTRODUCTION

When it is stated that the fertility of a soil has decreased, the reference is made to its initial level, as for example to the natural fertility or fertility that the soil, already taken in culture, had years ago. The fertility of such a soil can be improved by knowing its characteristics well, as well as how they can be influenced by agro-pedo-ameliorative measures (Budoi et al., 1996).

The arable area for every human being of the planet was valued in 1990 at 0.3 ha, reaching 0.25 ha in 2000, estimated at 0.15 ha in 2050 and at only 0.10 ha in 2150, so that the requirements of mankind could be met only by innovative scientific technologies (Lal, 1992).

FAO data showed that the agricultural area is estimated at 1475 million ha; the area affected anthropically, by technologies is 552 million ha, to these are added another 10 million agricultural land affected by industrial activities in Europe, which means that the total degraded area reaches 562 million ha (38% of the agricultural area) of which 285 million ha is moderately degraded. In Romania, the most widespread soil degradation processes are: anthropogenic or secondary compaction (44%), water and wind erosion (47%), drought (48%), temporary excess water (25%), low humus reserve (50%), low phosphorus content (42%) and acidity state (23%) (Marinca et al., 2009). The soil can no longer be satisfactorily classified according to the size of the particles of which it consists, in a few Restricted Groups: sands, loams, clays, etc., having to take into account their history. The properties of the soil depend not only on the parental material, as shown by Dokuchaev (quoted by Russel, 1973), they depend on the climate, vegetation and other factors with which they interact (Topa et al., 2013). Soil degradation through the decline of chemical and physical fertility requires the increase of the organic matter content necessary to improve soil fertility, nutrient cycle, soil structure in order to ensure sustainable agriculture in the future (Swift, 2001). The influence of the tillage system on soil characteristics are important indicators for preserving soil fertility and assessing the sustainability of the agricultural system (Guş, 1997; Rusu, 2001; Mark et al., 2004; Jităreanu et al., 2006; Almagro et al., 2017; Biddoccu, et al., 2017; Martínez-Mena et al., 2020; 2021; Burtan et al., 2016; 2023).

MATERIALS AND METHODS

Soil sampling agrochemical middle of plowed horizon (0-20 cm) were composed of 15-20 individual samples from the surface sampling plots of ground. The parceling of land, was considered pedological complexity, uniformity of land utilization, crop structure so that each sample representing a plot as uniform (SRTS, 2012; MESP, 1987, vol. I-III). The results were analyzed and interpreted based on the standards contained in the catalog A.S.R.O. that are consistent with international standards.

Methods of analysis used to determine the chemical characteristics

Organic matter (humus): determined by volumetric wet oxidation method after Walkley-Black, the change Doughnut – STAS 7184 / 21-82. Carbonates - gasometric method using calcimetrul Scheibler after SR ISO 10693: 1998 (%). Nitrogen content was determined indirectly (by calculation) based on the humus content and degree of saturation with bases.

IN = humus x V / 100.

Accessible phosphorus (P mobile): after EgnerRiehm-Domingo and dosed with molybdenum blue colorimetric after Murphy-Riley method (reduction with ascorbic acid). Available potassium (K mobile): extraction after Egner-Riehm-Domingo and determination by flame photometry.

pH: determined potentiometrically with a combined glass and calomel electrode in an aqueous suspension to the Soil / Water 1/2.5 - SR 7184 / 13-2001. The acidity of the hydrolytic - extraction with sodium acetate to pH 8.2. Amount bases - Kappen method Schoffield Chirita by extraction with 0.05 normal hydrochloric acid

Methods of analysis used to determine the physical properties

The bulk density (BD): the method of the metal cylinder of known volume (100 cm^3) to the temporary humidity of the soil (g/cm^3). The total porosity (TP) by calculating (% by volume - % v / v). The coefficient of hygroscopic (CH) drying at 105°C of a sample of soil moistened in advance in equilibrium with an atmosphere saturated with water vapor (in the presence of a solution of H_2SO_4, 10%) - % by weight (% w/w). Wiping coefficient (WC): calculated by multiplying by 1.5 the hygroscopicity factor determined by the modified Mitscherlich method (no vacuum, witness evidence) - weight %.

RESULTS AND DISCUSSIONS

Characterization of physico-geographical conditions

The studied territory is located in the Bărăgan Plain, a geographical subunit located in the eastern part of the Romanian Plain, is distinguished by certain specific geographical features. The southern Bărăgan is subdivided by the Argova - Vanata valleys with its tributary Furciturii Valey in three subunits: Lehliu Plain in the West (composed of Copuzeanca field, Milotina field and Andolina field); Mărculesti Plain (composed of Thistle field in the North, consisting of sands and Jegaliei field from the old Danube floodplains-Balti at the levels of terraces 4 and especially, 3 and 2) (Posea et al., 2005). Hagieni field, Calarasi-terrace field and Făcăeni field. The geomorphology of the plain is represented by smooth interfluves called Fields (Hagieni, Jegalia, Thistles, Andolina), with altitudes of 35-40 m, covered by limo-like deposits, interrupted by small depressions called “crovs” and narrow valleys of “mostiste” type, signifying a territory with arid climatic conditions and a steppe vegetation. Fields represent the major type of relief. They have a general fluvio-lacustrine origin, but locally diversified, and are covered with ligness having a thickness of 8-40 m absolute altitudes average 40-60 m. Due to its geographical location, with a wide opening towards the east, the north-eastern part of Bărăgan is characterized by a temperate zone of the plain, with a high degree of continentalism, with the contrast of the thermal high in the winter (-2°C, -4°C in January and the lowest possible until it is below 30°C) in summer (from 22°C to 23°C in July and a maximum of more than 40°C). The extreme values recorded in Mărculesti over time, were 41.5°C (Mărculesti, august 10, 1951) in the air and 68.6°C on the ground (at the same station, July 16, 1966) in the warm season and -30°C in the cold season on January 8, 1938 in the air and of -25.9°C on the ground (Posea et al., 2005). Precipitation is distributed unevenly during the year, so on average 39% of the amount of precipitation falls in the cold season, and the remaining 61% in the warm season.

Currently, in the Steppe area, the few remains of Meadows consist of bearded grass (*Botriochloa ischaemum*), steppe fescue (*Festuca valesiaca*), pir crested (*Agropyrum cristatum*), *Koeleria cristata, Phleum phleoides*, and the heavily modified ones, bulbous thread (*Poa bulbosa*), wormwood (*Artemisia austriaca*), pir gros (*Cynodon dactylon*), alior (*Euphorbia nicdeensis*) and other species adapted to dryness. The forest appears in the form of small areas, where the phlox Oak predominates (*Quercus pedunculiflora*) - Pontic species, in association with fluffy Oak (*Quercus pubescens*), mojdrean (*Fraxinus ornus*), plop (*Populus* sp.), dogwood (*Ligustrum vulgare*), turkish cherry (*Prunus mahaleb*).

Figure 1. Representative profile, Perișoru, Călărași

Steppe thickets are represented by blackthorn (*Primus spinosa*), dwarf almond (*Amygdalus nana*), cherry dwarf (*Cerasus fruticosa*), rosehip (*Rosa gallica*), stubble BlackBerry (*Rubus caesius*) etc., among which isolated specimens of elm are also found.

The soil type is represented by typical chernozem (SRTS-2012 and WRB-SR - 2014) with the following composition of Horizons: Am-AC-Cca (Figure 1).

Morphological characteristics

Horizon Am (0-36 cm), dusty clay, dark brown, (10 YR 2/1 to wet and 10 YR 3/2 to dry), glomerular structure well developed, porous, permeable, frequent fine roots from cultivated vegetation, weak effervescence at the base of the horizon, beginning of hardpan at 30-48 cm, (at the separation limit between bioaccumulative horizon and transition horizon), gradual transition to the lower horizon.

Horizon AC (36-68 cm), medium clay, light brown, (10 YR 3/3 in wet and 10 YR 4/4 in dry), poorly developed glomerular structure in the upper half of the horizon, slightly friable, porous, loose, with accumulations of carbonates in the form of pseudomycelia, moderate effervescence.

Horizon Cca (68-120 cm), sandy clay dusty, yellowish (2.5 Y 5/4 in wet and 2.5 Y 6/6 in dry), unstructured, very friable, porous, loose, with accumulations of carbonates in the form of pseudomycelia and small crumpled concretions, strong effervescence.

Physico-chemical characterization

The physico-chemical characteristics of this type of soil, are consistent with the formation of physical and geographical conditions thereof.

Analytical data for tipical chernozem are shown in Table 1.

Soil reaction (pH) is neutral with values of (6.56); organic matter content is medium (3.26%); bulk density (BD g/cm^3) is low (1.25); total porosity (%) is high (54%); degree of subsidence (GT%) is unattached with negative values. Carbonates occur at the base of the bioaccumulative horizon. The degree of saturation in bases (V%) falls within the eubase range with values between 91-100%. The nitrogen index (NI%) is medium with values of 3.02%; mobile potassium (P_{AL} mg/kg) is medium with values of 151 mg/kg and mobile phosphorus (K_{AL} mg/kg) with values of 23 mg/kg. Being a soil with undifferentiated loamy texture on the profile, the hydrophysical indices show favorable values for the growth and development of plants, creating a very favorable aerohydric regime. The total water capacity (TWC%) shows high values perfectly correlated by the ratio of total porosity and apparent density. The humus reserve shows medium values on the first 36 cm from the soil surface (146.7%).

Table 1. Physico-chemical analysis at soil Am-AC-Cca, of studied territory

Horizon	**Am**	**AC**	**Cca**	**C**
Depth (cm)	0-36	36-68	68-120	120-185
Coarse sand gr. (2-0.2 mm)	8.3	16.3	16.7	20.6
Fine sand (0.2-0.02 mm)	26.8	29.7	25.6	31.2
Dust (0.02-0.002 mm)	38.7	28.6	39.5	30.4
Clay (< 0.002 mm)	26.2	25.4	18.2	17.8
Textural class	LP	LL	SS	SM
Reaction (pH)	6.56	7.15	8.35	8.57
Humus content (%)	3.26	2.11	0.89	0.56
Bulk density (g/cm^3)	1.25	1.27	1.31	-
Total porosity (%)	54	52	48	-
Degree of compaction GT (%)	uncompacted	uncompacted	weak	weak
Carbonates (%)	-	6.5	12.9	12.5
Percentage of base saturation (%)	94	100	100	-
Nitrogen indicator	3.02	2.26	0.89	-
Phosphorus mobile (ppm)	23	19	-	-
Potassium mobile (ppm)	151	120	-	-
Wilting coefficient (%)	10.5	10.0	9.5	-
Field capacity (%)	19.1	18.2	17.3	-
Usable water capacity (%)	8.6	8.2	7.8	-
Total capacity (%)	43	41	36	-
Humus reserve (t/ha)	146.7	86	61	-

Table 2. Calculation of the crop crediting mark for typical chernozem

Indicators	**Culture**							
	wheat	barley	mais	sunflower	potatoes	beet	soybeans	peas
Average temperature	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0
Average precipitation	0.9	0.9	0.8	0.9	0.7	0.7	0.9	0.9
Gleization	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stagnogleization	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Saliniz/alcalization	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Texture in 0-20 layer	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Edaphic volum	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Poluation	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Slope	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sliding	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Growndwater	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Inundability	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Humidity	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total porozity	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Carbonates (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Reaction in 0-20 layer	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Humus content	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bonitation notes	72	72	64	72	50	56	72	72
Medium UTS	*66*							

The study was carried out for the use of arable, for the eight crops according to the specific indicators (Table 2). In order to improve the physical characteristics (BD, TP, GT), but also to come up with an additional intake of nutrients, the following dose of semi-fermented manure is recommended, based on the humus content and the percentage of clay:

$$\text{Dose gg (t/ha)} = \left(15 + \frac{30}{3{,}6}\right) . \left(1{,}35 - \frac{8}{26{,}2}\right) . 1 = 27.6$$

The results of analysis based on agrochemical four samples taken from the sole, as well as agrochemical recommendations on fertilizer application rates in crops, they are shown in Table 3 (a and b).

Table 3(a). Dosing recommendations of chemical fertilizers and amendments based on agrochemicals analyses

No.	Physical block	Specification					
		Reaction, pH	**Humus content** (%)	**Phosphorus** (mg/kg)	**Potassium** (mg/kg)	**IN** (%)	**V** (%)
1.	BF 6	7.11	3.69	62	326	3.61	98
2.	BF 89	7.63	3.55	52	277	3.55	100
3.	BF 84	7.70	3.46	49	278	3.46	100
4.	BF 1181	7.38	3.47	60	332	3.33	96
5.	BF 1161	7.67	3.75	77	377	3.75	100
6.	BF 1158	7.72	3.64	73	298	3.64	100
7.	BF 1116	6.56	3.80	62	263	3.61	95
8.	BF 1019	7.55	3.71	63	264	3.71	100
9.	BF 677	7.22	4.05	96	238	4.05	100
10.	BF 585	6.89	3.90	83	288	3.70	95
11.	BF 583	6.54	3.74	78	251	3.44	92
12.	BF 543	6.50	3.81	56	225	3.58	94

Table 3(b)

Physical block	**Surface** (ha)	**Previous culture**	**Current culture**	**Production** (kg/ha)	**Nitrogen** (kg s.a./ha)	**P_2O_5** (kg s.a./ha)	**K_2O** (kg s.a./ha)
BF 6	91.36	Wheat	Maize	10 000	200	120	100
BF 89	102.09	Rape	Wheat	8000	200	100	80
BF 84	4.66	Wheat	Wheat	7000	170	80	70
BF 1181	240.77	Wheat	Rape	4000	120	70	70
BF 1161	112.35	Barley	Sunflower	4000	120	70	60
BF 1158	16.80	Wheat	Wheat	7000	170	80	70
BF 1116	91.05	Rape	Maize	10 000	210	120	110
BF 1019	239.88	Wheat	Maize	10 000	200	120	100
BF 677	5.33	Rape	Barley	7000	140	70	60
BF 585	13.93	Rape	Barley	7000	140	70	60
BF 583	18.20	Rape	Barley	7000	140	70	60
BF 543	80.15	Rape	Barley	7000	140	70	60

Fertilization for corn: in spring, 50-60 kg N/ha of complex fertilizers NP (1:2 or 1:1) / NPK (1:2:1 or 1:1:1) will be administered, with sowing, one at a time, 5-10 cm sideways from the sowing row and 5-6 cm below the seed level), and the difference in the nitrogen dose is administered at the execution of the slingshot with the cultivator.

Fertilization in wheat/barley: phosphorus and potassium are applied in full in autumn with plowing; complex NP/NPK fertilizers are used; nitrogen is applied in three stages:

- the first stage is recommended to take place in autumn, until the twinning period, when the fertilizer requirement is relatively low; nitrogen being slightly soluble, it is recommended to apply an amount of 30-40 kg N/ha, and the rest of the fertilizers to be applied in winter or spring, depending on the evolution of the crop and the amount of precipitation; in autumn, nitrogen fertilizers can be applied before or after sowing, but too much fertilizer should not be applied, as there is a risk of leaching (precipitation acts on fertilizers).
- the second stage of wheat fertilization takes place in early spring and is applied about 40-60 kg N/ha; in order to complete the economically optimal dose of nitrogen, nitrocalcar should be used in spring, in order to avoid acidification;
- the third stage of fertilization should be carried out at the appearance of the first node of the stem when the remaining amount of the recommended dose is applied.

Rape fertilization: in autumn, before sowing, NP/NPK complexes will be administered; when applying nitrogen in autumn, we take into account the amount of plant residues from the previous plant; their presence generates a lack of N, because the bacteria responsible for the nitrification process consume nitrogen before it is taken over by plants; thus, the nitrogen dose is increased by 5-7 kg/t of plant residues; however, in autumn, a quantity of more than 50 kg S.a.N/ha will not be administered, in order to avoid nitrogen leaching and weakening of the frost resistance of plants due to intense growth; until entering winter, normally developed rapeseed plants absorb around 40 kg N/ha; on these soles, in spring, to complete the economically optimal dose of nitrogen will be applied the remaining amount in the form of nitrocalcar; the application of sulfur in spring is very important, with the fraction of nitrogen, and before flowering it is necessary to administer boron and magnesium.

Fertilization in sunflower: it is sensitive to both the deficit and the excess of nitrogen, especially in the early stages, which will have negative repercussions on the growth and development processes; the effect of nitrogen deficiency can be seen with the advancement in vegetation of plants, which have aging leaves, yellow color, and when harvesting presenting small calatids with many dry seeds; it has a well-developed root system, is able to explore a large volume of soil, absorbing leached nitrogen, finding that the plant capitalizes quite little nitrogen from fertilizers; during the flowering period, it records a consumption of at least 3-4 kg nitrogen/ha/day, late nitrogen absorption failing to correct the effects of the deficiency in the early phases; excess nitrogen can harm the sunflower crop, causing; at the same time, the excess of nitrogen causes a lush growth of plants, prolongs the vegetation period at the expense of production and oil content, and also decreases the resistance of plants to attack pathogens and drought.

The administration of nitrogen doses calculated for sunflower culture is administered in two stages, namely: one half of the total amount, when preparing the seedbed or simultaneously with sowing and the rest of the amount is administered during mechanical slingshots (in the form of NP/NPK). The calculated doses may be reduced by 0.75-1.5 kg for each tonne of manure administered to the preceding plant or directly to the crop concerned.

CONCLUSIONS

The main limiting factor of the production potential is the deficient rainfall during the vegetation period, which is partially compensated by irrigation.

The soil cover of the studied area is consistent with the physical and geographical conditions of the area, being identified only one type of soil with zonal character (typical chernozem). Parental material is predominantly made up of loessoid deposits. The texture of this soil unit is loamy (middle) undifferentiated, throughout the depth of the soil profile.

The soil reaction is neutral-weakly alkaline, with pH values ranging from 6.45 to 7.72. The supply of nitrogen, represented by the nitrogen index (in) is medium, with values between 3.33 and 4.05%. The supply of mobile phosphorus is medium-high, with values ranging from 49-96 ppm. The mobile potash supply is medium-high, with values ranging from 225-377 ppm. The values of hydrophysical indices are within optimal limits, giving plants a very favorable aerohydric regime, due to the medium (loamy) soil texture, undifferentiated on the profile.

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THE ROLE OF IRON COMPOUNDS IN THE FORMATION OF THE FERTILITY OF HYDROMORPHIC SOILS OF THE FLOODPLAINS OF THE LEFT BANK FOREST STEPPE OF UKRAINE

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Abstract

The special role of iron compounds in the formation of the fertility of alluvial Fluvisols is an actual direction of research. The purpose of the work is to establish the laws of transformation, migration and accumulation of iron in floodplain soils, taking into account the peculiarities of the conditions of their formation and the influence of these processes on their fertility. These processes have an important genetic-diagnostic and agro-ecological role. The regularities of the content and profile distribution of non-silicate forms of iron in floodplain soils of the Left Bank Forest-Steppe of Ukraine with different degrees of hydromorphism are indicated in the article. Iron significantly affects the content of nutrients in plants, in particular, the content of phosphorus and potassium. Fixing them in a form inaccessible to plants can cause a deficiency in mineral nutrition of plants. The transformation of organic matter occurs with an increase in the content of the fulvic acid group under conditions of waterlogging. There is a sharp increase in the solubility of iron hydroxide at the same time. Collectively, all this leads to the migration of iron compounds along the soil profile and the formation under certain conditions of concretionary forms of iron that can impair soil fertility. The introduction of different doses of iron did not significantly affect the acid-alkaline balance of the soil solution. The pH remained within the neutral range.

***Key words**: floodplain, alluvial soils, iron.*

INTRODUCTION

Floodplain soils are found in all natural zones of Ukraine. The area of floodplain soils within agricultural lands is 5.3 hectares.

The pH of the soils of the floodplains of the small rivers of the Left Bank of the Forest Steppe is neutral or close to alkaline. They are layered, glazed, enriched with nutrients. The soils of the floodplains of small rivers of the Livoberezhny Forest Steppe were formed under the action of the humus-accumulative process. A characteristic feature of all types of floodplain soils is hydromorphism, expressed to one degree or another. The high fertility potential of these soils is due to their special function of a kind of trap (barrier, filter) on the way of migration and accumulation of many substances and elements in nature. Among them are such important biogens for plants as iron, calcium, nitrogen, phosphorus, etc. In the spring, flood waters bring various water-soluble and suspended mineral particles into the floodplain, which enrich the soil cover of the floodplain.

Under conditions of stagnation, introduced oxidizing compounds of iron are restored and turn into active forms of oxidized iron. The latter significantly affect the state of potential fertility, which is reflected in the productivity of meadow phytocenoses. Groundwater also becomes a source of iron supply to floodplain soils. In the absence of dissolved oxygen in groundwater, it is usually found in the form of soluble compounds with Fe^{2+} ions.

With the appearance of mobile forms of iron in the soil, the accumulation of iron phosphates, which are poorly soluble and scarcely available compounds for plant nutrition, can be traced (Truskavetskyi et al., 2020; Ginsburg, 1981; Truskavetskyi & Zubkovska, 2015). In the work of prominent scientists indicates the priority role of one and a half oxides in the absorption of phosphates. This is particularly evident in floodplain soils.

Iron is a necessary and irreplaceable element of mineral nutrition and an active regulator of oxidation-reduction (OR) processes in the soil. Recovery of iron occurs at Eh from +100 to -

600 Mv. In hydromorphic soils, under oxidizing conditions, recovery processes can occur, with a local reduction of ORP to 300-350 mV and below. In such cases, both forms of iron are constantly or periodically present in the soil, or divalent forms predominate.

It is worth noting that the solubility of iron compounds is affected by the pH of the medium. In a strongly acidic environment, the mobility of iron hydroxide increases and iron ions appear in the soil solution. In soils with a neutral or alkaline reaction (pH >7), its solubility is usually reduced, which can lead to the formation of insoluble forms of iron and reduce its availability to plants.

The content of organic matter plays an important role in soil fertility. In floodplain soils, a significant role in the fixation of humic substances is played by oxides and a half, in particular free compounds of iron, which are able to form strong iron-organic complexes with fulvic acids under a dynamic redox regime.

Various properties of iron determine the interest of researchers in it as a diagnostic indicator of many macro- and micro-processes of soil formation and as a factor in the fertility of floodplain soils (Zaidelman, 2017; Zonn, 1982; Qiu et al., 2016; Li et al., 2018; Khan et al., 2015).

Taking into account the active participation of iron in many soil processes and the insufficient study of this issue, there is a need for additional research on its role in the formation of floodplain soil fertility.

The purpose of our research was to determine the content of mobile forms of iron, the dynamics of redox conditions, the nature of the water-air regime, the level of acid-base balance and phosphate mode in the floodplain soils of the Left Bank Forest Steppe of Ukraine and the influence of these factors on their fertility.

MATERIALS AND METHODS

The research was conducted during the growing season at the sites of the stationary experiment on the Humi-Gleyic Fluvisol (N 49°46'16.6", E 35°55'60.0") drained by a network of open channels and Calcari-Gleyic Fluvisol (N 49°46'33.6", E 35°56'11.9") in the floodplain of the Vilkhovatka River (Kharkiv District, Kharkiv Region) (Figure 1). Soil samples were taken three times in different periods: spring, summer and autumn.

Figure 1. Aerial view of the floodplain of the Vilkhovatka River

In order to preserve oxidized forms of iron and prevent their oxidation, the analysis was carried out in soil samples preserved with toluene, which were urgently sent to the laboratory for determination of oxidized and oxidized forms of iron. Soil samples were filled with 1N H_2SO_4 solution in the ratio of 1 part of soil to 10 parts of acid.

Determination of iron oxides in the acid extract is carried out using o-phenanthroline by colorimetric method. Fe^{2+} and Fe^{3+} were determined simultaneously in two portions from the same acid extract. In the first portion, Fe^{2+} was determined, in the second, the sum of Fe^{2+} and Fe^{3+} was previously converted into Fe^{2+} with hydroxylamine. The content of Fe^{3+} is determined as the difference between the content of Fe^{2+} and Fe^{3+} and the value of Fe^{2+} multiplied by a factor of 1.11.

The redox potential was measured in the field using a portable pH-meter-millivoltmeter I-130 in the walls. The EMF was measured (in mV) between the EPV-1 thin-layer platinum electrode and the EVL-1M auxiliary silver chloride electrode, which were lowered into the soil ball, to determine the soil redox potential. The determinations were accompanied by the measurement of the following parameters: humidity, temperature, and soil acidity (pH).

In the version with fertilizers, ammonium nitrate was used as nitrogen fertilizers at the rate of 120 kg/ha based on the active substance. Simple superphosphate and potassium salt were also applied at the rate of 50 kg/ha per active substance.

Determination of the effect of iron on the phosphate function was carried out in a model experiment on the example of Calcari-Gleyic Fluvisol medium-loamy (content of particles < 0.01 mm - 38.61%) alluvial soil. The soil is low in humus (3.12%) with pH_{H_2O} 7.5. The experiment consists of two blocks with four options in each. The first block is carried out with optimal humidity (70% of the full moisture content). The second unit was artificially overmoistened (up to 90% of full moisture content). The background of both blocks contained complete organo-mineral fertilizer $N_{60}P_{120}K_{60}$+decomposed manure.

Iron hydroxide was added to these backgrounds in all blocks in the following versions with increasing doses of loads (400, 800 and 1600 mg/kg of soil). The study was carried out in 3 repetitions in containers containing 0.6 kg of soil each. The test culture is Badyory barley.

The determination of mobile phosphorus compounds was performed according to the modified Machigin method: extraction of mobile phosphorus compounds from the soil was carried out with a 1% solution of ammonium carbonate ($(NH_4)_2CO_3$) (pH 9.0) at a soil-solution ratio of 1:20 and a temperature of 25 ± 2°C. Phosphorus is determined photocolorimetrically by the color intensity of the phosphorus-molybdenum complex.

RESULTS AND DISCUSSIONS

The redox regime of the soil is closely related to water and thermal regimes. The highest value of field soil moisture is observed in the spring period (Table 1), when the soil is saturated with flood and melt water. In summer, it decreases by 1.4-5.3 times due to an increase in air temperature and increased transpiration, and in autumn it increases slightly due to precipitation. The surface 0-20 cm horizon remains the least moistened. Humidity increases in glaciated horizons as it approaches the level of groundwater. In May of the first year of research, the groundwater level was at a depth of 60-80 cm, already in August - 100-150 cm, and in October 80-100 cm.

Table 1. Dynamics of ORP, pH and moisture content in drained Humi-Gleyic Fluvisol

Version	Depth, cm	Spring			Summer			Autumn		
		pH	Eh, mV	moisture content, %	pH	Eh, mV	moisture content, %	pH	Eh, mV	moisture content, %
2009										
Control	0-20	7.2	498	20.2	7.1	528	4.6	7.1	512	4.9
	20-50	7.0	451	23.1	7.1	532	6.6	7.1	522	6.5
	50-65	6.8	447	39.4	6.8	489	7.4	6.9	478	8.7
$N_{120}P_{50}K_{50}$	0-20	7.0	505	20.7	6.8	494	4.2	6.9	504	6.7
	20-50	6.9	523	30.1	6.8	500	10.4	6.9	497	5.6
	50-65	6.8	456	45.5	6.7	472	32.0	6.8	468	15.3
2010										
Control	0-20	7.0	476	16.1	7.2	571	10.2	7.1	537	28.6
	20-50	6.8	506	21.0	7.0	506	13.9	7.1	552	29.2
	50-65	6.8	501	45.1	6.8	436	17.7	6.9	529	35.4
$N_{120}P_{50}K_{50}$	0-20	6.8	496	21.3	6.7	467	8.9	6.7	534	31.8
	20-50	6.9	503	22.8	6.7	474	16.1	6.7	510	27.4
	50-65	6.8	432	53.2	6.8	492	26.2	6.7	492	41.0
2011										
Control	0-20	7.0	524	25.2	7.0	518	10.8	7.1	482	12.3
	20-50	6.8	543	27.9	6.9	520	10.0	6.9	443	13.8
	50-65	6.9	561	30.3	6.8	504	12.3	6.8	445	24.2
$N_{120}P_{50}K_{50}$	0-20	6.8	539	29.3	6.8	523	15.1	6.8	509	15.3
	20-50	7.0	565	32.1	7.0	517	14.0	6.9	477	14.8
	50-65	6.9	586	43.2	6.9	506	18.9	7.0	490	25.5

Measurements of the soil temperature in the surface layer at a depth of 5-10 cm showed that the greatest warming of the soil, on average, to +18°C, occurs in summer, and the least in autumn, around +5°C, while in the spring the temperature was at the level of +8°C.

When the temperature rises in the spring-summer period, the humus horizons of the investigated hydromorphic soils dry out and are enriched with oxygen due to the establishment of oxidizing conditions during this period. At the same time, thanks to the presence of organic substances, which floodplain soils are rich in, the activity of microflora, which also requires oxygen, is activated.

The introduction of mineral fertilizers in doses of $N_{120}P_{50}K_{50}$ affects the indicator of oxidation-relative potential, if a gradual decrease in the Eh indicator is observed on the option of control over the sampling depths of soil samples (0-20, 20-50 and 50-65 cm), then the introduction of mineral fertilizers violates this regularity. For example, in the spring of 2009, during the control, the ORP index decreases layer by layer in the following order: 498, 451, and 447 mV, then when mineral fertilizers are applied in the same soil layers, this indicator is slightly higher, respectively 505, 523, and 456 mV. That is, in our opinion, it is obvious the fact that the introduction of mineral fertilizers increases the Eh indicator can be traced.

Certain redox conditions are necessary for the better development of meadow phytocenoses. Seasonal dynamics of ORP on the control variant increases in summer and autumn. The maximum value of Eh was determined at the level of 571-537 mV, which was recorded in the second year of research. Meadow grasses do not show any noticeable signs of suppression at such values of the ORP indicator. In the lower horizons, the indicator of redox potential decreases. Restorative processes can develop in separate zones. But they do not determine the general orientation of redox conditions both along the genetic horizons and over the entire soil profile.

It should be especially noted the developed microzonal redox reactions in peated layers at a depth of 50-65 cm in summer with a high content of mobile forms of iron and a decrease in pH during this period (Table 2). And although in the real soil and ecological situation there is no direct proportional relationship between the value of ORP and the content of mobile compounds of oxidized and oxidized iron, still sometimes, due to the decrease of ORP in the lower horizons of floodplain soils, a partial reduction of iron is also observed. We recorded these patterns in the 50-65 cm soil layer in the spring of the first year of research.

Table 2. The dynamics of the content of mobile oxidized and oxidized iron compounds in the Humi-Gleyic Fluvisol of the Vilkhovatka river floodplain

Version	Depth, cm	Content of acid-soluble iron, mg/kg of soil								
		Spring			Summer			Autumn		
		FeO	Fe_2O_3	∑	FeO	Fe_2O_3	∑	FeO	Fe_2O_3	∑
2009										
Control	0-20	353	2092	2445	296	2327	2623	185	2762	2947
	20-50	366	2146	2512	271	2576	2847	248	2713	2961
	50-65	813	1441	2254	196	2703	2899	434	2819	3253
$N_{120}P_{50}K_{50}$	0-20	214	2052	2266	145	2243	2388	189	2224	2413
	20-50	227	2236	2463	242	2709	2951	257	2540	2797
	50-65	691	1923	2614	573	2413	2986	604	2439	3043
2010										
Control	0-20	252	1994	2246	161	2373	2534	121	2591	2713
	20-50	396	2276	2672	361	2560	2921	221	2785	3002
	50-65	703	1673	2376	276	2823	3104	300	3056	3357
$N_{120}P_{50}K_{50}$	0-20	136	2285	2421	186	2858	3044	166	2082	3658
	20-50	506	1918	2424	442	2736	3178	313	3345	3658
	50-65	927	2973	3900	578	4426	5004	922	4037	4953
2011										
Control	0-20	302	2239	2541	154	2666	2820	167	3190	3357
	20-50	418	2356	2774	221	2921	3142	376	3517	3893
	50-65	871	1448	2319	301	2698	2999	476	3596	4072
$N_{120}P_{50}K_{50}$	0-20	172	1828	2000	175	2336	2511	123	2268	2391
	20-50	559	2354	2913	276	3260	3536	398	2780	3178
	50-65	914	1932	2846	585	5496	6081	1069	5480	6549

In the seasonal dynamics, an increase in the total content of mobile iron compounds was characteristic in the warm summer months, when favorable conditions are created for the mobilization of its potential reserves, the value of this indicator in the soil layer 0-20 cm in the control was 2623 mg/kg, in the lower horizon 20-50 cm it was equal to 2847 mg/kg, and for

50-65 depth it was at the level of 2899 mg/kg. In the autumn, the total content of mobile iron compounds in the meadow-swamp soil increases significantly compared to the summer period, in all horizons, both in the control and in the fertilized version.

Thus, in the spring of the second year of research, the content of total mobile iron did not exceed 2246 mg/kg of soil in the near-surface layer of the control variant, which is explained by a decrease in biological activity during this period. Moisture reserves in the period of summer measurements significantly decreased in all genetic horizons. Under such conditions, a redox environment is created, which prevents the formation of oxidized forms and stabilizes elements of variable valency, in particular iron.

Taking into account the fact that soil moisture actively affects its air, thermal, nutritional regimes, it is possible to draw a conclusion about the mandatory need to monitor its optimal regulation throughout the growing season.

Studies of the dynamics and profile distribution of oxidized and oxidized iron in the alluvial meadow-swamp soil showed that the content of oxidized iron is significantly higher than that of oxidized iron Fe^{2+} in the unfertilized control within the entire soil profile. When the air temperature increases and soil aeration improves in the summer, the amount of oxidized iron decreases and the amount of oxidized iron increases, which is well monitored by the Fe^{2+}/Fe^{3+} ratio.

The content of oxidized forms increases noticeably with depth. The increased content of oxidized iron at a depth of 50-65 cm is facilitated by the presence of a well-decomposed peat layer, as well as the elevation of the capillary border to this horizon and the deposition of mobile iron compounds on the border of the contrast of changes in redox conditions. This regularity can be traced both in the control and in the fertilized version of the field experiment.

The profile heterogeneity of the content of acid-soluble forms of iron, which is clearly visible in the studied soil, is caused, first of all, by the contrast of ORP, as well as the granulometric and mineralogical composition of the genetic horizons of the Fluvisol.

According to the granulometric composition, the studied Fluvisol is light loam (the content of physical clay (< 0.01 mm) in the 0-20 cm layer is 29%). The content of humus in the upper horizon is 5.5%. The soil profile has a distinct stratification, which creates conditions for the redistribution of mobile iron compounds and the formation of its accumulation sites. Up to a depth of 65 cm, the soil has a high ORP throughout the growing season (431-571 mV).

The research carried out in 2017-2020 on meadow-swamp light loam drained soil established a decrease in the level of groundwater, which was established respectively at a depth of 90-100 cm in spring, 120-165 cm in summer and 100-120 cm in autumn. Which is on average 20 cm more than in previous years of research. Such changes, in our opinion, are probably related to modern climatic fluctuations due to climate change.

Thus, the changes that have occurred in the temporal dimension (over the past 12 years) may indicate the influence of climatic factors (increased average annual air temperatures, fluctuations in the amount of precipitation, increased frequency of abnormal weather phenomena), which is reflected in the water regime of floodplain soils.

Compared to previous years, in 2017, the direction of redox processes was established in the direction of oxidation, which significantly affected the redistribution of mobile forms of iron with a noticeable increase in the content of its oxidized form (Table 3).

Table 3. The content of mobile oxidized and oxidized iron compounds in the Humi-Gleyic Fluvisol of the floodplain of the Vilkhovatka River (2017)

Depth, cm	Content of acid-soluble iron, mg/kg of soil								
	Spring			Summer			Autumn		
	FeO	Fe_2O_3	∑	FeO	Fe_2O_3	∑	FeO	Fe_2O_3	∑
0-20	291	2130	2421	123	2804	2927	118	2702	2820
20-50	387	1848	2235	198	2336	2534	482	5131	5613
50-65	1291	1923	3214	1150	5866	7016	1059	4952	6011

The contrasting mode of wetting of the studied alluvial soil affected the content of oxidized and oxidized forms of iron and indicators of redox potential. Observations of the dynamics of oxidation-reduction processes in the alluvial floodplain during 2009-2020 showed their dynamics in individual years and seasons. Higher values of ORP are typical for the dry year of 2010.

Contrasting changes in the regimes of "overwetting - drying" (oxidation-reduction processes) in temporal and spatial dimensions affect the state and behavior of the main biogenic elements (phosphorus, nitrogen, potassium). Changes in the phosphate regime due to the strengthening of the antagonistic interaction between phosphorus and iron are especially noticeable.

Floodplain soils are usually distinguished by a sufficiently high natural wealth of gross phosphorus with different contents of its mobile forms. This is due to the accumulation of phosphorus in alluvial sediments and residual phosphates in long-term fertilized floodplain soils. Despite the high biogenicity, organophosphates in floodplain virgin soils reach 30-40% of the gross phosphorus content, decreasing during their agricultural use. The main share of phosphates, similarly to zonal soils, consists of mineral compounds of phosphorus, which are salts of orthophosphoric acid and are represented by salts of calcium, sodium, iron, aluminum and other cations. A characteristic feature of phosphate soil compounds is their low solubility and weak dissociation into ions. Phosphate ions are well fixed by the solid phase of the soil and their migration in the soil is very limited.

The water regime is of great importance for the redistribution of phosphates in floodplain soils. It was established that the stronger the overwetting of the soil and the longer it lasts, the more mobile phosphates are contained in the soil. Simultaneously with the accumulation of mobile forms on fertilized soils, the formation of sparingly soluble compounds and gross phosphorus takes place.

Our laboratory model experiments showed that the introduction of iron hydroxide affected the mobility of phosphates (Table 4). When using organo-mineral fertilizers, the phosphorus content is 64 mg/kg of soil in option 1. At the same time, there is a clear trend towards its decrease in options with the introduction of iron - the content drops to 41 mg/kg of soil (option 4). Waterlogging also negatively affects the phosphate regime of soils, the content of mobile phosphorus decreased to 32 mg/kg of soil (option 8). This can be explained by the ability of oxidized iron to form iron phosphates under conditions of overwetting, remove phosphate ions from the soil solution and thereby worsen the phosphate regime of the soil.

Table 4. The effect of iron compounds on the mobility of phosphates and the biomass of barley seedlings (laboratory model experiment)

Research options	The content of mobile forms of iron mg/kg soil		P_2O_5, mg/kg of soil	Green mass, g/vessel
	Fe^{3+}	Fe^{2+}		
Optimal hydration				
1. $N_{60}P_{120}K_{60}$ + decomposed manure – background	5315	88	64	2.49
2. Fe_{400}	5573	138	55	2.08
3. Fe_{800}	5714	165	50	1.93
4. Fe_{1600}	6089	181	41	1.67
Overwetting				
5. background	5224	208	60	2.36
6. Fe_{400}	5469	285	53	1.96
7. Fe_{800}	5705	312	42	1.74
8. Fe_{1600}	5619	459	32	1.67

It should be noted that the additional application of iron, starting with 400 mg/kg of soil, contributed to a decrease in yield in all variants of the experiment, which increased as the dose of applied iron increased, but the greatest decrease in yield - 1.67 g/pot, was obtained at the dose of applied iron iron 1600 mg/kg of soil.

CONCLUSIONS

The redox regime significantly affects the ratio of elements with variable valence in the soil, primarily iron. With the predominance of reduction processes, the solubility of iron increases and conditions are created for its migration. When reductive processes change to oxidizing ones, the segregation of iron hydroxides and the formation of iron neoplasms occurs, which affects soil fertility. The seasonal change of ORP and the hydrothermal regime of the floodplain-swamp soil determines the dynamics of the ratio of the content of oxidized and oxidized forms of iron. With the improvement of hydrothermal conditions and, accordingly, the biogenicity of the soil, the content of mobile forms of iron increases with a decrease in the Fe^{2+}/Fe^{3+} ratio.

Free (non-silicate) acid-soluble forms of iron significantly influence the behavior of phosphorus in floodplain medium loam soil. Waterlogging of meadow soil leads to the transformation of oxidized iron into oxidized, more mobile and active forms. The latter bind phosphate ions and precipitate, thereby reducing their availability to plants.

With the simultaneous application of iron compounds with organic fertilizer (peat) and optimal moistening, the activity of phosphate ions in the soil solution increases significantly, which is associated with the formation of migratory complex organo-phosphate compounds.

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THE EFFECT OF HISTORICAL POLLUTION ON MICROBIAL FUNCTIONAL KINETICS IN BIOREMEDIATED SOILS FROM BAIA MARE

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Abstract

This industry is a key contributor to environmental disturbances, with residual impacts such as contaminated water sources and heavy metal presence affecting local ecosystems. Microorganisms exhibit a high susceptibility to contamination by heavy metals and play a crucial role in the recycling of materials and the energy dynamics within the ecosystem. The revised Biolog-Ecoplate approach involves employing soil contaminated with heavy metals from Baia Mare at five polluted locations - Craica, Romplumb, Colonia Topitorilor, Ferneziu, and Urbis. The kinetics has been noted, referring to the percentage growth rate from 24 hours to the next 24 hours. The overall microbial functional profile exhibits multiple fast changes within a time frame of 24 hours, leading to a specific site-microbial activity. The results offer insights into significant characteristics of soil microbial functional communities affected by the presence of heavy metal pollution in all analyzed locations.

Key words*: functional microbial communities, biolog ecoplate, heavy metal toxicity, microbial community structure.*

INTRODUCTION

The progress in mining, metallurgy, industrialization, and urbanization has caused the introduction of heavy metals like lead (Pb), mercury (Hg), chromium (Cr), and cadmium (Cd), as well as metalloids such as arsenic (As) into the environment (Mishra et al., 2023). Many industrial byproducts that contain harmful heavy metals and metalloids can dissolve in water and combine with soil and water. As a result, the ecological environment is severely affected (Ahmed et al., 2022).

With rapid urbanization and industrialization in developing country, relatively and intensive short-term human activities will bring many organic pollutants (such as polycyclic aromatic hydrocarbons and phenols) and inorganic pollutants (such as heavy metals, arsenic compounds) into the urban environment (Li et al., 2018). Some heavy metals and metalloids (like As, Pb, Cd, and Hg) are not necessary for biological functions and can be harmful (Gall et al., 2015). These substances have been classified as dangerous by the United States Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2007; Khalid et al., 2017; Rai, 2018a).

On the other hand, certain heavy metals like Cu, Fe, Zn, and even Cr (III) are essential for metabolic processes in living organisms (Marschner, 2012). The relationship between soil, food crops, and the environment illustrates the interaction between abiotic and biotic factors (Rai et al., 2019).

Heavy metals are recognized for their ability to decrease or inhibit the functioning of soil enzymes, disrupt the transformation of carbon, nitrogen, and organic matter, and diminish both the biodiversity and biomass of microorganisms in soil (Giller et al., 2009). Consequently, this can result in the presence of particular microorganisms that are tolerant to heavy metals in soil environments (Giller et al., 2009).

Over the past few years, global concern has risen regarding soil contamination by heavy metals (HMs) because of their elevated toxicity, resistance to biodegradation, and prolonged accumulation (Fajardo et al., 2019). These heavy metals not only impact soil fertility but also interfere with the bacterial community, resulting in a reduction in biodiversity (Pan et al., 2020). Heavy metal pollution-induced stress can alter the attributes of bacterial communities

(Lin et al., 2016). Sensitive soil bacteria may experience a decline in both diversity and population, whereas resilient bacteria can easily adapt and proliferate, leading to the establishment of a distinct bacterial community structure. The presence of heavy metals continually influences both bacterial biomass and activity (Liu et al., 2020).

In contrast to plants and animals, soil microorganisms are more sensitive to fluctuations in heavy metal levels due to their ability to react and adapt quickly to such stressors (Giller et al., 1998).

Heavy metals, soil, and bacteria have complex interactions. The presence of heavy metals significantly influences the microbial community structure, especially in soils with moderate to severe contamination (Li et al., 2017a). However, acidic wastewater from mining adds extra stress for microorganisms, as they have to cope with both heavy metals and acidity, further disrupting soil nutrient cycling (Pereira et al., 2014).

Hence, it is essential to comprehend how soil microbial diversity and composition changes under different degrees of heavy metal contamination (Azarbad et al., 2015).

Removing heavy metals from the environment is a significant challenge, as their decomposition, much like other pollutants, cannot be achieved through biological or chemical means (Sharma et al., 2023).

The purpose of this article was to investigate the kinetics and dynamics of microorganisms in soils polluted with heavy metals in Baia Mare, focusing on five pilot sites, utilizing the EcoPlate method to provide comprehensive insights into microbial activity and community structure.

MATERIALS AND METHODS

Soil samples were collected in 2023 from an ongoing experiment initiated in 2019 across five sites located inside the town of Baia Mare (47°39′ N 23°34′ E) in North-West Romania, covering a total area of 7.3 hectares of brownfields.

The locations exhibit varying degrees of soil heavy metal contamination due to human-caused pollution, primarily stemming from mining, metallurgical activities, and urban development.

For EcoPlate examinations, soil samples and protocols adhered to the approach outlined by (Weber et al., 2009), arranging all substrates according to their chemical resemblance. The solution introduced into EcoPlates underwent dilution up to 10^{-4}, and measurements were taken at 590 nm using a plate reader. The entire procedure spanned five days, reaching the plateau phase wherein no further increments were noted in the readings.

The EcoPlates functional guilds and groups are analyzed according to Stoian et al., 2022:

- CH - Carbohydrates; P - Polymers; CX - Carboxylic & acetic acids; AA - Amino acids; AM -Amines/amides;
- Water - W; Pyruvic acid methyl ester - CH1; Tween 40 - P1; Tween 80 - P2;α-Cyclodextrin - P3; Glycogen - P4; d-Cellobiose - CH2; α-d-Lactose - CH3; β-Methyl-d-glucoside - CH4; d-Xylose - CH5; i-Erythritol - CH6; d-Mannitol - CH7; N-Acetyl-d-glucosamine - CH8; d-Glucosaminic acid - CX1; Glucose-1-phosphate - CH9; d,l-α-Glycerol phosphate - CH10; d-Galactonic acid γ-lactone - CX2; d-Galacturonic acid - CX3; 2-Hydroxy benzoic acid - CX4; 4-Hydroxy benzoic acid - CX5; γ-Hydroxy butyric acid - CX6; Itaconic acid - CX7; α-Keto butyric acid - CX8; d-Malic acid - CX9; l-Arginine - AA1; l-Asparagine - AA2; l-Phenylalanine - AA3; l-Serine - AA4; l-Threonine - AA5; Glycyl-l-glutamic acid - AA6; Phenylethylamine - AM1; Putrescine - AM2.

The data analysis primarily adhered to traditional methods (Garland, 1997), calculating the Least Significant Difference (LSD), a statistical test used to determine if there are significant differences between the means± standard errors between in terms of various microbial parameters or characteristics affected by heavy metal pollution. Data analysis was performed in RStudio, version 2022.02.3 (RStudio Team. RStudio, 2019), with packages "psych" (Revelle, 2019; Corcoz et al., 2022a) and "agricolae" (de Mendiburu, 2020; Corcoz et al., 2022b).

RESULTS AND DISCUSSIONS

Among the water group (WAT), it is observed that the highest level recorded is 107.51 in 3_CR. On the other hand, the lowest level is recorded in 3_FR, being 94.84, indicating a considerable difference between the two extremes. As for reading 4, there is a maximum level of 103.98 and a minimum of 87.91. These findings highlight significant differences between 4_ROMP and 4_CT. In relation to reading 5, the levels range between 101.99 and 99.14, with no significant difference between them. In the amine group, phenyltinamine or AM1, it is highlighted that 3_CT records the highest value, i.e. 144.76. In contrast, the lowest value, i.e. 102.87, is observed for 3_CR, thus illustrating that there is no significant difference between 3_CT and 3_CR. Regarding reading 4, the values for 4_FR and 4_URB are significantly different. In the context of reading 5, the maximum value is 116.36 for 5_ROMP, while the minimum is recorded at 5_CT, where it is observed that there is no significant difference between them. Among the putrescine or AM2 group, Within the putrescine or AM2 group, we observe that the highest value is recorded at 3_CT. On the other hand, the lowest value is identified at 3_URB, showing significant differences from the maximum value, but not from the other groups. Therefore, significant differences are found between 3_CT and 3_URB after 48 hours of incubation. For reading 4, the highest value is represented by 4_FR, while the lowest value is identified at 4_URB. In this context, it can be concluded that there are significant differences between these values after 72 hours of incubation. Concerning reading 5, the highest value is observed at 5_URB, while the lowest is recorded at 5_CT. Comparing these two values, it can be seen that there are no relevant differences.

Within the pyruvic acid methyl ester family, CH1, 3_FR highlights with the highest value, marking a significant difference compared to the other groups. On the other hand, 3_URB has the lowest value, being clearly different from the groups with high values, but not from those with medium or low values. Therefore, 3_URB and 3_FR are significantly different, suggesting possible environmental variations or pollution influences. For 4_CR and 4_CT, there is a significant difference between them, with the values for 4_CT being considerably lower than those for 4_CR. In contrast, 4_FR, 4_ROMP and 4_URB do not show significant variations between them or from the other groups (4_CR and 4_CT). 5_ROMP stands out with the highest mean value, marking a significant difference from all other groups. On the other hand, 5_CT has the lowest mean value, differing from the high value groups, but similar to the medium value groups. In comparison, 5_ROMP and 5_CT are significantly different, with the values for 5_CT being much lower than those for 5_ROMP. Finally, 5_CR, 5_FR and 5_URB do not differ significantly from each other or from the other groups.

Table 1. Dynamics of basal and amines/amides functional groups in long-term contaminated sites

	WAT	AM1	AM2
3_CR	107.51±0.76a	102.87±3.36c	149.53±36.87ab
3_CT	103.01±3.25ab	144.76±18.14bc	179.93±14.29a
3_FR	94.84±2.71c	140.86±11.48bc	134.49±12.76abc
3_ROMP	104.28±1.5ab	108.88±9.69c	150.88±46.24ab
3_URB	99.56±0.9bc	104.96±0.34c	103.06±1.52bc
4_CR	100±0.41bc	102.53±1.92c	113.04±12.82bc
4_CT	87.91±1.57d	146.23±13.8bc	132.42±2.9abc
4_FR	100.91±3.54b	219.91±12.35a	183.36±23a
4_ROMP	103.98±1.79ab	164.34±61.98b	121.44±13.04bc
4_URB	101.21±0.18b	102.39±0.75c	108.55±3.37bc
5_CR	100.1±0.33bc	111.08±5.57c	107.85±4bc
5_CT	101.74±1.98b	104.12±7.29c	93.62±3.11c
5_FR	99.22±1.14bc	110.46±2.72c	95.88±4.48c
5_ROMP	99.14±2.97bc	116.36±6.13bc	105.11±3.1bc
5_URB	101.99±2.16ab	105.66±1.07c	111.53±2.32bc

Note: Means±s.e. followed by different letters present significant differences at $p<0.05$ based on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

Regarding the compound CH2 or D - cellobiose, it is noted that the maximum value is recorded at 3_CT, showing a significant difference compared to the other groups. In contrast, the lowest value is recorded at 3_URB, indicating significant differences from the maximum value, but not from the other groups. Thus, significant differences between 3_URB and 3_CT after 48 hours of incubation are evident. Regarding reading 4, the maximum value is represented by 4_CR, with significant differences from the other groups, while the lowest value is identified in 4_CT. Therefore, it can be concluded that there are significant differences between these values after 72 hours of incubation. Concerning reading 5, it is

observed that the maximum value is recorded at 5_ROMP, while the lowest is recorded at 5_CT. Comparing these two values, significant differences are found, although no significant differences are identified between 5_ROMP and the other values.
In relation to the substrate α - d - lactose (CH3), the highest value recorded is 107.65, while the lowest value, 95.86, is identified for 3_FR. The difference between the two extremes does not seem to be significant. For reading 4, a maximum value of 176.08 and a minimum of 103.18 is noted. Analysing these data, we can observe significant differences between 4_CT and 4_CR, as well as between 4_CT and the other values. As for reading 5, the maximum value is recorded at 142.15 and the minimum at 97.47, and the difference between them is considerable.
For β-methyl-d-glucoside (CH4), the highest value recorded is found to be 166.34. In contrast, for 3_ROMP, we observe the lowest value, which is 100.53. This discrepancy is significant between the two extremes. Regarding the data from reading 4, we observe a maximum value of 154 and a minimum value of 104.43. Therefore, significant differences can be identified between 4_FR and 4_CT. Regarding reading 5, there is a maximum value of 142.15 and a minimum of 97.47 and the difference between them is significant.
In substrate d, the maximum value recorded for Xylose or CH5 is 127.15, associated with 3_CT. In contrast, the lowest value, 101.4, is identified in 3_URB, with no significant difference between the two extremes. Concerning reading 4, a maximum value of 133.97 and a minimum of 107.25 is observed. In the light of these findings, no significant differences are shown between 4_CR and 4_URB, or between 4_CR and the other values. For reading 5, the maximum value is 133.04 and the minimum is 97.21, marking a significant difference between them.

Table 2. Dynamics of carbohydrates functional guilds in long-term contaminated sites

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
3_CR	140.77±17.35abc	118.71±3.05de	103.67±1.07cd	136.41±30.23abcd	118.19±7.19abcd	111.28±3.38abcd	149.54±20.71bc	154.55±21.63bc	139.36±27.14a	110.96±2.79bc
3_CT	161.15±16.01a	212.49±33.17a	107.65±2.75cd	166.34±16.32a	127.15±6.46abc	111.18±4.41abcd	170.76±13.45b	186.82±16.83ab	126.92±8.3ab	112.2±3.46b
3_FR	161.46±14.53a	108.75±1.68e	95.86±1.74d	106.06±9.8cd	109.19±3.33abcd	101.79±0.58d	137.89±9.38bc	143.4±15.65bc	97.32±3.73c	134.92±5.43a
3_ROMP	124.37±21.27abc	106.02±1.6e	104.91±3.04cd	100.53±2.52d	102.88±1.14cd	107.46±1.76bcd	111.1±5.08c	107.6±1.13c	105.11±3.49bc	104.29±2.47bc
3_URB	103.7±0.96bc	102.28±1.33e	100.81±1.08cd	101.44±0.36cd	101.4±0.13d	104.91±0.54bcd	120.98±13.96c	105.52±1.87c	106.92±1.99bc	101.53±1bc
4_CR	155.15±4.48ab	201.5±55.43ab	103.18±0.24cd	112.32±7.44cd	133.97±26.12a	107.33±4.59bcd	225.09±16.24a	152.81±9.25bc	110.97±8.57bc	104.19±1.02bc
4_CT	96.48±2.13c	122.78±4.27de	176.08±19.8a	104.43±1.75cd	117.18±3.27abcd	146.33±8.94ab	113.32±2.91c	118.01±2.61c	126.82±9.69ab	107.59±4.87bc
4_FR	121.54±1.68abc	149.78±15.5bcde	114.78±15.57cd	154±32.22ab	128.86±7.05ab	139.54±1.29abcd	224.35±9.67a	222.37±50.81a	142.12±15.64a	107.65±10.72bc
4_ROMP	132.57±27abc	138.81±29.42cde	105.43±0.91cd	104.73±3.01cd	110.3±9.51abcd	144.21±40.58abc	150.37±31.03bc	108.27±1.67c	104.68±2.82bc	102.68±1.19bc
4_URB	115.95±10.84abc	134.75±9.8cde	105.7±2.54cd	115.23±10.53bcd	107.25±3.6bcd	104.31±1.62cd	130.03±25.39bc	113.4±7.26c	102.23±0.18bc	103.17±0.93bc
5_CR	132.3±23.68abc	177.82±14.46abcd	100.89±0.42cd	109.86±10.32cd	133.04±10.71a	125.76±22.17abcd	154.21±26.23bc	130.56±4.76c	101.12±0.75bc	103±1.41bc
5_CT	103.24±4.82bc	106.6±3.1e	149.55±14.35ab	97.47±0.93d	97.21±0.57d	127.56±3.99abcd	111.44±1.87c	104.74±2.45c	102.86±2.37bc	98.68±2.05c
5_FR	109.05±1.06abc	172.21±14.48abcd	181.38±17.32a	142.15±20.63abc	130.09±4.55ab	124.53±17.69abcd	133.34±2.8bc	121.58±13.17c	150.81±2a	107.04±8.56bc
5_ROMP	159.72±52.62a	185.87±26.49abc	133.34±30.89bc	99.55±3.1d	112.64±7.73abcd	152.68±23.29a	141.05±10.82bc	148.88±28.28bc	102.71±1.44bc	104.01±0.98bc
5_URB	119.93±2.76abc	140.64±10.39cde	127.05±6.46bcd	109.46±6.58cd	127.61±3.65abc	111.67±3.95abcd	121.2±7.5c	138.54±6.03bc	101.78±1.69bc	110.73±4.75bc

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

In substrate i - Erythriol (CH6), the highest value recorded is found to be 111.28 in 3_CR. In contrast, the lowest value is found in 3_FR, being 101.79, with an apparently insignificant difference between the two extremes. For reading 4, a maximum value of 146.33 and a minimum value of 104.31 are shown. Analysing these data, significant differences can be observed between 4_CT and 4_URB. For reading 5, the values recorded are 152.68 and 111.67 respectively, with a significant difference between them. In group d - Mannitol (CH7), the highest value is observed in 3_CT, showing a considerable difference compared to the other groups. In contrast, the lowest value occurs in 3_ROMP, indicating significant differences from the maximum value, but not from the other groups. Hence, significant differences are found between 3_URB and 3_CT after 48 hours of incubation. As for reading 4, the maximum value is recorded at 4_CR, while the minimum value is at 4_CT, with significant differences from the other non-overlapping groups. These differences are evident at 72 hours of incubation. For reading 5, the maximum value is recorded at 5_CR and the lowest at 5_CT. Analysis of these values shows no significant differences after 96 hours of incubation.

In the context of dataset N, it is observed that group 3_CT stands out with the highest value, indicating a significant discrepancy compared to the other groups. On the other hand, the 3_URB group records the lowest value, showing a significant difference between 3_CT and 3_URB, which may suggest variations in the environmental setting or the impact of pollution. Regarding reading 4, the values for 4_FR and 4_ROMP are significantly different, with the values for 4_ROMP being considerably lower than those for 4_FR. Regarding reading 5, although the maximum value is 148.88 and the minimum is observed at 5_CT, these differences are not significant.

In the glucose-1-phosphate group (CH9), it is observed that 3_CR records the highest value of 139.36, while the lowest value of 97.32 is recorded in 3_FR, showing a significant difference between these two. As for reading 4, the values for 4_FR and 4_URB are notably different. In the context of reading 5, the maximum value is 150.81 for 5_FR, while the minimum value is recorded in 5_CR, showing a significant difference between them. Significant differences are also shown between 5_FR and the other groups.

Analysing the presence of d,1-α-glycerol phosphate in comparison to the other substrates, it is observed that it shows low levels of uptake. Specifically, glycerol phosphate suggests that the microorganisms in the examined soil are less efficient in their metabolic process. The maximum value is recorded in 3_FR, while the minimum value appears in 3_URB, showing significant differences between them. For reading 4, no significant differences are found between 4_FR, which has the highest uptake, and 4_ROMP, which records the lowest value. This indicates that the substrate present, glycerol phosphate, shows low uptake compared to other carbohydrate types, suggesting that the community of microorganisms capable of metabolising it is relatively smaller than for other elements in the previous groups.

For the substrate d-glucosaminic acid (CX1), 3_CT is observed to have the highest value, while 3_URB has the lowest, indicating a significant difference between them, possibly reflecting variations in the environment or the influence of pollution. Regarding reading 4, the values for 4_CT and 4_ROMP are considerably different, with those for 4_ROMP being significantly lower than those for 4_FR. Regarding reading 5, although the maximum value is 169.34, the differences between the minimum values and those for 5_CT are not significant.

Regarding the γ-lactone group galactonic acid (CX2), it is observed that the highest value recorded is 167.58 at 3_C. In contrast, the lowest value is identified in 3_URB, with a value of 100.32, with a significant difference between the two extremes. As for reading 4, a maximum value of 180.87 and a minimum value of 103.65 are found. From these observations, significant differences can be identified between 4_CT and 4_URB. For reading 5, there is a maximum value of 109.84 and a minimum value of 95.57, with no significant difference between them.

In the case of the d-galacturonic acid or CX3 group, it is observed that the highest value is found at 3_FR, while the lowest value is identified at 3_URB, suggesting significant variations between these two points, but not compared to the other groups. Therefore, significant differences are found between 3_FR and 3_URB after 48 hours of incubation. For reading 4, the maximum value is registered at 4_CR, while the minimum value is at 4_CT. This discrepancy indicates significant differences between these two values after 72 hours of incubation. For reading 5, the highest value is observed at 5_CR, while the lowest is recorded at 5_CT. Comparing these two values, significant differences are observed, although no significant differences are identified between 5_CT and the other values.

In the case of the substrate d-glucosaminic acid (CX1), it is noted that 3_CT shows the highest value. In contrast, 3_URB has the lowest value,

showing a significant difference between 3_CT and 3_URB, which may reflect differences in the environment or the impact of pollution. For reading 4, the values for 4_CT and 4_ROMP are significantly different, with the values for 4_ROMP considerably lower than those for 4_FR. For reading 5, the maximum value is 169.34 and the minimum is observed for 5_CT, but these differences are not significant.
Regarding the -galactonic acid y-lactone group (CX2), the highest value observed is 167.58 in 3_C, while the lowest value of 100.32 occurs in 3_URB, indicating a significant difference between these two extreme values. For reading 4, the maximum value is 180.87 and the minimum is 103.65, showing significant differences between 4_CT and 4_URB. In reading 5, the maximum value recorded is 109.84 and the minimum is 95.57, but the difference between them is not considered significant.

Table 3. Dynamics of carboxylic acids functional groups in long-term contaminated sites

	CX1	CX2	CX3	CX4	CX5	CX6	CX7	CX8	CX9
3_CR	115.27±6.48c	112.14±6.81c	122.99±14.21cd	112.21±1.64b	174.65±40.5ab	184.4±48.12abcd	112.21±3.67c	100.48±9.11cde	110.8±7.88c
3_CT	135.51±1.86bc	167.58±16.25ab	171.95±11.8abc	121.12±8.61b	186.47±19.81a	205.68±28.86abc	165.01±26.8bc	96.21±3.16de	169.42±24.12abc
3_FR	112.99±1.12c	147.04±17.08b	200.7±3.64a	106.94±0.93b	142.48±15.86ab	252.2±40.35a	122.1±2.42c	95.27±6.04e	112.95±7.43c
3_ROMP	104.96±1.97c	105.47±2.33c	109.6±4.07d	109.05±1.26b	124.67±15.5ab	140.52±31.34cde	108.04±1.49c	116.42±4abc	107.51±2.29c
3_URB	104.3±2.31c	100.32±1.09c	103.06±0.44d	107.54±1.11b	105.54±1.11b	102.46±0.3e	102.95±0.39c	100.27±1.69cde	102.89±1.33c
4_CR	218.78±60.23a	112.86±11.33c	176.25±25.41ab	103.14±0.32b	185.1±34.9a	222.4±11.51ab	195.28±87.34b	115.05±7.9abcd	200.36±53.12ab
4_CT	141.1±7.43bc	108.09±0.75c	106.49±6.81d	159.96±18.41a	121.99±4.53ab	123.46±3.5de	162.98±8.75bc	123.37±5.63ab	119.95±5.26bc
4_FR	195.61±12.05ab	180.87±18.96a	175.85±11.04ab	123.87±23.21b	186.39±22.9a	183.49±24.03abcd	338.41±13a	133.13±12.97a	151.1±45.8abc
4_ROMP	106.09±1.08c	103.26±0.92c	148.21±38.9bcd	101.28±0.59b	166.3±60.38ab	158.57±48.63bcde	104.08±1.89c	93.46±1.56e	106.17±4.59c
4_URB	117.53±8.49c	103.65±1.22c	107.92±2.34d	106.3±1.36b	106.06±3.21b	107.35±2.72e	103.97±0.5c	100.81±0.21cde	104±2.83c
5_CR	169.34±47.75abc	107.59±2.26c	172.67±37.5abc	112.46±4.45b	138.86±35.43ab	131.96±21.16de	122.69±4.34c	118.3±5.93abc	135.66±27.22bc
5_CT	112.03±1.39c	95.57±1.7c	102.08±6.51d	109.28±3.84b	103.93±7.46b	106.32±2.92e	105.69±1.38c	109.52±5.69bcde	97.02±2.09c
5_FR	147.5±8.4bc	109.84±2.03c	107.98±3.93d	115.43±13.78b	113.55±6.4ab	113.86±5.84de	122.58±0.86c	101.71±4.16cde	220.34±58.59a
5_ROMP	116.2±13.58c	104.19±2.41c	124.12±21.83cd	101.79±2.51b	123.41±16.03ab	105.46±1.01e	107.2±1.18c	96.87±8.41de	108.41±3.78c
5_URB	154.99±45.63abc	101.97±3.45c	118.94±10.27d	106.72±0.82b	117.89±6.91ab	140.08±10.5cde	101.09±2.69c	104.6±8.39bcde	154.84±47.14abc

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

Within the d-galacturonic acid or CX3 group, the highest value was recorded at 3_FR, while the lowest value was observed at 3_URB. This shows significant differences between 3_FR and 3_URB after 48 hours of incubation, but not compared to the other groups. In reading 4, the maximum value was recorded at 4_CR and the minimum at 4_CT, indicating significant differences after 72 hours of incubation. For reading 5, the highest value was observed at 5_CR and the lowest at 5_CT, indicating significant differences between these two values, although no significant differences were found between 5_CT and the other values.
In the 2-hydroxy benzoic acid substrate (CX4), it is notable that there is no difference between the maximum value, 3_CT, and the minimum value, 3_URB, and regardless of the value, there are no significant differences in the other groups. Regarding reading 4, the highest value is indentified at 4_CT, with 159.96, showing significant differences from the other groups, while the lowest value is at 4_ROMP. Hence, it can be concluded that there are relevant differences between these values after 72 hours of incubation. Regarding reading 5, the highest value is observed at 5_FR and the lowest at 5_CT. Comparing these two values, no significant differences are observed after 96 hours of incubation.
For the substrate 4-hydroxy benzoic acid (CX5), the highest value recorded is 186.47 in 3_CT, while the lowest value of 107.54 is identified in 3_URB, showing a remarkable difference between these two extreme values. In reading 4, a maximum value of 186.39 and a minimum value of 106.06 are observed, marking notable differences between 4_FR and 4_URB. In

reading 5, the maximum value is 138.86 and the minimum is 103.93, but the difference between them is not considered significant.
In the y-hydroxy butyric acid or CX6 group, the highest value recorded is 252.2 and belongs to 3_FR. In contrast, the lowest value of 102.46 is observed in 3_URB, showing a significant difference between the two extremes. For reading 4, the values range from a maximum of 222.4 to a minimum of 107.35, indicating notable differences between 4_CR and 4_URB. For reading 5, the values range from a maximum of 131.96 to a minimum of 105.46, with no significant difference between them.
Within the itaconic acid group (CX7), 3_CT has the highest value of 165.01. In contrast, 3_URB has the lowest value of 102.95, indicating that there is no relevant variation between 3_CT and 3_URB. As for reading 4, the values for 4_CR and 4_URB are considerably different. For reading 5, the maximum value of 122.69 is observed at 5_CR and the minimum value at 5_URB, with no significant difference between them. Also, no significant differences are observed between the other groups.
Comparing the keto-butyric acid substrate or CX8 with other groups shows that it has lower absorption levels. This indicates that the microorganisms in the soil analysed are less efficient in metabolising this type of substance. At reading 3, the highest value is at 3_ROMP and the lowest is 95.27, with no significant differences. For reading 4, the highest value is 133.13 and the lowest is 93.46, showing significant differences between 4_FR and 4_ROMP. For reading 5, the maximum value is 118.3 and the minimum 96.87, with a significant difference between them.
Within the d-malic acid group (CX9), 3_CT has the highest mean value, while 3_URB has the lowest mean value. However, 3_URB is not significantly different from the groups with high, medium or low values, indicating that there are no notable differences in environmental or pollution impact between 3_CT and 3_URB. In contrast, 4_CR and 4_URB show significant differences, with the values for 4_URB being considerably lower than those for 4_CR. The groups 4_FR, 4_ROMP and 4_URB show no significant differences between them or from 4_CR and 4_CT. In group 5, 5_FR has the highest mean value, indicating a significant difference from all other groups. Group 5_CT has the lowest mean value and, although it differs from the groups with high values, it is similar to those with mean values. In comparison, 5_FR and 5_CT are significantly different, with the values for 5_CT being much lower than those for 5_FR. The groups 5_CR, 5_ROMP, 5_CT and 5_URB do not differ significantly from each other and from the other groups.
In the Tween 40 group (P1), 3_CR shows the highest value of 138.37. In contrast, the lowest value of 108.18 is recorded for 3_URB, indicating that the differences between 3_CR and 3_URB are not significant. On reading 4, significant differences are observed between 4_FR and 4_URB, suggesting possible environmental changes or the impact of pollution on soil microbiota. For reading 5, the maximum value is 146.97 for 5_FR and the minimum value is 110.35 for 5_CT, with no significant differences between them, suggesting greater homogeneity of environmental conditions or impact on soil microbiota.
In the Tween 80 or P2 group, values for 3_URB are observed to be much lower than those for 3_CT, reflecting variations in environmental conditions or the effects of pollution on the soil microbiota at those sites. In term of reading 4, there is a maximum value of 149.37 and a minimum of 113.66. In the light of these findings, no significant differences between 4_FR and 4_URB can be shown. For reading 5, there is a maximum value of 185.44 and a minimum of 125.33 and the discrepancy between them is significant.
As for the α-cyclodextrin or P3 group, 3_CT is observed to have the highest value, while 3_ROMP has the lowest value, suggesting that there are no significant differences between these two variables. Regarding reading 4, the values for 4_CT and 4_CR are significantly different. In the context of reading 5, 5_FR records the highest value, while 5_CR records the lowest value, with significant differences
Among the glycogen group (P4), 3_CT is shown to have the highest value, while 3_URB has the lowest value. This significant discrepancy between 3_CT and 3_URB may reflect variations in the environment or the effects of pollution. As for the readings for variant 4, the

values for 4_CR and 4_FR are considerably different, with 4_CR having considerably lower values than 4_FR. In reading 5, the highest value recorded is 152.1 and the lowest is observed at 5_CR, confirming the significant differences between these values.

It is observed that in the 1-Arginine or AA1 group, the highest value is recorded at 3_CT, while the lowest value is identified at 3_URB, showing significant differences between the two, but not with respect to the other groups. Significant differences between 3_URB and 3_CT after 48 hours of incubation are thus observed. As for reading 4, the highest value is recorded at 4_CR, with significant differences from the other groups whose letters do not coincide, while the lowest value is observed at 4_URB. In this situation, it can be concluded that there are significant differences between these values after 72 hours of incubation. Concerning reading 5, the highest value is recorded at 5_CR, while the lowest is observed at 5_CT. Comparing the two values, it is found that there are no significant differences between them.

Within the 1-Asparagine group (AA2), the highest value recorded is 364.34, while in 3_URB the lowest value is found, namely 103.45, indicating a significant difference between the two extremes. As for reading 4, there is a maximum value of 192.03 and a minimum of 102.95. Following these observations, no significant differences can be shown between 4_CR and 4_URB, as well as between 4_CR and the other values. In relation to reading 5, the maximum value recorded is 142.82, while the minimum is 88.46, but the difference between them is not significant.

Table 4. Dynamics of Polymers and Amino acids functional groups in long-term contaminated sites

	P1	P2	P3	P4	AA1	AA2	AA3	AA4	AA5	AA6
3_CR	138.37±17.5 3ab	132.67±18.98 abc	109.83±2.6b cd	115.38±3.89d e	134.14±13.95 cd	221.9±64.88b	108.02±3.84cde	131.08±22.9 3bc	103.62±2.85 d	108.26±3.02 cd
3_CT	128.21±2.04 ab	136.84±11.03 abc	125.29±6.18 bc	135.6±4.12bc d	194.14±25.06 abc	224.91±31.06 b	139.06±6.9abcd	173.8±4.71a b	113.31±8.61 bcd	107.71±5.33 cd
3_FR	112.23±3.38 b	121.74±5.74c	105.05±9.31 cd	123.17±5.51d e	146±7.69cd	364.34±69.34 a	106.39±2.35de	123.87±9.04 bc	101.41±2.24 d	102.78±0.66 d
3_RO MP	118.53±4.46 ab	119.82±23.33 c	105.82±4.09 cd	110.41±5.99d e	125.81±18.49 cd	158.21±50.54 bcd	89±18.11e	113.93±5.49 bc	107.41±1.96 d	107.66±2.07 cd
3_URB	108.18±1.14 b	102.91±2.77c	106.2±1.55c d	103.68±1.55e	101.41±1.41d	103.45±1.17c d	105.09±2.62de	107.42±2.55 bc	104.89±2.67 d	104.46±1.8d
4_CR	146.98±25.5 2ab	127.88±6.52b c	103.06±1.65 d	114.48±11.23 de	269.29±77.85 a	192.03±15.82 bc	128.86±12.17ab cde	146.56±22.4 9bc	101.25±0.31 d	105.33±1.72 cd
4_CT	140.34±8.4a b	129.53±7.46b c	162±7.28a	128.59±3.09b cde	129.4±4.36cd	121.4±2.21cd	166.08±16.78a	156.96±11.6 2bc	124.98±2.92 bc	117.42±3.11 cd
4_FR	170.45±22.0 9a	149.37±12.11 abc	156.86±16.5 3a	167.89±11.57 a	237.91±23.15 ab	145.04±14.61 bcd	151.4±17.46abc	238.79±62.6 8a	153.97±2.92 a	144.09±3.55 a
4_RO MP	115.98±23.3 6b	136.57±14.03 abc	111.26±6.18 bcd	115.02±2.09d e	164.34±51.36 bcd	147.89±44.91 bcd	103.64±3.46de	123.72±10.7 2bc	104.01±1.54 d	105.4±4.38c d
4_URB	100.87±11.0 6b	113.66±4.81c	108.03±2.68 cd	124.68±6.42d e	106.3±3.75d	102.95±1.82c d	107.86±4.21cde	107.43±2.06 bc	103.56±1.72 d	102.13±0.75 d
5_CR	144.96±35.3 5ab	181.35±55.92 ab	106.14±3.5c d	111.45±7.13d e	128.87±21.27 cd	142.82±38.37 bcd	158.3±11.89ab	130.09±17.0 8bc	127.86±9.42 b	106.25±1.37 cd
5_CT	120.98±3.64 ab	125.33±3.29c	106.34±2.76 cd	119.99±1.94d e	86.53±20.15d	88.46±14.37d	115.56±2.51bcd e	101.82±6.22 c	127.57±13.5 3b	136.77±19.0 1ab
5_FR	146.97±10.2 8ab	142.94±11.44 abc	168.97±11.4 a	151.2±3.36ab c	123.57±5.93c d	97.27±3.33cd	160.87±18.6a	144.23±29.1 5bc	112.28±1.94 cd	121.28±3.59 bc
5_RO MP	130.82±36.2 4ab	185.44±19.66 a	112.12±8.86 bcd	125.12±22.75 cde	116.03±16.06 cd	120.76±14.62 cd	140.77±36.78ab cd	165.49±37.9 bc	106.88±2.23 d	101.63±2.85 d
5_URB	142.87±12.3 ab	144.05±13.86 abc	129.3±5.29b	152.1±16.05a b	109.39±3.93d	115.02±1.25c d	131.04±21.03ab cde	108.62±3.63 bc	107.72±3.23 d	114.15±5.23 cd

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

In the 1-phenylalanine (AA3) group, it is observed that the highest value is recorded at 3_CT, while the lowest value is identified at 3_ROMP. These differences are significant compared to the maximum value, but not compared to the other groups. Thus, significant differences are found between 3_CT and 3_ROMP after 48 hours of incubation. For reading 4, the highest value is represented by 4_CT, while the lowest value is identified at 4_ROMP. In this case, it can be concluded that there are significant differences between these values after 72 hours of incubation. As for reading 5, the highest value is observed at 5_FR, while the lowest is recorded at 5_CT. Although there are significant differences between these two values, no significant differences are identified between 5_FR and the other values.

For the 1-Serine group (AA4), it is noted that 3_CT records the highest value, i.e. 173.8. In contrast, the lowest value of 107.42 is observed for 3_URB, indicating that there is no significant difference between 3_CT and 3_URB. Concerning reading 4, the values for 4_FR and 4_URB are significantly different. In the context of reading 5, the maximum value is 165.49 for 5_ROMP, while the minimum is recorded at 5_CT, where it is found that there is no significant difference between them.

Group 1-Threonine or AA5 shows no discrepancy between the highest value, 3_CT, and the lowest, 3_FR, while no significant differences are observed between groups at other value levels. Regarding reading 4, the highest value is recorded at 4_FR, totalling 153.97, indicating significant differences from the other groups, while the lowest value is evident at 4_CR. Therefore, it can be concluded that there are significant differences between these values at 72 hours of incubation. Regarding reading 5, the maximum value is observed at 5_CR, while the minimum is recorded at 5_ROMP. Analysing these two values, significant differences can be observed after 96 hours of incubation.

In relation to the glycyl-l-glutamic acid group or AA6, the highest value recorded is 108.26, while the lowest value of 102.78 is identified in 3_FR, but the difference between them does not seem to be significant. Regarding reading 4, a maximum value of 144.09 and a minimum of 102.13 is observed. Analysing these data, significant differences can be identified between 4_FR and 4_URB, as well as between 4_FR and the other values. For reading 5, the maximum value recorded is 136.77, while the minimum is 101.63, and the difference between them is significant.

Within the methyl ester group of pyruvic acid, or CH1, it is evident that CR has the highest value at 275.79. Conversely, the lowest value, 144.17, is seen in URB, highlighting a significant difference between CR and URB.

Regarding the substrate CH2, or D-cellobiose, it is notable that there is no difference between the highest value, CT, and the lowest value, URB. Additionally, regardless of the value, no significant differences are observed in other groups.

In the context of the α-d-lactose substrate (CH3), the highest recorded value is 277.83. In contrast, the lowest value, 135.36, is identified in URB, highlighting a significant difference between these two extremes.

In the context of β-methyl-D-glucoside (CH4), the greatest value is recorded for FR. On the other hand, the lowest value is recorded for ROMP, showing significant differences from the maximum value, but not from the other groups. Therefore, significant variations are observed between FR and ROMP.

In the context of the substrate D-xylose, or CH5, considering the second reading, with CR having the highest value and ROMP the lowest, we can observe significant differences between these values.

Within the context of the substrate i-erythritol (CH6), it is notable that ROMP shows the highest value. In contrast, URB has the lowest value, highlighting a significant difference between ROMP and URB. This may reflect environmental differences or the impact of pollution.

In the d-mannitol group (CH7), the higher value recorded is 494.31 in CR. In contrast, the lower value is 206.43 in URB, indicating a significant difference between these two extremes.

Within the context of the N-acetyl-D-glucosamine group (CH8), it is observed that the greatest value is recorded in FR. Conversely, the lowest value is identified in URB, indicating significant differences compared to the maximum value. Therefore, significant differences are noted between FR and URB after 24 hours of incubation.

Among the glucose-1-phosphate (CH9) group, FR has the maximum value, indicating a significant difference from the other groups. URB has the minimum value, being substantially different from the groups with high values, but not from those with medium or low values. Thus, URB and FR are significantly different.

For the substrate d,1-α-glycerol phosphate (CH10), it can be observed that the highest recorded value is 154.31 in FR. In contrast, the lowest value is identified in ROMP, reaching 111.34, with a significant difference between these extremes being evident.

In the case of the substrate Acid d-glucosamine (CX1), it can be observed that the maximum value recorded is 368.48 in CR. In contrast, the lowest value is found in ROMP, namely 129.19, showing a significant difference between these two extremes.

Table 5. Dynamics of carbohydrates functional groups from 24 to 96 hours in long-term contaminated sites

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
CR	275.79±4.64a	412.74±89.3a	107.90±0.56c	168.51±40.7ab	206.75±35.2a	150.67±29.1ab	494.31±33.9b	312.11±55.7ab	161.73±46.3ab	119.14±4.55b
CT	160.63±18.8bc	277.64±44.1ab	277.83±16.0a	169.36±16.9ab	145.25±11.3b	206.84±11.3ab	216.94±25.0b	230.78±21.0ab	168.03±27.3ab	118.71±0.58b
FR	214.09±20.0abc	283.77±49.7ab	204.54±43.3ab	219.58±29.2a	183.18±13.8ab	176.90±25.2ab	409.80±8.88ab	377.82±84.1a	209.85±30.5a	154.31±15.0a
ROMP	237.05±52.1ab	277.23±72.8ab	148.05±35.7bc	104.61±2.67b	127.78±13.2b	238.26±71.4a	246.06±72.0ab	174.44±36.0b	112.83±2.29b	111.34±2.23b
URB	144.17±13.6c	192.19±8.81b	135.36±7.40bc	127.55±11.4b	138.72±5.34b	122.28±5.71b	206.43±78.6ab	166.94±19.7b	111.17±0.90b	115.93±4.51b

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR - Ferneziu, ROMP - Romplumb, URB - Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

Within the group of galactonic acid γ-lactone (CX2), it is observed that the highest value is recorded in FR. Conversely, the lowest value is identified in URB, indicating significant differences compared to the maximum value. Therefore, significant differences are noted between FR and URB.

In the d-galacturonic acid or CX3 group, according to the second reading, we observe significant differences between the maximum and minimum values, represented by FR and URB, respectively.

In the substrate 2-hydroxy benzoic acid (CX4), it is observed that CT has the highest value. In contrast, ROMP has the lowest value, highlighting a notable difference between ROMP and CT. This may indicate environmental differences or pollution effects.

In the case of 4-hydroxy benzoic acid (CX5) substrate, CR presents the highest level, while URB shows the lowest level. This shows substantial distinctions between CR and URB, with no discernible variation between the other groups.

Within the y-hydroxy butyric acid or CX6 group, there is a notable contrast in values. FR stands out with a value of 503.11, while URB lags behind with a value of only 153.75, showing a substantial disparity between the two.

In the CX7 itaconic acid category, FR is the one with the highest average, while URB is far behind FR in value. Consequently, FR and URB show notable differences.

Examining the substrate keto-butyric acid or CX8, relative to the other groups, shows that it has lower levels of uptake. Specifically, this substrate indicates that the microorganisms in the soil analysed are less efficient in metabolising this substance. Hence, at reading 2, the highest value is observed at CR and the lowest is 104.78, with no significant differences.

In the d-malic acid group (CX9), it is observed that the highest value is recorded at FR. In contrast, the lowest value occurs at ROMP, showing significant differences from the maximum value, but not from the other groups that have overlapping letters. Therefore, significant differences are found between FR and ROMP.

Table 6. Dynamics of carboxylic acids functional groups from 24 to 96 hours in long-term contaminated sites

	CX1	CX2	CX3	CX4	CX5	CX6	CX7	CX8	CX9
CR	368.48±41.3a	138.65±25.7bc	346.78±41.8ab	130.28±6.86ab	390.31±33.6a	502.10±72.6a	263.91±112.85bc	135.34±9.84a	273.87±45.8ab
CT	214.44±13.6b	173.47±18.7b	190.05±31.6bc	212.11±31.2a	241.82±44.9ab	273.13±48.5ab	280.20±32.9b	129.77±8.48a	200.55±40.6abc
FR	323.72±3.97a	289.01±31.4a	380.27±22.3a	159.91±50.6ab	309.53±69.3ab	503.11±19.1a	507.17±28.5a	129.22±17.5a	313.77±17.7a
ROMP	129.19±14.4b	113.35±1.35bc	227.10±108.95abc	112.39±2.46b	271.69±126.14ab	265.51±138.19b	120.55±3.50bc	104.78±5.78a	123.92±8.72c
URB	190.80±58.8b	105.93±2.38c	132.85±14.9c	122.00±2.06b	131.64±6.05b	153.75±10.1b	108.19±2.80c	105.98±10.1a	169.73±58.9bc

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR - Ferneziu, ROMP - Romplumb, URB - Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

In the second reading of the Tween 40 group (P1), it is observed that FR records the highest value.

On the other hand, the lowest value appears at URB, showing notable differences compared to the maximum value.

Therefore, after 24 hours of incubation, significant differences are observed between FR and URB.

In the Tween 80 or P2 group, ROMP shows the highest numbers while URB shows the lowest numbers, indicating minimal contrast between ROMP and URB.

In group α - Cyclodextrin or P3, FR is found to have the highest figure of 274.79, while CR has the lowest value of 120.16. This highlights a significant discrepancy between FR and CR.

Regarding the glycogen group (P4), it is observed that FR has the highest value while CR has the lowest value, indicating a significant difference between the two. This difference can be attributed to variations in the environment or the impact of pollution.

In the Arginine or AA1 group (Group 1), there's a notable contrast in levels. The highest measurement, 437.79, is observed in the CR condition, whereas the lowest, 117.64, is found in URB. This variation between the highest and lowest values is considerable.

In the analysis of group 1-Aspargine (AA2), it is noted that the highest concentration is recorded in CR. In contrast, the lowest level is recorded in URB, indicating significant differences compared to the maximum value, but not compared to the other groups having the same associated letter. Therefore, significant differences are evident between URB and CR.

In the 1-phenylalanine group (AA3) category, the highest value recorded reached 267.04, while the lowest value, 116.10, was observed in ROMP. This notable difference between the highest and lowest value is relevant.

In category 1-Serine (AA4), FR has the highest value of 382.11, while URB has the lowest value of 125.71. This highlights the significant differences between FR and URB.

In the first category, threonine or AA5, in reading 2, CT shows the highest value, 182.92, which differs markedly from the values of the other groups. In contrast, the lowest value is observed in URB. Consequently, it suggests notable distinctions between these values.

We can identify significant differences between FR and ROMP within the glycyl-l-glutamic acid or AA6 group, with a maximum value of 179.39 and a minimum of 115.00.

Table 7. Dynamics of polymers and amino acids functional groups from 24 to 96 hours in long-term contaminated sites

	P1	P2	P3	P4	AA1	AA2	AA3	AA4	AA5	AA6
CR	271.88±38.4a	298.05±81.2a	120.16±5.69c	150.62±30.0b	437.79±115.31a	534.45±109.65a	224.75±39.3ab	239.62±41.2bc	134.55±12.8ab	121.13±3.96b
CT	217.21±11.2ab	225.21±33.3a	214.76±3.02b	209.65±12.9b	218.58±64.5ab	240.21±53.9ab	267.04±30.8a	278.36±31.1ab	182.92±31.4a	172.99±25.6a
FR	281.25±43.8a	257.48±18.7a	274.79±28.6a	313.39±31.2a	428.69±48.3a	493.98±38.9a	262.76±54.8a	382.11±30.3a	175.12±1.93a	179.39±3.29a
ROMP	165.47±38.7b	298.63±57.6a	132.62±16.0c	160.14±33.6b	246.49±94.8ab	313.74±171.69ab	116.10±3.74b	241.13±73.5bc	119.62±6.32b	115.00±1.58b
URB	156.46±25.0b	170.30±26.0a	148.34±7.39c	196.07±20.0b	117.64±2.32b	122.52±3.38b	149.89±29.3b	125.71±8.59c	116.97±4.58b	121.53±2.61b

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR - Ferneziu, ROMP - Romplumb, URB - Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

The results show that substrate water or WAT is highest in CR and lowest in CT. This discrepancy is significant compared to the maximum value recorded, but not so significant compared to the other groups with overlapping letters. Therefore, significant differences are observed between CR and CT.

In the amine category, phenyltinamine or AM1 has the highest average value, while URB has the lowest average value. Therefore, there is a significant difference between URB and FR.

As for the putrescine or AM2 group, the maximum value recorded is 236.55, while the lowest value of 124.76 is identified for URB. The difference between the two extremes appears to be negligible.

Table 8. Dynamics of basal and amines/amides functional groups from 24 to 96 hours in long-term contaminated sites

	WAT	AM1	AM2
CR	107.61±1.31a	117.20±7.73b	186.50±59.7a
CT	92.133±3.7925c	219.37±33.5ab	222.74±18.0a
FR	94.884±3.5595bc	343.34±42.0a	236.52±36.4a
ROMP	107.40±2.66a	229.54±115.98ab	201.18±78.7a
URB	102.77±2.61ab	113.57±2.02b	124.76±4.72a

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR - Ferneziu, ROMP - Romplumb, URB - Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

Regarding high carbohydrate levels, such as CH2, CH1 and CH1 in CR(2), CT(3), CR(4) and ROMP(5) soils show intense microbial activity, suggesting more efficient degradation of organic

matter. Lower CH1, CH3, CH4, CH5 values in ROMP(2), FR(3), CT(4), CT(5) soils may signal an inhibition of microbial activity due to the presence of heavy metals that are toxic to microorganisms.
The high polymer values, namely P1, P2 AND P4 in FR(2), CR(3), FR(4) and ROMP(5) soils, indicate a significant presence of microorganisms capable of degrading complex materials. At the same time, lower P2, P3 and P4 in CR(2), URB(3), URB(4) and CR(5) soils indicate a decrease in microbial diversity and capacity to break down polymeric substances due to heavy metal contamination.
Increased carboxylic and acetic acid concentrations, namely CX3, CX6, CX7 in FR(2), FR(3), FR(4) and FR(5) soils suggest active communities to stressful environments. The decreased CX2 and CX8 levels in ROMP(2), FR(3), ROMP(4), CT(5) soils may be a warning sign of inhibition due to heavy metal toxicity.
Elevated values of amino acids, AA1, AA2 and AA4 in FR(2), FR(3), CR(4), ROMP(5) soils indicate active protein synthesis and good metabolic health of microbial communities. Lower values, AA1, AA3 and AA6 in ROMP(2), ROMP(3), URB(4) and CT(5) soils may show metabolic stress caused by metal contaminants, affecting protein synthesis.
The high values of amines and amides, AM1, AM2 in FR(2), CT(3), ROMP(4) and ROMP(5) soils indicate a microbial activity of protein degradation. Smaller concentrations, AM1 and AM2 in URB(2), URB(3), URB(4) and CT(5) soils reveal an inhibition of microbial function caused by the presence of heavy metals.

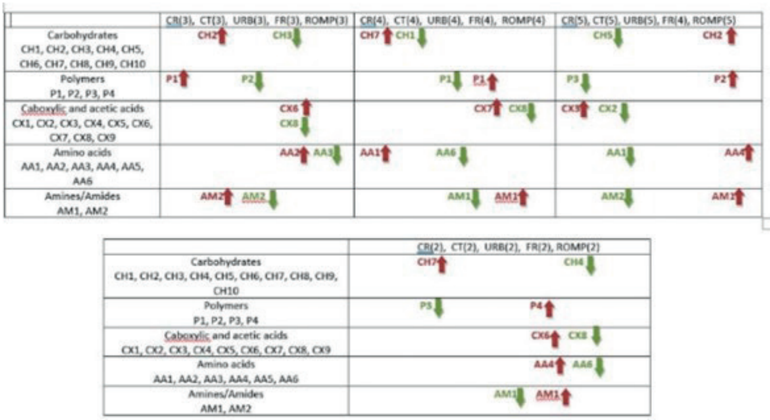

Figure 1. Trends of functional groups asociated with contaminated sites

CONCLUSIONS

The presence of heavy metals significantly alters the functional kinetics of microorganisms in Baia Mare soils. They reduce microbial diversity, moreover they disrupt nutrient cycling and decrease soil fertility. Microorganisms show different responses to contamination. Susceptible ones reduce their diversity and population, while resistant species adapt and proliferate.
This paper highlights significant differences in microbial activity between polluted sites in Baia Mare, such as Craica and Colonia Topitorilor, which show varying microbial functional profiles and growth rates.
The method used effectively measures the functional responses of the microbial community to contamination, while providing detailed insights into how different substrates metabolise in polluted environments.
The research highlights the need to understand soil microbial dynamics in the face of heavy metal pollution, which is essential for developing effective bioremediation strategies and ensuring soil and thus environmental health.

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PEDOLOGICAL STUDY IN THE SOUTHWESTERN PART OF MEHEDINȚI COUNTY, VRANCEA AREA

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Abstract

The town of Vrancea, Mehedinți County, belongs to Burila Mare Commune and it is located in the south-western part of the county wich is part of the west Oltenia Plain being located on the fluvial terraces of the Danube. On the studied territory there were formed and occur as soil units, Reddish Preluvosol on carbonate deposits, mollic Reddish Preluvosol on carbonate deposits, mollic Psamosol on aeolian deposits. The specific meso-relief of the reddish preluvosol is represented by the Danube terraces - the II Corabia terrace, altitude 15-22 m, and the psamosol - the Danube terraces - the IV terrace - Flamanda, with an altitude between 50 and 60 m. Preluvosols are characterized by a low content of humus, 2.70-2.93%, slightly acidic reaction, pH 6.13-6.43, low content in nitrogen and medium in phosphorus and potassium. Calcium carbonates are present in a high percentage, in the Cca horizon, and the chemical neoformations represented by $CaCO_3$ concretions have been identified. Psamosols have a medium humus content, 2.64%, a slightly acidic reaction, pH 5.83-6.23, medium-low nitrogen content, medium phosphorus and weak potassium.

***Key words**: soil profile, soil horizon, humus, soil reaction, fertility.*

INTRODUCTION

The soil, the layer on the surface or "Earth's epidermis" (Lavelle & Spain, 2002; Raducu et al., 2022), represents the ecological environment for plant life. This quality, this complex and fundamental function, is determined both by its intrinsic properties and by the pedogenesis factors (climate, relief, bedrock, etc.), which contributed to its evolution. The properties of soils, as well as their geomorphological distribution, are totally influenced by these factors.

The local conditions of relief, groundwater, lithology, determine the variety of soils in a certain area, which proves that the distribution of soils is determined not only by bioclimatic zonation but also by local factors, which sometimes play a fundamental role in the distribution of soils in a certain area (Amuza et al., 2023).

As a life support for plants, the soil has a fertility, which is influenced by the natural factors that acted in its genesis and evolution. The relief, through its components, altitude and exposure, largely determines its variety. (Tărău et al., 2007).

Plant growth and fruiting are influenced by the physical, hydro - physical and chemical properties of the soil. The aero-hydric regime, microbiological activity but also the transformation of nutrients in the soil is indirectly influenced by the physical properties of the soil (Drăgănescu, 2002).

In order to be able to ensure good conditions for the growth and fruiting of plants, the soil components must also be maintained in the optimal natural ratio, any disturbance causing a decrease in productive capacity (Popescu, 2020).

Soil is a natural and precious resource that mankind has inherited over time. He had a close connection with humans, throughout the history of mankind, through its cultivation, in order to obtain the products necessary for life. As the need to obtain higher yields productions, through the development of civilizations, it became more and more necessary, in order to increase the productivity of the soil, the application of fertilizers, pesticides and in this way, the beneficial intervention of man was felt (Datta et al., 2016).

The humans, through their intervention, however, modified the natural framework in which the soil was formed and thus the soil processes were disturbed (Popescu, 2018).

By using the soil for agricultural needs, soil works have influenced the physical properties, especially the structure, porosity, resistance to penetration, plasticity, adhesiveness (Cârciu et al., 2019; Cornavski, 2010). In this way, the structure of the soil was degraded, by doing agricultural works at unfavorable moments of humidity or by performing a large number of passes with agricultural machineries (Mihalache, 2014).

By changing the structure of the soil, its total porosity changes, it decreases, and in this way creates unfavorable conditions for the growth and development of plants (Mușat et al., 2023), in the sense that the microbiological activity is reduced, the permeability for water and air is small, and the root system of the plants develops hard. In specialized literature, it is known that the root system of plants develops properly, at a value of bulk density between 1.0 and 1.4 g/cm^3. By decreasing the porosity of the soil, as a result of the degradation of its structure, compaction is evident and it influences all other physical properties of the soil (Richard et al., 2001).

Soil compaction is closely interdependent with total porosity, with soil densities, all of which, in turn, are decisively influenced by the texture, the content in organic matter and of course the quality of the agricultural works performed (Canarache, 1990).

As it is well known, the good conditions for plant development are when the soil has total porosity between 50 and 55% of the soil volume, capillary porosity 30-35% of the soil volume or 60-70% of the total porosity, and aeration porosity 15-20% of the soil volume or 30 – 40% of the total porosity (Popescu, 2019).

When the human intervention is beneficial, i.e. the soil works are executed properly, thoughtfully, all its physical properties are favorable, causing the total porosity to increase, the apparent density to decrease. Also, it positively influences the thermal regime of the soil, the microbiological activity, but also its chemical properties. In order to be able to ensure good conditions for the growth and fruiting of plants, the soil components must also be maintained in the optimal natural ratio, any disturbance causing a decrease in the productive capacity (Popescu, 2020).

The decrease in soil fertility implies numerous shortcomings related to its health and quality, to the level of production. This aspect should be a wake-up call for all those who work cultivate the soil, for all agricultural workers, who must intervene to preserve and increase the fertility of the soil, to reduce or eliminate its degradation. (Zafiu & Mihalache, 2021; Mihalache et al., 2012; 2015; Marin et al., 2015; Marin et al., 2015).

Anthropogenic activity, in general, decisively influences large spaces, the intensity of relief modeling, which in turn, as we said before, determines soil variability. It can be noted, through an overview of soil conservation, that farmer has intervened on it, according to his requirements and needs. For the territory under study, it can be said that human influence in this area had negative and positive consequences. Over time, the studied area has benefited from radical land improvement works. We can mention here the construction of the Crivina - Vanju Mare irrigation-drainage system, which covers the territory of Burila Mare - Vrancea, Mehedinti County, and which led to the transformation of waterlogged soils in arable land and replacing of the former fens into cropping soil.

Desiccation and drainage led to the exploitation of the physical and chemical properties of the soil. By removing excess moisture, through deep loosening, the aero - hydric regime was improved, which determined positive aspects regarding the mineralization of organic matter, humification, structuring, mitigation or removal of gleization process.

The negative influence of human intervention in the reference area on the soil was made through massive deforestation of the protective zones and by carrying out tillage in inadequate humidity conditions, which led to the modification of the physical properties of the soil and especially the structure.

Starting from all these aspects, a pedological study was carried out in the area of Vrancea town in Mehedinți County, in order to establish the natural conditions in which the soils were formed in this area and what are their physical and chemical properties by using them in the agricultural production process.

MATERIALS AND METHODS

The pedological study was carried out in the south-western part of Mehedinti County, in the area of Vrancea locality, in a heterogeneous natural areal.

In this sense, the natural conditions of soil formation in this area were studied, a survey of the territory was carried out, on the occasion of which the location points of the soil profiles were set up, depending on the characteristics of the relief, so that each profile of soil to be as representative as possible, for as large a surface as possible, according to the methodology established by the Institute of Research for Pedology, Agrochemistry and Environment Protection Bucharest (IRPAEP Bucharest).
After digging the soil profiles, they were studied in the field, from a morphological point of view (Munteanu and Florea, 2009).
Soil profiles were made and studied in 2022 year.
The soil samples collected from the land with a modified soil structure and in natural structure, were prepared and analyzed in the laboratories of the Office for Pedology and Agrochemistry (OPA) Dolj.
The following analytical methods were used:
1. For chemical properties:
- The humus content was determined by the Walkley-Blak method;
- The pH value by the potentiometric method, in aqueous solution;
- $CaCO_3$ content by the gasometric method, using the Scheilbler calcimeter;
- The nitrogen content was determined by Kyeldahl method;
- Soluble potassium, by Egner-Riehn-Domingo method;
- Soluble phosphorus, by Egner-Riehn-Domingo method;
- Hydrolytic acidity, by Kappen method;
- The exchange capacity for bases, by Kappen method;
- The degree of saturation in bases, by calculation with the relationship V% = SB/T x 100
2. For physical properties:
- The size fractions were determined by wet sieving, siphoning and pipetting, and the soil texture was established with the texture triangle;
- The bulk density was determined by the 100 cm^3 metal cylinder method;
- The density was determined by the pycnometer method;
- The total porosity was determined by calculation;
- The hygroscopicity coefficient was determined by drying the soil sample at 105^0C, after saturating it with water in a desiccator with water vapor, created by a 10% sulfuric acid solution;
- The wilting point, by calculation according to the value of the hygroscopicity coefficient;
- The field capacity, by the centrifugation method;
The interpretation of the results was carried out according to the methodology in force (Institute of Research for Pedology and Agrochemistry, IRPA, 1987).

RESULTS AND DISCUSSIONS

The town of Vrancea, Mehedinti County, belongs to the Burila Mare commune and is located in the south-western part of the county. From a geomorphological point of view, the territory is part of the Western Oltenia Plain, and is located on the fluvial terraces of the Danube, the Corabia terrace, the Băilești terrace and the Flamanda terrace (Coteț, 1957).
Hydrologically, the studied territory belongs to the Danube hydrographic basin, comprising size particles ranging from clays, sandy clays to dune sands.
The climate regime is characterized by average annual temperatures of 11.7^0C and average annual precipitation of 520.1 mm. The drought was also present in this area, as in fact in all of Oltenia region, in recent years (Bonea, 2020). The natural vegetation specific to the area is that of oak forests.
In heterogeneous natural conditions, there were identified the reddish preluvosoil, the mollic reddish preluvosol, soils specific to the terrace meso-relief - Corabia II terrace, with an altitude of 15-20 m, formed on carbonate deposits and the psammosoil, on the IV-a Flamanda terrace, with an altitude between 50-60 m (Florea & Munteanu, 2012).
Soil profile 1 – Reddish Preluvosoil (Figure 1)
Relief: plain
Meso-relief: 2nd terrace of the Danube, Corabia terrace;
Bedrock: loessoid deposits;
Groundwater depth: over 10 m;
Global drainage: imperfect;
Use: arable, barley crop.

Figure 1. Reddish Preluvosoil

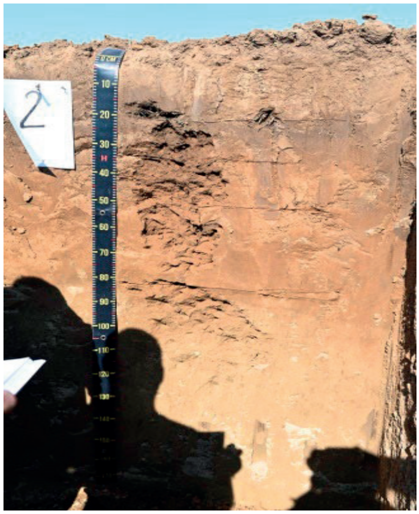

Figure 2. Mollic reddish Preluvosoil

Morphological characterization:
Ao horizon, 0-20 cm, grayish brown color, 7.5YR 4/4, loamy-clay texture, granular structure, compacted, clay films on the surface of structural aggregates, dense spots of iron and manganese, does not make effervesce, gradual passing to the next horizon;
AB horizon, 20-40 cm, grey-brown color, 7.5YR, fine porous, medium compacted, prismatic structure, dense spots of iron and manganese, does not make effervesce with HCl 1/3, clear passage to the next horizon;
Bt horizon, 40-80 cm, reddish brown color, 7.5YR,4/3, clay-clay texture, very compacted, prismatic structure, many iron and manganese concretions, does not make effervesce, gradual transition to the next horizon;
BC horizon, 80-105 cm, light brown color, 7.5YR 5/3, low compacted, low structured, low effervescence;
Cca horizon, below 105 cm, yellowish brown color, 6/3 10 YR, unstructured, very frequent carbonate concretions, strong effervescence.

Soil profile 2 - mollic reddish Preluvosoil (Figure 2)
Relief: plain;
Meso-relief: second Danube terrace, Corabia terrace, altitude 15-22 m;
Bedrock: loessoid deposits;
Groundwater depth: over 10 m;
Global drainage: imperfect;
Use: arable, sunflower crop.

Morphological characterization:
Ap horizon, 0-21 cm, grayish brown color, dark, 7.5YR 4/2, granular structure, loamy-clay texture, fine porous, good biological activity, does not make effervesce, gradual transition to the next horizon;
Am horizon, 21-43 cm, grayish brown color, 7.5YR , loamy-clay texture, medium compacted, fine porous, good biological activity (coprolites and cervotocins), frequent spots of iron oxides and concretions of iron and manganese, gradual passage to the next horizon;
AB horizon, 43-52 cm, grayish brown color, 7.5YR, loamy-clay texture, medium granular structure, low biological activity, and frequent iron oxide stains, clear passage to the next horizon;
Bt horizon, 52-86 cm, reddish brown color, 7.5YR, well-formed prismatic structure, clayey texture, very compact, frequent spots of iron and manganese oxides, frequent ferromanganese concretions, from 2 mm to 6 mm, does not make effervescence, gradual transition to the next horizon;
Cca horizon, below 86 cm, brownish yellow color, unstructured, frequent spots of calcium carbonate and carbonate concretions, strong effervescence.

Soil profile 3 – Mollic psamosoil (Figure 3)
Relief: plain, with a wind-drift appearance;
Meso-relief: 4th terrace of the Danube, Flamanda terrace, altitude 60-60 m;
Bedrock: aeolian sandy deposits;

Groundwater depth: over 10 m;
Overall drainage: very good;
Use: arable, wheat crop

Figure 3. Mollic psamosoil

Morphological characterization

Am horizon, 0-27 cm, dark gray color, 10YR2.5/1, poorly developed structure, friable in wet state, low cohesive, dense roots, does not make effervesce, gradual transition to the next horizon;

AC horizon, 27-40 cm, light gray color, 10 YR, livid, unstructured, non-cohesive, frequent roots, clear transition to the next horizon;

Cn1 horizon, 40-75 cm, very pale brown color, 10YR 7/2, moist, unstructured - monogranular, non-cohesive, does not make effervesce, frequent spots of white mica ore, and quartz grains;

Cn2 horizon, below 75 cm, light color, unstructured, non-cohesive, does not make effervesce.

Physical Properties – Reddish Preluvosoil, soil unit 1 (S.U.1)

In the reddish preluvosoil (S.U.1), from a particle size point of view, the coarse fraction, fine sand, predominates in the soil composition, with a percentage of 48.74%, in the Ao horizon and reaches 58.4% in the Cca horizon. It is followed by clay, with 31.4% in the first horizon and 17.6% in the last, and by the silt fraction, 17.7% and 12.4% respectively in the same horizons (Figure 4).

The soil is loosened in the surface horizon where the bulk density has the value of 1.26 g/cm^3 (Figure 5), the density of 2.32 g/cm^3 (Figure 6) which indicates a low compaction degree and it becomes compacted in the Bt clay accumulation horizon, where the values are the highest (1.66 g/cm^3 and 2.69 g/cm^3, respectively).

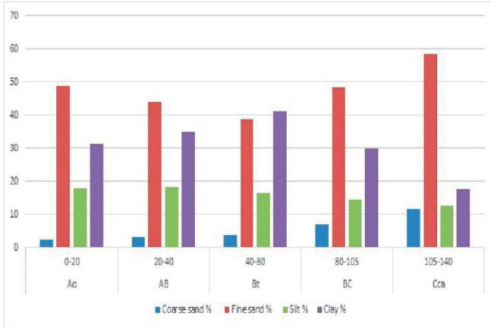

Figure 4. Particle size composition with S.U.1

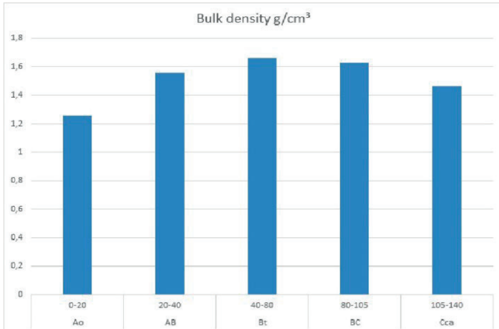

Figure 5. Bulk density with S.U.1

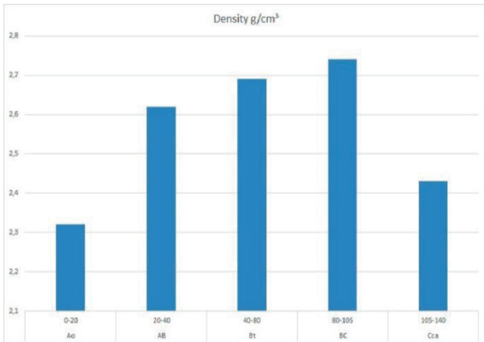

Figure 6. Density with S.U.2

Interpreting the values obtained by the hydro - physical indices for the reddish preluvosoil on carbonate deposits, it can be seen that they are in full concordance with the content of fine fractions of soil (Figure 7).

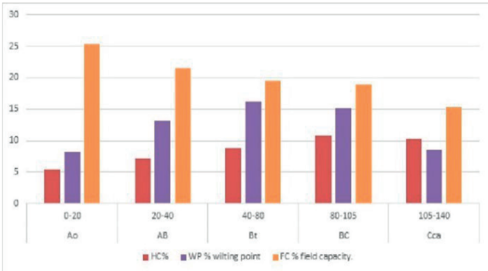

Figure 7. Hydro - physical indices with S.U.1

Chemical Properties – Reddish Preluvosoil (S.U.1)

Analyzing the humus content, at soil unit 1, in the area of Vrancea, Mehedinti County (Figure 8), it can be seen that it is low in the horizons Ao (2.7%), AB (1.58%), Bt (1.52%) and very low in BC (1.10%) and Cca (0.61%) deep horizons.

The soil reaction is low acidic in the first three horizons (pH 6.13-6.61), neutral in the BC horizon (pH 6.86) and low alkaline, pH 8.14, in the Cca horizon.

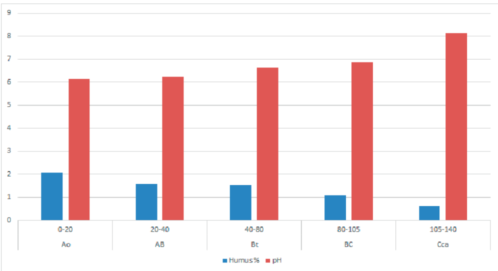

Figure 8. Humus content and pH values at S.U.1

The total nitrogen content in this soil is low in all horizons, decreasing with soil depth from 0.107% in the Ao horizon to 0.032% in the Cca horizon.

The soil is average supplied with soluble phosphorus in the Ao, AB, Bt and BC horizons, values that decrease from 33.0 ppm, in the Ao horizon, to 18.8 ppm, in the BC horizon. The content of soluble phosphorus is low (9.7 ppm), Cca in the horizon.

Regarding the potassium content, in this soil unit, it can be found that it is average in the surface horizons, in Ao it is 162 ppm and in AB 154 ppm, low in the Bt and BC horizons (112, respectively 88 ppm) and very low (52 ppm), in the bedrock material (Figure 9)

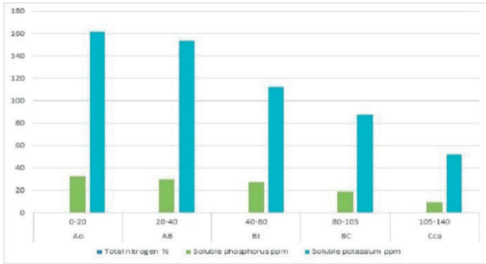

Figure 9. Nitrogen, phosphorus and potassium content with S.U.1

The base saturation degree (BS %), is in the eubasic range, in the first 4 horizons and in the deep horizon, Cca, the soil is saturated in bases, BS % = 100, and calcium carbonate ($CaCO_3$) is present with this soil unit, only at the level of the Cca horizon, in a percentage of 14.9 (Figure 10), as a result of the presence of calcium carbonate concretions in the bedrock material (Figure 11).

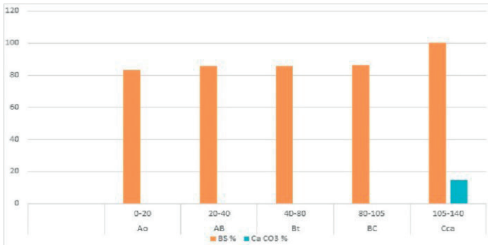

Figure 10. Base saturation (BS %) and calcium carbonate content with S.U.1


Figure 11. The presence of calcium carbonate concretions in the Cca horizon, at S.U.1

The mollic reddish preluvosoil (S.U.2), is generally similar both in terms of physical and chemical properties, both were formed on carbonate deposits, an aspect illustrated by the presence in this soil unit, in the Cca horizon, of calcium carbonates, in a slightly lower percentage, 13.6%, compared to 14.9%, in the reddish preluvosoil (Figure 12) and of chemical neoformations represented by $CaCO_3$ concretions (Figure 13).

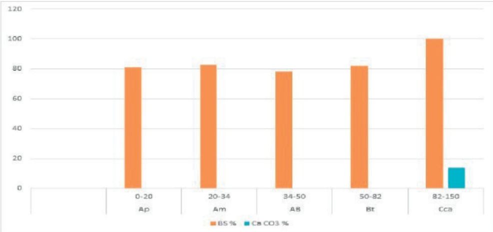

Figure 12. Base saturation (BS %) and calcium carbonate content with S.U. 2


Photo. 13. The presence of calcium carbonate concretions in the Cca horizon, at S.U. 2

Physical Properties – Mollic Psamosoil (S.U.3)

The particle size percentage that predominates is the sand fraction that exceeds 74% in all horizons. Coarse sand reaches a percentage of 66.09% in the Ao horizon, 53.5% in the AC horizon, 65.4% in Cn1 and 66.1% in depth. Fine sand increases on the soil profile, from 18.57% n the Ao horizon to 26.29% in the AC horizon. Silt has a value of 6.54% in the surface horizon, increases to 7.12% in the AC horizon and decreases to 4.10% in Cn1. The fine fraction, clay, registers the lowest values, except for the surface horizon, Ao, where it reaches 8.1% (Figure 14). This particle size composition gives the soil a sandy-clay texture in the first two horizons and sandy in the bedrock material.

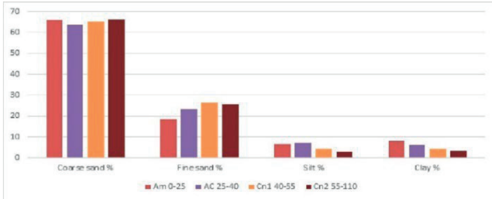

Figure 14. Particle size composition with S.U.3

Through the graphic representation of the bulk density of the mollic psamosoil on aeolian deposits (Figure 15), it is found that the soil is loosened in the surface horizons, where the value is 1.16, respectively 1.42 g/cm^3, and becomes compacted in depth, where values of 1.56 g/cm^3 are recorded in the Cn1 horizon and 1.56 g/cm^3 in Cn2.

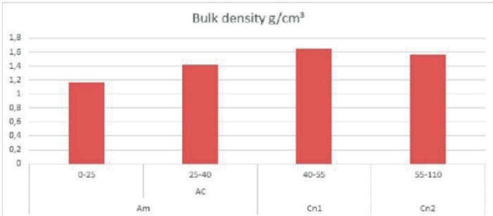

Figure 15. Bulk density with S.U. 3

The small content in clay fractions makes the hydro - physical indices very low. HC %, registers slightly above the value of 1, namely, 1.12-1.23%, and the value even becomes subunit (0.7%), in the Cn2 horizon. In the same sense, the values of the field capacity for water of the soil are highlighted, 15.23%, in the Ao horizon and 10.9%, in Cn2 (Figure 16).

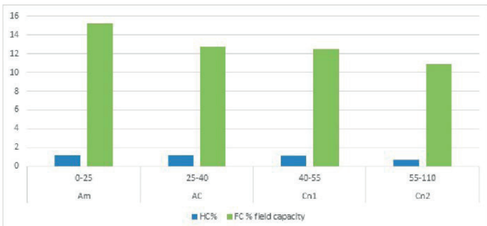

Figure 16. Hydro - physical indices with S.U.3

Chemical Properties – Mollic Psamosoil (S.U.3)

From a chemical point of view, the mollic psamosoil on aeolian deposits (S.U.3) is characterized by a medium humus content in the surface horizon, Ao (2.64%), low in the AC

horizon, 1.29% and very low in the bedrock material, 1.10% in the Cn1 horizon and 0.75% in the Cn2.
The soil reaction is slightly acidic throughout the depth of the soil profile, the pH value increases from 5.83, in the surface horizon, to 6.23, in the Cn2 bedrock material (Figure 17).

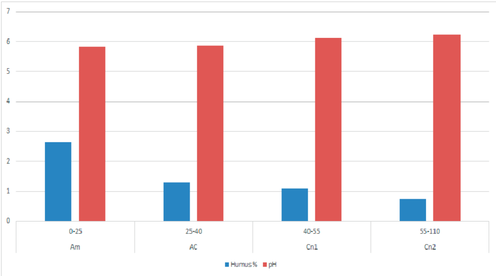

Figure 17. Humus content and pH values at S.U.3

From the point of view of the content of nutrients, the mollic psamosoil on aeolian deposits is characterized by a medium-low content in total nitrogen, in the Ao horizon, 0.135% and low - very low in the underlying horizons, the percentage reaching 0.039 in Cn2 %. The supply of soluble phosphorus is in the middle range, in the first three horizons (29.7, 25.3, 20.1 ppm) and small (16.0), in the Cn2 horizon. As for phosphorus, the values recorded for this nutrient show a poor supply of the soil, throughout its depth. Soluble potassium, decreases on the soil profile, from 130 ppm, in the Ao horizon, to 72 ppm, in the Cn2 horizon (Figure 18).
As it can be seen from Figure 19, the mollic psamosoil on aeolian deposits does not contain calcium carbonate and it has a base saturation degree between 68.1 and 83.9%.
Based on the processing of data regarding the natural conditions and properties of the soils in the studied area, the quality classes for arable use were established, which has a significant importance in Vrancea locality, Mehedinti County. The surface occupied by the three soil units was classified into two classes: the third quality class, which includes the surface occupied by the reddish preluvosoil on carbonate deposits and the mollic reddish preluvosoil on carbonate deposits, and the fourth class of quality, in which the surface on which the mollic psammosoil is found spread on aeolian deposits is included.

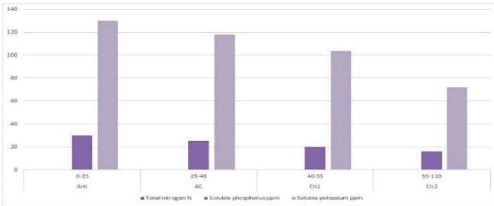

Figure 18. Nitrogen, phosphorus and potassium content with S.U.3

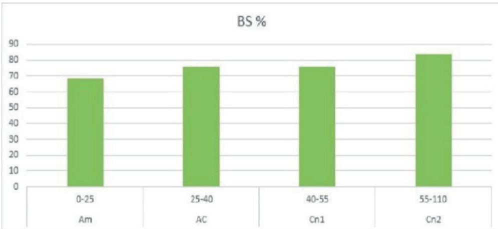

Figure 19. Base saturation (BS %) and calcium carbonate content with S.U.3

The object of agricultural activity, in the farm that operates in this territory, as well as for private producers, on the arable surfaces is the crops of wheat, barley, sunflower and corn.
The favorability classes for these crops are presented as follows: wheat, the 5th favorability class, for the soil units reddish preluvosoil on carbonate deposits and mollic reddish preluvosoil on carbonate deposits and the 8th favorability class, for the mollic psamosoil on aeolian deposits; barley, the 5th favorability class, for the soil units reddish preluvosoil on carbonate deposits and mollic reddish preluvosoil on carbonate deposits and the 8th favorability class, for the mollic psamosoil on aeolian deposits; corn, class VIIth of favorability, for the soil units reddish preluvosoil on carbonate deposits and mollic reddish preluvosoil on carbonate deposits and class IXth of favorability, for mollic psamosoil on aeolian deposits; sunflower, the 6th favorability class, for the reddish preluvosoil soil unit on carbonate deposits and the mollic reddish preluvosoil on carbonate deposits and the 8th favorability class, for the mollic psamosoil on aeolian deposits.
In relation to the nature and intensity of the restrictive factors, for agricultural production, the lands were grouped into suitability classes for arable use: class II, lands with reduced limitations in the case of arable use. In this class are included the soil units, reddish preluvosoil

on carbonate deposits and mollic reddish preluvosoil on carbonate deposits and class III, land with moderate limitations in case of arable use. Mollic psamosoil on aeolian deposits is, also, included in this class.

CONCLUSIONS

The territory of the town of Vrancea, Mehedinti County, is part of the large unit of the Romanian Plain (Western Oltenia Plain) being located on the Danube terraces.

The predominant bedrock, on which the soils within the studied territory have evolved, is varied, from leossoid deposits to sandy loams to dune sands.

From a hydrographic point of view, the studied territory is part of the Danube hydrographic basin.

Climatologically, the studied territory is characterized by a temperate climate, with the manifestation of the phenomenon of drought.

Through the interaction of soil genesis factors, the following soil types were formed on the researched territory of Vrancea locality: reddish preluvosoil on carbonate deposits, reddish mollic preluvosoil on carbonate deposits and mollic psamosoil on aeolian deposits.

In relation to the pedogenetic factors and environmental conditions, bonitation marks were calculated and quality classes were established. For arable use, the surface of Vrancea town, Mehedinti County, was classified into two quality classes: class III, for preluvosoils and class IV, for psamosoil.

The crops cultivated in the area are in the Vth class of favorability (wheat and barley) and the VIth and VIIth classes (sunflower and corn), on preluvosolis, the VIIIth class (wheat, barley and sunflower) and class IXth (maize), on psamosoil.

Depending on the intensity of the restrictive factors for agricultural production, the lands were grouped into two suitability classes for arable use. In class II, lands with reduced limitations, where the two preluvosoil units were placed, and class III, lands with moderate limitations, for psamosoil.

From the consideration of the restrictive factors, the ameliorative requirements and the necessary measures to optimize exploitation result.

The way of carrying out soil tillage is the main means of preventing or mitigating the physical degradation of the soil. Poor structuration and compaction of the soil, the most common forms of physical degradation, can be greatly reduced if the following measures are made: performing soil tillage only in optimal humidity conditions; the reduction to the minimum, to the strictly necessary, of seedbed, crop maintenance, etc.; preservation and incorporation of vegetable debris into the soil; the inclusion of some improving plants in the crop rotation. The physical degradation of the soil takes place through the phenomenon of soil displacement known as soil erosion, a complex phenomenon in which soil material is removed by moving water and drift wind. Wind erosion affects psamosoil arable land. The establishment of protective forest strips in the perimeters affected by wind erosion is very effective in preventing deflation.

Chemical degradation refers to unwanted changes in some important chemical or physical -chemical properties of the soil. The most important chemical degradation process is soil acidification. The correction of the acid reaction of the soil is done by lime as amendment, in order to change the acid reaction and the low level of bases saturation in the soil.

Fertilization by organic and mineral fertilizers has a particularly important role for increasing the humus content and replenishing the nutrient pool.

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STUDY ON SOIL RESOURCES AND THE LIMITING FACTORS OF LAND USE IN DRAGANESTI AREA, PRAHOVA COUNTY

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Abstract

The paper presents the pedological and agrochemical study elaborated on the territory of Dăgăneşti commune, Prahova county, it is intended for the foundation of the county (national) soil-land monitoring system for agricultural areas. The paper inventories soil resources, identifies and describes the limiting or restrictive factors of land use for agricultural production. The influence of relief on pedogenesis is generally manifested by altitude and, on small surfaces, by slope. In the presented case, the relief has little direct influence due to the morphological characteristics of the terrain: low altitudes and slopes, lack of exposure. Knowing the nature and intensity of the limiting factors of agricultural production is absolutely appropriate in the situation where the decision is made to reduce or eliminate their negative effects.

Key words: *evolution, assessment, soil, fertility, limiting factors.*

INTRODUCTION

The studied territory is located in the south of Prahova county, at a distance of about 35 km from the municipality of Ploiesti.
The total area of Drăgăneşti commune is 8756 ha, of which 7514 ha were studied (mapped) (OSPA PRAHOVA, 2019), the studied territory is located in the south of Prahova county, at a distance of about 35 km from Ploiesti municipality (Chiuciu et al., 2017). It borders the following administrative territories:
- to the north - the administrative territories of Tomşani, Colceag;
- to the east - the administrative territories of Fulga, Ciorani;
- to the south - the administrative territory of Rădulesti (Ialomiţa county);
- to the west - the administrative territories of Dumbrava, Rîfov, Gherghiţa.

Locally, three important geomorphological units are defined:
a) high plain;
b) Prahova river meadow;
c) Cricovul Sarat river meadow.

The geological layers that served as parent rocks for the current soils in the researched perimeter, are of Lower Pleistocene age and are represented by sedimentary rocks made up of silty-psamo-psephytic materials, newer or older depending on the distance from the two rivers that delimits the said interfluve (Enescu et al., 2018).
The predominant material in the soils of the high plain is reworked loess (Ioniță et al., 2013). The parent rocks on which they evaluated the soils in the meadow are alluvial in nature with textures that vary from coarse to fine, and the rocks in the major basin are recent deposits consisting of coarse alluvium (Mihalache, 2015).

MATERIALS AND METHODS

The average temperature per season is as follows (Figure 1):
- in winter – the average temperature is -0.5°C.
- spring – the average temperature is +10.8°C.
- summer – the average temperature is +21.1°C.
- autumn – the average temperature is +11.3°C.

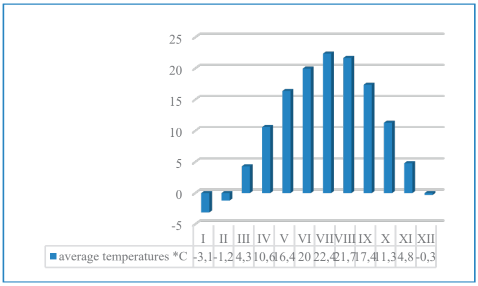

Figure 1. Average monthly temperatures

Average annual precipitation is in the range of 500 - 600 mm (ANM, 2022) (Figure 2).

The average annual measurements recorded in Ploiesti are 588 mm, in Armăşeşti 466 mm, in the Ciupelniţa rainfall point 621.0 mm (1966-1988) and the Drăgăneşti rainfall point 593.4 mm (1963-1988).

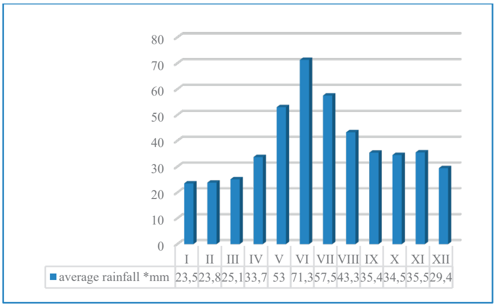

Figure 2. Average monthly precipitation

From the point of view of land use, the mapped surface is structured as follows:

- arable 6807.0 ha;
- pastures 685.0 ha;
- hay fields 7.0 ha;
- vines 11.0 ha;
- orchards 4.0 ha.

During the study, 358 main and secondary profiles were made, collecting a number of 295 pedological samples and 307 agrochemical samples analyzed in the O.S.P.A. laboratory Prahova, by the following methods:

a) determination of soil reaction - pH – the potentiometric method

b) determination of carbonates – $CaCO_3$ (%)– SCHEIBLER gas volumetric method

c) determination of soluble salts - method of determination in aqueous soil extract

d) humus determination (%) – the WALKLEY-BLACK method modified GOGOAŞA.

e) nitrogen index - IN – by calculation according to the content in humus and the degree of saturation in bases (IN=H%*V%/100).

f) determination of mobile phosphorus – P_2O_5 (ppm) – in lactic acetate extract according to EGNER-RIEHM-DOMINGO

g) determination of mobile potassium – K_2O (ppm) – the flame-photometric method

h) granulometric analysis (texture) – pipetting, sieving – the KACINSKI method (ICPA, 1987).

TERRITORIAL LAND UNIT (U.S.) No. 1. **FLUVISOLS** *entic calcareous gleic saline, moderately salinized glaciated weakly proxicalcalcareous*, LL/LN on coarse fluvial deposits, weakly-moderately polluted with animal droppings.

Location: Prahova County, Drăgăneşti Administrative Territory.

Distribution: in the southwest of the commune, along the river Prahova.

Aspect of the land surface: high alluvial plateau, slope 01%.

Natural conditions in which it occurs: drift plain, groundwater Q4 1.41 – 2.00 m, cover with nitrophilous vegetation and sedges.

CHARACTERISTICS OF THE SOIL

Horizon Ațsc 0-15 cm, yellow color 5Y 7/6, monogranular structure, clay texture, strong effervescence, very low porosity, very compact, moist, skeleton below 2%;

Horizon ACksc 15-35 cm, yellow color 5Y 7/6, monogranular structure, sandy loam texture, strong effervescence, very low porosity, very compact, moist, skeleton below 2%;

Horizon Ckgsc 35-70 cm, yellow color 7.5YR 6/8 with oxidation spots 10R 5/8, monogranular structure, sandy loam texture, strong effervescence, medium porosity, weakly compact, moist, skeleton below 2%;

Horizon CkGosc from 70 cm, yellow color 7.5YR 6/8 with oxidation spots 10R 5/8, monogranular structure, loamy sand texture, strong effervescence, medium porosity, weakly compact, moist, skeleton below 2%.

The physical and chemical characteristics of Fluvisols entic calcareous gleic saline, moderately salinized glaciated weakly proxicalcalcareous are presented in Table 1 and Table 2.

TERRITORIAL LAND UNIT (U.S.) No. 2. **PHAEOZEMS** *Alluvial cambic, LA/LA on medium-fine fluvial deposits*

Location: Prahova County, Drăgăneşti Administrative Territory.

Distribution: in the north of the territory, on the sides of the Albeşti - Urziceni road and in the east of Braitaru village up to the Cricovul Sărat river.

Aspect of the land surface: high alluvial plateau, slope 01%, 07%.

Natural conditions in which it occurs: drift plain, groundwater Q5 3.01 – 5.00 m.

Table 1. Physical characteristics of the soil

Depth	Soil reaction (pH)	($CaCO_3$)	Humus (H)	Phosphorus (P)	Potassium (K)	Coarse sand (Ng)	Fine sand (Nf)	Loam (P I+II)	Clay (A)	Psyhical clay (Af)
cm		%	%	ppm	ppm	%	%	%	%	%
0-15	7.68	7.7	2.16	24.6	236.0	18.9	40.0	20.2	20.9	32.9
20-30	7.54	7.2	1.26	16.0	116.0	10.3	58.7	17.7	13.3	22.8
50-60	7.85	8.3	-	-	-	3.3	70.4	12.8	13.5	17.2
90-100	7.95	8.3	-	-	-	1.9	73.8	17.4	6.9	18.4

Table 2. Chemical characteristics of the soil

Depth	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Total salts
cm	mg/100 g soil								
0-15	-	51.8	25.9	52.8	44.0	3.7	6.2	-	177.8
20-30	-	42.7	26.6	105.6	55.0	9.6	4.5	-	233.8
50-60	-	44.5	19.5	235.2	47.6	15.0	1.2	-	379.4
90-100	-	39.7	21.3	67.2	42.4	6.0	1.2	-	184.4

CHARACTERISTICS OF THE SOIL (Table 3)

Horizon Ap 0-21 cm, dark brown color 10YR 3/3, disturbed structure, clay loam texture, no effervescence, low porosity, moderately compact, dry;

Horizon Am 21-35 cm, dark brown color 10YR 3/3, granular structure, clay loam texture, no effervescence, low porosity, moderately compact, dry;

Horizon ABv 35-50 cm, dark brown color 10YR 3/3, small angular polyhedral structure, clay loam texture, no effervescence, low porosity, moderately compact, dry;

.

Horizon Bv 50-70 cm, dark yellowish brown color 10YR 4/4, medium polyhedral structure, clay loam texture, no effervescence, low porosity, moderately compact, reed;

Horizon BCn 70-90 cm, yellowish brown color 10YR 5/8, massive structure, clay loam texture, no effervescence, very low porosity, very compact, stiff;

Horizon Cn from 90 cm, yellowish brown color 10YR 5/8, monogranular structure, clay loam texture, no effervescence, very small porosity, very compact, reash (Table 4).

Table 3. Physical characteristics of the soil

Depth	Soil reaction (pH)	($CaCO_3$)	Humus (H)	Phosphorus (P)	Potassium (K)	Coarse sand (Ng)	Fine sand (Nf)	Loam (P I+II)	Clay (A)	Psyhical clay (Af)
cm		%	%	ppm	ppm	%	%	%	%	%
0-20	6.84	-	2.76	51.0	148.0	1.0	32.5	28.9	37.6	53.7
35-45	7.12	-	2.94	26.4	144.0	0.7	32.9	25.8	40.6	58.4
60-70	7.49	-	-	-	-	0.5	35.6	26.3	37.6	53.6
90-100	7.86	-	-	-	-	0.8	38.2	23.0	38.0	48.3

Table 4. Chemical characteristics of the soil

Depth	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Total salts
cm	mg/100 g soil								
0-20	-	-	-	-	-	-	-	-	-
35-45	-	-	-	-	-	-	-	-	-
60-70	-	51.9	23.0	24.0	25.0	1.5	1.6	13.8	158.4
90-100	-	36.6	35.5	10.6	15.0	4.5	-	16.1	107.4

TERRITORIAL LAND UNIT (U.S.) No. 3. **GLEYOSOL** calcareous salsodic alluvium, salinized/moderatel yalkalized / weakly proxicalcareous, LA/LA on fine fluvial deposits.
Location: Prahova County, Drăgăneşti Administrative Territory.
Distribution: in the center of the studied territory and in the south of the village of Cornu de Jos.
Aspect of the land surface: high alluvial plateau – micro depression, slope 07%.
Natural conditions in which it occurs: drifting plain, water table Q3 1.01-2.00 m, water ponding for a large period of the year and in some areas permanently, reed cover (Table 5).
CHARACTERISTICS OF THE SOIL
Horizon Aţgsc 0-06 cm, marbled brown color 7.5Y 3/2, massive structure, clay loam texture, moderate effervescence, low porosity, moderately compact, soft;
Horizon AmGoxsc 06-26 cm, marbled brown color 7.5Y 3/21 with oxidation spots 10R 5/8, massive structure, clay loam texture, moderate effervescence, low porosity, moderately compact, soft;
Horizon ACGoxscac 27-45 cm, marbled brown color 7.5Y 3/21 with oxidation spots 10R 5/8, massive structure, clay loam texture, strong effervescence, low porosity, moderately compact, jiggly;
Horizon CkGrsc 45-75 cm, dark burgundy color 5P 3/1 with oxidation spots 10R 5/8, massive structure, clay loam texture, strong effervescence, low porosity, moderately compact, moist;
Horizon Ck1Grscac 75-100 cm, dark burgundy color 5P 3/1, massive structure, clay texture, moderate effervescence, low porosity, moderately compact, moist;
Horizon Ck2Grscac from 100 cm, dark burgundy color 5P 1.7/1, massive structure, clay texture, weak effervescence, low porosity, moderately compact, wet (Table 6).

Table 5. Physical characteristics of the Gleysols

Depth	Soil reaction pH (pH)	($CaCO_3$)	Humus (H)	Phosphorus (P)	Potassium (K)	Coarse sand (Ng)	Fine sand (Nf)	Loam (P I+II)	Clay (A)	Psyhical clay (Af)
cm		%	%	ppm	ppm	%	%	%	%	%
0-20	7.98	3.8	4.32	30.8	236.0	0.2	23.7	37.8	38.3	61.2
30-40	7.97	4.6	3.84	22.4	172.0	0.2	18.7	38.6	42.5	71.2
60-70	8.10	6.1	-	14.9	216.0	0.3	19.7	41.4	38.6	71.1
90-100	7.84	2.5	-	-	-	0.2	17.7	30.5	51.9	79.2
110-120	7.82	1.7	2.82	-	-	0.3	12.0	28.9	58.8	83.4

Table 6. Chemical characteristics of the Gleysols

Depth	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Total salts
cm	mg/100 g soil								
0-20	-	54.9	85.2	145.4	84.0	14.4	5.1	18.4	460.0
30-40	-	61.0	55.0	139.2	68.0	14.4	2.3	18.4	380.0
60-70	-	506	63.9	193.9	37.4	50.0	2.0	23.0	420.8
90-100	-	70.1	69.2	117.1	71.0	5.6	3.5	25.3	400.1
110-120	-	70.2	94.1	72.0	70.0	7.7	3.1	25.3	374.8

TERRITORIAL LAND UNIT (U.S.) No. 4. ***TECHNOSOLS*** spoliic aline calcareous lithic, weakly salinized proxicalcareous epilithic, LL/LLq3 on anthropogenic deposits (permeable gravels).
Location: Prahova County, Drăgăneşti Administrative Territory.
Distribution: in the southeast of the studied territory.
The aspect of the land surface: high alluvial plateau – filling, slope 0.1%.
Natural conditions in which it occurs: drift plain, groundwater Q4 4.01-10.00 m (Table 7).

CHARACTERISTICS OF THE SOIL
Horizon Aţ 0-03 cm, light yellowish brown color 10YR 6/4, monogranular structure, clay

texture, strong effervescence, low porosity, moderately compact, dry.

Horizon ACk 03-20 cm, light yellowish brown color 10YR 6/4, monogranular structure, clay texture, strong effervescence, low porosity, moderately compact, dry.

Horizon CkR 20-40 cm, light yellowish brown color 10YR 6/4, monogranular structure, clay texture, strong effervescence, very high porosity, very compact, dry, skeleton 70%.

Horizon R from 40 cm, permeable gravels (Table 8).

Table 7. Physical characteristics of the Tehnosols

Depth	Soil reaction (pH)	($CaCO_3$)	Humus (H)	Phosphorus (P)	Potassium (K)	Coarse sand (Ng)	Fine sand (Nf)	Loam (P I+II)	Clay (A)	Psyhical clay (Af)
cm		%	%	ppm	ppm	%	%	%	%	%
0-20	8.10	7.0	1.44	29.9	224.0	11.4	37.4	28.6	22.6	40.8

Table 8. Chemical characteristics of the Tehnosols

Depth	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Total salts
cm	mg/100 g soil								
0-20	-	54.9	24.8	57.6	48.0	1.4	3.9	7.0	200.0

RESULTS AND DISCUSSIONS

Following the laboratory analyzes regarding the soil reaction, we have the following situation in Table 9.

The communal territory of Drăgănești is mostly occupied by moderately and weakly acidic soils, representing 3211 ha (89%). Strongly acidic soils are found on approximately 157 ha (4.0%). Neutral soils occupy 26.0 Ha (0.5%), and slightly alkaline soils 208 Ha (6%). On acidic surfaces, calcareous amendment accompanied by organic fertilization is required. It is not recommended to use chemical fertilizers with an acidic physiological reaction such as nitrate and ammonium sulfate. It is recommended to apply nitrolimestone and complex fertilizers 22:22:0, 16:48:0 that provide nitrogen fertilization and correct soil acidity.

These measures will be taken after the application of the other agropedo-ameliorative measures to stabilize and reduce excess moisture (Ilie, 2019).

Table 9. Soil reaction (pH)

Soil reaction	Surface	
	ha	%
strongly acid soils	157.0	4.0
moderately acid soils	2532	70.0
weakly acid soils	679	19.0
neutral soils	26	0.5
weakly alkaline soils	208	6.0
moderately strongly alkaline soils	26	0.5
TOTAL:	3628	100.00

The main source of nitrogen in the soil is humified organic matter. The evaluation of the state of soil provision with this element was made on the basis of the humus content. It correlates directly with the degree of saturation in bases (V%), resulting in the nitrogen index (IN = (H% x V%)/100). The provision of soils with nitrogen is weak and moderate. To correct this condition, organic fertilization is required in doses established according to the morphological situation of each type of soil and the values resulting from the analyses. In addition, chemical fertilizers will be used, respecting the appropriate agricultural techniques, and in accordance with the Code of Good Agricultural Practices. We find a good nitrogen supply situation on the surface of approximately 350 Ha (10%), following the values of the other agrochemical indices correlated with the pH value, we conclude that the correct fertilization of the soil has been achieved here (Table 10).

Table 10. Humus H% content and nitrogen supply (IN)

Nitrogen supply (IN)	Surface	
	ha	%
poorly supplied soils	2027	56
moderately supplied soils	1251	34
well supplied soils	350	10
TOTAL:	3628	100.00

The Riehm index is a synthetic indicator that characterizes the reaction of the soil and the state of supply with the main nutritional elements (phosphorus, potassium) (Cerbari et Stagarescu, 2016). The Riehm index for phosphorus is calculated by adding the percentages from well and very well supplied soils with half of the percentages of moderately supplied soils (Table 11). The result is a value of 55.76%, characterizing a representativeness of soils moderately-well supplied with mobile phosphorus.

Table 11. Mobile phosphorus (P) supply status

Insurance with mobile phosphorus (ppm P)	Surface	
	ha	%
very poorly insured soils	26.0	0.5
poorly secured soils	731.0	20.0
moderately safe soils	1697.0	47.0
well secured soils	810	22.0
very well safe soils/excessive for some crops	364	10.5
TOTAL:	3628	100

The Riehm index for potassium is calculated by adding the percentages from well and very well supplied soils with half of the percentages of moderately supplied soils. The result is a value of 99.5%, which represents soils well and f. well supplied with potassium for the studied territory. Most of the soils that belong to the communal territory of Drăgănești are well and very well provided with potassium and occupy 3602 Ha (99.5%), and the moderately provided 26.0 ha (0.5%). To correct the deficiency, as well as on well and very well-supplied soils, potassium chemical fertilization is recommended, with doses corresponding to the respective situation (Table 12).

Table 12. Supply status with accessible forms of potassium

Providing soils with mobile potassium (ppm K)	Surface	
	ha	%
moderately safe soils	26.0	0.5
well secured soils	1201	33.0
very well secured soils	2401	66.5
TOTAL:	3628	100

CONCLUSIONS

According to the research and depending on the intensity of the limiting factors, 2363 ha of the arable surface, representing 72.62% of the surface of the Drăgănești commune, are lands with severe limitations in the case of arable use that require intensive measures of development and/or improvement.

In class II a of pretability are classified 511 ha of the arable area of the commune, respectively 15.7%, land with reduced limitations, with good pretability to arable.

Only 0.77% of the arable area of Drăgănești includes land classified as arable class I, very good land for crops, without any restrictions.
There is also an area of 167 ha with very severe land limitations when used in arable, unpretentious in conditions of non-arrangement or complex measures.
In case of the pre-tability of the soils from Drăgănești locality for the use of pastures and hay meadows, we can notice that there are no lands in first class of pretability, the largest area being 103 ha, respectively, it is occupied with lands of class IV a, land with severe limitations on pastures.
In the II-th class we have 70 ha of land with reduced limitations as pastures and hay meadows, in class III a we have 73 ha of land which for their use as pastures the limitations are moderate, and in class a we also have 73 ha, land with severe limitations for use as pastures and hay meadows.
As regards the pretability of the viticulture use, we also note that the largest land area used for the vineyards is classified in class IV a of pre-ability, as, respectively 55 ha (42.97% of the vineyard area), followed by 44.5 ha in the first class iI of the pretability, respectively 34.775 of the vineyard area, 24 Ha are in the pretability class III a and only 4.5 ha are with very severe limitations when used as vineyards.

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THE CALCIUM CARBONATE EVOLUTION INFLUENCING THE PHISICO-CHEMICAL CHARACTERISTICS OF THE LONG TERM IRRIGATED CHERNOZEMS AND THEIR CLASSIFICATION

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Abstract

The evolution of the soil architecture after the matrix lost the $CaCO_3$ under the water irrigation influence had important consequences on the evolution of the physico-chemical characteristics of the long term irrigated soils. The analytical data results showed that, in the case of the horizons with calcium carbonate pedofeatures, the bulk density reached higher values, comparing to the horizons free of $CaCO_3$. The paper represents also an alarm signal for the soil scientists that classified the long-term irrigated Chernozems from the Romanian Plain (with $CaCO_3$ washed deeper than 125 cm), to the Phaeozems, which represents a regrettable mistake. Between Chernozems and Phaeozems there are very important differences concerning: the quality and the quantity of the organic matter; the plasmic material mobility; the leaching process with clay coatings formation; $CaCO_3$ morphology, etc. The leaching of the $CaCO_3$ to >125 cm depth in the long-term irrigated Chernozems from the Romanian Plain is due to the anthropogenic influence, and it is not pedogenetic, that is why it is not a pertinent condition to classify these Chernozems as Phaeozems.

***Key words**: Chernozems, long-term irrigation, bulk density, calcium carbonate, Phaeozems.*

INTRODUCTION

The Chernozems are the most fertile and the most friendly soils with the farmers, having a great power of self-restoration of the structure and self-conservation of the fertility.

As showed Huang et al. (2009), the Chernozem had been classified as modern soil by agricultural pedologists, while the geoscientist classified them as paleosols. The high value of Chernozem for biomass production and the environment influenced the decision of the first soil chosen for the "Soil of the Year" (proclaimed for the first time in Germany in 2005, on the occasion of the World Soil Day) was Chernozems (as showed Altermann et al., 2005).

The researches of the Chernozems and Phaeozems extent in Central Germany during the Neolithic period had been achieved by Suchodoletza et al. (2015), using the sedimentologic and micromorphological properties. The decisive factor for the recent and former spatial distribution of Chernozems and Phaeozems (located in the Luvisol region) is the carbonate dynamics under the relatively dry climate of Central Germany (Suchodoletza et al., 2019).

The former distribution of Chernozems and Phaeozems in regions that are covered by other soil types today, their formation and degradation were studied by many authors during the time (citated by Huang et al., 2009; Lorz & Saile, 2011; Suchodoletza et al., 2019).

Apart from a sub-continental climate, the main factor determining the recent and former distribution of Chernozems and Phaeozems in Central Germany was obviously the carbonate content of the parent material (Suchodoletza et al., 2015).

Important generalizations concerning the evolution of Chernozems in space and time have been also performed by different authors (Ivanov & Tabanakova, 2009; Eckmeier et al., 2007).

The climate changes influenced differently the development of the different subtypes of Chernozems in the Late Holocene (Lisietskii et al., 2013).

The researches of Bobrovsky and Leiko (2019) stated that in the dark-humus soils (Haplic Phaeozems) no traditional features of the

Chernozem «steppe» stages of soil formation during the Holocene were found.

Reviewing the hypothesis of a predominantly anthropogenic pedogenesis of Chernozems in Northern Germany during the Early Neolithic, Lorz and Saile (2011) by the aim of an interdisciplinary research (involving geosciences, palaeobotany, and archaeology) rejected the hypothesis, but validated the theory of their natural formation.

The Chernozems area in Eurasian occupies a special place in the history of human society, as well as in the history of soil science (Chendev et al., 2010).

Labaz et al. (2019) showed that diverse chernozemic soils featured by thick mollic horizon, rich in humus, dark-coloured, structural, and saturated with base cations are relatively common in the loess-belt of SW Poland, being defined as soils developed from parent materials predominately rich in carbonates (Eckmeier et al., 2007; Chendev et al., 2010; Suchodoletza et al., 2019), under grassy meadow vegetation supported by high ground-water table. These soils contain larger accumulations of secondary (pedogenic) carbonates in the calcic horizons.

The paper objective was to emphasise evolution of the physico-chemical characteristics of the long term irrigated Chernozems. The paper also focused on the correct classification of these soils, avoiding any regrettable mistake.

MATERIALS AND METHODS

The investigated territory is located in the Eastern part of the Romanian Plain (Southern Baragan Plain) in Marculesti area, where the climate is temperate continental, with long and warm summers and droughty periods in late summers and early falls. The average annual temperature is 10.6°C and the average annual precipitation is 480 mm. De Martonne aridity index is 23, while the evapotranspiration reaches 700 mm. The water table is at > 10 m depth.

The soil is Vermic Chernozem (according to SRTS-2012 and WRB-SR-2014) formed in carbonate loess like deposits. The studied site is located in the steppe bioclimatic zone (danubian steppe subzone).

Six soil profiles of Chernozems were studied: P1a and P1b - irrigated with carbonates; P2a and P2b - non-irrigated with carbonates; P3a and P3b - irrigated without carbonates.

For a better understanding of the irrigated soils classification, a Phaeozem were also studied, being located in Suceava Tableland characterized by a mean annual temperature of 9.6°C and a mean annual precipitation of 518 mm. According to the Romanian pedoclimatic micro-zonation (Methodology ICPA-1987), the territory belongs to the IIIO-CM - Chernozem-like micro-zone, with cool-humid climate, in the regions with undulated relief.

The soil had been sampled for the physical, chemical and micromorphological analyses and further analyzed and data results interpreted according to the ICPA Methodology-1987.

For the micromorphological investigation, the oriented large (6 x 9 cm) thin (25-30 μm) sections were prepared from the undisturbed soil samples collected from each pedogenetic horizon (in order that the investigation results be statistically covered), after air drayed and impregnated with epoxidic resins. In order to describe and interpret the soil important characteristics and features at the microscopic level, investigation had been proceed by the aim of: microfilm reader Carl Zeiss Jena DL at 5-20X; petrologic microscope Amplival at 50-100X; and Stereomicroscope Nikon SM2800 at 1-6 X; in plain (PPL) and polarized (XPL) light, and using the terminology of Bullock et al. (1985).

RESULTS AND DISCUSSIONS

The bulk density is a very sensitive property of the soil and also very dynamic, changing proportional with the root grow, biological (macro– and mezofauna) activity, wetting–drying processes etc.

In these conditions, it could give precious information about the soil status at a certain period of time, as well as about the processes that induce changes in the soil evolution.

The four studied Chernozem profiles (P1a and P1b; P3a and P3b) were irrigated and consequently, under the influence of the irrigation water, many transformations of the soil constituents occurred.

The most sensitive is calcium carbonate (as well as other salt accumulated mainly in the deeper horizons). Its dissolution and removal from the soil profiles strongly influenced the physical properties, as showed the data results.
The presence of salts, as calcium carbonate, in the deeper horizons, as result of a non-transpercolative water regime, the horizon is more compacted, with higher values of the bulk density (Figure 1).

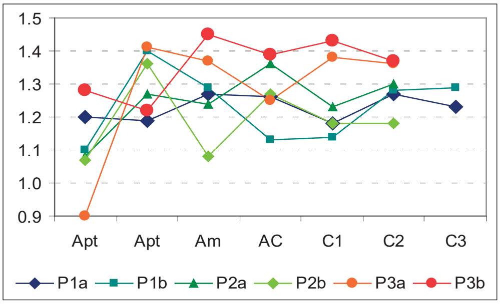

Figure 1. The bulk density (g/cm^3) of the studied Chernozems

In this respect, the analytical data results showed important differences between the bulk densities (BD) of the studied Chernozems (Figure 1).
Detailing the data results (Figure 2), in the profile P1a ("irrigated with carbonates"), the $CaCO_3$ appear from 35 cm depth and its content increased from 9.5% (into the Ck horizon) to 18.5% in the deepest Cca horizon.

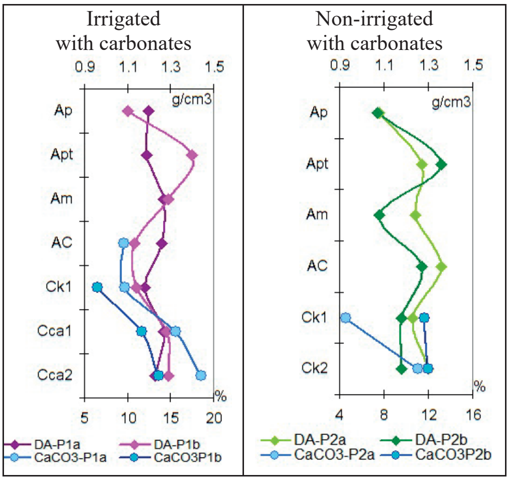

Figure 2. The bulk density (g/cm^3) values and the calcium carbonate content of the P1 and P2 Chernozems

In the Cca horizons, the BD values increased to 1.26-1.27 g/cm^3, while in the top horizons the BD is smaller (1.19-1.20 g/cm^3).
In the irrigated P1b profile, the $CaCO_3$ appear at 60 cm depth and the content is smaller (comparing to P1a) 6.4% in the Ck horizon, increasing to 13.5% in the deeper calcic horizon (Figure 2).
In this profile, the BD registered the same dynamic as in P1a: smaller values (1.1-1.4 g/cm^3) in the top horizons, and higher values (1.28-1.29 g/cm^3) in the deeper calcic horizons, where both fine silt-sized $CaCO_3$ crystals (or crypto-crystals) impregnated the matrix and calcium carbonate pedofeatures (nodules and concretions) had been formed.
It showed be underlined that in Chernozems, the biological activity is very high, improving continuously the poral space and strongly influencing the BD (transforming it in a very dynamic soil parameter).
In P2 soil profiles ("non-irrigated with carbonates"), the $CaCO_3$ appear deeper (comparing to P1 profiles): at 70 cm depth in P2a, where the $CaCO_3$ content is 11.6%; and at 80 cm in P2b, where the $CaCO_3$ content is 4.5 - 11.0%.
In P2a the BD is 1.08 g/cm^3 to the top horizon and reached 1.30 g/cm^3 in the Ck horizon, while in the P2b the BD is 1.07 g/cm^3 in the top horizon and increased to 1.18 g/cm^3 in the bottom Ck_2 horizon.
In what concerning the P3 soil profiles ("irrigated without carbonates"), the irrigation for long time (more than fifty years) (Figure 3), removed the $CaCO_3$ from the soil profiles, under 125 cm depth.
Consequently, in the deeper horizons (of P3a) free of $CaCO_3$, the BD is lower (1.36 g/cm^3), comparing to the higher value (1.41 g/cm^3) of the upper horizon of the soil profile (Figure 3).
In the Cn horizon of the P3b, the BD value is 1.37 g/cm^3, comparing to 1.45 g/cm^3 reached in the higher horizon.
As pointed out the analytical data, both P3a and P3b soil profiles are more compacted, with higher values of the BD, and specially in the deeper horizons, where the $CaCO_3$ had been leached from the soil profile.
The evolution of the soil architecture after the matrix lost the $CaCO_3$ under the water irrigation influence had important consequences on the evolution of the physico-chemical characteristics of the long term irrigated soils.

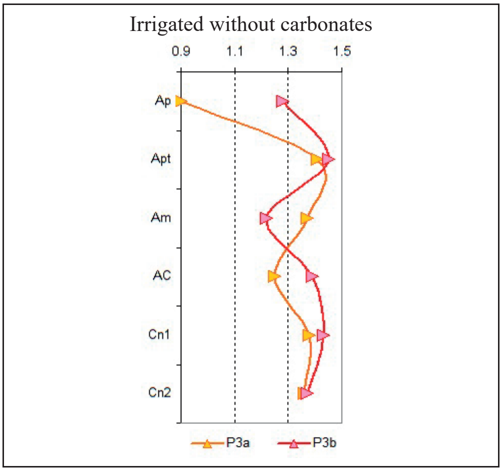

Figure 3. The bulk density (g/cm^3) of the P3 Chernozems without $CaCO_3$

Soil bulk density is a physical parameter that depends on the soil constituents, as granulometry and organic matter as well as on their spatial arrangements.

The results emphasize that in the case of horizons with calcite crystals (and/or crypto-crystals) embedded into the soil matrix, the bulk density is also influenced.

Together with the $CaCO_3$, different salts also accumulated as a result of a non-transpercolative water regime, but in very low quantities.

The analytical data of the cation exchange capacity (Figure 4) showed among the Ca^{2+} and Mg^{2+} cations, the presence of Na^+.

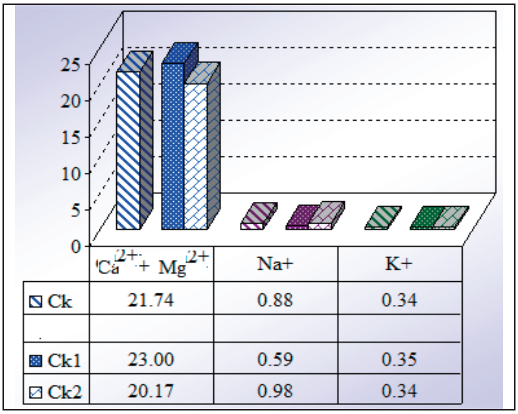

Figure 4. The cations (me/100 g soil) dominating the exchange cation capacity of the P2a (Ck horizon) and P2b (Ck_1 and Ck_2 horizons)

The very low quantities of Na salts also accumulate in the deeper horizons of the Chernozems highlighted by the analytical data that showed (Figure 5) an increased of Na^+ cations from the lower values (0.16 me/100 g soil) in the top horizons to the higher values (0.90-0.98 me/100 g soil) in the bottom profile. This cation, even in very low quantities, during the high humidity seasons could locally disperse the matrix and change, at the microscopic scale, the soil architecture, and further the bulk density.

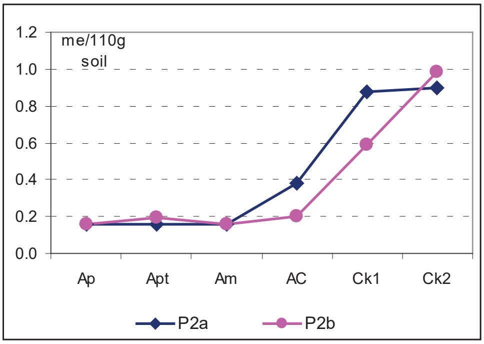

Figure 5. The level of Na^+ cations along the P2a and P2b soil profiles

Concluding, it could be underlined that the calcium carbonate (secondary and/or inherited from the parent material) in the deeper calcic horizons induced a higher compaction emphasised by the higher values of the BD.

Another important problem concerning the long term irrigation is the leaching of the $CaCO_3$ under 125 cm depth, the diagnostic condition for both Chernozems and Phaeozems: for naming a mollisol as Chernozem the condition is the "presence of $CaCO_3$ (secondary carbonates) in the first 125 cm"; while for naming a mollisol as Phaeozem, the condition is "without Cca horizon or concentrations of secondary carbonates in the first 125 cm".

In this respect, the paper represents also an alarm signal for the soil scientists which could classified the long-term irrigated Chernozems (with $CaCO_3$ washed deeper than 125 cm) located in the Romanian Plain, to Phaeozems, which would represent a regrettable mistake.

Detailing, it is very important to underline that between Chernozems and Phaeozems there are

many important differences that clearly showed their different pedogenesis and evolution, as for example: the quality and the quantity of the organic matter; the plasmic material mobility; the leaching process with the formation of clay coatings (their quantity and quality respectively); $CaCO_3$ morphology, etc.
In this respect, the main differences are the quantity and the quality of the organic matter; as well as the difference at the chromatic level: in Chernozems the organic matter is black-very dark brown, while in the Phaeozems, the organic matter is darker, black with metallic chroma (showing higher moisture regime).
Micromorphological investigation showed that plasmic material had a very low mobility in Chernozems. Even if the irrigated Chernozems (with $CaCO_3$ washed deeper than 125 cm), the plasmic material is still stable, and very thick and rare impure clay coatings formed. In contrast, in Phaeozems, many illuvial coatings varieties formed: from impure clay coatings with abundant organic impurities (micro-particles ≤ 0.02 μm) to coatings with very rare or no impurities ("argillane"); and also impure clay pore infillings with crescentic fabric. These coatings varieties represent sequences of their evolution in time, as well as the polyphasic evolution of the soil together with its environment.
Plasma of the Chernozems (even irrigated) is more stable which showed that only the $CaCO_3$ from the calcite pedofeatures and the fine silt-sized $CaCO_3$ crystals (or crypto-crystals impregnating the matrix) were removed, while the Ca^{2+} ions which reinforces the structural edifice of the soil (by binding the clay to the organic matter) have not been removed.
In what concerning the calcium carbonate pedofeatures, in Chernozems pseudo-mycelium formed, which show the fluctuation of the secondary $CaCO_3$ under the evapotranspiration effect; also the matrix around the $CaCO_3$ nodules has very strong effervescence (when applying dilute 1 *M* HCl solution). Contrasting, in Phaeozems only the core of the nodules has effervescence, while the surrounding matrix does not show effervescence (which pointed out the leaching of secondary carbonates under more humid conditions).
In this respect, for naming soils in the classification it is necessary to taking into account all the characteristics of the soils and their pedogenesis, and not only a specific characteristic.
Concluding, the presence of the $CaCO_3$ to > 125 cm depth is due to the anthropogenic influence, and it is not pedogenetic, that is why it is not a pertinent condition to classify the Chernozems as Phaeozems.

CONCLUSIONS

The researches results pointed out that calcium carbonate (secondary and/or inherited from the parent material) induced a higher compaction to the calcic horizons, with consequences on the bulk density (BD) which registered higher values into the calcic horizons, comparing to the horizons free of $CaCO_3$ (with smaller values of BD).
This aspect showed that under the irrigation water influence, the soil matrix lost the $CaCO_3$ with important consequences on the soil architecture and on the evolution of the physico-chemical characteristics of the long term irrigated soils.
Another important problem concerning the long term irrigation is the leaching of the $CaCO_3$ under 125 cm depth, the diagnostic condition for Phaeozems.
But, it should taking into account that the presence of the $CaCO_3$ to > 125 cm depth in the long-term irrigated Chernozems from the Romanian Plain is due to the anthropogenic influence, and it is not pedogenetic, that is why it is not a pertinent condition to classify these Chernozems as Phaeozems, which represents a regrettable mistake.

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METHODS OF REMEDIATION OF SALINE SOILS IN LONG-TERM EXPERIENCES AND THROUGH RICE CULTIVATION AT ARDS BRAILA

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Abstract

The paper presents results of some long-term experiences at the Braila Agricultural Development Research Station, with different mineral and organic fertilizers, as well as through rice cultivation. Through agrochemical analyses of the soil as well as through the analysis of the productions, it was found that the optimization of the fertilization system can be done both by the method and by the time of application, which are factors for increasing the efficiency of the fertilizing elements and the economy of fertilizers. The localized application of a small amount of N23P23 or N13P26K13 kg/ha s.a. fertilizers, the so-called starter fertilization, determined the increase in the efficiency of the use of fertilizers for all fertilizing elements. Thus, for phosphorus at equivalent doses, production increases of up to 18% were achieved, for nitrogen up to 5% and for potassium up to 4%. Also, rice cultivation has proven to be the fastest and most effective method of improving and capitalizing on saline soils, especially under the conditions of climate change.

***Key words**: soil salinization, rice, crops.*

INTRODUCTION

The soil is the basis of the existence of terrestrial life (Hera, 2016), and represents 29% of the Earth's surface, of which only 6.4% is intended for agriculture. However, food production is carried out predominantly on this surface, i.e. in a percentage of 95%, while only 5% food production is carried out on the 71% of the surface covered by water. That is why it is very important to preserve soil fertility through conservative technologies, no matter how much one would like to increase production per hectare.

To satisfy the ever-increasing need for food and in the conditions where the agricultural land surfaces are reduced year by year, and their fertility is substantially degraded, it is essential to increase the productions per surface unit through technological methods continuously adapted to the climatic conditions and through maintaining soil fertility.

The long-term experiences with fertilizers can ensure an efficiency of the consumption of fertilizers to obtain superior agricultural productions from a quantitative and qualitative point of view.

It was found that the doses of fertilizers applied to obtain maximum or economically optimal productions fluctuate widely, from year to year, depending on the climatic conditions (Hera, 1972; Hera and Borlan, 1975).

Salinity has affected almost 1,000 million hectares of land, of which approximately 77 million hectares are cultivated. Globally it reached 19.5% and 2.1% respectively in irrigated and non-irrigated fields. At the same time, the current population is approximately 7.7 billion inhabitants, and by 2020 it is estimated that it will increase to approx. 8 billion, which will further worsen food security (Asharf, 2009; https://www.worldometers.info/ro/).

Salinity is a major abiotic stress originating from irrigation sources and groundwater. Thus, salinity reduces plant growth and development, yield of sensitive crops by inducing physiological dysfunction (Shannon et al., 1994; Khan and Panda, 2008). The harmful effects of salinity are associated with water deficit, ionic imbalance, mineral nutrition, stomatal behaviour, and the efficiency of the photosynthesis process (Bohnert et al., 1995).

Thus, salinity has come to affect almost every aspect of plant physiology and biochemistry (Khan et al., 2002). All soils contain a wide range of soluble salts, some of which are essential for plant growth and development. Thus, Ca^{2+}, Mg^{2+} and Na^{+} are the most common cations, and Cl^{-}, SO_4^{2-} and HCO_3^{-} are the anions associated with soil salinity.

Jacoby (1999) stated that for saline environments, NaCl is the most important constituent, and Tucker (1999) says that Na^{+} and Cl^{-} are considered necessary for some plants, for example, salt-tolerant plants.

Exposure of plants to salinity can cause several changes, morphologically, physiologically, and biochemically, due to excessive ions and deficient water (Maskri et al., 2010).

In various research, a change was observed in the growth processes, photosynthesis, and protein synthesis, but also in lipid metabolism (Parida and Das, 2004); high concentrations of salts in plants generate changes related to plant productivity (Hasegawa et al., 2000), nutritional imbalances (Ashraf, 2009), as well as the accumulation of osmotic protective compounds (proline).

Occupying just over 15% of the world's arable surface, rice (*Oryza sativa* L.) is one of the most important agricultural crops in the world and represents the basic food for Asia and Africa, given that Asia produces and consumes over 90% of the global amount of rice. FAO Statistics shows that rice feeds 2/3 of the world's population (over 66%).

Simplifying the technology and making each element more efficient can lead to impressive economic results. Rice could become a representative crop for Romania and for dry areas that have water resources for irrigation, including areas with saline soils. The technology of rice cultivation and flood irrigation, associated with the 3-year rotation, could solve the problem of salinized soils in a natural way and without additional expenses. Romania is at the Northern limit of rice cultivation in Europe. The purpose of introducing and maintaining rice in culture was to cover the needs for current consumption, from the national production.

MATERIALS AND METHODS

The long-term experiments with fertilizers carried out at SCDA Braila were in the three experimental centres (Chiscani, Silistraru and IMB), for two research directions:

- evaluation of NP interaction and the effect on crops and indicators within the soil-plant system;
- evaluation of the effect of organo-mineral fertilization, by applying manure in interaction with NPK mineral fertilizers, on crops and the evolution of soil fertility indicators.

Thus, it was possible to study the evolution of production yields, in different agricultural conditions, with the aim of elaborating the basis for the scientific substantiation of the application of organo-mineral fertilizers to different agricultural crops.

The following aspects were analysed:

- the average productions obtained under the influence of the application of different doses of NP fertilization;
- the average effect of nitrogen application on production;
- the average effect of phosphorus application on production;
- the effect of applying different doses of nitrogen on the agrochemical indices of the soil;
- the effect of applying different doses of phosphorus on the agrochemical indices of the soil;
- average results regarding the export of NPK in grains;
- the effect of applying different doses of fractionated and unfractionated fertilizers to the corn crop.

Also, the improvement of the elements of rice cultivation technology and the introduction of the 3-year rotation led to a decrease in soil salinity and an increase in soil fertility indices.

RESULTS AND DISCUSSIONS

The average productions obtained in soybean fell between the values of 2660 kg/ha at N30P0 and 3220 kg/ha at N120P40, the fertilization effect being maximum at doses of 120 kg/ha N s.a. and 40 kg/ha P_2O_5 (Figure 1).

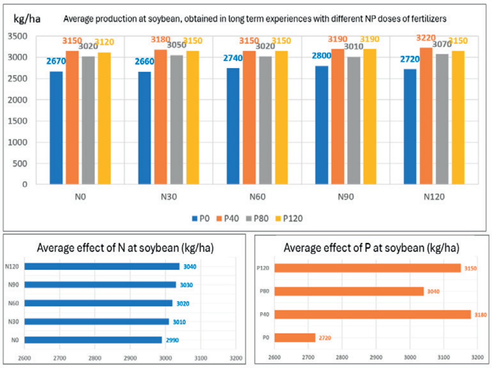

Figure 1. The graphs with average effect
of different doses of NP fertilizers

Regarding the influence of fertilization with different doses of nitrogen and phosphorus on the soybean crop, it was found that fertilization with a dose of 120 kg s.a. of phosphorus increases most of the soil fertility indices, while the increase of the nitrogen dose in the soybean crop did not have a significant influence on the other indices (Figures 2, 3).

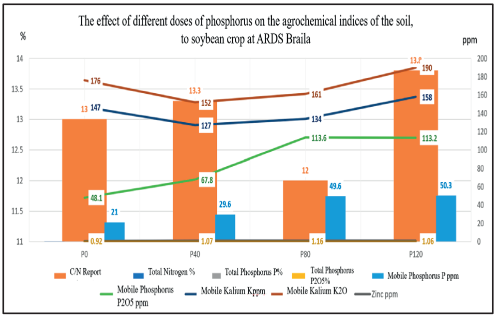

Figure 2. The effect of different doses of phosphorus
on the agrochemical indices of the soil,
to soybean crop at ARDS Braila

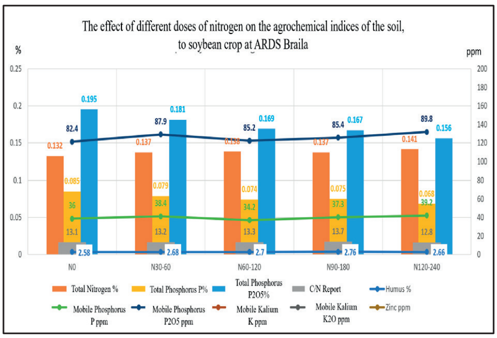

Figure 3. The effect of different doses of nitrogen
on the agrochemical indices of the soil,
to soybean crop at ARDS Braila

For a more detailed analysis of the influence of long-term fertilization with NP, the average results regarding the export of NPK in soybean and corn crops were analysed, both by analysing the percentage content in grains and by calculating the export of active substance in kg/ ha. The statistical data highlighted the fact that when fertilizing with different doses of phosphorus, the highest percentage content of nitrogen and kalium in grains was recorded when fertilizing with phosphorus at a dose of 80 kg s.a./ha, and for the content of phosphorus in grains, the best result was when fertilizing with P40 (Figure 4).

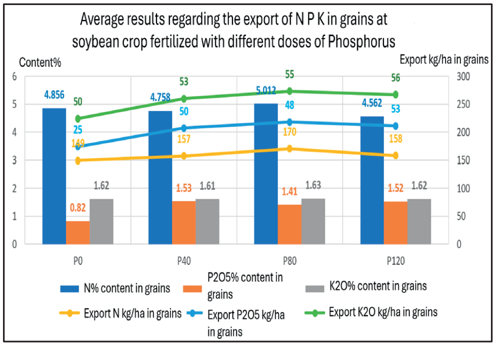

Figure 4. The effect of Phosphorus fertilization on N P K
export in grains soybean at ARDS Braila

Regarding fertilization with different doses of nitrogen, the highest export of nutrients in grains was recorded at the dose of 120-240 kg/ha, both for nitrogen (170 kg s.a./ha) and for phosphorus (46 kg s.a./ha) and potassium (54 kg s.a./ha) (Figure 5).

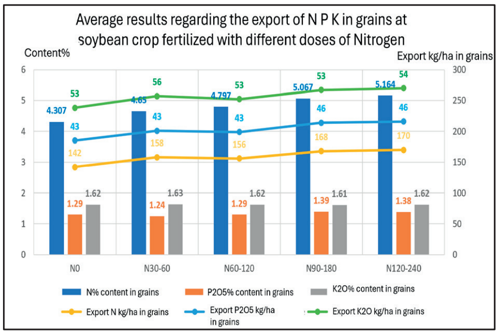

Figure 5. The effect of Nitrogen fertilization on N P K
export in grains soybean at ARDS Braila

The average productions obtained in the long-term corn experiments at the Chiscani experimental centre were between the value of 7510 kg/ha for the control variant (N0P0) and 11720 kg/ha for the N180P80 variant (Figure 6).

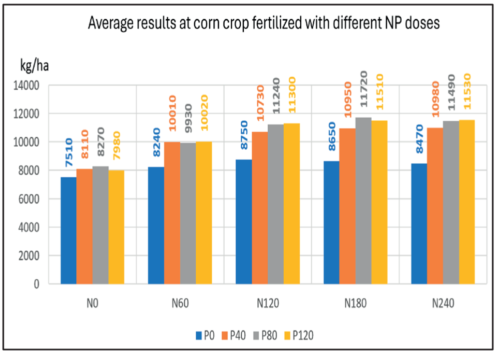

Figure 6. The graph of absolute average productions compared to the untreated control in the corn crop

The calculation of the percentage differences in corn production, compared to the untreated control, showed that the best results were obtained, in descending order, by the variants N180, N240 and N120 in combination with P80 (Figure 7).

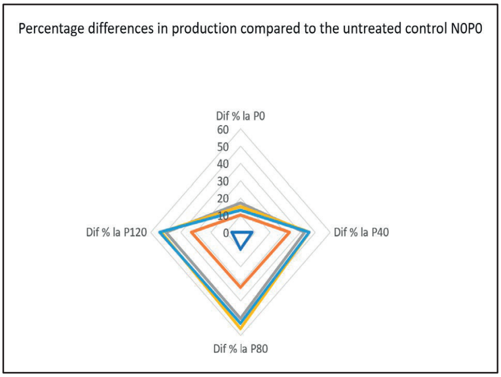

Figure 7. The graph of average absolute and percentage productions compared to the untreated control in the corn crop, at SCDA Braila

The average effect of nitrogen fertilization on production was the most increased in the corn crops at the dose of 180 kg/ha N s.a. with 80 kg/ha P s.a., followed in descending order by the dose of 240 kg/ha N s.a. with 120 kg/ha P s.a., and 120 kg/ha N s.a. with 40 kg/ha P s.a. (Figures 8, 9).

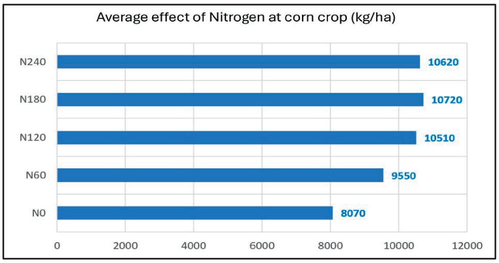

Figure 8. The graphs of average effect of Nitrogen fertilization at corn crop

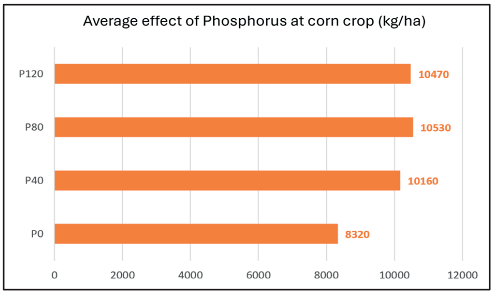

Figure 9. The graphs of average effect of Phosphorus fertilization at corn crop

The export of N P K macro elements in grain production was the highest when fertilized with P40-P80 and N120-N180, with N export values in grains between 113 kg/ha when fertilized with P40 and 115 kg/ha when fertilized with N120, followed in descending order by the export of P in grains between 69 kg/ha P2O5, when fertilized with P80 and 65 kg/ha when fertilized with N180 and N240, then by the export of K of 39 kg/ha K2O, when fertilized with N240 and 38 kg/ha K2O, when fertilized with P80-120 (Figures 10, 11).

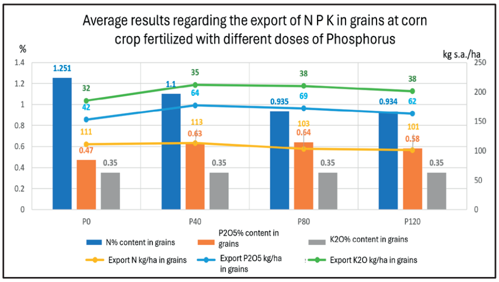

Figure 10. The effect of Phosphorus fertilization on NPK export in grains soybean at ARDS Braila

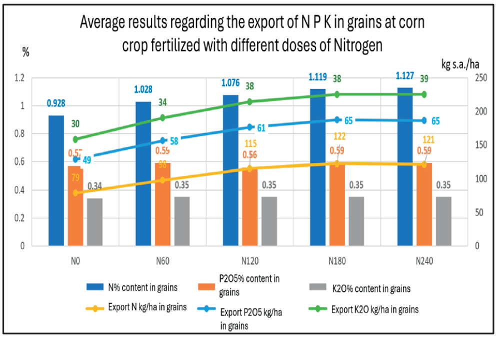

Figure 11. The effect of Nitrogen fertilization on NPK export in grains soybean at ARDS Braila

Regarding the effect of fertilization with nitrogen and phosphorus in long-term experiments, on the agrochemical indices of the soil, significant increases in the content of nitrogen, phosphorus and potassium were observed when applying the dose of 120 kg/ha P s.a. and 60 kg/ha N s.a., but the humus content was not influenced by the increase in the doses of chemical fertilizers (Figures 12, 13).

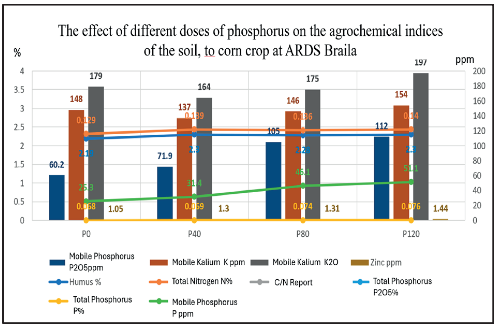

Figure 12. The average effect of chemical fertilization with phosphorus in long-term experiments, on the agrochemical indices of the soil, at ARDS Braila

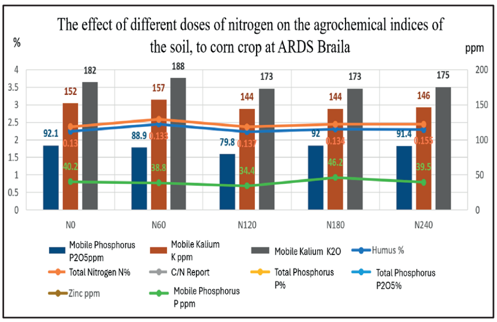

Figure 13. The average effect of chemical fertilization with nitrogen in long-term experiments, on the agrochemical indices of the soil, at ARDS Braila

In rice cultivation, the most important technology is on the plots with laser-guided machines, which ensure a very good levelling and a deviation from the average elevation of +/- 2-3 cm.

Therefore, the levelling creates possibilities to reduce the production cost through the economy due to the decrease in the thickness of the irrigation water layer. At the same time, the possibility of increasing the yields of the rice plots is created.

At the same time, merging the rice plots, by eliminating the ridges and bringing them to a level of 10 cm, the plots can be merged up to 4-8 hectares, and the application of crop rotation thus becomes more economically profitable.

Intelligent pumping systems, with minimal maintenance needs, sensors, and remote controls, can reduce electricity costs by 50% per total irrigation season.

The practice of crop rotation in rice cultivation technology has shown that production increases by 41% compared to monoculture rice. At the same time, the monoculture of rice affects the fertility of the soil and increases the maintenance cost of the crop.

CONCLUSIONS

The production increase in agricultural crops is conditioned by the application of mineral and organic fertilizers, in accordance with the pedological conditions and the specific consumption of the plants.

The protection, resilience and increase of soil fertility is done through a correct and rational application of fertilizers, to ensure the necessary plant production, but also to increase soil fertility.

In long-term experiences with mineral fertilizers, there is a tendency to reduce the content of C-organic, N-organic and humus, which is why the periodic use of organic fertilizers is recommended, C+NPK fertilization systems being much more sustainable (organic + NPK) than only NP or NPK.

Considering the current inflationary situation and the war in the neighbourhood, which will continue to have a negative impact on Romania's agriculture, the fertilization plan must be simplified, complying with the standard of the maximum amounts of nitrogen (170 kg/ha s.a.)

that can be applied on agricultural land and to ensure a uniform distribution of fertilizers on the land, to maintain soil fertility.

The calculation of the doses of fertilizers must be done correctly, both for economic reasons and for environmental protection requirements, the forecasted production must be realistic, considering both the local pedoclimatic conditions and the productive potential of the cultivated varieties and hybrids.

Currently, through the introduction of digital technologies in the agricultural field, which allow the scanning of lands with the help of satellites and drones, the application of fertilizers can be done variably, so that the crops grow uniformly over the entire surface, and the productions are increased and uniform, and the fertility of the soil to be durable.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Agriculture and Rural Development Romania, by Sectorial Plan ADER 2026, and was financed from the project: "Measures and recommendations for reducing the risk of salinization and soil erosion under the influence of climate change" – Contract No. ADER 20.1.3./17.07.2023.

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STUDY OF AGRONOMIC CHARACTERISTICS OF AN ASSORTMENT OF SWEET CORN HYBRIDS

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Abstract

Sweet corn is a relatively new crop for our country and with certain peculiarities compared to conventional corn cultivation technology. The aim of this study is to contribute to the improvement of cultivation technology in relation to pedoclimatic conditions in the Berzovia area, by studying the relationship between cob weight and production of sweet corn cobs according to the maturity group in an assortment of 11 sweet corn hybrids compared with the local hybrid Estival. The study was carried out in the Ramna area, characterized by an early spring, the minimum germination temperature of 10°C, taking place in the first days of April, a fact that allows obtaining early harvests. The results highlighted that the highest cob weight values were obtained in hybrids: Driver F1, Accentuate F1 and 11-Sweet Thing, between 336 and 368 g. Small cob weight values of approximately 232-255 g were obtained in hybrids the extra-early Estival and Spirit F1, the early Legend F1, Tyson F1, Starshine F1 and the semi-early hybrid Landmark F1. Regarding the production of sweet corn, SF201 F1, Jubilee F1, Driver F1, Accentuate F1 and Sweet Thing hybrids were well above the experience average in terms of cob production, surpassing the field average with gains between 2392.43 kg/ha and 5224.59 kg/ha, or in other words: the five hybrids surpassed the control by: 12.56%, 16.50%, 27.43%, 17.68%, respectively 16.76%.

Key words: *sweet corn, production, climatic conditions, vegetation period.*

INTRODUCTION

Arising as the result of a naturally occurring recessive mutation in the genes that control the conversion of sugar to starch within the endosperm, sweet corn (*Zea mays* L. *Saccharata* Sturt) is an important food grain and the second largest processing crop, second only to tomato (Mehta B.K. et al., 2017; Budak F. and Aydemir S.K., 2018; Soare, 2019).

The Iroquois gave the first recorded sweet corn (called "Papoon") to European settlers in 1779 (Williams M.M., 2008).

In the last decade we have seen an increase in consumer demand for fresh sweet corn between June and September, especially in the United States, where the vast majority of the world's acreage is grown, but also among consumers in Europe and Asia (Imbrea et al., 2014). Sweet corn has also become an important large-scale cash crop for export to Europe and other major world markets in temperate zones (David et al, 2006; Leneschi et al., 2018).

The production of sweet corn for the fresh market can be a profitable option for growers with small acreage, provided they can secure a good market and use their own labor (Imbrea et al., 2014).

Another advantage of this culture is represented by the short vegetation period and the fact that by choosing the assortment of hybrids the effects of climate change can be counteracted (Hatfield J.L. et al., 2011; Smuleac et al., 2020).

Another advantage of this culture is represented by the good utilization of chemical fertilizers (Esiyok D., 2004; Bozokalfa M.K., 2008; Amanullah A.M. et al., 2010; Bhatt P.S., 2012).

MATERIALS AND METHODS

The biological material was represented by an assortment consisting of 11 hybrids of sweet corn, with different vegetation periods and which are currently sold in our country.

The research was carried out on a eutric-gleic alluvial soil, moderately glaciated, extremely

deep, medium loam/coarse sandy loam, developed on medium (clay) non-carbonate fluvial deposits.
The experiment was set up according to the Latin rectangle method, and the observations were made at the waxy milk physiological maturity, at 10 plants/repetition, respectively 30 plants/experimental hybrid.
The observations were carried out at the waxy milk physiological maturity, at 10 plants/ repetition, respectively 30 plants/hybrid/ experimental year.

RESULTS AND DISCUSSIONS

The results regarding cob weight according to hybrid in the 2020-2021 experimental cycle are presented in Table 1 and Figure 1.
The 11 hybrids tested in the 2020-2021 experimental cycle, in terms of cob weight, had the following behavior, compared to the field average:
- Summer hybrids, Spirit F1, Legend F1, Tyson F1 and Starshine F1, and the hybrid Landmark F1 they had cob weight values below the average of the experience, that is, the difference from the average of the experience is negative;
- SF201 F1, Jubilee F1, Driver F1 hybrids, Accented F1 and Sweet Thingthey were higher than the experience average, they exceeded the field average with gains between 36.64-78.70 g, or in other words: the five hybrids exceeded the control by: 12.68%, 16.40%, 27.24%, 18.00%, and 16.41%, respectively.

Table 1. Results regarding the weight of the cob depending on the hybrid

A Hybrid factor	Cob weight		Difference [g]	Significance
	Mr	%		
a1. Summery	231.61	80.17	-57.28	000
a2. Spirit F1	243.15	84.17	-45.73	000
a3. Legend F1	255.31	88.38	-33.58	000
a4. Tyson F1	242.23	83.85	-46.65	000
a5. Starshine F1	251.49	87.05	-37.40	000
a6. SF201 F1	325.52	112.68	36.64	***
a7. F1 Jubilees	336.27	116.40	47.39	***
a8. Landmark F1	247.43	85.65	-41.45	000
a9. F1 driver	367.58	127.24	78.69	***
a10. Accented F1	340.83	117.98	51.95	***
a11. Sweet Thing	336.29	116.41	47.41	***
Field average	288.88	100.0	Mt	

DL 5% = 1.091 g; DL 1% = 1.483 g; DL 0.1% = 1.995 g.

Following the figure above, it can be seen that hybrid 9-Driver F1, 10-Accentuate F1 and 11-Sweet Thing have the highest values [336-368 g] compared to the other hybrids, and hybrid 1 - Estival has the lowest approximate value 232 g.

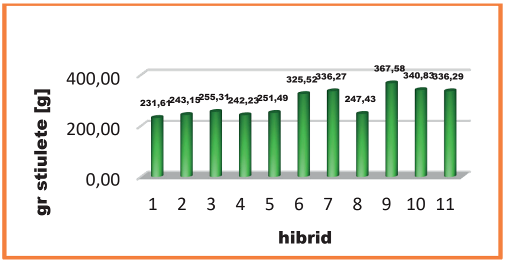

Figure 1. Variation of cob weight depending on hybrid

Low values of cob weight, approximately 232-255 g, were obtained in extra-early hybrids [1-Estival, 2-Spirit F1], early [3-Legend F1, 4-Tyson F1, 5-Starshine F1] and hybrid 8- Landmark F1-hybrid semi-early are inferior to the other hybrids studied.
The contribution of the experimental factors to the weight of the cob of sweet corn during the experimental period is presented in Figure 2. The factor A [year] contributes to the variation of the weight of the cob with 7.49%, factor B [hybrid] contributes with 92.3%, and the interaction A x B with 0.21%.

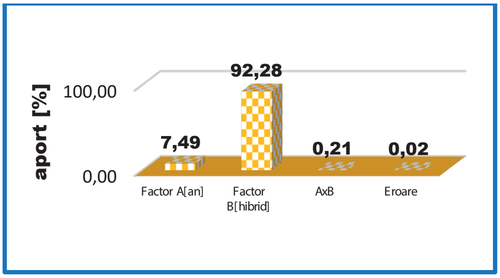

Figure 2. Contribution of factors A [year], B [hybrid] and A x B interaction

The results regarding the production of sweet corn cobs according to the hybrid in the experimental cycle 2020-2021, are presented in Table 2 and Figure 3.

Table 2. Results regarding the production of the cob

Factor A [hybrid]	Production		Difference [kg]	Significance
	kg/ha	%		
a1. Summery	15248.88	80.07	-3795.83	000
a2. Spirit F1	16016.28	84.10	-3028.43	000
a3. Legend F1	16812.76	88.28	-2231.94	000
a4. Tyson F1	16058.72	84.32	-2985.99	000
a5. Starshine F1	16484.26	86.56	-2560.45	000
a6. SF201 F1	21437.14	112.56	2392.43	***
a7. F1 Jubilees	22186.88	116.50	3142.18	***
a8. Landmark F1	16328.23	85.74	-2716.48	000
a9. F1 driver	24269.30	127.43	5224.59	***
a10. Accented F1	22412.57	117.68	3367.87	***
a11. Sweet Thing	22236.76	116.76	3192.05	***
Field average	19044.71	100.0	Mt	

DL 5% = 93,248 kg, DL 1% = 126,744 kg, DL 0.1% = 170,491 kg

The production of cobs in the 11 hybrids tested compared to the production of the control - the average of the field, presented the following behavior:
- Summer hybrids, Spirit F1, Legend F1, Tyson F1 and Starshine F1, and the hybrid 8 Landmark F1 they had production values below the average of the experience, i.e. the difference from the average of the experience is negative;

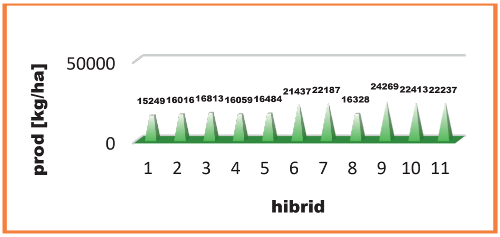

Figure 3. Variation of cob production depending on hybrid

- SF201 F1, Jubilee F1, Driver F1 hybrids, Accented F1 and 11 Sweet Thing were clearly higher than the average of the experience, they exceeded the average of the field with increases between 2392.43 kg/ha and 5224.59 kg/ha, or in other words: the five hybrids exceeded the control by: 12.56%, 16.50%, 27.43%, 17.68%, respectively 16.76%.
Following the figure above, it can be seen that the hybrid 9-Driver F1- 24270 kg/ha - semi-early hybrid has the highest value compared to the other hybrids, and the hybrid 1-Estival - 15250 kg/ha, has the lowest value.
The contribution of the experimental factors to the production of sweet corn cobs is presented in Figure 4.
The factor A [year] contributes to the production variation with 11.94%, the factor B [hybrid] contributes with 87.70%, the interaction A x B with 0.33%.
So the biggest contribution is the B [hybrid] factor, followed by the A [year] factor.

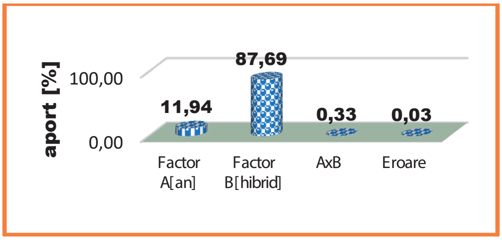

Figure 4. Contribution of factors A [year], B [hybrid] and A x B interaction

Calculating the correlation coefficient helps us establish the linear dependence between the two studied variables, while the regression coefficient a1 shows us how much the dependent variable [production] increases/ decreases by increasing the independent variable [cob weight] by one unit.

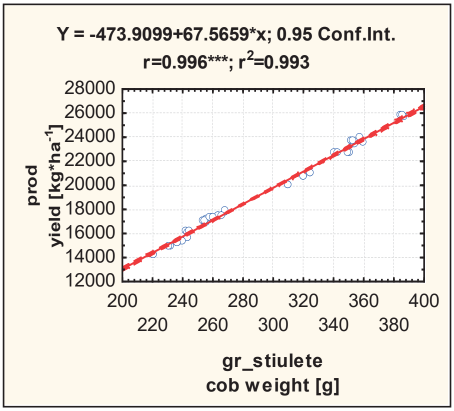

Figure 5. Correlation between cob weight and production

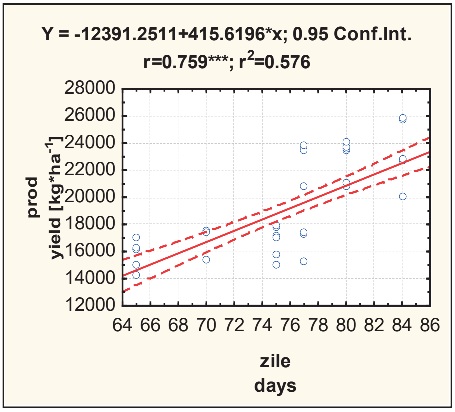

Figure 6. Correlation between the growing season of hybrids and production

Analyzing the values presented in Figures 5 and 6, it is found that cob production correlates positively with any of the 2 independent variables [cob weight and vegetation period], the correlation coefficients vary between 0.67 and 0.99, the correlation is from close to very closely, the coefficients are statistically assured at α = 0.001 level.
According to the coefficient of partial determination [d = r2*100], cob production was influenced by 99.3% by cob weight and 57.6% by vegetation period.

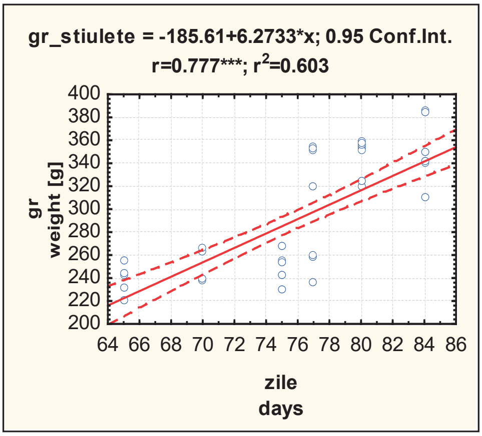

Figure 7. Correlation between growing season and cob weight

The data presented in Figure 7 show us that there is a positive correlation between the weight of the cob and the vegetation period, by increasing the value of one of them leads to the increase of the values of the other variable, i.e. the variables are directly proportional.

TEST DUNCAN pt α5% - factor B DL 5% = 90.50 kg

Date originale			Date sorate			
Mean 1 =	15250.	I	Mean 9 =	24270.	A	Mean1 b1-Estival
Mean 2 =	16020.	H	Mean 10 =	22410.	B	Mean2 b2-Spirit F1
Mean 3 =	16810.	E	Mean 11 =	22240.	C	Mean3 b3-Legend F1
Mean 4 =	16060.	H	Mean 7 =	22190.	C	Mean4 b4-Tyson F1
Mean 5 =	16480.	F	Mean 6 =	21440.	D	Mean5 b5-Starshine F1
Mean 6 =	21440.	D	Mean 3 =	16810.	E	Mean6 b6-SF201 F1
Mean 7 =	22190.	C	Mean 5 =	16480.	F	Mean7 b7-Jubilee F1
Mean 8 =	16330.	G	Mean 8 =	16330.	G	Mean8 b8-Landmark F1
Mean 9 =	24270.	A	Mean 4 =	16060.	H	Mean9 b9-Driver F1
Mean 10 =	22410.	B	Mean 2 =	16020.	H	Mean10 b10-Accentuate F1
Mean 11 =	22240.	C	Mean 1 =	15250.	I	Mean11 b11- Sweet Thing

Figure 8. Duncan test for B factor

Following the 55 comparisons [C112], classes A - I were obtained.

The highest production value - 24270 kg/ha, was obtained at b9[Driver F1] - class A, which differs significantly from other hybrids.

The lowest value of 15250 kg/ha was obtained at b1 [Estival] - class I, a value that differs significantly from the other hybrids.

It should be noted that in the case of hybrids:

- **b11** [Sweet Thing] and b7 [Jubilee F1], are part of the same homogeneity class - class C. We can say that the hybrid 11 [Sweet Thing] and the hybrid 7 [Jubilee F1], do not differ significantly from each other, i.e. in the two hybrids, a homogeneous production value of approximately 22,200 kg/ha is obtained
- **b2** [Spirit F1] and b4 [Tyson F1], are part of the same homogeneity class - class H. We can say that the Spirit F1 hybrid and the Tyson F1 hybrid do not differ significantly from each other, i.e. the two hybrids have a homogeneous value of the production of 16600 kg/ha.

TEST DUNCAN pt α5% - interacțiunea AxB DL 5% = 128 kg

Mean 1 =	14310.	P	Mean 20 =	25780.	A	Mean1 a1b1-Estival
Mean 2 =	15010.	O	Mean 21 =	23810.	B	Mean2 a1b2-Spirit F1
Mean 3 =	15760.	L	Mean 22 =	23650.	C	Mean3 a1b3-Legend F1
Mean 4 =	15010.	O	Mean 18 =	23590.	C	Mean4 a1b4-Tyson F1
Mean 5 =	15460.	M	Mean 17 =	22770.	D	Mean5 a1b5-Starshine F1
Mean 6 =	20110.	G	Mean 9 =	22760.	D	Mean6 a1b6-SF201 F1
Mean 7 =	20790.	F	Mean 10 =	21010.	E	Mean7 a1b7-Jubilee F1
Mean 8 =	15270.	N	Mean 11 =	20830.	F	Mean8 a1b8-Landmark F1
Mean 9 =	22760.	D	Mean 7 =	20790.	F	Mean9 a1b9-Driver F1
Mean 10 =	21010.	E	Mean 6 =	20110.	G	Mean10 a1b10-Accentuate F1
Mean 11 =	20830.	F	Mean 14 =	17870.	H	Mean11 a1b11- Sweet Thing
Mean 12 =	16190.	K	Mean 16 =	17510.	I	Mean12 a2b1-Estival
Mean 13 =	17030.	J	Mean 19 =	17390.	I	Mean13 a2b2-Spirit F1
Mean 14 =	17870.	H	Mean 15 =	17100.	J	Mean14 a2b3-Legend F1
Mean 15 =	17100.	J	Mean 13 =	17030.	J	Mean15 a2b4-Tyson F1
Mean 16 =	17510.	I	Mean 12 =	16190.	K	Mean16 a2b5-Starshine F1
Mean 17 =	22770.	D	Mean 3 =	15760.	L	Mean17 a2b6-SF201 F1
Mean 18 =	23590.	C	Mean 5 =	15460.	M	Mean18 a2b7-Jubilee F1
Mean 19 =	17390.	I	Mean 8 =	15270.	N	Mean19 a2b8-Landmark F1
Mean 20 =	25780.	A	Mean 4 =	15010.	O	Mean20 a2b9-Driver F1
Mean 21 =	23810.	B	Mean 2 =	15010.	O	Mean21 a2b10-Accentuate F1
Mean 22 =	23650.	C	Mean 1 =	14310.	P	Mean22 a2b11-Sweet Thing

Figure 9. Duncan test for A x B interaction

From the same homogeneity class [that is, similar values were obtained in the respective hybrids] are:

- Mean22 a2b11-Sweet Thing and Mean18 a2b7-Jubilee F1 [hybrid 11, year 2021 and hybrid 7 year 2021], about 23600 kg/ha - class C;
- Mean17 a2b6-SF201 F1 and Mean9 a1b9-Driver F1 [hybrid 6, year 2021 and hybrid 9 year 2020] with production of 22770 kg/ha-class D;
- Mean11 a1b11- Sweet Thing [Sweet Thing, year 2020] and Mean7 a1b7 - Jubilee F1 [Jubilee F1, year 2020], belong to the same homogeneity class - class F. We can say that hybrid 11 [Sweet Thing] and hybrid 7, [Jubilee F1], do not differ significantly from each other, i.e. the two hybrids obtain a homogeneous production value of 20800 kg/ha in 2020;
- Mean16 a2b5-Starshine F1 and Mean19 a2b8-Landmark F1 [hybrid 5, year 2021 and hybrid 8 year 2021], i.e. hybrid 5 Starshine F year 2021 and hybrid 8-Landmark F1 year 2021 gave a similar yield of 17400 kg/ha - first class;
- Mean13 a2b2 - Spirit F1 and Mean15 a2b4 - Tyson F1, belong to the same homogeneity class - class J. We can say that the Spirit F1 hybrid and the Tyson F1 hybrid do not differ significantly from each other, that is, the two hybrids have a homogeneous value of production, approximately 17000 kg/ha, in 2021.
- Mean2 a1b2-Spirit F1 and Mean4 a1b4-Tyson F1, are part of the same homogeneity class - class O. So the Spirit F1 hybrid and

the Tyson F1 hybrid do not differ significantly from each other, that is, the two hybrids have a homogeneous production value of 15000 kg/ha, in 2020.

CONCLUSIONS

The success of a sweet corn crop depends on the choice of an assortment of hybrids with different vegetation periods, which allow production to be staggered and ensure availability for the market in the months of June-August, the period in which the highest demand for fresh consumption is met

The highest cob weight values were obtained in the hybrids: Driver F1, Accentuate F1 and 11-Sweet Thing, between 336 and 368 g. Low cob weight values of approximately 232-255 g were obtained in the hybrids the extra-early Estival and Spirit F1, the early Legend F1, Tyson F1, Starshine F1 and the semi-early hybrid Landmark F1.

The contribution of the experimental factors to the realization of the sweet corn cob weight during the experimental period showed that Factor A [experimental year] contributes to the variation of the cob weight by 7.49%, factor B [the experimented hybrid] contributes by 92.3%, and the A x B interaction by 0.21%.

The largest cob production of 24270 kg/ha was achieved with the Driver F1 semi-early hybrid, and the lowest of 15250 kg/ha, with the Estival hybrid.

The contribution of the experimental factors to the realization of the production of sweet corn cobs was in percentage of 11.94% for Factor A [experimental year], 87.70% for factor B [experimental hybrid] and 0.33% for the A x B interaction.

Among the 11 hybrids tested, the semi-early hybrid Driver F1 had the best performance both in terms of agronomic characteristics and cob production.

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THE EFFECT OF CROP ROTATION AND SEED INOCULATION ON SOYBEAN YIELD, YIELD ELEMENTS AND SEED QUALITY

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Abstract

In order to evaluate the effect of crop rotation and seed inoculation on soybean yield, yield elements and seed quality, an experiment was conducted in Amati village, Satu Mare County, in 2020, using three early soybean varieties (Onix, Felix, Cristina TD). Crop rotation (two graduations) and seed treatment (three graduations) factors were studied. The experiment was based on a split plot design. Yield, yield elements (plant size, insertion of the first pod, number of pods/plant, number of grains/plant, weight of grains/plant, size of grains and seed quality (protein and fat) were determined, highlighting the influence of genotype and of seed treatment on studied parameters. In terms of crop rotation, generally no influence was identified. Cristina TD was a high yielding genotype with 5361 kg/ha obtained in the experimental variant when soybean was included in rotation and Nitragin product was applied.

Key words: *crop rotation, inoculation, quality, soybean, yield.*

INTRODUCTION

The global climate scenario faces a drastic depletion of soil nutrients due to various anthropogenic activities, burning of fossil fuels and excessive use of agrochemicals (Sarfraz et al., 2019), and the use in agricultural systems of legumes living in symbiosis with nitrogen-fixing bacteria can be a good way to increase soil fertility and production.
Soybean is the most important protein (Joiţa-Păcureanu et al., 2023; Badea et al., 2023) and oil plant in the world, but Europe relies mainly on imports due to the small area of legume cultivation (only 1.5% of agricultural land) compared to the global needs (Watson et al., 2017).
Due to its widespread use in many industries, soybeans are classified as a strategic crop (Guzeler and Yildirim, 2016). The value of soybean seeds depends on their oil content and fatty acid composition (Carrera and Dardanelli, 2017; Kurt and Baloch, 2023). The chemical composition of seeds is primarily genetically determined (Tamagno et al., 2020) but also by environmental and technological factors that intervene in soybean production.
Soybean yield depends largely on the availability of nitrogen that affects plant growth and development (Salvagiotti et al., 2008), but through its ability to absorb atmospheric nitrogen, soybean becomes an important crop in agricultural rotations (Moldovan et al., 2022; Ionescu et al., 2022).
Due to the growing interest in biological methods to improve soil fertility in recent years as well as increasing crop production and quality, interest in inoculating soybeans with bacteria is growing (Fatima et al., 2007). Inoculation of seeds with symbiotic bacteria, especially in soils naturally lacking them, but also optimal fertilization with nutrients are considered particularly important elements in soybean cultivation (Jarecki, 2023) and Salvagiotti et al. (2008) argued that when cultivating high-yielding varieties, fertilization with a small dose of nitrogen might be necessary. Inoculation of soybeans with bacteria of the genus *Bradyrhizobium japonicum* in combination with the optimal

level of fertilization results in the most effective biological fixation of N (Grossman et al., 2011).
Although soybean is a crop that requires fertilization in the early stages of development, until the formation of active nodosities, a high amount of N can slow down the process of symbiosis. The activity of developing and fixing nitrogen by the nodules on the roots is suppressed when the nodulated roots are exposed to a high concentration of nitrogen. Mendes et al. (2003) reported that the average number of nodules was 50% lower for plants to which an additional 40 kg/ha N was applied.
To obtain a high soybean yield, a good nodulation and a high and long-lasting activity are very important, because the availability of nitrogen in the soil is generally insufficient to support the growth of soybeans, and mineral nitrogen is lost within a few weeks of application (Șimon et al., 2022).
In the current context of climate change and the need to reduce the effects of global warming, the implementation of crop systems that reduce the amount of chemical fertilizers and have a positive impact on long-term agricultural sustainability (Lemessa and Wakjira, 2015) is of real importance.

MATERIALS AND METHODS

In order to evaluate the influence of crop rotation, inoculant with bacteria applied to soybeans seeds before sowing, and genotype on yield and quality of this crop, a polifactorial experiment (A x B x C) was conducted in 2020, in Amati village, Satu-Mare County.
The agricultural area of the experiment is characterized by lower fertility potential for soil with a clay-loam texture.
The biological material studied consisted of three commercial soybean varieties created at the Agricultural Research and Development Station (ARDS) Turda. Felix, Onix and Cristina TD are early soybean varieties that are cultivated on large areas in Romania.
The experiment was conducted according to the method of split plot design, each experimental variant having an area of 700 m^2.
The varieties were sown in two different rotations, a rotation in which soybeans was included five years ago and a rotation in which the soybean crop followed after four years of fodder plants.
The seed inoculation factor with products based on nitrogen-fixing bacteria had the following three graduations: the control variant (without treatment), the variant in which Poliriz S was applied and the variant in which the product Nitragin was applied. The application of the products was carried out on the day of sowing, respecting the recommendations of the companies.
Mechanized sowing of Felix, Onix and Cristina TD soybean varieties, with narrow row spacing and chemical weed control by using pre-emergence and post-emergence herbicides were practiced.
At the end of the growing season, 10 plants from each experimental variant were invasively analyzed for: plant size, insertion of the first pod, number of pods/plant, number of grains/plant, weight of grains/plant, size of grains (TKW). The yield was estimated based on the weight of grains /plant and number of plants/m^2, at the end of the maturity.
For each experimental variant, the protein and fat content of soybeans was determined using a near-infrared method.
The experimental data were statistically processed using Polifact program for Anova, Past4 for chemometric analysis and Pearson coefficients, respectively Excel for graphic presentation.

RESULTS AND DISCUSSIONS

Worldwide, in the context of current climate change, soybean breeding programs have as a priority the creation of high-performance varieties, adapted to environmental conditions, which meet the dynamic requirements of the market. Also, an important aspect pursued in increasing productivity is represented by the improvement of crop technologies applied to this crop plant. Based on data collected (https://www.meteoblue.com/ro) the temperatures recorded in the experimental field in 2020 (Figure 1), close to the multiannual average in the summer months, as well as the rainfall in the same calendar period positively influenced the reproductive stages of soybean plants and, of course, the yield obtained in the three soybean varieties analyzed.

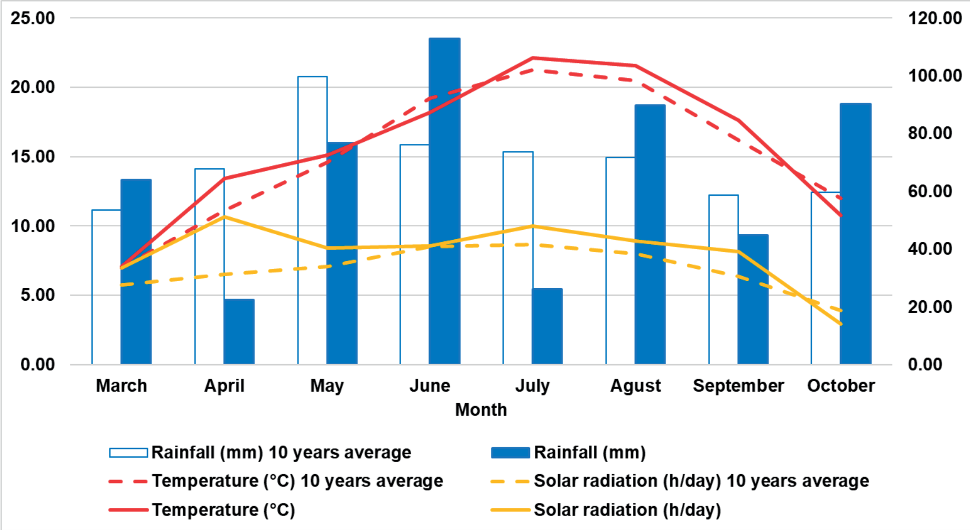

Figure 1. Monthly temperature, solar radiation average and rainfall in Amați (2020) compared to the ten year average (2010-2019)

Based on the values obtained in F test, the influence of each factor analyzed during the experiment on: production, plant height, insertion of the first pod, number of pods/plant, number of grains/plant, weight of grains/plant and thousand kernel weight was established (Table 1). It can be seen that, in general, production and productivity elements did not vary according to crop rotation, but were very significantly influenced by genetic factor (soybean genotype). In terms of seed inoculation factor, it seems that it had a very significant influence on production, insertion of the first pod, number of pods and grains/plant and on the size of grains.

The results obtained by Szpunar-Krok et al. (2023) show that inoculation of seeds with nitrogen-physicalizing bacteria resulted in a higher number of seeds and a higher seed weight/plant compared to uninoculated seeds, with the use of seeds inoculated with Nitragin resulting in an increase in seed number and weight per plant by up to 3.9% and 2.7%, respectively, compared to uninoculated seeds, however, there was no statistically significant effect of inoculation on the value of the obtained mass of 1000 seeds.

Other authors such as Namozov et al. (2022) showed that inoculation increased the number of pods by 16.5%, the number of grains/pod by 14.3%, the weight of pods by 20.7%, and the MMB by 10.5%, compared to the variant without inoculation.

The soybean yield varied depending on the genotype studied, the Cristina TD variety being the most productive, regardless of the rotation studied or the type of inoculation experimented. In both crop rotations, the application of seed treatment with products containing nitrogen-fixing bacteria led to higher yields than the control variant. It would seem that for all varieties the highest yields were obtained in the experimental variant in which seed treatment with Nitragin product was applied.

When soybean was included in crop rotation (Figure 2), the obtained yield varied between 4181 kg/ha (Felix) and 5361 kg/ha (Cristina TD, in the experimental variant where Nitragic was applied). For Felix variety were obtained similar values for yield, regardless of seed treatment. When soybean was new in crop rotation (Figure 3), the obtained yield varied between 4228 kg/ha (Felix) and 5232 kg/ha (Cristina TD, in the experimental variant where Nitragic was applied). Experimental date highlights the yields obtained in the Cristina TD variety, which had a small variation depending on the inoculant used.

Table 1. ANOVA Test for yield and yield elements as influenced by crop rotation, seed inoculant and soybean genotype

Cause of variability	Yield	Height	Insertion	Number of pods/plant	Number of seeds/plant	Seed weight/ plant	TKW MMB
Rotation (A)	ns	ns	*	***	ns	ns	ns
Seed inoculation (B)	***	ns	***	***	***	ns	***
A x B	***	ns	***	***	***	ns	ns
Soybean genotype (S)	***	***	***	***	***	***	***
A x S	***	ns	ns	***	***	ns	ns
B x S	***	ns	**	***	***	ns	ns
A x B x S	***	ns	ns	***	***	ns	ns

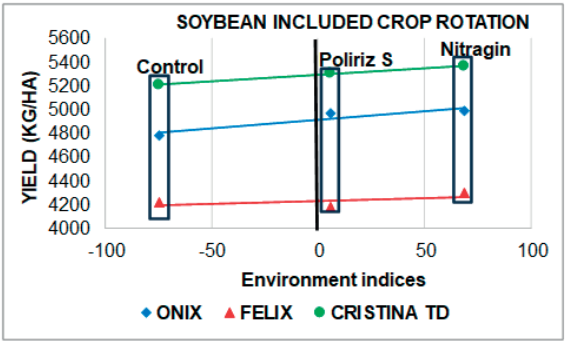

Figure 2. Yield obtained at three soybean varieties depending on seed inoculation

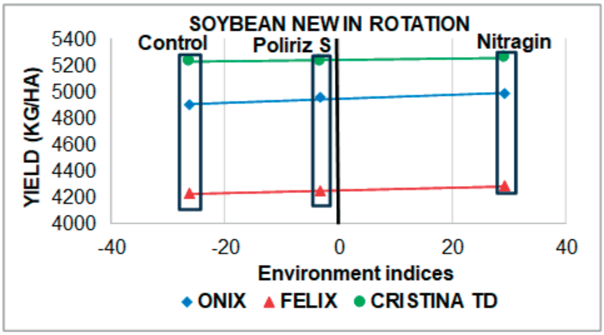

Figure 3. Yield obtained at three soybean varieties depending on seed inoculation

Because crop rotation did not have an influence on obtained yield and productivity elements analyzed, only the results obtained in the experimental variant in which soybean was included in the five years rotation will be following presented in the paper. The principal component analysis (PCA), confirmed by the cluster analysis (Figure 4) point out the experimental variant in which the product Nitragin was applied, with high values obtained for most of the agronomic parameters: production (4843 kg/ha), plant size (117 cm), insertion of the first pod (17 cm), number of pods/plant (38), number of seeds/plant (97), weight of grains/plant (16.32 g), thousand kernel weight (170 g). Similar results were obtained by Vollmann et al. et al. (2011), which confirmed that nodulation had a significant effect on biometric traits of plants, including some yield components.

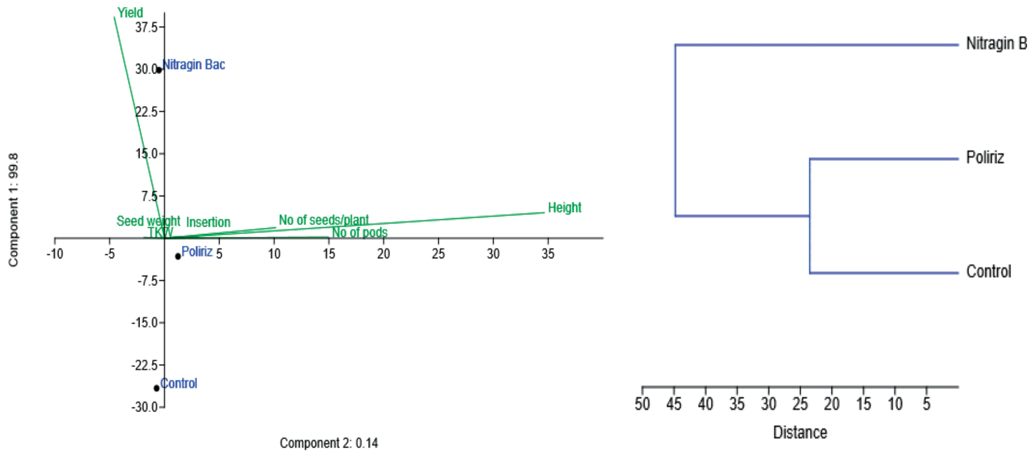

Figure 4. Principal component analysis (PCA) and Cluster analysis

Based on the plot of correlations (Figure 5), the close positive relationship between grain yield and: plant height (r = 0.96), grain weight/plant (r = 0.98) and grain size (r = 0.89) are highlighted. Regarding the negative correlations established between the studied parameters, it is noted the relation between production and protein content (r = -0.62), oil content and number of pod/plant (r = -0.98), respectively between TKW and protein content (r = -0.91).

Data obtained by Szpunar-Krok et al. (2023) in a similar experiment showed that soybean production was positively correlated with plant density after sprouting, weight of 1000 grains, and seed/plant mass. Other authors stated that next to production elements that vary depending on factors that intervene during the growing season, the protein and oil content is strongly influenced by environmental conditions (Popa et al., 2023).

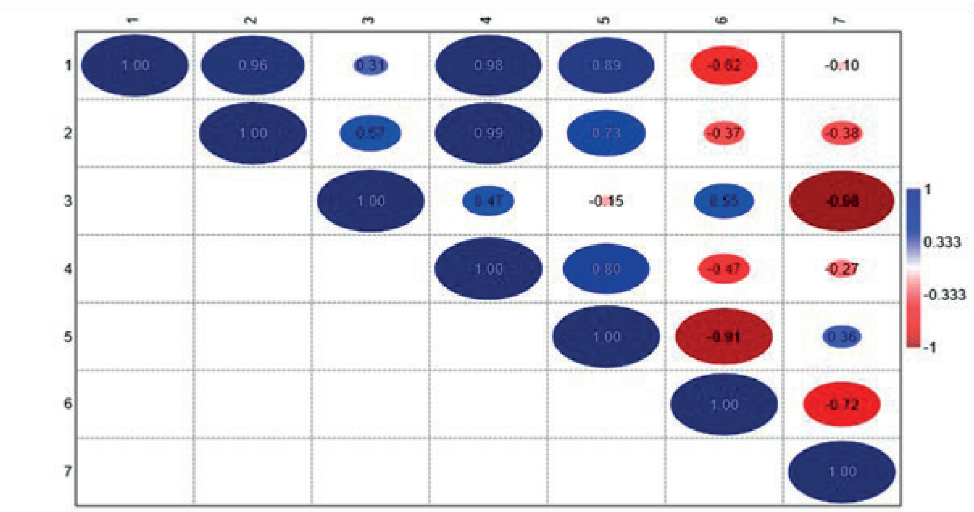

Figure 5. Person coefficient analysis between yield (1), yield elements [height (2), no of pods/plant (3), seed weight/plant (4), TKW (5)) and seed quality (protein (6), oil (7)], when soybean was included in crop rotation

CONCLUSIONS

The results obtained in this study indicated that, soybean yield could be increased by applying bacteria inoculation regardless of crop rotation.

Inoculant applied to soybean seeds determined different results for studied varieties in terms of yield and yield elements. A positive yield response to Nitragin seed inoculant when soybean was included in 5 years crop rotation was observed at Cristina TD variety with the highest value for this parameter (5361 kg/ha). A slightly increase, a lack of response or negative influence on yield has been observed with seed inoculants applications to Felix soybean variety.

The variety Cristina TD in combination with Nitragin BAC inoculant, in both crop rotations, can be recommended for cultivation in the North West part of Romania.

In terms of Pearson coefficient, were identified close positive relationship between grain yield and: plant height (r=0.96), grain weight/plant (r=0.98) and grain size (r=0.89) are highlighted. Regarding the negative correlations established between the studied parameters, it is noted the relation between production and protein content (r = -0.62), oil content and number of pod/plant (r = -0.98), respectively between TKW and protein content (r = -0.91).

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THE INFLUENCE OF TILLAGE SYSTEM AND FERTILIZATION ON TWINNING AND THE OBTAINING OF PRODUCTIVE SIBLINGS IN THE GRAIN SORGHUM IN THE CONDITIONS OF SĂRĂŢENI, IALOMIŢA

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Abstract

The lack of precipitation puts more and more emphasis on the cultivation technology of the agricultural land, for which it is necessary to adapt the cultivation technology according to the climatic changes. Sorghum can be a substitute for corn when conditions do not allow its cultivation. The number and quality of the productive brothers obtained in the sorghum culture depends a lot on the pedoclimatic conditions, but also on the technology applied in the culture. Our research aimed to evaluate the twinning potential of sorghum under the conditions of South-East Romania (Sărăţeni Locality, Ialomiţa County), analyzing the interaction between different variants of the basic soil work and some fertilization funds based on nitrogen, phosphorus and foliar fertilizers. In this way, it will be possible to establish the optimal variants for stimulating twinning, but also obtaining productive brothers who have reached maturity. The results of the three years of research show that they were different in terms of precipitation, 2021 being a year with high precipitation and 2022-2023, two years with a water deficit, so that the influence of climatic conditions on twinning can be observed. The deeper the loosening of the soil was, the higher the twinning was, tillage by scarification at 35 cm and at 45 cm excelled at all four fertilizations, thus having the highest growth rates of the number of productive siblings in sorghum culture. The most favorable combination of technological factors that ensured a maximum twinning yield in 2022 of 3.6 productive brothers per plant, was represented by tillage by scarifying at 35 cm and a fertilization of N_{100}*+*P_{50}*+Foliar (Borocal).*

Key words: *Sorghum bicolor L., soil tillage, fertilization, twinning, productive siblings.*

INTRODUCTION

Sorghum is the fifth most produced cereal crop after maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) globally (FAOSAT).

It is a staple food crop in Africa and Asia and a vital source of industrial feedstock for the manufacture of feed, bioethanol and syrup (Hossain et al., 2022).

Sorghum is not pretentious to the preceding plant, succeeding even in monoculture. It is not recommended to cultivate it in repeated culture because it leads to a sharp impoverishment of the soil in the main nutritional elements. Water stress, poor soils and reduced fertilization are the main yield-limiting factors (Aleminew, 2015).

The reduced requirements for the preceding plant are explained by its ability to take both water and nutrients from the soil. Fertilization is an essential technological factor in improving yield (Oprea et al., 2016). Fertilization with nitrogen and phosphorus has a positive influence on the number of shoots/ha, but large sowing plots cause their number to decrease (Pochiscanu, 2015). In favorable humidity conditions, sorghum reacts favorably to nitrogen fertilization both in terms of yield and protein content, and in dry areas phosphorus has a positive effect (Coclea et al., 2013). Along with the deeper cultivation of the soil, the height of the sorghum plants also increases, regardless of the way in which it was fertilized (Bănică, 2023).

Research on the influence of culture technology on twinning, carried out under different conditions, shows that it reacts favorably to mineral fertilization (Oprea et al., 2017). Sorghum requires well-drained and deep fertile soils. This will improve root growth and expansion for better absorption of moisture into the soil, the best soil pH being 5.4-8.4. (https://www.yara.co).

In this context, the objective of the research carried out and provided in the paper is to take into account the main technological elements that influence the formation of productive brothers in the culture of sorghum for grains: the basic work of the soil and fertilization with a view to an optimal level of yield in correlation with the conditions from the town of Sărăţeni, Ialomiţa county.

MATERIALS AND METHODS

The researches were carried out in the town of Sărăţeni-Ialomiţa county (44°38′11″N 26°55′41″E) between the years 2020-2023 within the Bănică Ion Individual Enterprise on a cambic chernozem.

The rainfall regime was high in 2021, with a total of 673.6 mm, thus favoring obtaining a high production through the high number of siblings reaching maturity. Both at sowing and in the twinning phases, there was an amount of precipitation above normal. During the period of vegetative development of plants (May-August), 283.4 mm were recorded in 2021, and 96 mm in 2022, the difference between the two years being 184 mm (Table 1).

The year 2023 was an average year between the two years, accumulating 177 mm.

The average monthly temperature for the period of vegetative growth is 20.4°C, and the average for the period 2020-2023 is 21.5°C, which shows us that the studied area is consistent with global warming.

Table 1. Climatic conditions during sorghum plant's vegetative period at Sărăţeni-Ialomiţa

	Temperature (ºC)				Rainfall (mm)			
	Normal (1981-2010)	2020-2021	2021-2022	2022-2023	Normal (1981-2010)	2020-2021	2021-2022	2022-2023
May	17.6	17.0	17.8	16.8	50.5	36.0	15.0	43.6
Jun	21.4	20.7	22.7	21.1	71.2	175.0	26.0	42.6
July	23.2	24.9	25.5	25.7	61.9	33.0	31.0	46.2
August	22.6	23.6	24.8	25.9	46.8	36.0	24.0	32.4
September	17.3	16.8	17.2	21.5	47.8	3.4	0.0	12.2
Avg./Sum	20.4	20.6	21.6	22.4	278.2	283.4	96	177

The experiment was placed in randomized blocks, in 3 repetitions with the analyzed factors: Factor A - basic soil work with graduations: a_1 - Plow at 25 cm; a_2 - Scarified at 35 cm; a_3 - Scarified at 45 cm; a_4 - Disc 10 cm; and factor B - fertilizations with graduations: b_1 - N_0P_0 (Control); b_2 - $N_{100}P_0$; b_3 - $N_{100}P_{50}$; b_4 - $N_{100}P_{50}$ + Foliar; b_5 - Borocal 1.5 l/ha (Foliar).

The biological material studied was the hybrid ES Abanus, a simple hybrid with white grains, excellent vigor at the start of vegetation with a compact panicle and very good tolerance to drought and shaking.

It shows high tolerance to *Fusarium*, the plant size is small with very good resistance to falling.

Tillage and fertilization were applied according to the graduations.

The predecessor plant was corn.

Chemical fertilizers were administered before sowing, and foliar fertilization was applied to the vegetation.

Sowing was carried out at a density of 230.000 grains/ha, and after sowing pre-emergent herbicide was used with the herbicide Dual Gold 960 EC in a dose of 1.2 l/ha.

RESULTS AND DISCUSSIONS

The influence of fertilization and tillage on the initial number of siblings

The ability to twin is a hereditary characteristic, but it varies a lot depending on the vegetation and climatic conditions, at the same time it is an important element of productivity. In the research carried out, the fertilizations applied in the sorghum culture stimulated twinning regardless of the method of tillage, with increases between 0.3 brothers (Foliar - Borocal 1.5 l/ha - Plow 25 cm) and 2.3 brothers (b_4 - $N_{100}P_{50}$ - a_2 - Scarified 35 cm) (Table 2). The most pronounced twinning was obtained by applying a fertilization based on $N_{100}P_{50}$ and loosening the soil at 35 cm. The nitrogen deficiency delays the growth of the plants, thus reducing the number of siblings. Foliar fertilizations resulted in reduced twinning, the minimum was 1.7 brothers per plant in the variant where the soil was tilled and 2.1 brothers per plant, where the soil benefited from a scarification at 45 cm superficial work with the disc, the twinning manifested itself in an excellent way with a number of 2.8 siblings per plant in the variants fertilized with $N_{100}P_0$ and 3.4 siblings in the variants with N_{100} P_{50}.

Tilling the soil scarification at 35 and 45 cm favored twinning in the sorghum culture, compared to the control plowed at 25 cm compared to which there was a difference of 0.6 and 0.9 brothers per plant in the agrofund $N_{100}P_{50}$ + Foliar, with an average of 3.8 and 3.5 brothers (Table 3).

Table 2. The influence of agrofund and tillage on the initial number of siblings (2021-2023 average)

Variant	b_1 - N_0P_0			b_2 - $N_{100}P_0$			b_3 - $N_{100}P_{50}$			b_4 - $N_{100}P_{50}$ + Foliar			b_5 - Foliar (Borocal 1.5 l/ha)		
	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.
a_1 - Plow 25 cm	**1.4**	100	C	**2.3**	164.2	0.9	**2.6**	185.7	1.2	**2.9**	207.1	1.5	**1.9**	135.7	0.3
a_2 - Scarified 35 cm	**1.4**	100	C	**3.**	214.2	1.6	**3.1**	221.4	1.7	**3.8**	271.4	2.4	**2.1**	150	0.7
a_3 - Scarified 45 cm	**1.5**	100	C	**2.7**	180	1.3	**2.9**	193.3	1.4	**3.5**	233.3	2	**2.1**	140	0.6
a_4 - Disc 10 cm	**1.1**	100	C	**2.8**	254.5	1.7	**2.8**	254.5	1.7	**3.4**	309	2.3	**1.7**	154.5	0.6

Table 3. The influence of the basic soil work on the initial number of brothers by different types of fertilization (2021-2023)

Variant	b_1 - N_0P_0			b_2 - $N_{100}P_0$			b_3 - $N_{100}P_{50}$			b_4 - $N_{100}P_{50}$ + Foliar			b_5 - Foliar (Borocal 1.5 l/ha)		
	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.
a_1 - Plow 25 cm	**1.4**	100	Ct	**2.3**	100	Ct	**2.6**	100	Ct	**2.9**	100	Ct	**1.9**	100	Ct
a_2 - Scarified 35 cm	**1.4**	100	0	**3.**	130.4	0.7	**3.1**	119.21	1.7	**3.8**	131	0.9	**2.1**	110.51	0.2
a_3 - Scarified 45 cm	**1.5**	107.1	0.1	**2.7**	117.3	0.4	**2.9**	111.5	0.3	**3.5**	120.6	0.6	**2.1**	10.5	0.2
a_4 - Disc 10 cm	**1.1**	78.5	-0.3	**2.8**	121.7	0.6	**2.8**	107.6	0.2	**3.4**	117.2	0.5	**1.7**	89.4	- 0.2

The influence of fertilization and tillage on the number of productive brothers

Even if sorghum had a high capacity for twinning, not all the siblings that appeared in the first phases of vegetation reached maturity and were productive. The dynamic increase in the number of panicles was not as pronounced as that of the number of siblings per plant. In the case of the variants without fertilization, regardless of the method of tillage, the maximum number of siblings that reached maturity was 0.4 (Scarified 35 cm).

Fertilization based on $N_{100}P_{50}$ influenced the twinning productivity with a minimum of 1.1 panicles when the soil was worked superficially by discus and a maximum of 2 productive brothers by scarification at 45 cm of the land.

Fertilizations based on foliar fertilizers were above the non-fertilized controls, but with the lowest values among all the fertilization options. The maximum number was recorded by tilling the soil at 35 cm: 0.6 productive brothers (Table.4).

Table 4. The influence of the agrofund and tillage on the number of productive brothers (2021-2023 average)

Variant	b_1 - N_0P_0			b_2 - $N_{100}P_0$			b_3 - $N_{100}P_{50}$			b_4 - $N_{100}P_{50}$ + Foliar			b_5 - Foliar (Borocal 1.5 l/ha)		
	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.
a_1- Plow 25 cm	**0.3**	100	C	**1.1**	366.6	0.8	**1.4**	466.6	1.1	**1.6**	533.3	1.3	**0.5**	166.6	0.2
a_2-Scarified 35 cm	**0.4**	100	C	**1.6**	400	1.2	**1.9**	475	1.5	**2.1**	525	1.7	**0.6**	150	0.2
a_3-Scarified 45 cm	**0.3**	100	C	**1.6**	533.3	1.3	**2**	666.6	1.7	**2.1**	700	1.8	**0.4**	133.3	0.1
a_4-Disc 12 cm	**0.2**	100	C	**0.9**	450	1.2	**1.1**	550	0.9	**1.2**	600	1	**0.3**	150	0.1

Analyzing the soil tillage, with the deeper loosening, the number of productive panicles per plant was continuously increasing. Fertilization with nitrogen, phosphorus, foliar fertilizers, together with scarification at 45 cm of the land brought the highest increase of productive brothers - 0.6 brothers.

The variant with the best yield in terms of productive brothers reaching maturity was the scarification of the land at 45 cm along with a fertilization with $N_{100}P_{50}$ + Foliar, thus obtaining 2.1 productive brothers on average per plant (Table 5).

Table 5. The influence of fertilization and tillage on the number of productive brothers (2021-2023 average)

Variant	b_1 - N_0P_0			b_2 - $N_{100}P_0$			b_3 - $N_{100}P_{50}$			b_4 - $N_{100}P_{50}$ + Foliar			b_5 - Foliar (Borocal 1.5 l/ha)		
	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.	No.	%	Dif.
a_1 - Plow 25 cm	**0.3**	100	Ct	**1.1**	100	Ct	**1.4**	100	Ct	**1.6**	100	Ct	**0.5**	100	Ct
a_2 - Scarified 35 cm	**0.4**	133.31	0.1	**1.6**	145.4	0.5	**1.9**	135.7	0.5	**2.1**	131.2	0.5	**0.6**	120	0.1
a_3 - Scarified 45 cm	**0.3**	100	0	**1.6**	145.4	0.5	**2**	142.8	0.6	**2.1**	131.2	0.5	**0.4**	80	-0.1
a_4 - Disc 10 cm	**0.2**	66.6	-0.1	**0.9**	81.8	-0.2	**1.1**	78.5	-0.3	**1.2**	75	-0.4	**0.3**	60	-0.2

The influence of fertilization and tillage on the height of the productive brothers

At the time of the sorghum harvest, after the completion of the vegetative growth, depending on the agrofund applied, the height of the productive brothers from the sorghum culture was between 70.1 cm in the unfertilized version - discussed and 92.4 cm in the foliar fertilized version - scarified at 45 cm. The application of fertilizers determined the obtaining of more vigorous brothers, thus influencing their productivity (Table 6).

The biggest differences compared to the non-fertilized variants were brought by the application of nitrogen + phosphorus + foliar fertilizations, the largest being 10.8 cm for the Disc 10 cm + $N_{100}P_{50}$ + Foliar variant. Through the application of phosphorus-based fertilizers, progressive values were recorded for all soil works, but they were best utilized in the case of deep works - scarified at 35 and 45 cm (81.1 cm and 84.7 cm).

Fertilization with $N_{100}P_0$ brought differences of up to 5.1 cm (Scarified 45 cm), but the superficial processing did not positively capitalize this agrofund compared to the plowed control. In the case of foliar fertilizers, the height of the brothers was not constant, values between 72.1 (a_4 - Disc 10 cm) and 82.4 cm (Scarified 45 cm) were recorded.

Table 6. The influence of fertilization on the height of the productive brothers in different tillage systems

Variant	b_1 - N_0P_0			b_2 - $N_{100}P_0$			b_3 - $N_{100}P_{50}$			b_4 - $N_{100}P_{50}$+Foliar			b_5 - Foliar (Borocal 1.5 l/ha)		
	cm	%	Dif.	cm	%	Dif.	cm	%	Dif.	cm	%	Dif.	cm	%	Dif.
a_1 - Plow 25 cm	**74.6**	100	C	**77.7**	104.1	3.1	79.5	106.5	4.9	80.4	107.7	5.8	79.8	106.9	5.2
a_2 - Scarified 35 cm	**75.6**	100	C	**78.6**	104.2	3	81.1	107.7	5.6	82.9	110	7.3	80.9	107	5.3
a_3-Scarified 45 cm	**82.1**	100	C	**82.8**	100.8	0.7	84.7	103	2.5	88.8	97	6.6	82.4	100.3	0.3
a_4 - Disc 12	**70.1**	100	C	**72.1**	107.7	2	77.8	111	7.7	79.7	96.4	10.8	72.1	102.7	3.5

The deeper the tillage, the higher the height of the productive brothers, regardless of the agrofund applied. Scarification of the soil at 45 cm brought the biggest differences compared to the Plowed control at 25 cm: 8.4 cm ($N_{100}P_{50}$ + Foliar) (Table 7).

The superficial work of the soil brought negative differences compared to the work by ploughing, regardless of fertilization, with values between -1.7 cm ($N_{100}P_{50}$) and -7.7 cm (Foliar Borocal 1.5 l/ha).

Table 7. The influence of tillage on the height of the productive brothers by different types of fertilization (2021-2023)

Variant	b_1 - N_0P_0			b_2 - $N_{100}P_0$			b_3 - $N_{100}P_{50}$			b_4 - $N_{100}P_{50}$ + Foliar			b_5 - Foliar (Borocal 1.5 l/ha)		
	cm	%	Dif.	cm	%	Dif.	cm	%	Dif.	cm	%	Dif.	cm	%	Dif.
a_1 - Plow 25 cm	**74.6**	100	C	**77.7**	100	Ct	79.5	100	Ct	80.4	100	Ct	79.8	100	Ct
a_2 - Scarified 35 cm	**75.6**	101.3	1	**78.6**	101.1	0.9	81.1	102	1.6	82.9	103	2.5	80.9	101.3	0.1
a_3 - Scarified 45 cm	**82.1**	110	7.5	**82.8**	106.5	5.1	84.7	106.5	5.2	88.8	110.4	8.4	82.4	103.6	2.6
a_4 - Disc 10 cm	**70.1**	93.9	-4.5	**72.1**	92.7	-5.6	77.8	97.8	-1.7	79.7	99.1	-0.7	72.1	90.3	-7.7

CONCLUSIONS

Following the research carried out, the following more important conclusions can be synthesized regarding the influence of tillage systems and fertilization in grain sorghum culture regarding twinning:

Under the conditions of a cambic chernoziom from the Sărăţeni-Ialomiţa area, the highest height of the productive brothers in a sorghum crop was obtained in the variants fertilized with foliar fertilizers 92.4 cm together with tillage by scarification at 45 cm.

The most pronounced twinning was obtained by applying a fertilization based on $N_{100}P_{50}$ + Foliar and loosening the soil at 35 cm: 3.8 siblings. The variant with the best yield in terms of productive brothers reaching maturity was the scarification of the land at 45 cm along with a fertilization with $N_{100}P_{50}$ + Foliar, thus obtaining 2.1 productive brothers on average per plant.

Even if the discussion of the land emphasized twinning in all the fertilization options, not all brothers reached maturity, productive.

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CORRELATION DEPENDENCES OF QUANTITATIVE TRAITS IN WINTER PEA GENOTYPES

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Abstract

*In the past, majority of the plant breeding programmes were focused mostly on developing the cultivars with high yields. Recently, stable, quality and reliable yields under various environmental conditions have consistently gained importance over solely increased yield. The experiment was conducted during the period 2019-2021 at the National Agriculture Research Development Institute Fundulea in order to establish the phenotypic and genotypic correlations between the main quantitative traits in eight winter pea genotypes. The highest positive correlation was found between plant height and grain yield (r = 0.99***), the number of pods per plant with number of grains per plant (r = 0.90***) and grain yield (r = 0.87***) and plant height (r = 0.80***), the grains weight per plant with earliness (r = 0.83***); the highest negative correlation was registred between earliness and plant height (r = -0.83***), grain yield and earliness (r = -0.82***). Pea breeding should be focused on traits with consistent heritability and a positive effect on seed yield when selecting high-yielding genotypes, and on allowing for more widespread use of pea in various agricultural production systems.*

Key words: *winter peas, seed yield, correlation, yield component.*

INTRODUCTION

Pea (*Pisum sativum* L.) is an important and wide spread legume crop. There is a great demand to develop high yielding cultivars in order to achieve future crop improvement of both, quantitative and qualitative traits (Kalapchieva et al, 2021; Singh et al., 2018).

Yield potential is the genetically determined ability of a crop to generate optimal yield in a given growth environment. Yield potential is thought to be partially determined by seed size, and numerous studies have tried to understand the relationship between seed size and yield in pea and other pulses with contradictory results (Biger, 2009; Gusmao, 2013; Smitchger et al., 2018). Grain yield is associated with harvest index, which is the ratio of grain yield to plant biomass. The source-sink relationship in peas is dependent on seed size, pod length, plant height, leaf size, stem width, stem wall thickness, leaf thickness, stipule size and other factors (Smitchger, J. & Weeden, F.N., 2018).

The fundamental goal of pea breeders is to increase seed yield to maximize plant productivity and allow for more widespread use of pea in various agricultural production systems. Seed yield is a complex trait that is quantitatively inherited, and its expression depends on genetic factors, environment and interaction (G x E) (Tan et al., 2012). Many studies have shown a significant influence of environment and genotype-by-environment interaction on seed yield and the yield components on their phenotypic performance (Bocianowski, 2019; Tiwari & Lavanya, 2012; Uhlarik et al., 2022). Otherwise, associations between yield components may be used as the best guide to achieve a yield increase through indirect selection. Phenotypic and genotypic correlation values provide a reliable information about the relationship between two or more rather than two independent variables. Highlighting the relation between the various traits and seed productivity would be of great assistance toward a successful selection and screening program. Correlations between yield and its components in different legumes have been reported. (Kamandi et al, 2015; Kour & Agarwal, 2016; Kumar & Lavania, 2014). The phenotypic correlation is conditioned by the relationship among individual characters and the influence of environmental factors. The genotypic correlation is a function of the pleiotropic action of the genes involved and their related inheritance. Linked genes have additive genes are of greatest value in breeding (Kosev & Georgieva, 2022). In this study, grain yield and some yield component, some physiological characters and correlation among these traits were investigated in eight winter pea genotypes.

MATERIALS AND METHODS

The study was conducted in the experimental fields of the National Agriculture Research Development Institute Fundulea, during the period 2019-2021. Eight genotypes of winter peas, Enduro, James, Lavinia F, Ghittia F, 13008MT28-1, 12004MT2, 12034MT1-2, 12038MT2 were tested. The experiment was performed by randomized complete block design, three repetitions with a working plot area of 6 m$^{2.}$ The sowing was done out at 11.10.2019, 21.10.2020. The pea was grown according to the technology adopted for the culture. The main morphological (biometric) characteristics of the aboveground biomass were measured at the technological maturity of plants. For this, 5 plants were used from the three replications of the trial. Plant height (cm), the total number of pods per plant, number of grains per plant, the grains weight per plant (g), earliness, winter hardiness and grain yield were measured. The results obtained were statistically evaluated using ANOVA and correlation analysis.

Regarding meteorological conditions, NARDI Fundulea area is characterized by a continental temperate climate, with uneven distribution of rainfall by months. The data regarding temperature and rainfall registered during the years of testing, delivered by the Weather station of NARDI Fundulea, are presented in Table 1. Weather conditions over two years during winter peas vegetation period and especially during the grain filling period, were very different.

Table 1. Rainfall distribution and average temperatures during winter peas vegetation at NARDI Fundulea (2019-2021)

Month	2019/2020		2020/2021	
	Temperature (°C, mean values)	Rainfall (mm)	Temperature (°C, mean values)	Rainfall (mm)
October 2019/2020	12.8	38.2	14.7	28.6
November 2019/2020	6.2	33.2	6.1	20
December 2019/2020	4	12.8	3.9	77.6
January 2020/2021	0.9	2.0	1.6	77
February 2020/2021	5.2	16.0	3.2	16.2
March 2020/2021	8.3	16.6	5.1	59
April 2020/2021	12.3	29.8	9.7	31
May 2020/2021	16.8	14.0	17.2	56
June 2020/2021	21.7	57.8	21.1	135
Total rainfall		**219.8**	-	**500.4**

Source: The weather station NARDI Fundulea

As shown in Table 1, rainfall was higher and unevenly distributed during the vegetation period of 2021, in contrast to 2020 characterized by less rainfall. Overall monthly temperatures were positive on average, not allowing a good selection of genotypes according to their frost tolerance.

RESULTS AND DISCUSSIONS

Application of the two-way analysis of variance (Table 2) on the characteristics of the pea genotypes showed statistically significant effects of the environments (years) and genotypes in all investigated parameters excepting the both, number of seed per plant and grain yield. Genotypic influence on the expression of the height of the plant and the number of pods and seeds per plant was prevalent.

Table 2. Analysis of variance for stability for yield components in winter pea genotypes - NARDI Fundulea (2019-2021)

	Plant heigth			Number of pods per plant		Number of grains per plant		The grains weigh per plant (g)		Earliness		Grain yield	
Source of variation	df	F	P-value	F	P-value	F	P-value	F	P-Value	F	P-value	F	P-value
Genotypes	7	100.7**	0.000	2.52**	0.12	1.01	0.49	1.36	0.035	23**	0.0002	1.63	0.27
Years	2	289.5**	0.000	18.7**	0.003	20.8**	0.002	176.5**	0.000	25.2**	0.0015	105.2**	0.000
Interaction	7	-	-	-	-	-	-	-	-	-	-	-	-
Total	15	-	-	-	-	-	-	-	-	-	-	-	-

In Table 3 the correlation indexes between these characters over all genotypes are presented.
The values of the genotypic correlation coefficients with a positive sign were slightly higher than the phenotypic ones, that suggests a less pronounced effect on the manifestation of the studied traits.
Significant positive genotypic relations were evidential with respect to plant height and grain yield (r = 0.99***), number of pods per plant with number of grains per plant (r = 0.90***) and grain yield (r = 0.87***) and plant height (r = 0.80***), the grains weigh per plant with earliness (r = 0.83***). The number of grains per plant was significant positively correlated with grain yield (r = 0.69**) and plant height (r = 0.64*). The value of r = 0.68* indicates the significant and positively relation between winter hardiness and earliness. Our results suggest that these traits would contribute to increasing grain production and should be taken into account in the selection of genotypes. There was a strong negative correlation between earliness and plant height (r = -0.83***), grain yield and earliness (r = -0.82***), the grains weight per plant with grain yield (r = -0.66*) and plant height (r = -0.62*). Timmerman-Vaughn et al. (2005) indicated that grain weight is negatively correlated with seed yield. The results obtained in our study support the conclusions made by Adetiloye et al. (2017) in cowpea.

Table 3. Correlation coefficients between winter pea yield component

	Number of pods per plant	Number of seeds per plant	The grains weight per plant (g)	Plant heigth	Earliness	Grain yield	Winter hardi ness
Number of pods per plant	X						
Number of seeds per plant	0.90***	X					
The grains weight per plant (g)	-0.45	-0.07	X				
Plant heigth	0.80***	0.64*	-0.62*	X			
Earliness	-0.48	-0.24	0.83***	-0.83 ***	X		
Grain yield	0.87***	0.69**	-0.66*	0.99 ***	-0.82 ***	X	
Winter hardiness	0.42	0.34	-0.52*	0.47	0.68*	0.51*	X

The authors established significant positive genotypic correlations between productivity and pods number, pod length and seeds number per plant. The carried out assessment on the dependencies in white lupine accessions was in line with the studies of Machado et al. (2017) in soybean genotypes correlations concerning the pods number and seed production per plant. Similar finding have been reported by other researchers (Tiwari & Lavanya, 2012; Govardhan et al. 2013 and Kosev & Georgieva, 2022).
According to the results of experiments at NARDI Fundulea, extensive phenotypic variation was observed in the analyzed pea genotypes. Means value of plant height, number of pods per plant, number of seeds per plant, seed weight per plant and grain yield were lower, which is likely to be the consequence of drought in the flowering period in May 2020 (Table 1). For all characteristics, statistically significant differences were observed for mean value between two season, except for earliness and winter hardiness (Table 4). The results obtained in our study partially support the conclusions made by Uhlarik et al. (2022). The highest coefficients of variation (CV) were observed for grain yield (102% by James), grain weight per plant (82% by 12034MT1-2), plant height (53 % by James), number of seeds per plant (51% by 12034MT1-2), number of pods

per plant (37 % by James). The lowest CV was observed for winter hardiness and earliness.

The results of this study should contribute to a better knowledge of variability and seed yield stability of winter pea genotypes. These phenotypic results could improve pea breeding by developing new cultivar scarring favorable traits to attain more sustainable production and higher yields. Last but not least, such work should promote the broader use of pea as a grain legume within diverse agricultural systems to provide multiple beneficial economic advantages, in line with the principles of sustainability within diverse national agricultural systems.

Table 4. Descriptive measures of winter peas genotypes at NARDI Fundulea

No.	Genotype	X_{av}	X_{opt}	X_{lim}	Stress tolerance	Std.	C.V.%
Plant height							
1.	JAMES	46.,5	64	29	-35	24.7	53
2.	ENDURO	50.5	63	38	-25	17.7	35
3.	LAVINIA F	51.5	66	37	-29	20.5	40
4.	GHITTIA F	58	79	37	-42	29.7	51
5.	13008MT28-1	54	70	38	-32	22.6	42
6.	12004MT2	56.5	73	40	-33	23.3	41
7.	12034MT1-2	128.5	147	110	-37	26.2	20
8.	12038MT2	49	63	35	-28	19.8	40
Number of pods per plant							
1.	JAMES	13.25	16.7	9.8	-6.9	4.9	37
2.	ENDURO	14	14.2	14	-0.2	0.14	0
3.	LAVINIA	12.25	14.5	10	-4.5	3.2	26
4.	GHITTIA F	12.15	13	11.3	-1.7	1.2	10
5.	13008MT28-1	13.8	15.3	12.3	-3	2.1	15
6.	12004MT2	11.1	13.5	8.7	-4.8	3.4	31
7.	12034MT1-2	17	20	14	-6	4.2	25
8.	12038MT2	11.45	12.4	10.5	-1.9	1.3	12
Number of seeds per plant							
1.	JAMES	48	63	33	-30	21.2	44
2.	ENDURO	59	66	52	-14	9.9	17
3.	LAVINIA	51	65	37	-28	19.8	39
4.	GHITTIA F	48.5	49	48	-1	0.7	1.4
5.	13008MT28-1	52	64	40	-24	17	33
6.	12004MT2	41.5	48	35	-13	9.2	22
7.	12034MT1-2	62.5	85	40	-45	31.8	51
8.	12038MT2	47.5	56	39	-17	12	25
The grains weigh per plant (g)							
1.	JAMES	8.3	13	3.6	-9.4	6.6	81
2.	ENDURO	10.35	14	6.7	-7.3	5.2	50
3.	LAVINIA	10.3	15	5.6	-9.40	6.6	65
4.	GHITTIA F	9.75	12	7.5	-4.5	3.2	33
5.	13008MT28-1	9	13	5	-8	5.7	63
6.	12004MT2	8.75	12	5.5	-6.5	4.6	53
7.	12034MT1-2	7.6	12	3.2	-8.8	6.2	82
8.	12038MT2	9	13	5	-8	5.7	63
Earlines							
1.	JAMES	118	121	115	-6	4.2	4
2.	ENDURO	118	122	114	-8	5.7	5
3.	LAVINIA	117.5	119	116	-3	2.1	2
4.	GHITTIA F	118	121	115	-6	4.2	4

No.	Genotype	X_{av}	X_{opt}	X_{lim}	Stress tolerance	Std.	C.V.%
5.	13008MT28-1	119.5	122	117	-5	3.5	3
6.	12004MT2	118	121	115	-6	4.2	4
7.	12034MT1-2	136.5	137	136	-1	0.7	1
8.	12038MT2	117	120	114	-6	4.2	4
Grain yield							
1.	JAMES	3413.5	5875	952	-4923	3481	102
2.	ENDURO	4171.5	6667	1676	-4991	3529	85
3.	LAVINIA	3567.5	6083	1052	-5031	3557.5	100
4.	GHITTIA F	3498	5208	1788	-3420	2418	70
5.	13008MT28-1	3270.5	4875	1666	-3209	2269	70
6.	12004MT2	3104.5	5208	1001	-4207	2975	96
7.	12034MT1-2	1808	2917	699	-2218	156.4	87
8.	12038MT2	2883.5	4375	1392	2983	2109.3	73
Winter hardiness							
1.	JAMES	1.5	1.5	1	-0.5	0.4	3
2.	ENDURO	1.5	2	1	-1	0.7	6
3.	LAVINIA	1.5	2	1.5	-0.5	0.4	3
4.	GHITTIA F	1.5	1.5	1.5	0	0	0
5.	13008MT28-1	2	2	1.5	-0.5	0.4	3
6.	12004MT2	1.5	2	1	-1	0.7	6
7.	12034MT1-2	2	3	1.5	-1.5	1	7
8.	12038MT2	2	3	1.5	-1.5	1	7

X_{av} - average value of trait (2019-2021), X_{opt} - maximal value of trait (in optimal conditions), X_{lim} - minimal value of trait (in limit conditions), Std.- standard deviation, C.V. - coefficient of variation (%).

CONCLUSIONS

- Investigated of eight winter pea genotypes for yield and physiological parameters under field condition in Fundulea region, Romania revealed that yield and yield components in pea is a complex character largely depending on genotypes, environmental, agronomical and physiological characters.
- Grain yield and some yield components, some physiological characters and correlation among these traits varied in winter pea genotypes analysed.
- The genotypic correlation was higher than the phenotypic one for all the traits that indicates a minor role of environment on the expression of the genes involved.
- Pea is a sustainable crop with multiple economic advantages.

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PHENOTYPIC DIVERSITY OF COMMON BEAN (*Phaseolus vulgaris* L.) GERMPLASM, A POTENTIAL CROP FOR ENSURING FOOD SECURITY

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Abstract

Bean crop is of great economic interest, being among the most used grain legumes in human nutrition. Also, it can use like organic crop, with great ecological plasticity, cultivated from the plains to high altitudes, also being a good precursor for other crops, leaving the soil rich in nitrogen. Therefore, we must give priority to the phenotypic characterization of local bean germplasm to provide breeders with valuable genetic sources, useful in creating of productive advanced cultivars, resistant to adverse environmental factors, to ensure food security. The objective of this study was to evaluate variability of morpho-physiological and agronomic traits of some common bean landraces (Phaseolus vulgaris L.), well adapted to pedoclimatic conditions from different regions sites of the country, conditioned by the genetic factors of the species. These populations could be used in prebreeding programs to improve some morpho-physiological characteristics very useful for future new creations. We must give priority to the phenotypic characterization of local bean germplasm to provide breeders with valuable genetic sources, useful in creating of productive advanced cultivars, to ensure food security.

Key words: *common bean landraces, variability, variation, phenophasic, morphological and agronomic traits.*

INTRODUCTION

Phaseolus vulgaris L. (common bean), a traditional grain legume in Romania is the most important cultivated species of the genus. Is one of the most remarkable crops for its seed nutritional value, as it has a high protein content and is considered to be one of the main sources of protein in the human diet (Abdollahi et al., 2016). It was introduced to our country at the beginning of the 17th century, and until 1989, it was cultivated on large areas (168,000 ha) (Bîlteanu, 1989). It has a high economic interest, being among the most used legumes in human nutrition. In general, seeds are used for their high content of digestible proteins rich in essential amino acids, but pods are also used; and sometimes young shoots are used as a salad.

There are several varieties, classified according to the shape of the grain: *compressus, oblongus, ellipticus, sphaericus*, according to the type of growth: nanus (small type), vulgaris (climbing type) and various intermediate biotypes between varieties (Musango et al., 2016). Within the varieties, there are different types differentiated by color as follows, unicolor (white, black, brown, beige, etc.), punctatus (dotted), maculatus (spotted), variegatus (motley) and zebrinus (striped) (Muntean, 1995; Ciofu R. et al., 2003; Stoilova, et al., 2005; Abdollahi et al., 2016).

The objective of this study was to evaluate the variability of morpho-physiological and agronomic traits of some common bean landraces (*Phaseolus vulgaris* L.), well adapted to pedoclimatic conditions from different regions sites of the country, conditioned by the genetic factors of the species. All these local landraces were collected from local farmers, who prefer and grow them in their gardens due to the multiple uses in food and the adaptation to biotic and abiotic stress factors, in the context of current climate changes (Stoilova et al., 2013; Szilagyi et al., 2011).

The study was carried out at the Suceava Genebank, during period 2016-2018 and included 122 bean local varieties. Morpho-physiological and agronomic traits were evaluated during the growing season according to bean descriptors edited by ECP/GR. The investigations carried out showed differences between the populations for all the analyzed traits. All the local bean varieties collected showed high variability, representing potentially valuable genetic resources for breeders of this species.

The landraces (common and dwarf beans) which was studied in this paper, is grown only in peasant gardens, microfarm because it is preferred by the rural population due to the quality of pods, long harvest period and production far superior to large grain beans.

The main objective of this study was to analyze variability of morphological descriptors and variation of the main agronomic traits of locally adapted varieties from different regions sites of the country, conditioned by the genetic factors of the species. Also, the aim of this study was to use agro-morphological traits to assess the genetic diversity and relatedness among 122 *Phaseolus vulgaris* L. accessions collected from different eco-geographical areas.

We must give priority to the phenotypic characterization of local bean germplasm to provide breeders with valuable genetic sources, useful in creating of productive advanced cultivars, to ensure food security (Boros et al., 2014).

Finally, possessing a good knowledge of the available genetic diversity will permit not only a good management of the bean germplasm, but also facilitate its valorization (Dutta et al., 2016; Bareke, 2019). These results represent the starting point from which the genetic diversity of our collection was assessed, thereby making it possible to optimize new breeding strategies for planning future breeding programs to individuate specific traits useful in the environment (Scarano et al., 2014; Guidoti, et al., 2018).

MATERIALS AND METHODS

In the present study, both morphological and phenotypical characterization were employed to highlight bean germplasm diversity and to determine the genetic relationships between 122 bean accessions obtained from different regions.

The study for the assessment of phenotypic variability of *Phaseolus vulgaris* L. genotypes, for different morphological and agronomic traits was conducted in the experimental field of the Suceava Gene Bank.

The biological material used in this study consisted of a total of 122 populations belonging to the species *Phaseolus vulgaris* L., collected from regions with different ecological conditions (Table 1). The experimental design and field management was laid out in blocks, with length row of 2 m, and the distance between rows of 70 cm on a cernoziomoid soil (3-5% humus).

During the growing period, data related to phenology, morphological and agronomic.

Table 1. Origin of bean local landraces analyzed

Collecting counties	No. of samples	Collecting counties	No. of samples
Constanța	1	Cluj	2
Suceava	24	Vaslui	2
Dolj	4	Mehedinți	2
Alba	15	Argeș	2
Iași	18	Caraș Severin	2
Hunedoara	4	Bistrița Năsăud	5
Brașov	9	Arad	2
Neamț	8	Maramureș	2
Vâlcea	3	Covasna	2
Vrancea	3	Satu Mare	2
Bihor	5	Bacău	5

traits were characterized in accordance with the IPGRI descriptor list (2009). Data on analyzed traits were collected from ten sample plants from each plot, selected randomly. In order to highlight the landraces variability, the following morpho-physiological descriptors were determined in the field and in the lab:

- Biometric descriptors (Leaf length, Pod length, No. of days from emergence to flowering, No. of days from flowering to maturity, No. of days from emergence to maturity);
- IPGRI descriptors by using codes (growth type, color of the flower banner, color of the fins, color of the immature pod, cross section through the pod, curvature of the immature pod, color of the ripe pod, presence of fibers on the pod, seed shape, seed size, presence of stripes/spots on the seeds, no. of colors on the seeds, base color of the seed, secondary color of the seed, distribution of the secondary color of the seed).

All analyses were conducted using SPSS 20 and GraphPad Prism 9.4.

The following estimators were calculated for biometric descriptors mentioned: average (x), variation amplitude (min.-max.), standard deviation (ƅ) and F-ANOVA test (p/0.05), presented in tables and dendrograms by statistical analysis techniques such as: one-way ANOVA and Hierarchical Cluster Analysis.

RESULTS AND DISCUSSIONS

The following estimators were calculated for biometric descriptors: average (x), amplitude of variation, variance (s2) and coefficients of variation (s %) (Fowler J.and Cohen L., 1990). The two morphological descriptors (leaf length and pod length) and the 3 physiological ones (no. of days from emergence to flowering, no. of days from flowering to maturity and no. of days from emergence to maturity.) are presented in Table 2. The interpretation of the results is based on the determination of the coefficient of variation, as an expression of the diversity of the studied biological material. From the Table 2, a large coefficient of variation it is observed for the descriptors: pod length and no. of days from flowering to maturity. Mean values of the coefficient of variation were recorded for the descriptor leaf length and minimum values were recorded for the descriptors: no. of days from emergence to flowering and no. of days from emergence to maturity. Table 2 also shows that the bean sample with the shortest vegetation period coming from Fundulea, Călărași (SVGB-2447) and the latest comes from Brodina, Suceava (SVGB-2286).

Referring the pod length, it can notice a variation amplitude between 6.05 cm, accession coming from Sânpetru, Brașov (SVGB-19935) and 16.35 cm, accession coming from Călărași, Dolj (SVGB-2788).

Table 2. Amplitude of variation in biometrics descriptors recorded on the bean samples analyzed

Descriptors	Average	Maxim values	Accession name	Minim values	Accession name	Stand. dev.	Variance	Coeff. of variation
Leaf length	7.69	11.14	SVGB-2504	5.28	SVGB-12577	1.16	1.335	10.41
Pod length	10.46	16.35	SVGB-2788	6.05	SVGB-19935	2.215	4.91	21.17
No. of days from emergence to flowering	45.8	51	SVGB-19614	34	SVGB-14248 SVGB-2951 SVGB-2111 SVGB-2788	3.213	10.32	7.00
No. of days from flowering to maturity	44.62	78	SVGB-19330	32	SVGB-2453 SVGB-15410	9.47	89.70	21.22
No. of days from emergence to maturity	92.48	104	SVGB-2286	76	SVGB-2447	7.094	50.33	7.67

To highlighting the links between the type of growth and the analyzed biometric descriptors (Table 3), it was calculated the F factor ANOVA which presented high values, statistically differentiating function by analyzed descriptors. In the Table 3 it is noticed the significant correlations between growth type and no. of days from flowering to maturity, and the no. of days from emergence to maturity.

Table 3. Simple linear regression of the morpho-physiological descriptors investigated

Growth type	No. of days from emergence to flowering	No. of days from flowering to maturity	No. of days from emergence to maturity	Leaf length	Pod length
Is slope significantly non-zero?					
F	0.4756	6.143	6.006	1.643	0.3751
DFn, DFd	1,108	1,108	1,108	1,108	1,108
P value	0.4919	0.0147	0.0159	0.2027	0.5415
Deviation from zero?	Not Significant	Significant	Significant	Not Significant	Not Significant

To emphasize the correlations between growth type and those two physiological traits (number of the days from flowering to maturity and number of the days from emergence to maturity) it assigned the regression lines. Growth type was positively correlated to number of the days from emergence to maturity (r = 0.435***) and also was positively correlated to number of the days from flowering to maturity (r = 0.317**) (Figure 1).

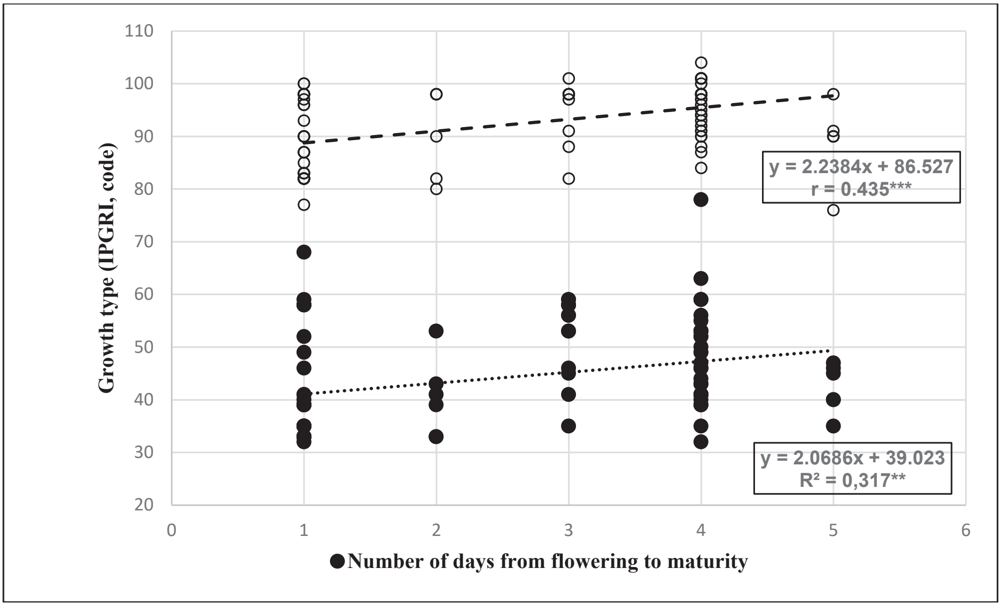

Figure 1. Relationships between growth type and the days from emergence to maturity and from flowering to maturity

All analyzed descriptors to those 122 local populations of *Phaseolus* were statistically classified into 4 clusters (Table 4) by the method of average linkage between groups, highlighting the similarities between members by different values of the Pearson correlation coefficients. So, it observes in dendrogram (Figure 2) their similarities, by strongly significant correlations between the color of the flower banner and color of the fins (0.777***); the base color of the seed and secondary color of the seed (0.510***); number of colors/seed and presence of streaks/spots on the seed (0.560***); number of colors/seed and distribution of secondary seed (0.777***); number of the days from emergence to maturity and number of the days from flowering to maturity (0.624***). Also there are significant correlations between presence of streaks/spots on the seed and base color of the seed (0.303*), shape of the seed and size of the seed (0.323*) and number of the days from emergence to maturity and leaf length (0.443**).

Table 4. Classification of morpho-physiological traits in clusters, by the method of average linkage between groups and Pearson correlation distance

Cluster number	Cluster membership (descriptors)	No. membership in dendogram	Cluster combined from agglomeration schedule to method average linkage between groups		Pearson correlation coefficients
			Cluster 1	Cluster 2	
1	Colour of the flower banner	7	7	8	0.777
1	Colour of the fins	8	7	10	0.206
1	Cross section through the pod	10	10	11	0.239
1	Curvature of the immature pod	11	7	12	0.100
3	Color of the ripe pod	12	2	9	0.246
2	No. of colours/seed	17	18	19	**0.510**
2	Distribution of secondary seed colour	20	17	16	**0.560**
2	Presence of streaks/spots on the seed	16	17	20	**0.777**
2	The base color of the seed	18	16	18	**0.303**
2	Secondary color of the seed	19	7	16	0.092
3	Pod length	2	1	2	0.134
3	Colour of immature pod	9	2	13	0.052
3	Shape of the seeds	14	14	15	**0.323**
3	Size of the seeds	15	14	13	0.237
3	Presence of fibers on the pods	13	2	4	0.044
3	No. of days from emergence to flowering	4	2	7	0.031
4	No. of days from emergence to maturity	5	5	6	**0.624**
4	No. of days from flowering to maturity	6	5	1	**0.443**
4	Leaf length	1	1	3	0.288
4	Growth type	3	3	4	0.053

In Dendogram using Average linkage (Figure 2) it observes the similarities of the descriptors, being grouping in 4 clusters, such as:
Cluster 1: Color of the flower banner, Color of the fins, Cross section through the pod, Curvature of the immature pod;
Cluster 2: No. of colors/seed, Distribution of secondary seed color, Presence of streaks/spots on the seed, The base color of the seed, Secondary color of the seed;
Cluster 3: Color of the ripe pod, Pod length, Color of immature pod, Shape of the seeds, Size of the seeds, Presence of fibers on the pods, No. of days from emergence to flowering;
Cluster 4: No. of days from emergence to maturity, No. of days from flowering to maturity, Leaf length, Growth type.

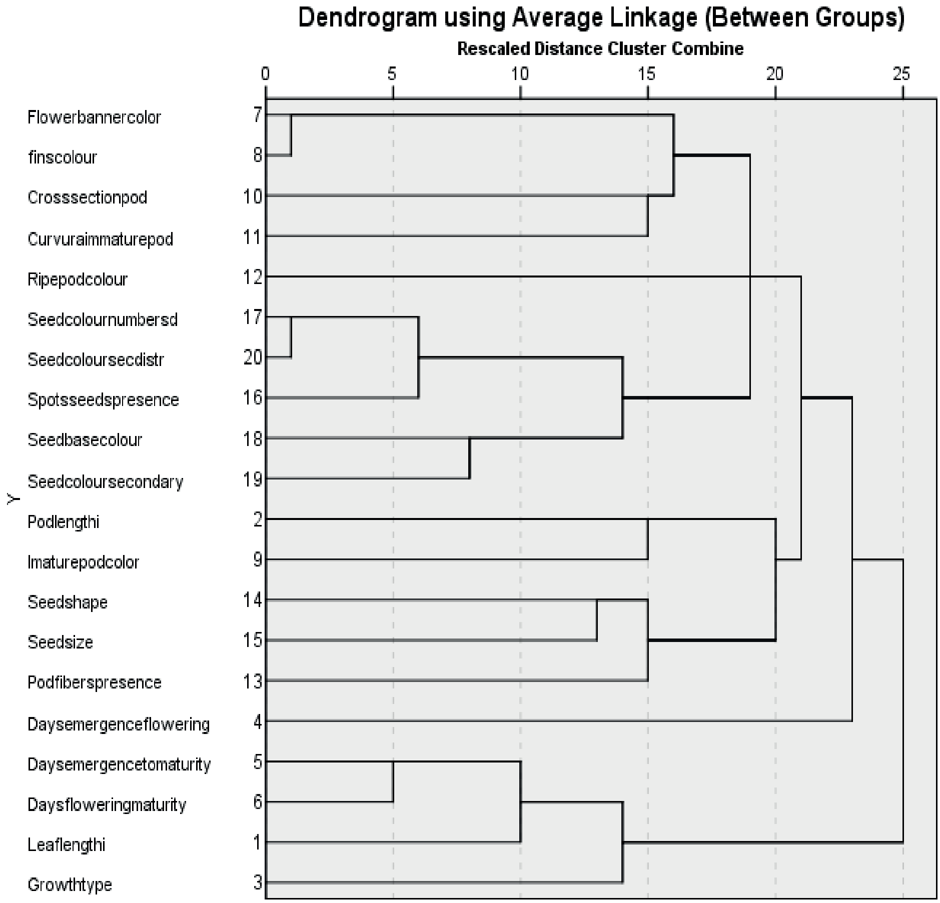

Figure 2. Hierarchical clustering of 122 common bean genotypes based on Pearson correlation using data from 20 morpho-physiological traits

The Ward Linkage dendrogram (Figure 3) built on the Euclidean distance showed the maximum distance in the third cluster (C3) represented by the following members: SVGB-14244, SVGB-14236, SVGB-2463, SVGB-2012; SVGB-15335, SVGB-2486, SVGB-14226, SVGB-2468, SVGB-244, SVGB-14247, SVGB-20313, SVGB-14239, SVGB-9059, SVGB-2457, SVGB-14648. Therefore, 13 % of the total studied members presented a high heteropgenity of the morpho-physiological traits, which could be used in the prebreeding programs to improve some morpho-physiological characteristics.

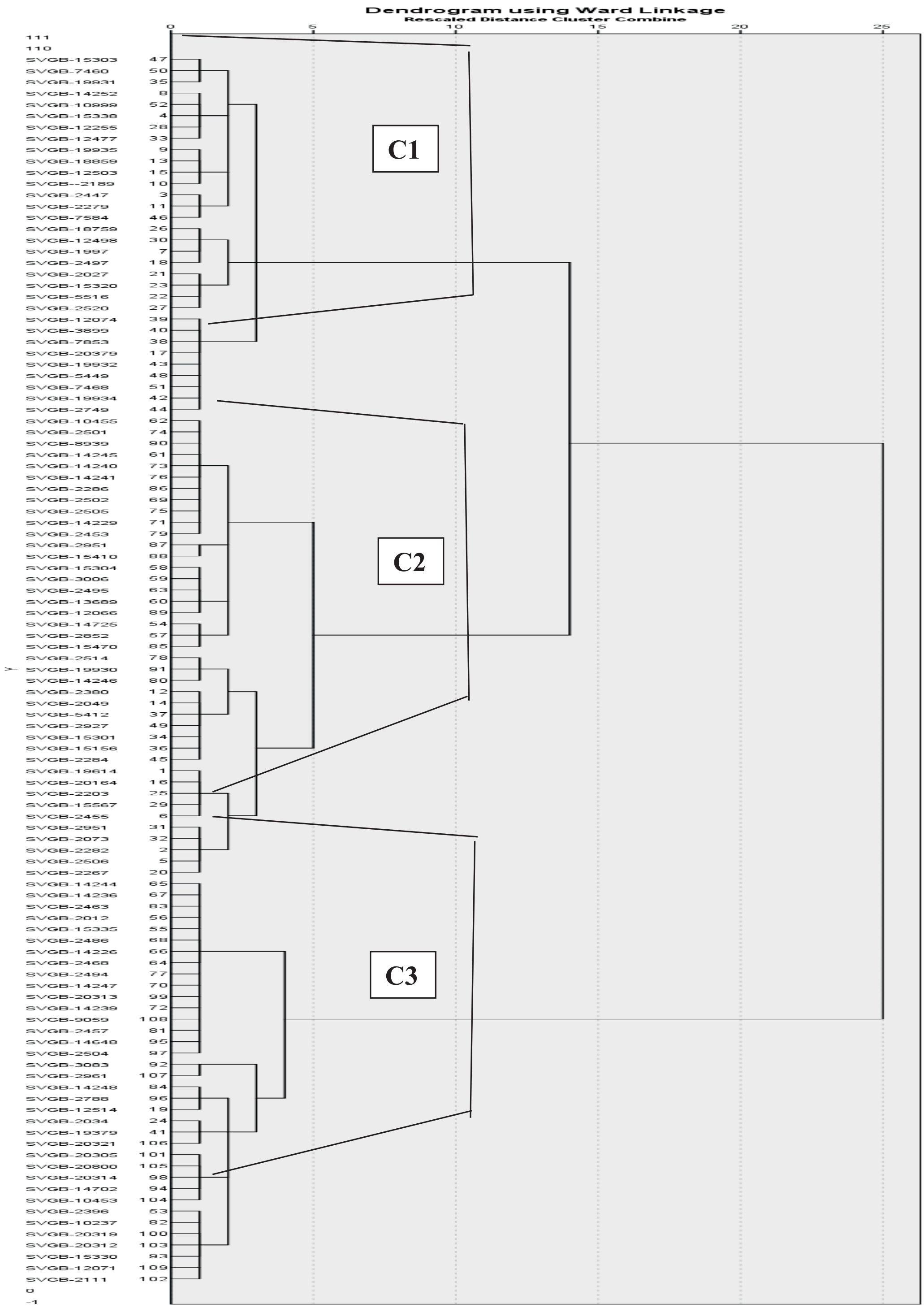

Figure 3 - Ward Dendogram based on Euclidean distance using the data from phenotypic descriptors recorded in 122 common bean populations

CONCLUSIONS

From this study it could be concluded that the bean populations represent a diversified biological material with a high variability.

The greatest variability of the studied populations was observed for the descriptors pod length, and numbers of the days from flowering to maturity.

The significant correlation coefficients were obtained between growth type and number of days from flowering to maturity, and the number of days from emergence to maturity, that mean the importance of the growth type in the obtaining of the earlier cultivars.

Concerning the Pearson correlation coefficients, it observes the strongly significant correlations between the color of the flower banner and color of the fins; the base color of the seed and secondary color of the seed; the number of colors/seed and presence of streaks/spots on the seed; the number of colors/seed and distribution of secondary seed; the number of the days from emergence to maturity and number of the days from flowering to maturity.

The Ward Linkage dendrogram built on the Euclidean distance showed the maximum heterogeneity of morpho-physiological descriptors to the following local populations: SVGB-14244, SVGB-14236, SVGB-2463, SVGB-2012; SVGB-15335, SVGB-2486, SVGB-14226, SVGB-2468, SVGB-244, SVGB-14247, SVGB-20313, SVGB-14239, SVGB-9059, SVGB-2457, SVGB-14648. These populations could be utilized in prebreeding programs to enhance specific morpho-physiological characteristics, which would prove beneficial for future breeding endeavors.

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THE YIELD AND GRAINS QUALITY OF SOME MAIZE HYBRIDS CREATED AT ARDS TURDA

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Abstract

Maize, due to its superior productions and multiple uses, is one of the main crops. 34 maize hybrids created at the Agricultural Research and Development Station (ARDS) Turda were tested in 3 experimental years (2020-2022). The grain yield and their quality (protein, starch, fats and fibers) were analyzed. The three years were very different from the point of view of favorability, in the first two years optimal conditions were met for achieving a higher production and a better quality of the grains, while in the year 2022 the negative influence is evident both on the production as well as on the quality of the grains. It stands out both for the average productions and for the highest productions in each of the three experimental years the hybrids Turda 344, Turda 3356, Turda 2020 and Turda 380 (12,764 kg/ha average and highest yield in 2021, of 16,280 kg/ha and in 2022, of 9,366 kg/ha). Turda 100 had the highest starch content (76.18%), while for fat and fiber were noted the hybrids Turda 199 (5.22%) and Saturn (3.63%).

Key words: *fat, fiber, protein, starch, yield.*

INTRODUCTION

Maize (*Zea mays*) one of the main crop plants, has experienced impressive increases in cultivated areas in the last two decades, occupying the second place in this respect (after wheat), but in terms of the total production achieved, it is in the first place (FAOSTAT, n.d.). These increases are due both to market demand and its multiple uses (human food, animal feed, industry, medicinal purpose, etc.) and to the increase in productions obtained per surface unit. However, the production is variable depending on the climatic conditions, both throughout the vegetation period, and with particular importance in certain phenophases (emergence, anthesis, grain filling) (Șimon et al., 2023), thus, the breeders must consider when creating new lines and hybrids, that they can cope with the stress factors as well as possible (Horablaga et al., 2023).

The discovery of the phenomenon of heterosis, the introduction of hybrids into the crop, the continuous improvement of the production capacity as well as the cultivation methods determined the obtaining of much higher productions than those obtained in the past (Paril et al., 2024).

However, due to the desire to obtain very high productions, the quality improvement must not be omitted. The quality of the maize grains is of particular importance, depending on the uses for which the crop will be used. The quality of maize grains can also be improved through other methods such as biofortification (Garg et al., 2018; Harjes et al., 2008; Hossain et al., 2022), the use of chemical fertilizers (Leonte et al., 2023; B.-G. Yu et al., 2022), biostimulators (Abdo et al., 2022; Luță et al., 2022), with good results.

The use of maize for the production of ethanol, biodegradable polymers and nutritional products has led to the reorientation of breeding programs towards modifying the starch, protein and oil content of the kernels (Niu et al., 2023; Pajić et al., 2010; J.-K. Yu & Moon, 2021), while the use of maize as raw material for hemicellulose and ethanol, but also for animal feed aims to increase the fiber content (Gáspár et al., 2005, 2007).

The aim of this work is to evaluate the yield obtained by 34 hybrids created at ARDS Turda and the grain quality, in three experimental years.

MATERIALS AND METHODS

In the present study, 34 maize hybrids created at the Agricultural Research and Development Station (ARDS) Turda were tested regarding the yield and grain quality, in three years (2020-2022). The hybrids were created and registered at the ARDS Turda in the last 50 years, and regarding their growing period, they are early or semi-early.

The experiment was carried out in the Maize breeding field, the crop was placed according to the randomized blocks method, in three repetitions, at 70,000 plants/ha density. The yield was determined from two 5 m long rows, while the grain quality was determined from grains obtained from 10 random cobs, using the spectrophotometer Bruker Tango NIR.

The crop management was the same and included: three years crop rotation soybean - winter wheat - maize; autumn plowing; mineral fertilization (400 kg/ha NPK 27:13.5:0); pre-emergent (1.5 l/ha a.s. metolachlor 960 g/l) and post-emergence (2 l/ha a.s. tembotrione (44 g/l) and isoxadiphen-ethyl (22 g/l) herbicides.

The data were processed statistically by variance analysis using ANOVA.

RESULTS AND DISCUSSIONS

Climatic conditions

The data from the Turda meteorological Station indicate that the three experimental years were different from the meteorological point of view and implicitly the favorability for maize crop. In the last decade, an increase in temperatures was observed in the area of the city of Turda (Haş et al., 2022; Simon, 2022), being noted that in the years studied, the temperatures exceeded the multi-year average during the maize growing season. In 2020, it was warm and with precipitation above the average of the area, throughout the growing season, thus favorable conditions for maize were met. This year, the richest precipitations were recorded in the months of May and June, the water requirement being ensured from the first stages of development, and the normal level of precipitation in July and August determined the optimal achievement of anthesis and grain filling.

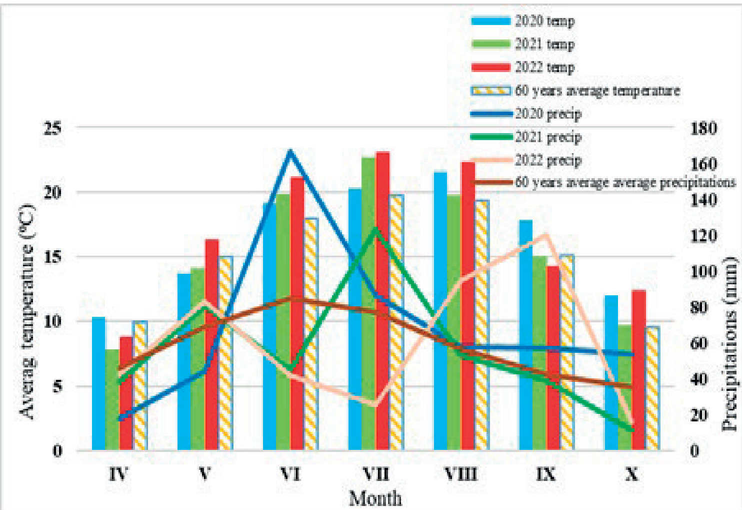

Figure 1. Meteorological conditions, Turda, 2020-2022

The year 2021, one of the most favorable for the maize crop in the last decades, was characterized by high temperatures, associated with a high precipitation, especially in June and July, the period when this plant needs the most water for obtaining high productions.

The extreme temperatures of 2022 associated with a lack of precipitation have made this year to be considered one of the most unfavorable for the maize crop and not only, droughts being reported in several areas of the country (Ciornei et al., 2023), as well as the calamity of agricultural crops. The high temperatures in the summer months (in some days over 35°C) (Simon, 2022), the deficit regarding the amount of precipitation or even the lack of it for a larger number of days, determined the obtaining of much lower productions, the precipitation being recorded late, only towards the end August and September.

Analysis of variances for yield and quality

The analysis of the variance (Table 1) shows the significant influence that both years, hybrids and the interaction between those factors have on production and some grain quality indices.

Table 1. Analysis of variance regarding maize yield and grain quality for 34 hybrids, Turda, 2020-2022

Variability source	DF	Yield kg/ha	Protein %	Starch %	Fat %	Fiber %
		F test				
Total	203					
Years (Y)	2	781 **	898 **	313 **	122 **	137 **
Hybrid (H)	33	33 **	143 **	18 **	14 **	26 **
Y x H	66	4 **	20 **	3 **	2 **	4 **

**significant at p<0.01 probability levels

Year influence on yield and quality

The influence of the different climatic conditions of the three years is evident in the case of yield (Table 2).

Table 2. The influence of the year on maize grain yield, Turda, 2020-2022

Year	Yield (kg/ha)	% average	± average
Average	*8,878*	*100*	-
2020	9,952	112	1,074***
2021	10,624	119	1,746***
2022	6,058	68	-2,820^{000}
		p<0.05 p<0.01 p<0.001	346 573 1,073

***/^{000} significant at p<0.001, positive, respectively negative values

In the favorable year, 2021, the average yield of the 34 hybrids considerably exceeded the average of the three years. High yield was also achieved in 2020, close to those of 2021. In 2022, the lack of precipitation associated with extreme temperatures led to some of the lowest productions in the last decade, in the case of the present study, the average of the 34 hybrids representing only 68% of the three-year average. The values regarding protein, starch, fat and fiber content (Table 3) were very close in 2020 and 2021. Same as in the case of yield, the year 2022 had an unfavorable influence on the quality of the grains, the accumulation of biochemical constituents in the grain being affected by the extreme temperatures in July and August.

Table 3. The influence of the year on maize grain quality (%), Turda, 2020-2022

Year	Protein %	Starch %	Fat %	Fiber %
Average	*8,48*	*72.79*	*4.38*	*3.03*
2020	9.02**	73.44*	4.63*	3.14*
2021	9.02**	73.43*	4.59^{-}	3.10^{-}
2022	7.41^{00}	71.51^{00}	3.92^{0}	2.84^{0}
p<0.05 p<0.01 p<0.001	0.19 0.43 1.38	0.38 0.88 2.80	0.22 0.51 1.62	0.09 0.20 0.63

*, **/$^{0, 00}$significant at p<0.05 and p<0.01, positive, respectively negative values.

Hybrid influence on yield and quality

The yield varied significantly depending on the hybrid studied (Table 4), with average values of the three years between 6692 kg/ha (HS105) and 12764 kg/ha (Turda 380). The productive superiority of the more recent creations is obvious, the hybrids approved after 2010 being noted: Turda 248 (2012), Marius TD (2013), Turda 332 (2014), Turda 344 (2017), Turda 335 (2021), Turda 2020 (2021) and Turda 380 (2022). These hybrids stood out both due to the yield obtained during the test period (Has et al., 2014; Haş et al., 2018; Haș et al., 2021; Varga et al., 2022) and due to their behavior in various stress conditions (Chețan et al., 2023; Popa et al., 2023; Simon et al., 2023; C. Vana et al., 2022; C. D. Vana et al., 2024).

The hybrids created during the beginning of the improvement of this crop, at Turda, can hardly cope with the cultivation conditions of recent years, but those with a shorter vegetation period could be an option for the successive crop in areas where the climatic conditions allow such agricultural practices. The new hybrids are better adapted and yield much higher.

Table 4. The influence of the hybrid on maize grain yield, Turda, 2020-2022

No.	Hybrid	Yield (kg/ha)	± average
0.	*Average*	*8,878*	-
1.	HD 115	6,898	-1,980 000
2.	HS 105	6,692	-2,186 000
3.	HS 105A	7,285	-1,593 000
4.	Turda 100	6,757	-2,121 000
5.	Turda SU 181	8,102	-776 -
6.	Turda SU 182	8,947	69 -
7.	Turda 145	8,622	-256 -
8.	Doina	7,865	-1,013 0
9.	Turda Mold 188	8,184	-694 -
10.	Turda 165	8,166	-712 -
11.	Turda 199	7,505	-1,373 00
12.	HD 211	7,813	-1,065 0
13.	Turda 228	7,455	-1,423 000
14.	Turda 200	8,136	-742 -
15.	Turda 213	7,734	-1,144 00
16.	Turda 160	7,782	-1,096 0
17.	Elan	7,525	-1,353 00
18.	Turda 200 Plus	8,336	-542 -
19.	Turda Super	8,696	-182 -
20.	Turda SU 210	8,626	-252 -
21.	Turda Star	10,431	1,553 ***
22.	Turda 215	7,544	-1,334 00
23.	Turda 248	10,531	1,653 ***
24.	Turda 165	7,101	-1,777 000
25.	Turda 260	9,191	313 -
26.	Saturn	9,806	928 *
27.	Turda 201	8,899	21 -
28.	Turda Favorit	10,180	1,302 **
29.	Marius TD	10,218	1,340 **
30.	Turda 332	11,036	2,158 ***
31.	Turda 344	12,279	3,401 ***
32.	Turda 335	12,287	3,409 ***
33.	Turda 2020	12,506	3,628 ***
34.	Turda 380	12,764	3,886 ***
		p<0.05	832
		p<0.01	1098
		p<0.001	1411

*, **, ***/0, 00, 000 significant at p<0.05, p<0.01 and p<0.001, positive, respectively negative values

Table 5. The influence of the hybrid on protein and starch, Turda, 2020-2022

No.	Hybrid	Protein (%)	Starch (%)
0.	*Average*	*8.48* -	*72.79* -
1.	HD 115	9.29 ***	71.69 0
2.	HS 105	9.03 ***	72.64 -
3.	HS 105A	8.85 ***	72.35 -
4.	Turda 100	9.35 ***	76.18 ***
5.	Turda SU 181	8.80 ***	73.11 -
6.	Turda SU 182	8.70 *	74.85 ***
7.	Turda 145	8.01 000	74.45 ***
8.	Doina	8.75 **	72.28 -
9.	Turda Mold 188	8.59 -	72.06 -
10.	Turda 165	8.25 00	72.83 -
11.	Turda 199	8.91 ***	72.09 -
12.	HD 211	9.51 ***	70.94 000
13.	Turda 228	9.90 ***	70.34 000
14.	Turda 200	8.86 ***	72.66 -
15.	Turda 213	8.59 -	70.56 000
16.	Turda 160	9.52 ***	74.95 ***
17.	Elan	9.04 ***	72.52 -
18.	Turda 200 Plus	8.21 00	74.06 **
19.	Turda Super	8.64 -	71.28 00
20.	Turda SU 210	8.63 -	72.92 -
21.	Turda Star	8.72 **	71.21 000
22.	Turda 215	9.13 ***	74.29 **
23.	Turda 248	7.30 000	73.67 -
24.	Turda 165	8.75 **	73.00 -
25.	Turda 260	8.98 ***	71.11 000
26.	Saturn	8.06 000	70.52 000
27.	Turda 201	7.89 000	73.82 *
28.	Turda Favorit	8.11 000	72.25 -
29.	Marius TD	7.03 000	73.64 -
30.	Turda 332	7.40 000	73.88 *
31.	Turda 344	7.27 000	73.69 *
32.	Turda 335	7.36 000	72.82 -
33.	Turda 2020	7.56 000	73.22 -
34.	Turda 380	7.36 000	73.04 -
	p<0.05	0.17	0.90
	p<0.01	0.23	1.19
	p<0.001	0.30	1.53

*, **, ***/0, 00, 000 significant at p<0.05 p<0.01 and p<0.001, positive, respectively negative values

A regression was observed regarding the protein content of the new hybrids, which is lower than that of the old hybrids. This aspect is also due to the increase in the frequency of genotypes from the dent type germplasm, productive but with a lower protein content, compared to the flint type genotypes, which stand out for their superior quality (Table 5). The lower values of the protein content are also due to the unfavorable influence of the year 2022, when the values were significantly reduced.

The hybrids that stood out for their high productions were also noted for their higher starch content. The Turda 100 hybrid is worth noting, which statistically significantly exceeded the experimental average for both protein content and starch, for the latter having the highest value among the 34 hybrids analyzed (76.18%). Among the hybrids created in recent years, it stands out for its starch content, Turda 332 and Turda 344.

Table 6. The influence of the hybrid on fat and fiber, Turda, 2020-2022

No.	Hybrid	Fat (%)	Fiber (%)
0.	*Average*	*4.38* -	*3.03* -
1.	HD 115	4.39 -	2.86 0
2.	HS 105	4.88 *	3.02 -
3.	HS 105A	4.77 *	2.80 0
4.	Turda 100	3.62 000	3.08 -
5.	Turda SU 181	4.35 -	2.85 0
6.	Turda SU 182	3.60 000	2.11 000
7.	Turda 145	5.03 **	3.44 ***
8.	Doina	4.32 -	2.93 -
9.	Turda Mold 188	4.04 -	3.31 **
10.	Turda 165	4.82 *	3.06 -
11.	Turda 199	5.22 ***	3.52 ***
12.	HD 211	4.06 -	2.62 000
13.	Turda 228	5.10 ***	2.88 -
14.	Turda 200	4.02 -	2.94 -
15.	Turda 213	5.18 ***	2.75 00
16.	Turda 160	3.65 000	2.66 000
17.	Elan	4.87 *	3.47 ***
18.	Turda 200 Plus	4.04 -	2.97 -
19.	Turda Super	4.16 -	2.93 -
20.	Turda SU 210	3.76 00	2.83 0
21.	Turda Star	4.55 -	2.97 -
22.	Turda 215	4.18 -	3.23 *
23.	Turda 248	4.49 -	3.09 -
24.	Turda 165	5.17 ***	3.31 **
25.	Turda 260	4.99 **	3.17 -
26.	Saturn	5.07 ***	3.63 ***
27.	Turda 201	4.31 -	3.16 -
28.	Turda Favorit	4.03 -	2.61 000
29.	Marius TD	4.57 -	3.21 *
30.	Turda 332	4.06 -	3.52 ***
31.	Turda 344	3.65 000	2.74 00
32.	Turda 335	4.15 -	3.24 *
33.	Turda 2020	3.94 0	2.91 -
34.	Turda 380	3.86 00	3.16 -
	p<0.05 p<0.01 p<0.001	0.38 0.51 0.65	0.17 0.23 0.29

*, **,***/0, 00, 000 significant at $p<0.05$, $p<0.01$ and $p<0.001$, positive, respectively negative values

The fat and fiber content of the 34 hybrids varied significantly (Table 6), with average values between 3.60 (Turda Su182) and 5.22 (Turda 199), respectively between 2.11 (Turda Su182) and 3.63 (Saturn).

Hybrid x year interaction influence on yield and quality

Each of the 34 studied hybrids obtained the lowest production in 2022, in some cases the values representing approximately half of the production obtained in the previous year (Figure 2). In the case of old hybrids, the yield in 2020 and 2021 had very close values, the differences between the two being several hundred kg/ha. However, the hybrids Doina, Turda Mold 188 and Turda 200 are worth noting, which obtained higher productions in 2020 than in 2021.

In the case of recently created hybrids, the superiority of the productions obtained in 2021 is statistically significant, both compared to the experimental average and to each of the other two years. The hybrids created in the last years (Turda 344, Turda 335, Turda 2020 and Turda 380) stand out for productions that exceeded 10,000 kg/ha in 2020 and 2021, and in 2022 they managed to yield between 6,712 kg/ha (Turda 332) and 9,366 kg/ha (Turda 380), demonstrating their increased adaptability to stress factors. Besides obtaining the highest production in unfavorable conditions, the Turda 380 hybrid stood out by obtaining the highest production in the most favorable year, exceeding 16,000 kg/ha.

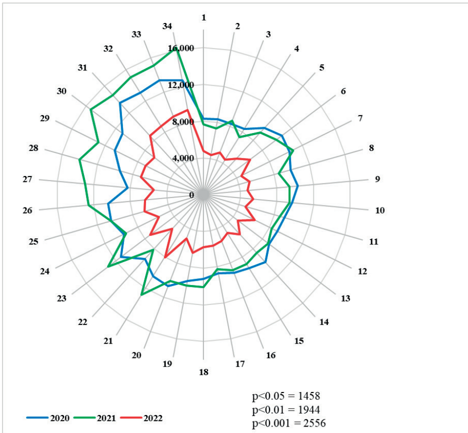

Figure 2. The influence of hybrid x year interaction on grain yield (kg/ha)

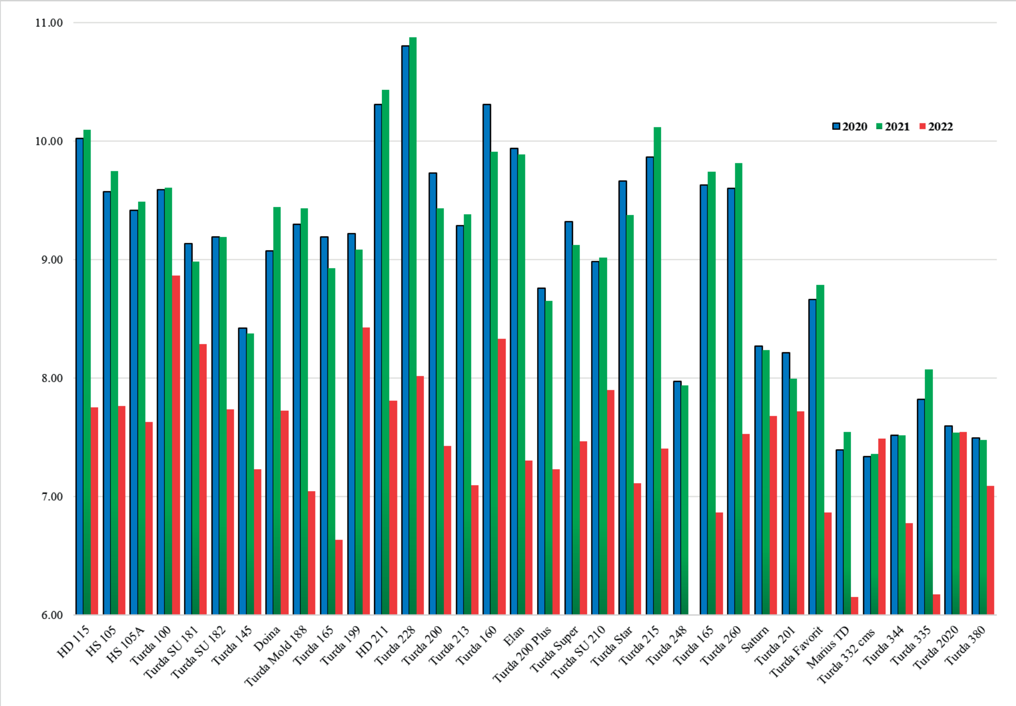

Figure 3. The influence of hybrid x year interaction on protein content (%)

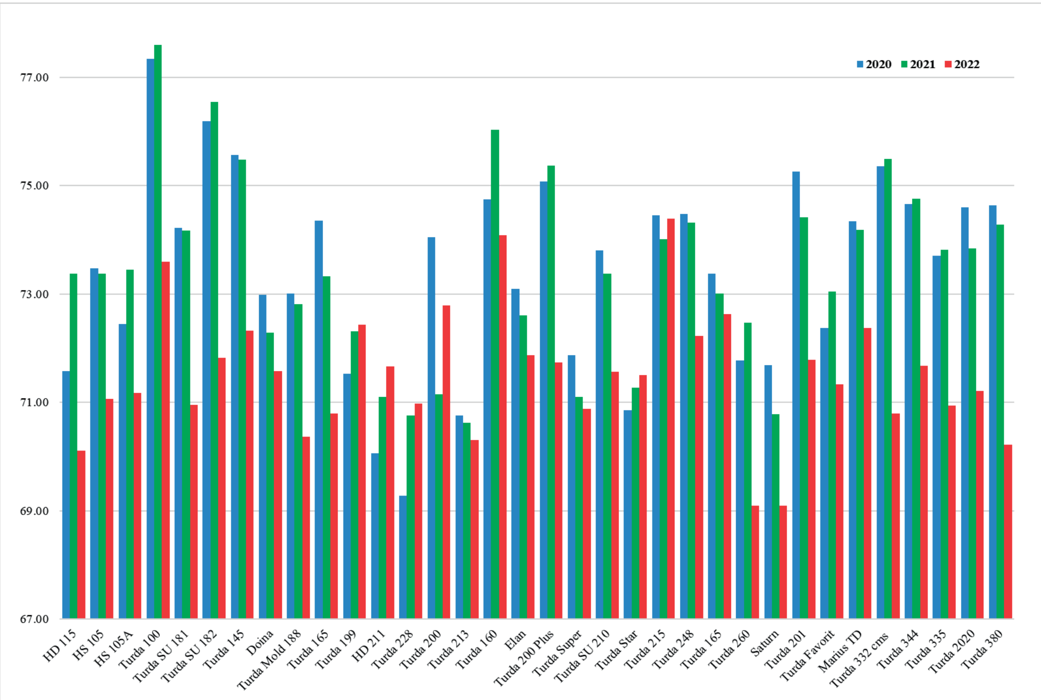

Figure 4. The influence of hybrid x year interaction on starch content (%)

The influence of unfavorable environmental conditions was evident in the case of proteins (Figure 3), for which much lower values were recorded in 2022 compared to the other two experimental years, for all hybrids. The statistically significant differences were up to 1.88%, compared to the experimental average ($p<0.001 = 0.87$). For most hybrids, in 2021, the accumulation of the highest amount of protein was achieved, the hybrid Turda 228 with 10.87% being remarkable. For this hybrid, the highest protein content was also obtained in 2020, of 10.80%.

The favorability of the years 2020 and 2021 is also reflected in the case of starch (Figure 4), which had significantly higher values in those two years, compared to 2022. Turda 100 had the highest starch content, 77.34% (2020), respectively 77.59% (2021). Higher values were also determined for the more recently created hybrids, Turda 380, Turda 2020, Turda 332, Turda 344.

The positive correlation between starch and yield is well-known, while both are negatively correlated with protein. Figure 5 shows the negative relationship between protein and yield, respectively starch in the case of the studied hybrids.

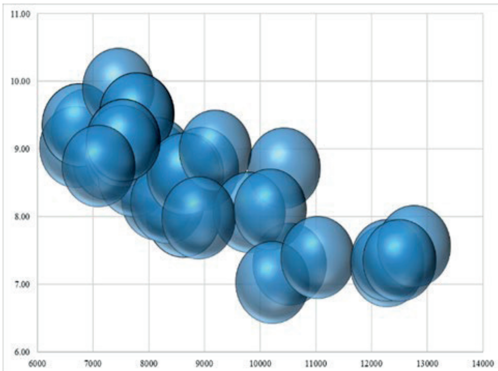

Figure 5. The relation between yield, protein and starch

CONCLUSIONS

Maize is a crop strongly influenced by climatic conditions, both in terms of quality and in terms of grain quality. However, under optimal cultivation conditions, the productions of this plant are impressive. In the case of the 34 studied hybrids, the highest yields were obtained by the majority of hybrids in 2021, followed almost by 2020 while in 2022 the average yield represented only 68% of the three-year average. Turda 380 obtained the highest average yield (12,764 kg/ha), including the highest one in the unfavorable year 2022, of 9,366 kg/ha, followed by Turda 2020, Turda 335, Turda 344 and Turda 332.

Due to the dent type germplasm, the new creations stand out for high productions and a rich starch content, while the older creations recorded higher values for the other biochemical constituents. Turda 228 had the highest protein content, standing out for the higher percentages of 2021 and 2020 (10.87% and 10.80%).

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RESEARCH ON THE INFLUENCE OF CLIMATIC PARAMETERS ON THE GEOGRAPHICAL DISTRIBUTION OF THE PEST *Tanymecus dilaticollis* Gyll. IN THE ROMANIAN PLAIN

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Abstract

The aim of the work was to determine the influence of recent climate changes on geographical distribution of the pest Tanymecus dilaticollis Gyll. in the main maize and sunflower growing areas from south and southeastern Romania. In the period 2021-2022, monitoring of the adults activity in laboratory and field conditions, adults abundance and climatic parameters were carried out in 60 farms. The results showed that the increase in temperature favour the activity of Tanymecus dilaticollis, the adult active period longevity was found to expanded by approximately 20%. The pest adult's abundance during the maize and sunflower early vegetation period varied from 3.67 to 27.24 adults per square meters in 2021 and from 7.21 to 16.27 adults per square meters in 2022.

Key words*: pest, maize, climatic parameters, Tanymecus dilaticollis.*

INTRODUCTION

Maize (*Zea mays*) is one of the world's three most important cereals along with wheat and rice. It is currently produced on nearly 100 million hectares in 125 developing countries and is among the three most widely grown crops in 75 of those countries (https://www.genebanks.org/resources/crops/maize/).With a total maize grains production of 22 million tonnes between 2021-2022 Romania occupy 2nd place in European Union, after France and 3rd place if we include Ukraine, validating that is one of the most important crops from our country (FAOSTAT database, 2024). At the same time maize yield per hectare may decrease due to biotic stresses such as pathogens, weeds or pests.

Maize leaf weevil [*Tanymecus dilaticollis* (Gyllenhal, 1834)] is a polyphagous species, which is among the most important insect pests of maize and sunflowers in Romania, Bulgaria, Serbia and Hungary (Toshova et al., 2021). Damages are caused by adults through feeding on maize leaves. In some particular conditions like monocropping or the case of high weevils' densities, maize plants are destroyed, and farmers must sow again (Čamprag et al., 1969; Paulian, 1978; Bărbulescu et al., 2001). The pest attack is dangerous when maize is in early vegetation stages, from plants emergence (BBCH 10) until four leaves stage (BBCH 14) (Georgescu et al., 2021). Romania faces an increasing number of major problems each year related to the infestation of the pest Maize Leaf Weevil, which infests large areas of arable land and can cause significant production and economic losses for Romanian farmers as well as implicitly for the national budget if specific chemical control measures are not applied.

The farmers need viable alternative in Romania to prevent yield losses caused by soil pests affecting areas of more than 2 million hectares (with a tendency to expand) of maize and sunflower, with very high densities per square meter of the pest *Tanymecus dilaticollis.* Some recent studies have already demonstrated good results of the insecticide activity of entomopathogenic fungi *Beauveria* spp. on the maize weevil, *Tanymecus dilaticollis*, under laboratory and small-scale trials in field conditions in Bulgaria (Toshova et al., 2021;) and Romania (Fătu et al., 2023) but the researches should be confirmed under large scale plots. Some corrective treatments with insecticide applied in crop early vegetation until five leaves stage are available but not always easy to apply and involves permanent surveillance, frequent applications and high costs to control pest populations. This is the reason why up to now the solution for the effective control of these pests remains the treatment of seeds with neonicotinoid insecticides (North et al., 2018). On the other hand, there is an urgent need of reducing insecticide use in Europe in general, and neonicotinoids in particular, and proper implementation of the IPM strategies as required by the European Directive 128/2009/EC on the Sustainable Use of Pesticides.

Badiu et al. (2019) demonstrated that pest population density significantly influences attack intensity and percentage of attacked plants, which means that, including when systemic treatments are applied, the extremely high insect population density in the monitored locations will induce significant attack intensities and crop losses. The bio-ecology of *T. dillaticolis* and the distribution of pests were the subject of systematic research conducted in the 1960's and 1970's by Paulian for his PhD thesis (Paulian, 1972).

Air temperature plays a major role in the regulation of insects physiological functions like respiration, metabolism, growth and reproduction (Harvey et al., 2020), and, consecutively, the climate changes and warming temperatures can influence their behavior, longevity and population dynamics (Stange & Aires, 2010). It is anticipated that climate change to have an impact on Maize Leaf weevil populations. Badiu et al. (2019) mentioned that an increase in average temperatures over the period of winter diapause results in an increase in insect populations per unit area assessed during the vegetation period.

The aim of the study was to evaluate the influence of climatic factors on the *Tanymecus dilaticollis* adult longevity and populations densities during two successive years (2021-2022) at large surfaces in the main maize growing area of the Romanian Plain.

MATERIALS AND METHODS

Field Survey area

The study was carried out in the south, south-eastern region of Romania, more precisely in the South-East, South-Muntenia and Bucharest-Ilfov Development Regions (Figure 1).

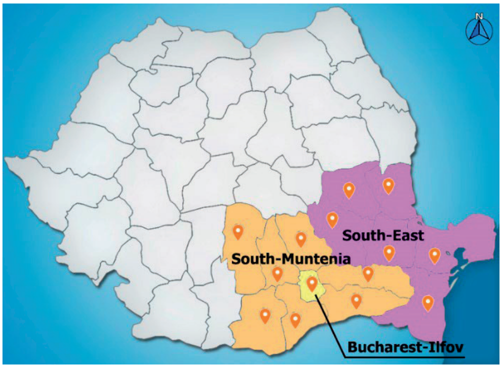

Figure 1. Area of study on Romania map

The choice of the locations was justified by the importance of this region for agricultural production, together with the favourable climatic and soil conditions for maize cultivation, as well as for the target pest *Tanymecus dilaticollis*.

Data collection

Between 2021 and 2022, a team from the Research - Development Institute for Plant Protection (R.D.I.P.P.) Bucharest and a group of farmers, associations and agricultural entities were involved in a comprehensive study dedicated to soil pest monitoring. The choice of this group was determined by their high level of expertise and involvement in the agricultural field, as well as the access they had to the agricultural land relevant to our research objectives. An original worksheet was designed by R.D.I.P.P. team of researchers to record relevant observations regarding the presence and intensity of soil pest attack and served as a data collection tool.

At the beginning of the survey, the participants were trained for conducting soil pest monitoring according to standardized protocols and were guided through the process of completing the worksheets. For each plot/parcel involved in survey, the following aspects were taken into account: locality, latitude, longitude, previous crop, current crop variety, crop vegetation stage and date of observation. The parcel identification process was carried out using current technologies, including mobile phones with GPS

applications, to precisely determine the coordinates of each parcel.
At least two main series of observations were performed in the field, focused on the assessment of soil pests present and all individuals that have been observed on 10-time standardised area of 1 square meter were counted, with attention paid to the identification and recording of the pest *Tanymecus dillaticolis*. Also, the number of plants per linear meter have been established, by accounting the number of plants on 10 linear meters in four different places of the parcel.

Data processing and analysis on the pest densities

The geographical distribution of pests density was achieved by vectorizing collection points and analyzing biological samples, namely the number of insects per square meter. Vectorization was performed by importing biological data along with geographic coordinates into the QGIS program. By interpolating the points using the IDW (Inverse Distance Weighted) function and assigning a color palette for five intervals of values, a raster GIS layer descrybing population density was generated. This type of raster was overlaid on the map of Romania, thus obtaining an overview image of the degree of *Tanymecus* infestation distribution.

Climatic influence factors on the populations of Tanymecus dillaticolis

Meteorological data were obtained from weather stations in the farm or from the local meteorological services in the area. These include: air temperature, soil temperature, and precipitation.
Adult active period longevity was determined under controlled temperature conditions. A series of 10 isolators were used at I.C.D.P.P. Bucharest with 10 insects each. The method involves as a first step obtaining an initial population of *T. dilaticollis* collected from the same field (a wheat crop in Belciugatele, Calarasi County) in early spring (29 March 2021) and rearing the adults under laboratory conditions at a temperature of 23 ± 2 degree Celsius (ºC) two weeks before using them in experiment. Each isolator consisted of a container with 5 maize plants covered with a transparent PVC cylindrical structure with an access area at the top that was covered with fine muslin cloth fitted by an elastic band, according to the method developed by Iamandei et al. (2024). The containers were first filled with sterilized soil and seeded with maize seed of P9537 Pioneer hybrid. Maize seeds were coated with fungicide Metalaxyl + Protioconazol + Lumidapt Kelta.
When maize plants were at BBCH 14, 10 *T. dilaticollis* adults (5 couple) were introduced into every isolator. Observation about adult activity have been performed daily, dead individuals were accounted and removed from isolators as soon as observed.
In field conditions, the influence of temperature on the *T. dilaticollis* was determined by analysing all captures at different atmospheric and soil temperatures. Further, the occurrence favorability of *T. dilaticollis* was estimated by relating the sum of the country-wide heat degrees to the Total thermal constant requirement to complete a generation, estimated to 2190-degree days (DD). WorldClim version 2.1 climate data for meteorological database covering the period 1950-2000 and the QGIS program were used to determine the sum of the degrees days.

RESULTS AND DISCUSSIONS

The study area coincide with the areas of greatest susceptibility to *T. dilaticollis* attack: the south and south-east of the country where the attack of the pest can compromise a crop in 2-3 days (Lup et al., 2017). Analysis of data comming from participating farmers showes that in these region there is a very high proportion of agricultural land occupied by maize, that creates big problems for them to achieve a proper crop rotation. Farmers therefore make short rotations, whereby maize returns to the same area after only 3 years, in some cases even after two years, which is not recommended by the multiannual surveys carried out in our country (Badiu et al., 2019; Iamandei & Rujescu, 2023).
According to our observations, the end of adults diapause was noted in Belciugatele (Calarasi County) during the last decade of February (25th of February) in 2021 and in mid-February (19th February) in 2022, when they started to feed on winter cereals, during

the sunny and warm period of the days. In April, when the sunflower and corn crops started to emerged at soil surfaces, the adults migrated to the respectivelly plots sometimes together with the populations of adults that have come out of diapause from the soil during a staggered process untill the beginning of May.
In 2021, the mean number of adults per square metre was between 3,6723 and 27,299 (Figure 2) while in 2022 ranged from 7.21 to 16.262 (Figure 3) in all cases, it exceeds the economic damage threshold of 3 adults/square meters, being up to 907 % higher (in 2021), respectively 542 % higher in 2022) than the limit from which crop protection treatments are recommended.

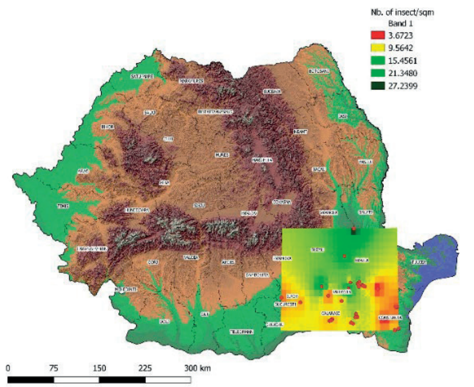

Figure 2. Number of insects/ sqm 2021

Our data confirmed the most recent data presented by Badiu et al. (2019) in their long-term study, stating that, including if systemic treatments are carried out, the extremely high population density of pest from the monitored region will induce higher attack intensities and significant harvest losses if contact insecticide are not applied in vegetation. The pest densities and variation from a year to another are influenced by weather conditions and previous crops, knowing that maize and/or sunflower, cultivated after a non-host plant species (annual leguminous, grassy cereals, rapeseed, flax etc.), leaves a reserve of up to 3 adults/sq.m in the soil while maize after maize provides a mean reserve of 10-15 adults/sq.m and up to 160 adults/sq.m. can be recorded, as reported in Braila County (Rosca & Istrate, 2009).

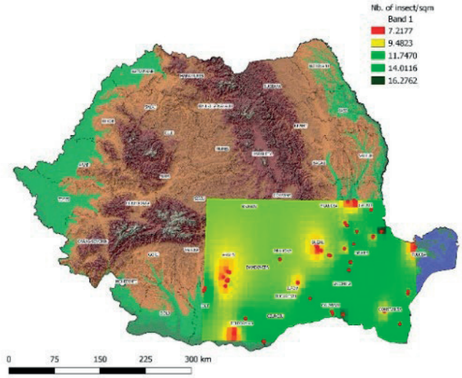

Figure 3. Number of insects/ sqm 2022

The corn leaf weevil is a thermophilic insect, in field the adults are very active, consuming intensively the seedlings both to ensure food and to cover water losses, when the average temperature during the day exceeds 20°C.
The analysis of laboratory data revealed that the adult active period mean longevity at an average temperature of 23 degrees Celsius, under controlled conditions, was of 95 days, with some individuals living up to 138 days (Figure 4). The active period it was found around two weeks longer than that observed by Paulian (1978, PhD thesis).

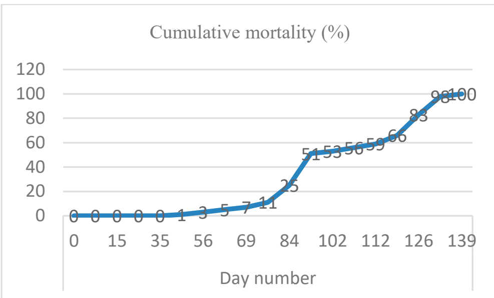

Figure 4. *Tanymecus dilaticollis* adults cumulative longevity under laboratory conditions, at 23 ± 2°C

In field conditions, the temperature value of 23°C was also significantly correlated with the moment of maximum activity of insects, confirmed by the highest number of captured insects, with a correlation coefficient R^2 of 0.73, demonstrating that temperature is the primary factor related to the distribution and biological activity of the adult (Figure 5).

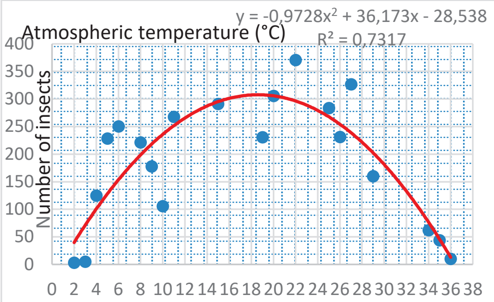

Figure 5. Correlation between the average atmospheric temperature and the number of captured insects

Given that the temperature was collected by meteorological stations at a height of 2 meters and the ground temperature is generally higher, it was necessary to make a correlation to determine the optimal temperature of biological activity of the pest. In this sense, the Khandaker equation ($T_{soil\ 10\ cm\ deep} = 6.224 + 0.842 * T_{air}$; r = 0.93; Islam et al., 2015) was applied to the atmospheric temperature of 21.3°C representing the parameter BIO10 (Average temperature of the hottest season) specific to Călăraşi county characterized by strong attacks of the pest, obtaining the value of 24.15^{0}C (Figure 6).

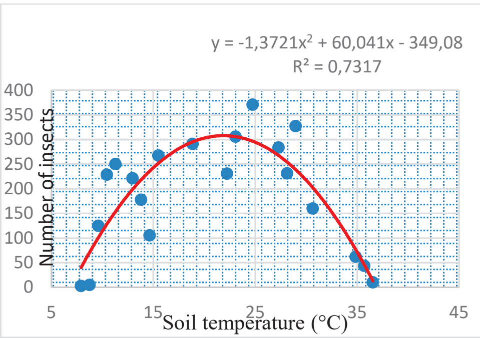

Figure 6. Correlation between the average soil temperature and the number of captured insects

Mass reproduction of the insect is favoured by higher temperatures and drought during the start of sunflower and maize vegetation period, in April and May. The area of maximum environmental suitability of the pest, estimated based on laboratory data and confirmed in the field conditions, overlaps with the area currently occupied by the two crops (Figure 7). Actually, some areas considered until recently unfavourable for Maize Leaf Weevil activity, such as Transylvania (Antonie I., 2012) register increasingly aggressive attacks in favourable years and increases of the temperatures explain the extending the pest areas to northern latitudes.

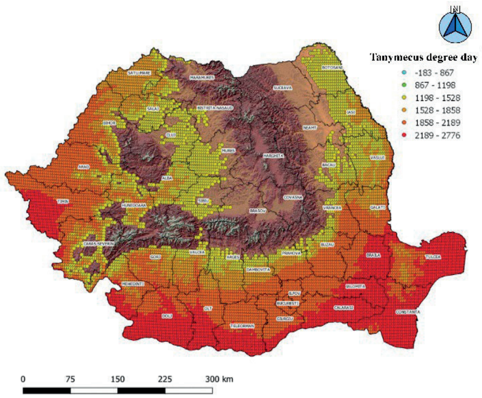

Figure 7. Thermal favourability of the pest *Tanymecus dilaticollis* in Romania

CONCLUSIONS

The recent climate changes, especially the increase in temperature in the months of April and May, with longer periods of drought, favour the activity of *Tanymecus dilaticollis* adults, the adult active period longevity was found to expanded by approximately 20%. The large-scale study of *T. dilaticollis* abundance during the maize and sunflower early vegetation period varied from 3.67 to 27.24 adults per square meters in 2021 and from 7.21 to 16.27 adults per square meters in 2022. Although the crops were established with seeds treated with systemic insecticides, the damage threshold was generally exceeded, and applications of treatments in the vegetation were mandatory. It is necessary to continue the research with a detailed analysis of the impact that other technological means might have on pest activity and the damages produced by their populations.

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RESEARCH ON WHEAT SEED GERMINATION AS A FUNCTION OF TEMPERATURE, UNDER THE INFLUENCE OF TREATMENT WITH BIOSTIMULATORS AND AT DIFFERENT LEVELS OF FERTILIZATION

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Abstract

During 2020-2021, a bifactorial experiment was set up on the ARDS Caracal cernozome to study the influence of biostimulator seed treatment (Ympact and Kerafol applied in different doses) on different levels of fertilization. The seed used in the first testing year-2021 and that obtained in 2022 was tested in terms of germination at different temperatures (1°C, 5°C, 10°C, 15°C and 20°C) as well as in terms of the number of days required for germination depending on the factors presented above. The Glosa wheat seed germinated at all temperatures, distinctly and very significantly more than at 1°C. However, there were no differences between the other temperatures, each taken as a control. The number of days required for germination is very significantly reduced from one temperature to another. At 1°C, wheat requires 33 days to germinate, while at 20°C, only 4 days. For the NPK fertilized variant in autumn + ammonium nitrate and foliar in spring, germination of the seed obtained is very little different between temperatures of 5, 10, 15 and 20°C and between the biostimulator treatment variants.

***Key words**: biostimulator, fertilization levels, germination, number of days required for germination, wheat.*

INTRODUCTION

Germination plays a substantial role in plant development, being the first stage of plant life. This process is easily influenced by environmental factors, which often have negative effects on germination, reducing plant growth, development and production (Moș et al., 2020). Seed quality is a particularly important aspect of wheat breeding technology, as uniform germination and high seedling vigour successfully contribute to the stability and productive performance of the crop (Vasilescu et al., 2019; Cardarelli et al., 2022). Seed germination is a contributing factor to high yield. Among abiotic factors, temperature is considered an important requirement for wheat germination, as it determines the amount of additional water and substrates needed for growth and development. Temperature is a factor that can alter germination because it can influence the rate of uptake of water and other substrates needed for growth and development (Wanjura and Buxtor, 1972; Essemine et al., 2002).

Temperature has a major impact on germination time (Guan et al., 2009). The effect of temperature on germination can be described as a scale with steps: minimum, optimum and maximum (Ostadian Bidgoli et al., 2018). The optimum temperature results in the most significant germination percentage (%) in the shortest time. Each germination stage has its own temperature; due to the complexity of the germination process, the temperature response may vary throughout the germination stages. Seed response to temperature is a function of cultivar, seed quality, time since harvest, and other factors (Dadach et al., 2015). Numerous studies have been conducted on temperature regulation of germination. Riley (1981) demonstrated that the energy status of cells and the behaviour of certain enzymes change with changing temperatures. ATP content and protein synthesis rate increase as temperature reaches the optimum and decrease as it goes in other directions (Riley, 1981). The antioxidant system greatly affected the germination of wheat seeds (Kunos et al., 2022).

Along with temperature, soil pH, water availability and soil moisture have the greatest influence on seed germination among abiotic factors (Rizzardi et al., 2009). Seed germination reflects seed size, abundance and distribution in the seedbed (Cone and Spruit, 1983; Bentsink and Koornneef, 2008; McCormick et al., 2016).

Germination of wheat seeds occurs only when the seed dormancy period has been completed, which, as Matei (2003) shows, in varieties grown in Romania, averages between 40 and 60 days. However, there are also cultivars with very short dormancy (up to 10 days), varieties with medium dormancy (between 30 and 45 days) and varieties with long dormancy (more than 45 days).

Grain germination depends on the grain's ability to use its more efficient reserves (Buriro et al., 2011) and is a contributing factor to grain yield formation. Rapid and uniform germination is essential for better plant growth and higher yield (Lupașcu et al., 2020).

Seed germination begins with water uptake by the dormant dry seed and is achieved by root emergence as the embryo axis lengthens (Fu et al., 2021). An orderly sequence of morphogenetic and physiological mechanisms, including energy transfer, nutrient uptake, and physiological and biochemical changes, are part of this process (Bewley and Black, 1994). The water uptake of a seed occurs in three stages: phase I - a rapid initial uptake, phase II - a plateau, and phase III - an increase in water uptake, but accompanied by the initiation of germination (Manz et al., 2005). Phase I germination, called imbibition, results in the softening and swelling of the seed coat at or near an ideal temperature (Xue et al., 2021).

Ghițău and Donțu (2010) investigated under laboratory conditions the effect of applying biostimulants in different concentrations on germination (energy and germination capacity), root length and coleoptile in winter wheat, Boema variety. In all treatments there was no difference in germination energy and germination capacity. The highest germination percentage occurred with the biostimulants: BCO-4K - 96.12%, BCO-2K+zinc acetate - 94.5% germination; BCO-4DMA - 95.5% germination; BCO-2DMA+zinc acetate - 93.87% germination.

The study of the influence of factors: years (Y)/culture (C)/treatment with *Bacillus* spp., *Trichoderma harzianum* (T), conducted by Poštić and co-workers (2021) showed that the year factor, Y x C interaction as well as Y x T interaction were not significant ($p \geq 0.05$). Instead, cultivar and treatments as well as their interaction on all determined traits were significant ($p \leq 0.05$ to $p \leq 0.01$).

Another study showed data on the reaction of prospective lines and released varieties of common winter wheat (*Triticum aestivum* L.) to different temperature levels (4, 14, 22°C).

Based on important growth and development traits (germination, root length, stem length) the researchers calculated vigour index, variance (genotypic, phenotypic), coefficients of variation, broad heritability and genetic advantage of traits. It was concluded that there is an opportunity for the studied genotypes to be involved in breeding programmes to improve cold hardiness (Lupașcu et al., 2020).

A study to investigate the germination performance of wheat seed under different moisture conditions, temperatures and seed densities was conducted by Khaeim et al. in 2022. The objectives of this study were (1) to determine the optimum range of water quantity for germination based on the volume of water at one milliliter intervals and the amount of water as a percentage of the mass of 1000 grains; (2) to determine the effects of temperature and germination time of wheat seeds; (3) to investigate the effect of seed number and seedling density in a Petri dish on germination percentage and seedling viability under the same amount of water. The results showed that germination in different percentages can occur in a wide range of water amounts starting from 0.65 ml, which represents 75% of the mass of 1000 seeds, and that the optimum range for germination is 4.45-7.00 ml, which represents 525-825% of this.

For laboratory experiments, a density of maximum 15 wheat seeds per 9 cm Petri dish was recommended. In general, 20°C was the ideal temperature for seedling development. Germination has a wider range, from 20°C to 30°C. The recommendation is made especially for breeding projects, when the number of seeds is limited (Khaeim et al., 2022).

Wheat yield in Romania varied after 2010 from 2,659 to 4,888 kg/ha and was influenced by the genetics used by growers (variety or hybrid), soil and climatic conditions (soil, climatic factors, climatic accidents) and technological factors (mainly crop rotation, fertilization, tillage, seed quality and sowing conditions, weed, disease and pest control, harvest conditions) (Dumbravă et al., 2019).

MATERIALS AND METHODS

A two-factor experiment was set up in 2020 and 2021 using the 3 replicate sub-divided plots method. The factors studied were: factor A - fertilization level with 4 gradations: a1 -fertilized with NPK in autumn (N50P50K0); a2- fertilized with NPK in autumn + ammonium nitrate in spring (N140P50K0); a3-fertilized with NPK in autumn + foliar in spring (N73P50K0); a4- fertilized with NPK in autumn + ammonium nitrate and foliar in spring (N163P50K0) and factor B - seed treatment with biostimulator with 6 gradations: b1- untreated with fungicide and biostimulator; b2- treated with fungicide but untreated with biostimulator; b3- treated with fungicide and YMPACT 0. 7 l/t; b4- treated with fungicide and YMPACT 0.35 l/t; b5- treated with fungicide and KERAFOL 1 l/t; b6- treated with fungicide and KERAFOL 0.5 l/t.

The seed used to set up the experiment in the first year of testing (2020-2021) and the seed obtained in 2022 (crop year 2021-2022) was tested for germination and number of days required for germination according to the factors presented above at different temperatures (1ºC, 5ºC, 10ºC, 15ºC and 20ºC) - factor C. Basically it was a single experiment, but for the germination study, the seed came from 2 sources - the first sample from seed used at sowing and the second from seed obtained at harvest.

The variety used - Glosa, registered in 2005, was obtained at INCDA Fundulea from the complex hybrid combination Delabrad "S"/Dor "S"//Bucur, by individual selection. In Romania, of all the wheat varieties in production, the most widespread is the Glosa variety, which occupies about 35% of the total cultivated area (Radu, 2023), enjoying great success among farmers in all areas of the country, with a very high adaptability and production potential superior to previous varieties.

Germination was determined in a temperature-controlled growth chamber (Snijders Scientific).

After homogenization and repeated splitting of the seed sample, 3 x 100 grains were randomly counted. In a washed and disinfected plastic tray of dimensions 40 x 25 x 7, a smooth layer of cotton wool was placed on which the grains were placed, i.e. 100 grains spaced in 10 rows and 10 columns (equivalent to one repetition). The three replicates for each sample were placed in different trays and a volume of 300 ml of water was sprayed evenly over each tray and then covered with clear food wrap. The placement of the trays in the growth chamber was randomized. They were checked periodically.

Germination, indicated by the appearance of the primary root followed by the appearance of the coleoptile, was expressed as a percentage of the total number of germinated or ungerminated grains of the initial 100.

(Numbers of sprouted grains*100)/100

(Numbers of ungerminated grains*100)/100.

RESULTS AND DISCUSSIONS

In the year 2021, without involving the influence of treatment, it was observed that the wheat seed of the variety Glosa, germinated at all temperatures distinctly and very significantly more than at 1ºC. In contrast, there were no differences between the other temperatures, each taken as a control (Table 1).

In contrast, the number of days required for germination decreases significantly from one temperature to another. At 1ºC, wheat requires 33 days to germinate, while at 20ºC, only 4 days (Table 2). It should be pointed out that the lower number of days for germination (very significant decreases) is a positive aspect for the crop.

Table 1. Germination of wheat seed as a result of temperature - 2021

Temperature (ºC)	Germination (%)	Difference + semnificance			
		C1	C2	C3	C4
1ºC (C1)	90.6	0.0			
5ºC (C2)	95.6	5.0**	0.0		
10ºC (C3)	96.1	5.5***	0.5	0.0	
15ºC (C4)	98.1	7.5***	2.5	2.0	0.0
20ºC	97.5	6.9***	1.9	1.4	-0.6
LSD 5%	2.9				
LSD 1%	3.9				
LSD 0.1%	5.1				

Table 2. Number of days needed for germination depending on temperature - 2021

Temperature (ºC)	No germination days	Difference + semnificance			
		C1	C2	C3	C4
1ºC (C1)	33	0			
5ºC (C2)	18	-15ººº	0		
10ºC (C3)	10	-23ººº	-8ººº	0	
15ºC (C4)	5	-28ººº	-13ººº	-5ººº	0
20ºC	4	-29ººº	-14ººº	-6ººº	-1ººº
LSD 5%	0.2				
LSD 1%	0.3				
LSD 0.1%	0.4				

The low values of the limit differences are due to the homogeneity of the number of days needed for germination between repetitions.
The influence of fungicide + biostimulator treatment on germination, regardless of temperature, is not shown by statistical calculation (Table 3).

Table 3. Germination according to wheat seed treatment - 2021

Seed treatment	Germination (%)	Difference C
b1-untreated with fungicide and biostimulator	94.5	0.0
b2-treated with fungicide but untreated with biostimulator	96.8	2.3
b3-treated with fungicide and YMPACT 0.7 l/t	95.9	1.4
b4-treated with fungicide and YMPACT 0.35 l/t	95.3	0.8
b5-treated with fungicide and KERAFOL 1 l/t	96.1	1.6
b6-treated with fungicide and KERAFOL 0.5 l/t	94.9	0.4
LSD 5%	3.3	
LSD 1%	4.6	
LSD 0.1%	6.7	

The number of days required for germination, although spread over 13-15 days, was very significantly higher or lower depending on the seed treatment. Therefore, the biostimulator YMPACT, irrespective of dose, stimulated seed germination compared to both untreated variants, while KERAFOL, irrespective of dose, slowed germination (Table 4).

Table 4. Number of days needed for germination depending on seed treatment-2021

Seed treatment	No of days required for germination	Diff. C
b1-untreated with fungicide and biostimulator	14	0
b2-treated with fungicide but untreated with biostimulator	14	0
b3-treated with fungicide and YMPACT 0.7 l/t	13	-1ooo
b4-treated with fungicide and YMPACT 0.35 l/t	13	-1ooo
b5-treated with fungicide and KERAFOL 1 l/t	15	+1***
b6-treated with fungicide and KERAFOL 0.5 l/t	15	+1***
LSD 5%	0.2	
LSD 1%	0.3	
LSD 0.1%	0.5	

The influence of germination temperature x treatment interaction on seed germination is differentiated (Table 5).
While in the fungicide-treated and non-biostimulator-treated variants germination is significantly and distinctly significantly higher at all temperatures compared to that at 1ºC, in the YMPACT-treated variants, germination is not statistically different from that at 1ºC.

Table 5. Germination as a result of temperature x seed treatment interaction - 2021

Temperature (ºC)	Germination (%)					
	b1	b2	b3	b4	b5	b6
1ºC (C)	90.7	89.7	93.0	92.0	90.3	88.0
5ºC	90.3	99.7**	96.0	92.7	97.7*	97.0*
10ºC	96.7	97.3*	96.0	96.3	96.7	93.7
15ºC	98.3*	98.0*	98.7	97.0	98.3*	98.0**
20ºC	96.3	99.3**	95.7	98.3	97.3	98.0**
LSD 5%	7.2					
LSD 1%	9.6					
LSD 0.1%	12.5					

This suggests that YMPACT biostimulator, helps the seed to germinate at the same values regardless of temperature. The same cannot be said for KERAFOL, where regardless of the dose, the seed germinates better at 5 and 15ºC, and at half the dose at 20ºC.
As the graph below shows, the lowest germination amplitude is at 15ºC and the highest at 5ºC. Although the fungicide-treated but not biostimulator-treated (b2) variant at 3 of the temperatures (5, 10 and 20ºC) has the highest germination values, this is not necessarily statistically assured (Figure 1).

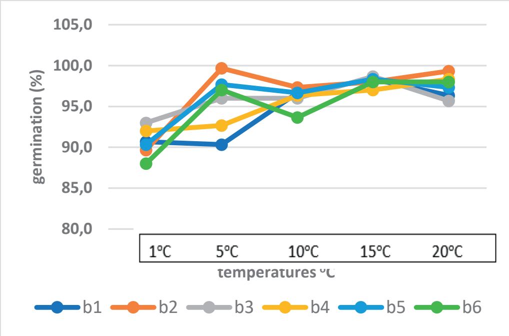

Figure 1. Germination amplitude at the same temperature based on seed treatment - 2021

In year 2022, the seed analysed came from the experiment located in the previous year and therefore the samples sum up all the factors studied, germination being done according to them. Consequently, it was observed that depending on the level of fertilization applied, the resulting seed germinated significantly, distinctly significantly and very significantly better than the variant in which only NPK complexes were administered in autumn (Table 6). This is due to the existing correlation between germination and mass of 1000 grains, the correlation coefficient r = 0.415 being significantly different at P 5% level (0.400). The larger the grain, the better it germinates.

In both variants where ammonium nitrate was applied in spring, germination was at the control level, regardless of the B-factor grading, i.e. the biostimulator did not influence germination. In contrast, in the absence of ammonium nitrate, YMPACT biostimulator at both doses highly significantly negatively influenced germination (only NPK in autumn) and the full dose - significantly negatively at NPK autumn + foliar in spring (Table 7).

Depending on the biostimulator treatment, the results showed that germination is reduced with statistical assurance in YMPACT treatment, both doses and KERAFOL treatment, 0.5 L/t dose.

Table 6. Germination as a function of fertilization in wheat - 2022

Fertilization	Germination (%)	Diff.+ Semnif. C	MMB
a1-fertilized with NPK in autumn (C)	94.8	0.0	36.61
a2-fertilixed with NPK in autumn + ammonium nitrate in spring	96.3	1.5*	37.19
a3-fertilixed with NPK in autumn + foliar in spring	96.7	1.9**	37.89
a4-fertilized with NPK in autumn + ammonium nitrate and foliar in spring	98.1	3.3***	36.95
LSD 5%	1.2		
LSD 1%	1.8		
LSD 0.1%	2.8		
Correlation coefficient germination - MMB			0.415

Table 7. Germination as a result of biostimulant treatment (Factor B) x fertilization (Factor A) and unilateral biostimulant treatment (Factor B) - 2022

Seed treatment	Germination (%)				
	a1	a2	a3	a4	average
Interactions	Treatment biostimulators (Factor B) x fertilization (Factor A)				Factor B
b1-untreated with fungicide and biostimulator	95.4	96.5	97.1	98.3	96.9
b2-treated with fungicide but untreated with biostimulator	94.8	96.6	97.0	98.0	96.6
b3-treated with fungicide and YMPACT 0.7 l/t	93.7^{ooo}	96.5	95.9$_{o}$	97.5	95.9^{oo}
b4-treated with fungicide and YMPACT 0.35 l/t	93.5^{ooo}	95.4	96.5	98.3	95.9^{oo}
b5-treated with fungicide and KERAFOL 1 l/t	95.6	97.3	97.7	98.6	97.3
b6-treated with fungicide and KERAFOL 0.5 l/t	95.7	95.7	95.7^{o}	97.9	96.3^{o}
LSD 5%	1.2				0.6
LSD 1%	1.7				0.8
LSD 0.1%	2.2				1.1

As in the previous year, germination is very significantly higher at all temperatures compared to the control, regardless of fertilization level (Table 8).

Table 8. Germination as a result of the interaction of temperature (Factor C) x fertilization (Factor A) and unilateral temperature (Factor C) - 2022

Temp (°C)	Germination (%)				
	a1	a2	a3	a4	average
Inter actions	temp (Factor C) x fertilization (Factor A)				Factor C
1°C (C)	89.9	92.4	92.1	93.5	92.0
5°C	95.4 ***	97.6 ***	97.8 ***	98.6 ***	97.4 ***
10°C	97.1 ***	97.8 ***	97.4 ***	99.5 ***	98.0 ***
15°C	96.4 ***	97.2 ***	98.4 ***	99.7 ***	97.9 ***
20°C	95.1 ***	96.6 ***	97.9 ***	99.2 ***	97.2 ***
LSD 5%	1.3				0.6
LSD 1%	1.7				0.9
LSD 0.1%	2.2				1.1

Seed obtained from the NPK-fertilized variety in autumn and ammonium nitrate-fertilized variety in spring required a significantly longer germination time compared to the control-NPK variety in autumn (Table 9).

Table 9. Number of days needed for germination depending on fertilization level - 2022

Fertilization	No days required germination	Dif+ Semnificance C
a1-fertilized with NPK in autumn (C)	16.6	0.0
a2-fertilized with NPK in autumn + ammonium nitrate in spring	17.7	1.1*
a3-fertilized with NPK in autumn + foliar in spring	17.1	0.5
a4-fertilized with NPK in autumn + ammonium nitrate and foliar in spring	16.5	-0.1
LSD 5%	1.0	
LSD 1%	1.4	
LSD 0.1%	2.3	

Interaction of biostimulator treatment (Factor B) x fertilization (Factor A) and unilateral biostimulator treatment does not influence the number of days needed for germination. The germination process occurs at the same time regardless of the variant differentiated by the level of fertilization from which the analysed seed originated (Table 10).

Irrespective of the level of fertilization, the number of days required for germination is very significantly lower at all temperatures tested than at 1°C. As in the previous year, the number of days needed to germinate was very significantly lower at all temperatures compared to the control, regardless of fertilization level (Table11). They decreased from 47 days needed for germination at 1°C to 4 days needed for germination at 20ºC.The values of the limit differences showed that the number of days required for germination decreased with statistical assurance from one germination to another.

Table 10. Number of days needed for germination depending on biostimulator treatment (Factor B) x fertilization (Factor A) and unilateral biostimulator treatment (Factor B) interaction - 2022

Seed treatment	No. days required for germination				
	a1	a2	a3	a4	average
Interacțiuni	Treatment biostimulators (Factor B) x fertilization (Factor A)				Factor B
b1-untreated with fungicide and biostimulator	16.7	16.8	17.1	16.5	16.8
b2-treated with fungicide but untreated with biostimulator	16.6	16.7	17.2	16.5	16.8
b3-treated with fungicide and YMPACT 0.7 l/t	16.7	16.9	17.3	16.5	16.8
b4-treated with fungicide and YMPACT 0.35 l/t	16.7	16.5	17.0	16.5	16.7
b5-treated with fungicide and KERAFOL 1 l/t	16.7	16.5	17.2	16.5	16.7
b6-treated with fungicide and KERAFOL 0.5 l/t	16.6	16.5	17.0	16.5	16.7
LSD 5%	1.0				0.5
LSD 1%	1.4				0.7
LSD 0.1%	1.8				0.9

Table 11. Number of days needed for germination as a result of the interaction of temperature (Factor C) x fertilization (Factor A) and unilateral temperature (Factor C) - 2022

Temperature (ºC)	No. days required for germination				
	a1	a2	a3	a4	average
Interactions	Temperature (Factor C) x fertilization				Factor C
1ºC	46.7	46.5	48.8	46.0	47.0
5ºC	17.4ººº	17.4ººº	18.0ººº	17.6ººº	17.6ººº
10ºC	10.2ººº	10.3ººº	9.6ººº	10.0ººº	10.0ººº
15ºC	5.0ººº	5.0ººº	5.0ººº	5.0ººº	5.0ººº
20ºC	4.0ººº	4.0ººº	4.0ººº	4.0ººº	4.0ººº
LSD 5%	1.0				0.5
LSD 1%	1.4				0.7
LSD 0.1%	1.8				0.9

CONCLUSIONS

The lowest germination amplitude is at 15ºC and the highest at 5ºC.

The number of days required for germination, although spread over the range 13-15 days, was very significantly higher or lower depending on the seed treatment. Thus, the biostimulator YMPACT, regardless of dose, stimulated seed germination compared to both untreated variants, while KERAFOL, regardless of dose, hindered germination. At 1ºC, wheat took 33 days to germinate, while at 20ºC, only 4 days. The results showed that the lower number of days for germination (very significant decreases) is a positive aspect for the crop.

In the NPK fertilized variant in autumn + ammonium nitrate and foliar in spring, the germination of the obtained seed is very little differentiated between temperatures of 5, 10, 15 and 20ºC, as well as between the biostimulator treatment variants.

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EFFECT OF SOWING DENSITY ON QUANTITY AND QUALITY OF PRODUCTION IN WINTER BARLEY (*Hordeum vulgare* L.)

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Abstract

Winter barley (Hordeum vulgare L.) is an important crop in Romania grown under different pedoclimatic conditions over whole country. The aim of this study was to establish the influence of four different sowing densities (300, 400, 500 and 600 seeds/m²) of Lucian variety and of Jallon hybrid (200, 250, 300 and 350 seeds/m²), under pedoclimatic conditions of Giurgiu county during 2021-2022. Assessments were conducted in order to evaluate production elements and the quality indexes (number of tillers, plants height, number of ears, number of grains/ears, the 1,000-grains weight, protein content and starch content). The results obtained showed that yield was obviously influnced by sowing density and by variety grown. Hybrid Jallon has provided the highest yield (7,875 kg/ha) when sown at 350 seeds on square meter. An yield of 6,487 kg/ha has been recorded when Lucian variety was sown at 600 seeds on square meter. Productivity elements and quality indicators of barley yield were strongly influenced more by sowing density than variety sown.

Key words*: winter barley, density, quality indexes, varieties, genotypes.*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest crop in the world and one of the most important cereal with many uses in livestock fodder, human nutrition and industry, being the fourth grown cereal in the world after wheat, corn and rice. Barley is grown under different pedoclimatic conditions that affect the overall performance, especially the grain yield and quality. Barley has been an excellent solution for farmers in recent years due to its many advantages, including the diversification of sown grass crops and the reduction of risks generated by drought or frost. In order to obtain a high quality barley, in addition to perform the technological links, choosing the right variety is the main factor that mostly influences profitability.

For barley crop, the cultivar has an important role in obtaining safe and stable yield, and its agronomic characteristics such as, tillers per plant and grain physical quality ensure better constancy and stability for yield (Friedt et al., 2011; Pérez-Ruiz et al., 2016). To achieve barley high yield for grain or green mass, stable and qualitatively superior, growing of several valuable genotypes, with high production capacity, is required with superior qualitative features, resistant to diseases and adapted to different environmental conditions (Bolandi et al., 2012; Vasilescu et al., 2020; Costantea et al., 2022).

Sowing density is an important factor which influence yield and quality of barley. Optimum densities vary greatly between areas, climatic conditions, soil, sowing time and cultivars. As cultivars differ genetically in yield components, individual cultivars must be tested at a wide range of seeding rates to determine the optimal seeding rate (Wiersma, 2002; Spasova; Dragica et al., 2013). For this reason, in scientific investigations, it is constantly investigated optimization of plant number per unit area (Zecevic et al., 2014).

Taking into account these aspects, the aim of our investigations was to assess the impact of two different genotypes and the sowing rate on the yield and some quality characteristics of winter barley under pedoclimatic conditions from the Giurgiu area.

MATERIALS AND METHODS

Research was carried out in field at the Oinacu village, Giurgiu county (43°58'452" N and 026° 05'164" E) and was performed under the influnce of the rainfall ammount of the area on a calcareous alluvial type of soil, moderately gleized, sandy texture (pH 7.3-7.5). The experiment used the method of subdivided plots into 3 replications, with the experimental plot area of 36 m^2 (3.6 x 10).

Two winter barley genotypes were sown in the field: the hybrid Jallon and the cultivar Lucian. Hybrid Jallon was sown at four different densities: 200, 250, 300 and 350 seeds/m^2 and the variety Lucian at: 300, 400, 500 and 600 seeds/m^2. Sunflower was the previous crop. Fertilization consisted on NPK fertilizer 20:20:0 application splitted in two stages: in the autumn at seedbed tillage (60 kg/ha) and after hibernation at the tillering stage in early spring (30 kg/ha). Seedbed was done before sowing by two soil harrowings. The sowing was performed mechanized in 08.10.2021. Distance between rows was 12.5 cm and sowing depth was 2-4 cm. Lucian variety has mid early maturity, medium height, good tillering capacity and long yellow awns. It proves good wintering resistance and damping-off resistance and tolerance to foliar diseases.

Jallon is a mid-early hybrid with an average height of 85-90 cm, with good damping-off resistance and drought tolerance, fast spring growth and excellent vigour, adapted to Romanian climatic conditions. It has a very well-developed root system, good tillering capacity, resulting in a greater number of fertile spikes and better disease tolerance.

Within the trial, several assessments were carried out during the growing season to observe how genotypes performed under the influence of other factors contributing to the growth and development of the winter barley crop.

In the field, observations and determinations were carried out regarding the percentage of emergence, number of tillers, plants height, number of spikes, number of kernels on spike.

The average percent of emergence was determined one month after sowing by counting the number of emerged plants per linear meter in each repetition, being reported on square metre. In the spring, tillering capacity was assessed by numbering the tillerings on linear meter in every repetition, on square meter reported.

Plant height was assessed by measuring with a graduated ruler before harvesting, from the soil surface to the top of the spike, excluding the awns. Measurements were made at three different places for each repetition.

Laboratory activity consisted on assessing the number of grans on spike, grains weight of spike (g), yield estimation (kg/ha) for field area under optimal moisture content of 14%, 1000 grains weight (g), protein content (%) and content of starch in barley grains (%). All the spikes were manually processed.

The total number of grains was divided by the number of spikes resulting in the number of kernels per spike. For the determination of TGW (1000 seed weight, g), samples of 1,000 grains each were counted from the spikes harvested from each experimental variant. The samples were weighed with an analytical balance and the weight was noted in grams.

Quality parameters (protein%, starch%) were determined by NARDI Fundulea (Barley Breeding Laboratory) with the help of Infratec 1241 grain analyzer.

All data were subjected to statistical analysis (ANOVA) provided by ARM-9 (P = .05, Student-Newman-Keuls) software. P-value was always inserted below every table to prove statistical differences between samples.

RESULTS AND DISCUSSIONS

From point of view of weather conditions, the year 2021-2022 was dried year compared to multiannual average recorded in Romania as shown by Weather station Giurgiu (Figure 1). The entire amount of rainfall recorded during December 2021 - June 2022 in barley crop resumed to only 154.7 mm. In October and November the rainfall amount was extremely low (in fact 8.7 and respectively 2.1 mm), but in December reached 91.5 mm to assure normal plant development in spring. During spring rainfall was extremely poor. Highest temperature degree was recorded on July (average 32.9°C) since the precipitation was also poor (4.9 mm).

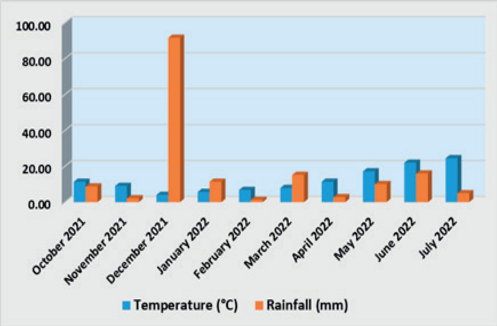

Figure 1. Precipitation and temperature evolution at Giurgiu, 2021-2022

It is well-known that in Romania winter barley gets to maturity stage with 7-10 days sooner than wheat because of its lower requirements for humidity (Panaitescu et al., 2011; Rizza et al., 2018; Berca et al., 2021; Vâtcă et al., 2021).

Table 1 shows the barley plants productivity (agronomic characteristics) as needed by the genotype grown and sowing density: number of tillers/m^2, plants height (cm), number of spikes/m^2, number of kernels on spikes and weight of kernels/spike (g). Out of data analysis it can be seen that for the barley genotypes studied the average shoot percentage did not vary much with seeding density. However, it decreased with increasing seeding density from 96.3% at 200 germinative seeds/m^2 to 91.1% at 350 germinative seeds/m^2 in the Jallon hybrid. In the case of Lucian variety, the germination percentage of barley plants decreased from 91.3% at a density of 300 germinative seeds/m^2 to 87.6% at a density of 600 germinative seeds/m^2.

Table 1. Influence of sowing density and genotypes on productivity elements in autumn barley crop, Giurgiu, 2022

No. crt.	Genotypes	Seed rate/m^2	Emergence percentage (pl/m^2)	No. of tillers/m^2	Plants height (cm)	No. spikes /m^2	No. of kernels on spikes	Weight of Grains/ spike (g)
1.	Jallon	200	95.50	433	19.16	421	38.41	1.533
		200	96.00	466	12.54	456	36.78	1.447
		200	97.50	491	12.55	484	34.56	1.351
		average	**96.33**	**463**	**14.75**	**454**	**36.58**	**1.444**
		250	94.00	551	11.06	540	25.04	0.991
		250	94.60	593	14.97	587	24.43	0.991
		250	95.40	599	11.40	585	24.88	0.966
		average	**94.66**	**581**	**12.47**	**571**	**32.26**	**1.266**
		300	93.50	674	13.50	664	32.52	1.285
		300	93.00	637	15.89	627	30.56	1.214
		300	93.50	677	17.10	660	30.08	1.164
		average	**93.33**	**663**	**15.49**	**650**	**31.05**	**1.221**
		350	91.00	782	13.71	775	27.38	1.081
		350	91.50	768	15.75	753	26.46	1.138
		350	91.00	804	22.83	792	27.19	1.077
		average	**91.17**	**785**	**17.43**	**773**	**28.01**	**1.099**
2.	Lucian	300	92.40	492	15.41	485	30.22	1.206
		300	90.60	475	16.27	467	32.42	1.310
		300	91.00	518	15.04	497	30.45	1.208
		average	**91.33**	**495**	**15.57**	**483**	**31.03**	**1.241**
		400	91.50	569	14.78	558	26.77	1.045
		400	91.00	582	15.55	569	26.97	1.067
		400	90.50	570	8.77	555	26.95	1.097
		average	**91.00**	**574**	**13.03**	**561**	**26.90**	**1.070**
		500	89.50	625	12.96	619	26.51	1.079
		500	89.00	654	12.76	643	25.60	1.020
		500	88.50	668	14.65	651	25.71	1.105
		average	**89.00**	**649**	**13.45**	**638**	**25.94**	**1.148**
		600	88.50	711	14.81	705	24.29	0.946
		600	87.00	736	14.16	724	22.48	0.898
		600	87.50	730	17.06	718	23.65	0.931
		average	**87.67**	**726**	**15.34**	**716**	**23.47**	**0.925**
*P-value**		-	0.000000001	0.0000001	0.0950	0.0000282	0.00000625	0.00000045
LSD			0.835	20.1029	3.5906	21.0946	2.2386	0.0952
Standard Deviation			0.568	13.6707	2.4418	14.3451	1.5223	0.0648

*Significant (p≤0.05)

However, the tillering capacity was strongly influenced by the seeding density. Thus, in the case of the Jallon hybrid the average number of tillers/m^2 was 463 at a density of 200 germinative seeds/m^2, 581 at 250 germinative seeds/m^2, 663 at a density of 300 germinative seeds/m^2 and 785 when 350 germinative seeds/m^2 were sown. In Lucian, variety the average number of tillers/m^2 also increased from 495 at 300 germinative seeds/m^2 to 726 when 600 germinative seeds/m^2 were sown.

Plant height was strongly influenced by density but also by genotype. Thus, in the case of the Jallon hybrid, the average height of the barley plants usually increased with the extension of sowing density, with the exception of the density of 250 germinating grains/m^2 where the lowest height was recorded 12.47 cm. Following the measurements, we found that for the Lucian variety, the average height of the barley plants did not vary so much depending on the density, with similar values being recorded for the lowest density, 15.57 cm and the highest density, 15.34 cm. The sowing density also greatly influenced the average number of spikes/m^2 for both autumn barley genotypes with the highest number of spikes being recorded at the highest sowing densities.

The number of grains/spike decreased with increasing sowing density with average values for the Jallon hybrid of 36.5 at 200 germinative seeds/m^2, 32.2 at 250 germinative seeds/m^2, 31.0 at 300 germinative seeds/m^2 and 28.0 when 350 germinative seeds/m^2 were sown.

The lowest number of grains/spike was recorded in Lucian field at the highest density tested with an average value of 23.47. Also the average weight of kernels/spike decreased with increasing sowing density from 1.44 g at the lowest sowing rate to 1.09 g at the highest density for the hybrid Jallon and from 1.24 g to 0.92 g in Lucian.

The influence of seeding density and genotype grown on barley yield is shown in Table 2.

Although the rainfall was very low the average yields obtained were satisfactory, the highest yield being obtained in the case of the Jallon hybrid at the density of 350 germinative seeds/m^2, respectively 7,876 kg/ha with 953 kg/ha more than the density of 250 germinative seeds/m^2 considered as the control sample. Although the critical periods with regard to water are from the straw formation stage to tasseling (similar requirements to wheat), at the same moisture regime, barley yields 20-25% higher than wheat (Bîlteanu, 1989).

Table 2. Influence of sowing density and genotype on barley yield, Giurgiu, 2022

Genotypes	Seed rate/m^2	Average yield		Dif. kg/ha
		kg/ha	%	
Jallon	200	6,923	100.0	-
	250	7,363	106.3	440
	300	7,575	109.4	652
	350	7,876	113.7	953
Lucian	300	5,870	100.0	-
	400	6,081	103.5	211
	500	6,274	106.8	404
	600	6,487	110.5	617
*P-value**		*0.0002*	-	-
LSD		*149.77*	-	-
Standard Deviation		*101.85*	-	-

*Significant ($p \leq 0.05$)

In the case of Lucian variety, the highest yield was recorded at the density of 600 germinative seeds/m^2, i.e. 6487 kg/ha, 617 kg more than the density of 300 germinative seeds/m^2 considered as control sample (Table 2).

Quality parameters of winter barley under sowing density and grown genotype influence (TGW – 1000 seed weight, g, protein content, starch content) are shown in the Table 3. Out of data analysis, it is noted that for the studied barley genotypes the TGW didn`t vary too much from point of view of sowing density. For Jallon variety the TGW average values were 42.3 g at a density of 200 germinative seeds/m^2, 42.0 g at 250 germinative seeds/m^2, 41.8 g at a density of 300 germinative seeds/m^2 and 41.7 g when 350 germinative seeds/m^2 were sown. For Lucian variety, the TGW average values were 40.1 g at a density of 300 germinative seeds/m^2, 39.7 g at 400 germinative seeds/m^2, 39.0 g at a density of 500 germinative seeds/m^2 and 38.5 g when 350 germinative seeds/m^2 were sown.

As regards the protein content of barley grains, the highest values were recorded for the hybrid Jallon at a density of 200 germinative seeds/m^2 (9.4%), and for the variety Lucian at the highest seeding rates, respectively of 15% at a density of 500 germinative seeds/m^2 and 14.2% at 600 germinable grains/m^2.

Starch content in grains averaged between 62.8 and 63.3% at Jallon and between 58.7 and 62.1% at Lucian (Table 3).

Table 3. Influence of sowing density and genotypes on quality parameters in autumn barley crop, Giurgiu, 2022

No. crt.	Genotypes	Seed rate (/m²)	TGW (g)	Protein (%)	Starch (%)
1.	Jallon	200	43.23	9.5	63.2
		200	42.41	9.5	62.5
		200	42.33	9.2	62.9
		average	**42.32**	**9.40**	**62.87**
		250	42.20	9.6	62.9
		250	41.98	8.9	63.4
		250	41.98	8.9	63.8
		average	**42.05**	**9.13**	**63.37**
		300	41.90	9.4	62.7
		300	41.72	9.0	62.9
		300	42.06	8.8	63.5
		average	**41.89**	**9.07**	**63.03**
		350	41.78	8.8	63.5
		350	41.73	8.7	63.0
		350	41.71	8.6	62.1
		average	**41.74**	**8.70**	**62.87**
2.	Lucian	300	40.08	12.1	61.7
		300	40.25	11.9	61.5
		300	40.20	10.2	63.2
		average	**40.18**	**11.4**	**62.13**
		400	39.99	14.3	61.0
		400	39.45	14.5	60.8
		400	39.87	11.3	60.5
		average	**39.77**	**13.37**	**60.77**
		500	38.64	15.4	59.4
		500	39.35	15.8	59.6
		500	39.04	14.0	60.0
		average	**39.01**	**15.07**	**59.67**
		600	38.36	14.3	57.0
		600	38.75	14.2	59.5
		600	38.57	14.1	59.6
		average	**38.56**	**14.20**	**58.70**
*P-value**		-	*0.0000019*	*0.00002*	*0.000015*
LSD		-	*0.3439*	*0.7742*	*0.8656*
Standard Deviation		-	*0.2339*	*0.5265*	*0.5887*

*Significant ($p \leq 0.05$)

CONCLUSIONS

Planting density is important factor which influence yield and quality of barley.

From climatic point of view, the 2021-2022 growing season year was unfavourable, extremely dried and hot.

Barley yields were strongly influenced by sowing density and genotype grown. The highest yield was recorded for the hybrid Jallon at a density of 350 germinative seeds/m^2, respectively 7,875 kg/ha and for the variety.

Lucian at a density of 600 germinative seeds/m^2, respectively 6,487 kg/ha.

Productivity elements and yield quality parameters of barley plants were greatly influenced by sowing density and less by the genotype grown.

The results achieved showed that for the barley genotypes studied, the average emergence percentage did not vary much with sowing density, but the tillering capacity, average number of spikes/m^2, and number of grains/spike was strongly influenced by sowing density.

Compared to the Lucian variety, where the height of the barley plants was approximately equal at the four sown densities, in the case of the Jallon hybrid, a greater variation of the plant height was found according to the density. Significant differences in the investigated quality components were established between seeding rates, with ecological factors playing an essential role in achieving quality yields.

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PHENOTYPIC DISTANCE OF BULGARIAN AND HUNGARIAN COMMON WINTER WHEAT GENOTYPES

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Abstract

The field experiment was conducted during the period 2020-2023 on an experimental field at Institute of Plant Genetic Resources "K. Malkov" in town of Sadovo, central southern Bulgaria. Five Hungarian, 4 standart varieties and 16 advanced lines winter common wheat were included in the experiment. The following traits were reported: grain yield, test weight, 1000 grains weight and plant heigth. The mathematical processing of the obtained results was carried out by Duncan`s multiple range test, mean, min and max values, CV and standard error, PC and Cluster analysis. Greater diversity was observed in the traits 1000 grains weight, test weight and height, while grain yield was less. The analyzes carried out will also help to select parental forms to create new varieties with the potential for high yield and quality.

Key words: *common winter wheat, phenotypic distance, cluster analysis, PC analysis.*

INTRODUCTION

Winter common wheat (*Triticum aestivum* L.) is a key component of human nutrition. Increasing global population and continuous urbanisation require an expansion of its production. Approximately one-fifth of human calories in various forms are supplied by wheat, which appears to be an important requirement for production security (Hongjie et al., 2019; Li et al., 2019; Nazarenko et al., 2020). The main goal of modern breeding is to create varieties combining high yield, quality and tolerance to biotic and abiotic stress factors (Uhr, 2015). Valuable hybrid populations have been created through hybridization programs and genetic studies. Trials of the resulting materials in given ecological regions help to create adapted high-yielding winter wheat cultivars (Tayyar, 2010; Muhe & Assefa, 2011; Tsenov et al., 2016; Lozada et al., 2021; Nazarenko et al., 2021). Varietal genotype has an important role in increasing yields and reducing its variability by year depending on growing regions. Microclimate has a significant influence in the expression of genetic possibilities related to grain productivity and quality (Tsenov et al., 2004; Uhr & Samodova, 2020). In this regard, the priority is to obtain high yields by breeding varieties of common winter wheat that can effectively use the environmental conditions and features of the ecological zone in which it is grown, to resist abiotic and biotic stress factors (Andrusevich et al., 2018). The search for new sources of genetic material for breeding programs on grain yield and quality requires the testing and use of domestic and foreign varieties of common winter wheat. An important component in the increasingly changing climatic stresses on plants is the selection of an appropriate varietal structure to ensure good performance in different ecological regions (Yanchev & Yordanova, 2005; Tsenov et al., 2021). Mannu et al., 2018 found that a good strategy for maintaining the yield potential of common winter wheat under global climate change is to optemize its phenology, which appears to be a major factor in adaptation to a given environment. The major phenological genes, together with the height determining genes, play an important role in breeding wheat lines with reduced lodging-resistant height and higher harvest index and hence high yield potential. The influence of these major adaptation and height genes should be considered in modern wheat breeding programs (Gasperini et al., 2012; Nazarenko et al, 2021; Lozada et al., 2021).

The use of different mathematical approaches such as the application of cluster analysis in combination with the application of principal component analysis (PCA) have been widely applied in research to identify and differentiate different lines and varieties of common winter wheat as well as to reveal the factors underlying existing phenotypic differences. The combination of the two analyses (Cluster Analysis and PCA) are applied in breeding when performing comparative studies on quantitative and qualitative traits between a larger number of lines and varieties. They allow interpretation and evaluation of their phenotypic similarity and dissimilarity depending on the objective of the study. The both analyses complement each other and their simultaneous use (conducting) gives us an in-depth information on the significance of studied traits when grouping the studied materials. These approaches allow us to group the different genotypes according to their similarity in the studied traits into approximately homogeneous clusters, which in turn allows for better selection and good combination in the future construction of an appropriate strategy to guide the selection process (Forkman et al., 2019; Gubatov & Delibaltova, 2020; Cheshkova et al., 2020; Ali et al., 2021; Reckling et al., 2021; Tsenov et al, 2022)

The main purpose of the study was to establish the phenotypic distance of Bulgarian and Hungarian genotypes of common winter wheat for use in their hybridization program.

MATERIALS AND METHODS

The competitive varietal trial was carried out in the experimental field of IPGR "K, Malkov", Sadovo in the period 2020-2023. The trial was carried out in four replications with an experimental plot area of 10 m^2. In the month of May 2022, there was a strong hailstorm and results were not counted. Twenty-five lines and varieties of common winter wheat were studied, including 4 standards, 5 Hungarian varieties and 16 advanced lines. To characterize the grain of the lines studied, the following parameters were recorded: grain yield (kg/da) plant height (cm), test weight (kg/hl) according to BSS ISO 7971:2000 and 1000 kernels weight (g) by weighing two samples of 500 grains (BSS ISO 520:2003). The analyses were carried out in the grain quality assessment laboratory. The Mathematical treatment used Duncan's multiple range test (DMRT), mean, minimum and maximum values, CV %. The coefficient of variation was used to define the variation of indicators (Dimova & Marinkov, 1999) as: weak - up to 10%, medium - greater than 10% and less than 20%, strong -above 20%,) and standard error.

Principle component and cluster analyses were applied to study the biological and economic traits based on the genotypes included in the experiment.

RESULTS AND DISCUSSIONS

For the period 2020-2023, the average monthly air temperature (Table 1) and precipitation amount (Table 2) were monitored to characterize each growing season. In the first year (2019-2020) less than normal rainfall was recorded in October during seedbed preparation, December, January and during germination, flowering, grain filling and maturing. In February, March and April, the rainfall is higher than normal and supports the picking and spindling. The average monthly temperature in April alone is (-0.4ºC) below normal and coincides with crop stem elongation. In November and December, temperatures fell below 5ºC and the plants can be said to be dormant. In 2020-2021, the average monthly temperature decreases below 5ºC only in January, and a negative deviation from the long-term norm of 0.2ºC, 0.5ºC and 1.3ºC is recorded in November, March and April. Temperatures in the winter months of December, January and March are positive and higher than normal. Below normal rainfall is recorded in November during and after sowing, May and July. In 2021-2022, below normal precipitation is recorded in November, January, March, April, May and July. Insufficient moisture is recorded during important phases of wheat development. Average monthly temperatures in October and March are below normal. In December and January temperatures are below 5ºC. Negative average monthly temperatures were not recorded during this growing season (vegetative period). In the last year, the average monthly temperature has been higher than 5°C degrees throughout the growing season. Negative deviations are recorded in April and May with -0.189 and -1.84°C. Moisture deficiency was recorded during seed preparation and sowing in October, December, February and March. There is a trend of rainfall deficiency during sowing preparation and May. Average monthly temperatures are lower than normal in March and April, with deviations of -0.2°C and no more than -1.3°C. Negative monthly mean temperatures were recorded during the period.

Table 1. Average monthly air temperature for the period 2020-2023

Years/Months	X	XI	XII	I	II	III	IV	V	VI	VII
Average monthly temperature 2019/2020	14.8	10.7	4.1	2.2	6.1	8.9	11.8	18.2	21.6	24.9
Multi-year values 1931–2000	12.6	6.9	2.1	-4.3	2.4	6.3	12.2	17.5	21.2	23.3
Deviation	2.2	3.8	2.0	6.5	3.7	2.6	-0.4	0.7	0.4	1.6
Average monthly temperature 2020/2021	15.2	6.7	5.6	3.3	5.9	5.8	10.9	18.5	22.3	26.6
Multi-year values 1931–2000	12.6	6.9	2.1	-4.3	2.4	6.3	12.2	17.5	21.2	23.3
Deviation	2.6	-0.2	3.5	7.6	3.5	-0.5	-1.3	1.0	1.1	3.3
Average monthly temperature 2021/2022	11.0	7.8	4.0	3.3	5.0	4.9	13.7	18.5	23.0	25.6
Multi-year values 1931–2000	12.6	6.9	2.1	-4.3	2.4	6.3	12.2	17.5	21.2	23.3
Deviation	-1.6	0.9	1.9	7.6	2.6	-1.5	1.5	0.9	1.8	2.3
Average monthly temperature 2022/2023	13.85	9.57	5.58	5.652	6.045	8.69	12.011	15.659	21.797	27.16
Multi-year values 1931–2000	12.6	6.9	2.1	-4.3	2.4	6.3	12.2	17.5	21.2	23.3
Deviation	1.25	2.67	3.48	9.952	3.645	2.39	-0.189	-1.841	0.597	3.86

Table 2. Amount of precipitation for the period 2020-2023

Years/Months	X	XI	XII	I	II	III	IV	V	VI	VII
Precipitation amount for the month - 2019/2020	14.2	80.7	24.9	2.1	49.4	91.5	93.8	40.1	45.9	38.1
Multi-year values	37.4	47.1	49.7	39.3	30.9	39	42.9	56.8	58.4	46.4
Deviation	-23.2	33.6	-24.8	-37.2	18.5	52.5	50.9	-16.7	-12.5	-8.3
Precipitation amount for the month - 2020/2021	72.7	6.3	55.7	96.4	32.8	42.5	78.5	32.7	60.4	19.9
Multi-year values	37.4	47.1	49.7	39.3	30.9	39	42.9	56.8	58.4	46.4
Deviation	35.3	-40.8	6.0	57.1	1.9	3.5	35.6	-24.1	2.0	-26.5
Precipitation amount for the month 2021/2022	167.9	11.9	96.1	30.3	57.9	22.3	31	39.8	159.7	8.3
Multi-year values	37.4	47.1	49.7	39.3	30.9	39	42.9	56.8	58.4	46.4
Deviation	130.5	-35.2	46.4	-9.0	27.0	-16.7	-11.9	-17.0	101.3	-38.1
Precipitation amount for the month - 2022/2023	1.8	49	47.6	54	1.2	34.8	55.3	80.9	80.2	63.5
Multi-year values	37.4	47.1	49.7	39.3	30.9	39	42.9	56.8	58.4	46.4
Deviation	-35.6	1.9	-2.1	14.7	-29.7	-4.2	12.4	24.1	21.8	17.1

Mean values of the studied traits and their corresponding evidences according to Duncan's multiple comparison test between genotypes are presented in the Table 3. The results show that for all four traits there are significant differences between the tested genotypes. Greater variation was observed in the traits thousand kernels weight, test weight and height, while there was less variation in grain yield. According to the coefficient of variation, plant height was characterized by the highest followed by thousand grains weight and grain yield. It is lowest for test weight. The three higher values are characterized as average in terms of its interpretation, which means that there is a well-defined genetic diversity in the sample of genotypes for the three traits. Grain yield averaged 716.3 kg/da over the three years. It ranged from 644.5 kg/da for the line MX 286/1759 to 864.4 kg/da for the Hungarian variety MV-Nemere. The standard variety Sadovo1 has an average of 673.3 kg/da, which places it in the middle of the sample of varieties and lines. Plant height averaged 97.6 cm over the years of testing. The highest average over the three years was line RU 33/3244 and the lowest was variety Avenue. For the trait thousand kernels weight, the highest value averaged over the years was line RU 134/1370 and the lowest was for variety Avenue. The trait is characterised by an average value of 45.4 g over the period. For the trait test weight, the average was 74.4 kg/hl, with the highest value for the line MX 286/1759 and the lowest for the line RU 135/1456.

Table 3. Duncan's multiple test, means, min., max., CV and standard error of 25 genotypes for 4 quantitative traits

Genotype	Yield	Sig	Plant Heigth	Sig	TKW	Sig	Test Weight	Sig
MX 270/ 28	735.4	abc	95.0	fghij	46.6	efghi	74.8	bcdefgh
MX 270/ 50	787.9	bc	96.0	ghij	49.0	ghij	74.2	bcdef
PY 129/3053	779.2	abc	99.0	ij	44.1	bcdef	76.5	efgh
PY 33/3244	699.1	ab	100.7	j	48.0	fghij	77.1	fgh
MX 270/ 3461	681.2	ab	94.0	fghij	49.3	ghij	77.2	fgh
MX 285/1058	752.2	abc	98.3	hij	41.5	bc	75.7	cdefgh
PY 48/2553	687.4	ab	88.7	cdefghi	41.8	bcd	74.9	bcdefgh
MX 286/1759	644.5	a	88.3	cdefgh	46.3	efghi	79.0	h
MX 286/1777	673.2	ab	88.0	cdefgh	43.0	bcde	77.6	fgh
Avenue	660.7	ab	68.7	a	35.8	a	73.8	abcdef
Anapurna	649.6	a	76.7	ab	40.7	b	75.4	cdefgh
Sadovo 1	673.3	ab	94.7	fghij	50.4	hij	76.0	defgh
Enola	653.9	ab	90.0	defghi	44.0	bcdef	78.7	gh
MX 272/3872	655.8	ab	94.7	fghij	50.4	ij	70.8	ab
MX 215/3	668.7	ab	84.3	bcdef	45.8	cdefg	74.3	bcdef
PY134/1343	689.6	ab	80.7	bcd	46.8	efghi	70.9	ab
PY177/486	754.7	abc	81.0	bcd	48.4	fghij	74.6	bcdefg
PY135/1456	695.2	ab	80.7	bcd	47.9	fghij	69.8	a
PY179/1400	766.6	abc	85.7	bcdefg	47.9	fghij	73.9	abcdef
PY134/1370	761.4	abc	78.7	bc	51.9	j	71.6	abc
MV-Nador	766.5	abc	76.7	ab	43.4	bcde	72.1	abcd
MV-Nemere	864.4	c	83.0	bcde	40.4	b	72.8	abcde
MV-Menrot	717.3	ab	87.7	cdefg	43.1	bcde	72.5	abcde
MV-Mente	726.1	ab	92.3	efghi	46.0	defgh	73.5	abcdef
MV-Kaplar	762.8	abc	85.7	bcdefg	41.8	bcd	71.9	abcd
mean	716.3	-	87.6	-	45.4 ±	-	74.4	-
± m	± 11	-	± 1.6	-	0.77	-	± 0.5	-
min.	644.5	-	68.7	-	35.8	-	69.8	-
max.	864.4	-	100.7	-	51.9	-	79.0	-
CV%	7.71	-	9.23	-	8.47	-	3.33	-

Mean values (in each column) followed by the same letters (in the subscript) are not significantly different at $p < 0.05$ according to Duncan's multiple range test (DMRT)

PC analysis was applied to study the biological and economic traits based on the genotypes included in Table 3. Figure 1 shows the PC analysis for the four traits studied. As can be seen from the figure, more than 70% of the total variation in phenotypic expression of genotypes across traits is due to the first two principal components, PC1 and PC2. This value is large and correct discussion of the results is possible. In the figure, grain yield (Y) is negatively correlated with test weight (TW) and plant height (H) and weakly positively correlated with thousand kernels weight (TKW). Thousand kernels weight is positively correlated with plant height and negatively correlated with test weight. Plant height is strongly positively correlated with thousand kernels weight and weakly positively correlated with test weight and negatively correlated with grain yield.

The locations of the common winter wheat genotypes from the CVT relative to the principal components PC1 and PC2 are presented in Figure 2. By comparing the two figures (Figure 1 and Figure 2) we can assess which genotypes are more strongly associated with which traits. It is noteworthy that most of the genotypes studied are associated with yield. It is noticeable that the individual varieties and lines are located in the middle of the coordinate system. This suggests that they are balanced with respect to the traits tested. Genotypes located at the periphery indicate that they have superiority in any of the traits tested. Impressive are the new lines, which are located around the center of the figure and should behave in a balanced manner in most respects.

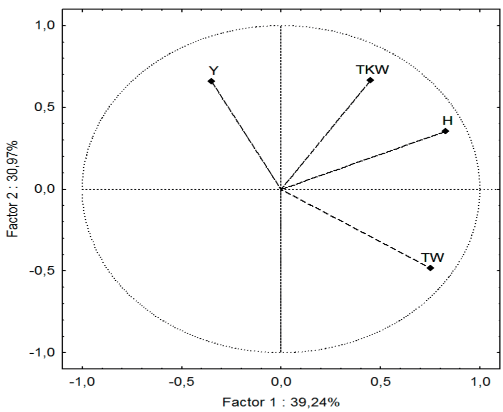

Figure 1. PC analysis of yield, 1000 kernels weigh, plant height and test weight

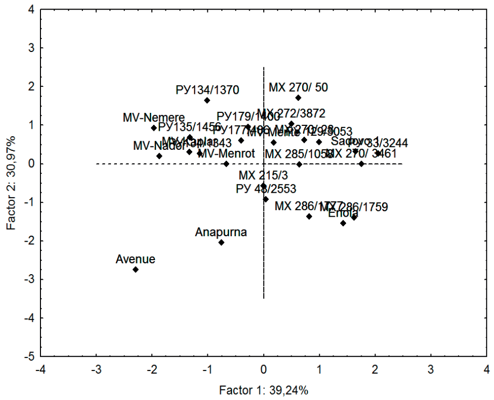

Figure 2. PC analysis of 25 common winter wheat genotypes

By comparing the two figures (Figure 1 and Figure 2) we can assess which genotypes are more strongly associated with which traits. It is noteworthy that a large proportion of the genotypes examined are associated with yield. This is where the lines suitable for presentation as new varieties are located. They are suitable for inclusion in the hybridisation scheme, as they would contribute to increasing productivity. The two standards, Avenue and Annapurna, occupy a neutral position in the coordinate system and are not characterized by any of the traits studied. It is noticeable that the individual varieties and lines are located in the middle of the coordinate system. This suggests that they are balanced with respect to the traits tested. The genotypes located at the periphery indicate that they have superiority in any of the traits. The new breeding lines that lie around the centre of the figure are impressive and should be more balanced on most traits.

The dendrogram shows that two major clusters with genetic distance are formed (Figure 3). The advanced lines are located in both clusters, subsequently splitting into two subclusters. In the leftmost cluster, newly established lines with RU 134/1370, RU 179/1400, RU 177/486, and RU 135/1456 and RU 134/1343 and MX 272/3872 are located, and in the second subcluster of the first cluster, foreign varieties are located. The right cluster has two smaller clusters. The left subcluster has the two Bulgarian standards and three newly established lines. This defines the lines as very close to the Bulgarian standards. In the right sub-cluster of cluster two are located for the most part lines of origin MX 270/28 and MX 270/50. For smaller and faster breeding progress we should cross genotypes located in the same cluster and vice versa for larger but slower progress cross genotypes from genetically distant clusters. According to this analysis, which gives a good idea of the genetic proximity and distance of the breeding materials, an appropriate strategy can be built to guide the breeding process. It is recommended to cross the Enola and Sadovo1 standards with genotypes from the other cluster to achieve higher but slower results.

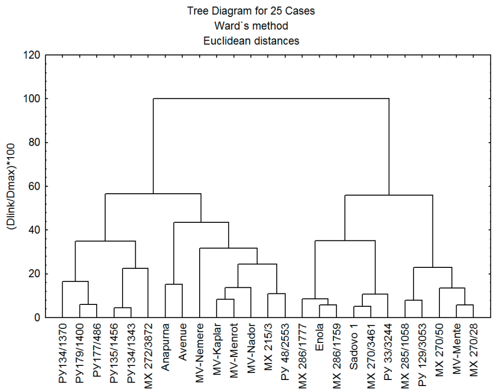

Figure 3. Cluster analysis of 25 common winter wheat genotypes

CONCLUSIONS

In the meteorological surveys, a trend of rainfall deficiency emerged during the preparation of sowing and the month of May. Monthly average temperatures are lower than normal in March and April, with deviations of -0.2°C and no more than -1.3°C. No negative monthly mean temperatures were recorded during the period.

The presented mean values of the tested traits and their corresponding evidences according to Duncan's multiple comparison test between genotypes show that for all four traits there are significant differences between the tested genotypes.

In the correlation analysis, grain yield was negatively correlated with test weight and plant height and weakly positive correlated with thousand kernels weight. Thousand kernels weight is positively correlated with plant height and negatively correlated with test weight.

Plant height is strongly positively correlated with thousand kernels weight and weakly positive correlated with test weight and negatively correlated with grain yield.

The PCA results show that more than 70% of the total variation in the phenotypic expression of the genotypes for the traits is due to the first two main components - PC1 and PC2 a large part of the studied genotypes are related to yield. This suggests that they are balanced with respect to the traits tested.

Cluster analysis, gives a good idea of the genetic proximity and remoteness of the breeding materials, an appropriate strategy can be built to guide the breeding process. It is recommended to cross the standards Enola and Sadovo1 with genotypes from the other cluster to achieve higher but slower results.

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GROWING POTATOES WITH REDUCED TILLAGE

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Abstract

The aim of the experiment is to study the possibility of growing potatoes with a limited number of soil cultivation, at the beginning of the vegetation. The experiment was conducted at the Vrazhdebna training field of the University of Forestry, on fluvisols. The experiment is laid out according to the growing distance of 70 x 30 cm, with the Soraya variety. The soil cultivation was carried out pre-planting, at the beginning of the experiment and consisted of cultivation at a depth of 20-25 cm and subsequent furrowing at 70 cm between the furrows. During the growing season of the plants, the weed communities in the experiment were recorded, after which the inter-row weeds were mowed. There are two variants - with one mowing and with two mowing of the weeds, with the shredded plant mass being used as mulch. The average yield from one plant with double mowing is 1.428 kg, while with the single mowed option it is 1.015 kg.

Key words*: potatoes, reduced tillage, weeds, yield.*

INTRODUCTION

Conventional intensive potato production can lead to soil erosion and moisture loss. Conservation practices such as reduced or no tillage, cover crops, or mulching are being tested as alternative methods.

Preservation of soil moisture at the beginning of the vegetation of plants and improvement of their development was achieved only by postponing the first tillage, which was carried out before the actual planting of potatoes (Griffin et al., 2009).

The influence of reduced tillage on potato yields is not unambiguous. Some authors found that potato yields when using reduced tillage did not differ from those of conventional production (Mundy et al., 1999), other authors registered a decrease in yields (Drakopoulos et al., 2018), while others found increases in yields using reduced tillage (Mosquera et al., 2019).

In no-till, different resources are used for weed control - herbicides, cover crops, or mulches, and different alternatives are being tested for organic production. Shehata et al. (2019) found that bio-degradable polyethylene mulches and so-called natural herbicides (acetic and citric acid) are a good alternative because higher yields of tubers are obtained compared to conventional production.

A variety of no-till is Strawponic, which is growing the potato tubers on the surface of the soil by covering them with straw mulch, the highest yield was obtained using an amount of 50 t/ha (Msheik et al., 2019).

Cover crops are used as living mulches. They can be cut shortly before the potatoes sprout and their residues used as mulch. The resulting potato yields are similar to or higher than conventionally produced (Boyd et al., 2001; Morse et al., 2006).

The yield and quality of potatoes are affected by the lack of moisture in the soil. Mulching potatoes helps to preserve moisture and thereby increase the yield and quality of the tubers. Waste materials such as straw or rice husks are readily available and widely used (Nowroz et al., 2021).

Mulching with grass clippings also has a positive impact on both soil and potato yields. It improves soil temperature and despite increased weed biomass, potato yields are also high (Dvořák et al., 2012).

When the grass mulch is laid immediately after planting the potatoes, the effect of reducing the weed biomass and increasing the yield and quality of the potatoes is greater compared to laying it in a later (after the second digging) phase (Dvořák et al., 2009).

On the other hand, weeds affect potato yields. Weeds that emerged a week after the potatoes and developed alongside them were found to reduce yields much more than variants where weeds emerged three weeks after the potatoes.

In the first case, yields are lower by 54%, and in the second - by 16%. Weeding affects the size and number of tubers (Nelson and Thoreson, 1981).
The purpose of the experiment is to investigate the possibility of growing potatoes with a limited number of soil treatments, as well as the possibility of using weeds as mulch.

MATERIALS AND METHODS

The research was conducted in 2023 under field conditions in the educational and experimental base of the University of Forestry, Sofia (42°70'76.1"N, 23°43'73.1"E) on alluvial-meadow soils. The groundwater level is high, on average 1.5-3 m from the surface. The content of humus in the layer 0-25 cm is 1.21%, and in the subsoil 25-50cm - 1.04%, the pH of the soil reaction in the arable soil layer (arable soil) is 6.8, and in the subsoil layer (subsoil) is 6.4, which is the optimal range for normal plant development.
The total area of the study is 700 m^2, divided into two equal parts of 350 m^2 each, in which the two tested options are located: variant A – with two mowing of the weed mass during the growing season; variant B – with one mowing at the end of vegetation. The used variety is "Soraya" with an average seed fraction of 35/55 mm and an average weight of 65 g, as it shows good quality indicators for the climatic region. The predecessor crop is cabbage (*Brassica oleracea* L. var. *capitata*), which is suitable for a potato crop. One pre-planting treatment was done at a depth of 20-25 cm and subsequent furrowing at 70 cm between the furrows on 09.05.2023. Planting was done on 10.05.2023 in strips with a depth of 10 cm, inter-row distance - 70 cm, and intra-row distance - 30 cm. No pre-planting or post-planting chemical preparations were introduced.
On 14.07.2023, in one-half of the experimental area (350 square meters), in a phase in which the vegetative mass grows, the generative organs of the potatoes form and grow, the first mowing of the weed vegetation, which overtakes the potatoes in its development, was carried out. The chopped plant mass from the weeds is left in the corresponding half of the area to mulch and enhance the accumulation of organic matter in the soil.
On 15.08.2023 a second mowing was carried out in the first half of the experimental area, and again the organic residues of the weed species were left for mulching.
In the last phase of development in which the potatoes are located, namely when they stop growing and forming tubers in the soil, weed vegetation was mowed in the second half of the experimental area and harvesting of potatoes was started in both halves of the area at the beginning of October 2023.
From each variant, 20 plants were randomly marked and collected. Average weight of tubers per plant and average weight per tuber were measured. The absolute dry mass was calculated in percentages and by variants. The percentage of sugars in the tubers was recorded with a refractometer. Statistical analysis was performed using ANOVA

RESULTS AND DISCUSSIONS

The meteorological situation during the experimental period (May-September) is characterized by more moderate temperatures at the beginning and higher in July and August. Lower temperatures (8°C) were recorded after planting the tubers, and the average monthly temperature from that point until the end of May was 14.3°C. Although in the third ten days of June, the average daily temperature rose above 20°C, the monthly average was 18.8°C. The highest average monthly temperatures are July (23.2°C) and August (22.7°C) (Figure 1).
The month of May is characterized by relatively frequent rainfall - there are 22 days of recorded rainfall, with a total amount of 70.2 mm. Although the total amount of precipitation in June was 161.4 mm, half of it (81.7 mm) fell on two days in the middle of the month, and the total number of rainy days was 15. July is the driest month, with only 6 rainy days and a total amount of precipitation of 11.8 mm, followed by the month of August with 8 rainy days (24.2 mm). In the month of September, the amount of precipitation increases almost twice (45.6 mm), compared to that in August, and the rainy days are collected at the beginning (4-6.9.2023) and in the middle of the month (16-20.09.2023) (Figure 2).

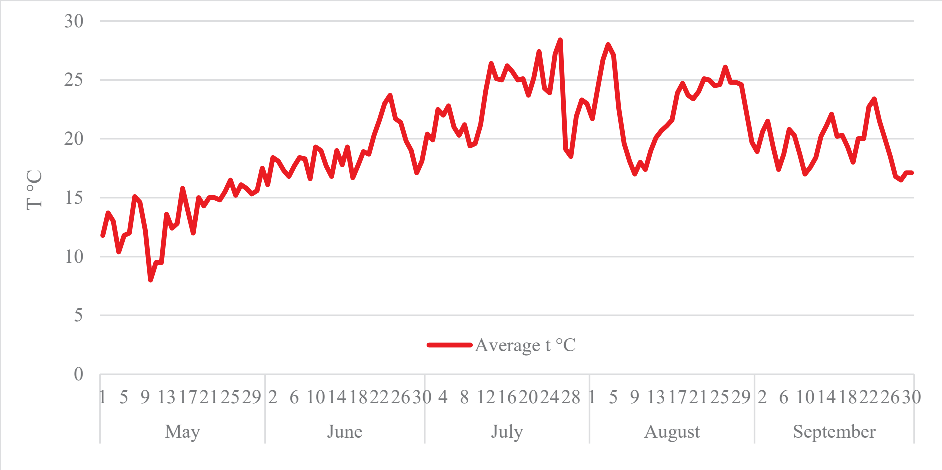

Figure 1. Average daily temperature during the experimental period

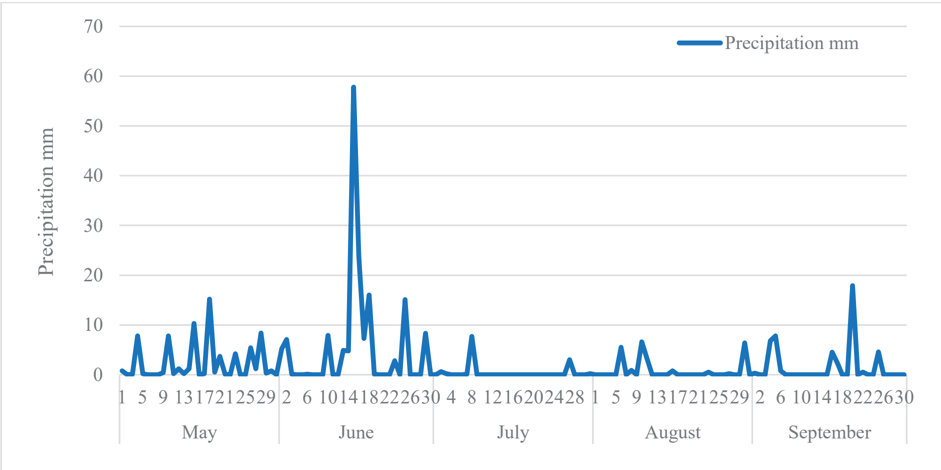

Figure 2. Daily precipitation during the experimental period

One month after planting the potatoes (June 14, 2023), the degree of weeding and the species diversity of the weeds were recorded.

The degree of weeding is between 65-70% of the area. A wide variety of wheat and broadleaf weeds are present, of which the most prevalent are: white goosefoot (*Chenopodium album* L.), meadow and sheep's fescue (*Festuca pratensis* L. & *Festuca ovina* L.), green field speedwell (*Veronica agrestis* L.), shepherd's purse (*Capsella bursa pastoris* L.), pigweed (*Amaranthus retroflexus* L.).

The number of other types of weeds reported throughout the area is not large and therefore they are summarized in one group, which represents about 32% of the total weed diversity of the experimental area (Table 1).

Table 1. The percentage ratio of weed species

Species	%
Chenopodium album L. (white goosefoot)	14
Festuca pratensis L. *(meadow fescue)*	12
Festuca ovina L. *(sheep's fescue)*	12
Veronica agrestis L. *(green field speedwell)*	10
Capsella bursa pastoris L. *(shepard's purse)*	10
Amaranthus retroflexus L. *(pigweed)*	10
Thlapsi arvence L., *Lepidium ruderale* L., *Lepidium campestre* L., *Galium tricorne* L., *Lathyrus hirsutus* L., *Spergula linicola* L., *Chenopodium poluspermum* L., *Setaria viridis* L., *Polygonum convolvulus* L., *Echinochloa crus galli* L., *Anthemis arvensis* L.	32

Approximately one month (14.07.2023) after the weeds were counted and after an analysis of the species present was made, they were mowed in variant A. Mowing was done with a trimmer in the inter-rows, and in-rows were removed manually, carefully. The grass clipping was left as mulching material.
One month later (15.08.2023) the mowing of newly grown weeds was repeated. After approximately one more month, (19.09.2023), a full mowing was done on both variants to prepare the areas for harvest.
At the end of the growing season, the yield of tubers per plant was recorded, and the average yield in variant A was higher than that of variant B (Figure 3).

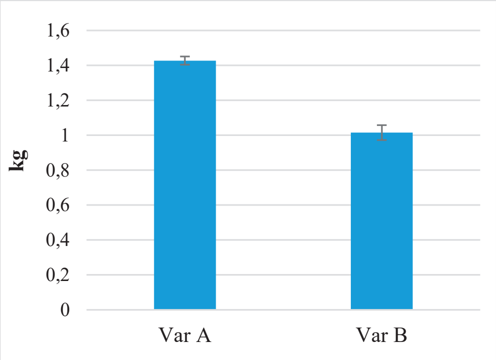

Figure 3. Average tuber weight per plant (kg). Error bars represent standard error

Data were processed with a one-way ANOVA to compare the effect of treatments on tuber yield per plant, under conditions of initial tillage combined with two mowings during the growing season (variant A) and variant B with a single mowing at the end of the growing season, before harvesting the plants. Mowing the inter-row weed vegetation and leaving the grass clipping as mulch had a significant effect on tuber yield per plant at the p<.05 level for the indicated conditions. [F (1.38) = 71.22, p < .001]. This result shows that the double mowing of the weeds in variant A has an effect on the yield of tubers from one plant, compared to variant B - with mowing at the end of the vegetation of the plants. In variant A, leaving plant residues from both mowings affected the yield per plant, probably due to the accumulation of organic residues of the weed species as mulch during potato development.

The mulching of variant A with the grass clipping also affected the average weight of a single potato, as again in variant B – without mowing, the potatoes were of lower weight (Figure 4).

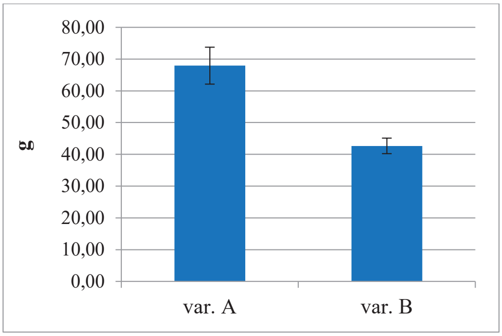

Figure 4. Average single potato tuber weight (g). Error bars represent standard error

The obtained results for the yield of potatoes, when growing them with mulching, are in agreement with the data of Dvořák et al. (2009; 2012), and Nowroz et al. (2021) for a higher yield when growing potatoes with mulching.
Similar to the average weight of individual tubers or the yield of tubers from one plant, the way of growing potatoes - by mowing the weeds during their vegetation (variant A) or once - at the end of the vegetation (variant B) also affects the content of dry matter.
One-way ANOVA test confirms that mowing in variant A also affected the average weight of a single tuber at the p<.05 level for the tested conditions [F (1.58) = 16.07, p < .001].
Growing potatoes with a single mowing of the weeds at the end of their growing season (variant B), besides producing smaller potatoes, they have a higher content of absolute dry matter - 26.64%. In the first option A - with mowing the weeds and laying them as mulch in the rows, the content of absolute dry matter is 20.84%.
The influence of the way of growing the plants and on the content of carbohydrates in the tubers is similar in the different variants (Figure 5).
The mean content of total sugars in potato tubers from variant A was 4.37% (SD = 0.29), while the percentage of reported average total sugar content was 5.63 in variant B - with weed cutting at the end of the growing season, and a standard deviation of 0.42. Variant B also had a higher percentage of dry matter.

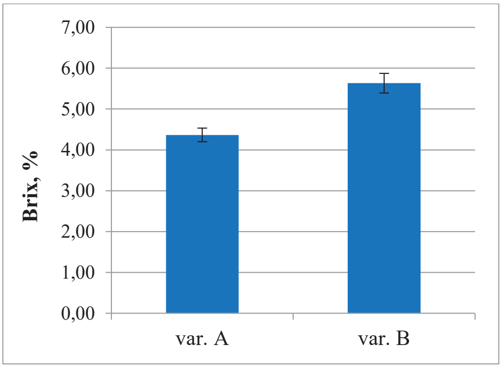

Figure 5. Content of total sugars (%) in potato tubers. Error bars represent standard error

When growing potatoes without irrigation and in drought conditions, the use of weeds as a mulching layer after their mowing during the growing season has a favorable effect on the yield and quality of the produce. This influence can be explained by the conservation of soil moisture. While mowing the weeds at the end of the growing season does not have this effect, as by then the weeds are competing with the potatoes for soil moisture. This results in smaller potatoes with an increased dry matter content, which corresponds to the results of Nelson and Thoreson (1981).

CONCLUSIONS

When growing potatoes without tilling the soil during their growing season and only with two mowing of the weeds in the rows, an average of 1.4 kg of potatoes per plant was obtained, with the average size of the tubers being about 68 g.

When weeds are left during the growing season and mowed at the end of the growing season, shortly before harvest, their influence is stronger, the potatoes are smaller (on average about 43 g) and the yield per plant is lower - approximately about a kilogram of a plant.

For small areas in urban and suburban conditions, growing potatoes without cultivation during the growing season and only with the initial preparation for planting is a good alternative. In this way, funds are saved, and by using the grass clippings from the weeds as a mulching layer, the environment for growing potatoes is improved - additional organic matter is added, the soil is protected from overheating and the moisture in it is preserved. To quantitatively substantiate these observations, it is necessary to deepen the research in this direction.

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THE INFLUENCE OF MINERAL FERTILIZERS ON THE DYNAMICS OF THE ACCUMULATION OF MAIN MACROELEMENTS IN THE SOIL AND IN WHEAT PLANTS

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Abstract

The experiment was carried out on chernozem during 2020-2021 in Dolj County, the wheat variety used is Glosa, an early autumn wheat, resistant to drought. The experiment was placed in the field according to the block method, in 4 repetitions with 8 variants. Following the use of different doses of fertilizers, the dynamics of nitric nitrogen, mobile phosphorus and mobile potassium in the soil, in the plant and in the grains were monitored at 3 determination dates, as well as the production obtained. After the analyzes carried out, it was found that the soil is rich in nitric nitrogen, ammoniacal nitrogen, the plants having at their disposal the nitrogen necessary for nutrition, and as regards the content of P and K, the soil is well supplied with these elements. Following the dynamics, the decrease in the content of main macroelements was observed, the younger the plants, the richer they are in these elements, and as the vegetative growth phase ends, the content decreases.

***Key words**: fertilizers, wheat, protein, production.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important crops, having an important place in field crops (Li et al., 2020). It is one of the most widely cultivated cereal grains in the world, and also a staple food for many people, providing a valuable source of energy and nutrients for a healthy diet (Păunescu et al., 2016; Shiferaw et al., 2013).

Wheat flour is used in the bakery and pasta industry, and bran and third-grade wheat is used as concentrated fodder in animal feed. The secondary products - straw and chaff - are used either as coarse fodder mixed with other fodder, or as bedding for animals (Shewry, 2009).

In order to obtain a quality harvest, it is important to plant wheat varieties adapted to local conditions, to use natural or chemical fertilizers depending on the needs and to respect the work technology recommended for wheat cultivation (Reynolds & Borlaug, 2006).

Glosa wheat variety ranks first among the seeds of cultivated areas in Romania (22476.46 ha) followed by FDL Miranda (6444.72 ha), Izvor (3131.06 ha) and the area sown with the Glosa variety (1238 ha) in 2017 represents half of the total area cultivated with wheat in Romania (Băcanu (Șerban) et al., 2018).

The Glosa variety has a wide adaptability, being stable and constant in terms of production and less affected by the fluctuation of climatic conditions.

According to specialists in agriculture, it is an early variety, with good resistance to fall, wintering, drought and heat or ear germination (Dobre, 2015).

In conclusion, wheat is an essential crop for human and animal food, and the proper management of the crop can ensure obtaining a quality and profitable harvest.

By applying the right combination of nutrients at the right time and in the right amounts, farmers can ensure that crops have access to the essential elements they need for healthy growth. This can help maximize the use of resources like water, sunlight, and other nutrients while minimizing waste and environmental impact (Srivastav, 2020).

In addition, balanced fertilization can help improve soil composition by replenishing nutrients that have been depleted through previous cropping cycles. This can help maintain the productivity of the soil over time and reduce the need for costly inputs like pesticides and herbicides.

Overall, the balanced application of fertilizers is essential for sustainable agriculture practices that prioritize both crop productivity and environmental stewardship (Pandey et al., 2020).

Regarding the soil requirements, wheat is the most demanding grain, distinctly exceeding the requirements of the other grains (Singh, 2017). The most suitable soils for wheat must offer optimal conditions for plant growth and development (Salim & Raza, 2020). The fertility of the soils intended for wheat cultivation must be high, because the poorly developed and somewhat predatory root system of this plant is not capable of extracting even the last remnants of nutrients from the soil (Popovici et al., 2017). Wheat is part of the crops that are very pretentious to fertilizers, although it does not extract large amounts of nutrients from the soil (Salim & Raza, 2020).

MATERIALS AND METHODS

The experiment was placed in the field according to the block method, in 4 repetitions with 8 variants, on a surface of 1600 m^2 (plot surface 10/5 m) located in the Segarcea area, Dolj county, on a clay- illuvial chernozem (SRCS 1980) or argic phaeozems (SRTS 2012) with the following profile and characteristics:

- Ap, Apt, A_m, AB, Bt_1, Bt_2, Bt_3, BC, Ck;
- subangular polyhedral structure, moderately compact, firm when wet, hard when dry, moderately plastic;
- medium clay loam texture on the Ap horizon and clay loam on the other horizons;
- weak acid reaction (pH = 6.10-6.56);
- the degree of nitrogen insurance is low to medium (Nt = 0.08-0.12%);
- well supplied with phosphorus accessible to plants: 55-61 ppm P and well provided with potassium (164-218 ppm K);
- humus content between 2.42-2.86%.

The treatments to which each variant was subjected are: V1 - unfertilized control, V2 - N_{60}, V3 - N_{100}, V4 - P_{40}, V5 - P_{80}, V6 - $N_{60}P_{40}$, V7 - $N_{100}P_{80}$, V8 - $N_{150}P_{100}$.

The fertilizers used to ensure the mentioned doses were ammonium nitrate with 34.5 N% and concentrated superphosphate with 40 P_2O_5%. The entire quantity of fertilizers was applied once at the beginning of October, before sowing, and was incorporated into the soil through a work with an agricultural combinator. On October 8, wheat of the Glosa variety was sown, the preceding plant was soybean - a leguminous crop that has the property of fixing nitrogen from the nodosity on the root.

Weather conditions during the experiment are summarized in Figure 1. The year 2020 was a favorable year for wheat cultivation, with precipitation and temperature values closer to the multiannual average. Thus, the precipitation exceeds the multi-year average exactly when it was necessary, namely in October, November, helping to good germination and growth, as well as in the months of May-June, helping to form the spike and the grain.

The temperatures were within accessible limits, registering slightly lower temperatures in the summer, thus helping the formation of the grain.

In 2021, higher temperatures were recorded in almost every month of the growing season except for May, which was 0.70°C lower. In June, during the formation of the grain, the temperatures were higher by 0.90°C, which led to its shrivelling.

Precipitation was also lower in 5 months out of the 9 months of the vegetation period, being very low at sowing, the sunrise did not occur on time, and 41.5 mm lower in April when the plants needed to form the spike.

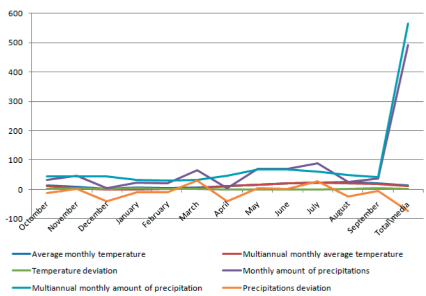

Figure 1. Temperatures and precipitations recorded during the wheat growing season in 2020-2021

The following determinations were made on the soil samples collected at a depth of 0-20 cm:

- determination of ammoniacal nitrogen from the soil by extraction in potassium sulphate and then by Nessler coloring, spectrophotometric determination at a wavelength of 525 nm,
- determination of nitric nitrogen by extraction in potassium sulphate and coloring with phenol

disulfonic acid, spectrophotometric determination at a wavelength of 420 nm,

- determination of mobile phosphorus in ammonium lactate acetate extract,
- determination of mobile potassium in ammonium lactate acetate extract by flamephotometry.

Regarding the plant samples, they were subjected to the following determinations: determination of total nitrogen, phosphorus and potassium in plants, by Kjedahl method modified by Gogoasa - disaggregation with sulphuric acid and perhydrol and then distillation.

Plant and soil samples were collected before heading stage, at heading and at harvest.

As part of the dynamics, chemical analyzes were carried out on the soil at a depth of 0-20 cm, in which nitric nitrogen, ammoniacal nitrogen, mobile phosphorus and mobile potassium were studied.

The chemical analyzes of the soil are particularly important, given the fact that only with their help, it is possible to determine the extent to which the plants use the fertilizers administered for fertilization. In order for the results to be as real as possible, 5 soil samples were taken from each experimental variant, from which an average sample was then made.

To determine the degree of supply of plants with assimilable nitrogen, it is necessary to determine the forms of nitrogen in the soil.

The samples of wheat grains were analyzed as follows:

- determination of nitrogen, phosphorus and potassium from wheat grains by Kjedahl method, disaggregation with concentrated H_2SO_4 and perhydrol, then distillation.
- interpretation of the data regarding the grain yield obtained in the year 2020/2021.

RESULTS AND DISCUSSIONS

Nitrogen is the most important nutritional element in the life of plants, it contributes to the increase of agricultural production (Sala, 2011), being able to affirm that without nitrogen there is no life, but also the lack of phosphorus and potassium in soil creates an improper environment for their development.

Following the dynamics of the formation of soluble nitrogen compounds, it is found that in general in the early spring the amount of nitric nitrogen is very small, then increases as the weather warms up to reach a maximum usually in April, May, so that afterwards decreases in summer during the dry period and increases again towards the beginning of autumn (Figure 2).

Due to the fact that the content of organic matter is higher in phaeozems, as well as the almost weak reaction, favorable conditions are created for the downgrading of the nitrification process, leading to an increase in the amount of nitric nitrogen.

During the vegetation period, the accumulation of nitrates in the soil does not occur at the same time as the plants' requirements, and for this reason, the application of chemical fertilizers is required to ensure the necessary nutrients for plant growth.

Wheat needs nitrogen in the spring for the completion of germination and for vegetative growth, or during this time it often happens that the soil has a small amount of nitrogen.

Analyzing the data obtained, it can be seen that, following the applied fertilizers, the nitric nitrogen in the soil during the heading stage (from 05.05 to 06.12.2021) is in sufficient quantity. On May 5, the NO_3^- content varied between 64-147 kg NO^{-3} /ha.

The highest amount of nitric nitrogen is recorded when it is applied $N_{150}P_{100}$ - 147 kg NO_3^-/ha and when N_{100} is applied - 141 kg NO_3^- /ha, situations when large amounts of nitrogen were used for fertilization.

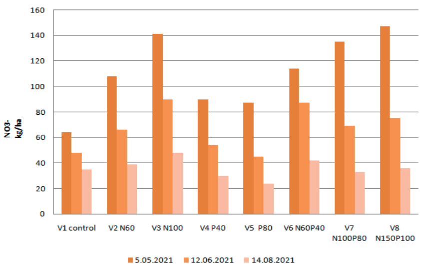

Figure 2. Nitric nitrogen content in the soil during the vegetation period

Moderate values of the nitric nitrogen content in the soil are also recorded in the situation where nitrogen was not used, but only phosphorus: P_{40}, P_{80}, respectively 90 kg NO_3^- /ha, respectively 87 kg NO_3^-/ha, due to the phosphorus-nitrogen interaction in the soil which has the ability to increase nitrogen efficiency, the contents recorded in nitric nitrogen are higher compared to the unfertilized control (V1 - 64 kg NO_3^- /ha).

On 12.06.2021, the date when the heading stage ended, the NO_3^- content of the soil decreases in all variants, between 45-90 kg NO_3^-/ha. This decrease is normal because the wheat plants needed large amounts of nitric nitrogen for nutrition.

The highest amount of nitrogen was registered at V3 (N_{100}), V6 ($N_{60}P_{40}$) and V8 ($N_{150}P_{100}$), variants where high doses of nitrogen were used for fertilization.

Except for the unfertilized control, in all other variants, the nitric nitrogen content in the soil, even if it has decreased compared to 05.05.2021, indicates a normal and good soil supply state.

Until the heading stage, wheat has the greatest need for nitrogen, after which the requirements decrease. The nitrogen in the soil decreases after this period, reaching at harvest (14.08) a nitric nitrogen content of the soil between 24 kg NO_3^-/ha (V5) - 48 kg NO_3^-/ha (V3), indicating a poor supply of the soil in nitric nitrogen, respectively a mediocre state of the soil in nitric nitrogen.

In conclusion, it can be observed, at all 3 determination dates, that the soil is well supplied with nitric nitrogen, the plants having the necessary nitrogen available for growth and development.

Nitric nitrogen is found in high quantities in the soil both due to the use of chemical fertilizers and the fact that the preceding plant was soybean, but also due to the increase in soil temperature which actively stimulates microorganisms and implicitly nitrifying bacteria.

Analyzing the obtained data as a result of the application of fertilizers, the ammoniacal nitrogen in the soil varies between 19.8-82.5 kg NH_4^+/ha on 05.05.2021, the condition of the soil's supply of ammoniacal nitrogen being very good, until heading stage, wheat has enough NH_4^+ which can be converted into nitrates (Figure 3).

The richest variant in ammoniacal nitrogen is V3 to which the dose of N_{100} was applied and V8 to which the dose of $N_{150}P_{100}$ was applied, with 75 kg NH_4^+/ha and, respectively, 82.5 kg NH_4^+/ha.

Until 12.06.2021, NH_4^+ is continuously decreasing in all variants, and the lowest NH_4^+ content is recorded at control - 9 kg NH_4^+/ha, the variant to which no fertilizers were applied. We can conclude that the plants had at their disposal a sufficient amount of ammoniacal nitrogen to later transform into nitrates.

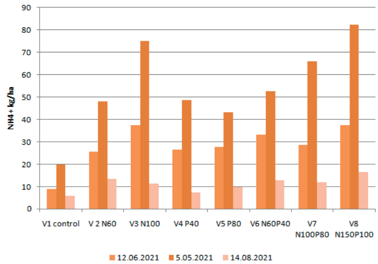

Figure 3. Ammoniacal nitrogen content in the soil during the vegetation period

Ammoniacal nitrogen decreases after heading stage, but in a smaller proportion than before heading stage. At harvest, the NH_4^+ content of the soil is low and is between 6-16.5 kg NH_4^+/ha, the soil having a weak to mediocre supply of ammoniacal nitrogen. This decrease is due to nitrification of ammoniacal nitrogen and consumption by plants.

The variant with the lowest content is unfertilized control -6 kg NH_4^+/ha, the highest content being recorded at the variant where $N_{150}P_{100}$ was applied -16.5 kg NH_4^+/ha.

In general, it is considered that the acidic environment favors the absorption of nitric nitrogen and the neutral and alkaline one favors the absorption of ammoniacal nitrogen (Tisdale et al., 1993; Lixandru et al., 1990).

Phosphorus in the soil, in an easily assimilable form, has a regime that fluctuates less during the vegetation period than that of nitrogen (Table 4). In the soil layer, usually, the total content of the soil in phosphorus is lower compared to nitrogen.

Before heading stage (May 5), the phosphorus content in soil varied within fairly large limits, recording from the control to V8 between 168 - 396 kg P/ha, the soil being well - very well supplied with phosphates accessible to plants and excessively supplied only for V8 variant.

At variants in which phosphorus is applied together with nitrogen, the highest phosphorus contents are registered, which oscillate between 254 and 396 kg P/ha, the supply of the soil with this element is very well and respectively excessive.

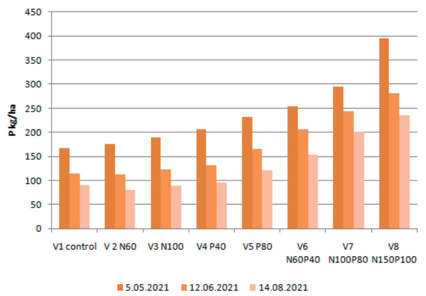

Figure 4. Phosphorus content in the soil during the vegetation period

At heading stage (12.06.2021) the phosphorus content of the soil registers lower values, being between 113 and 282 kg P/ha.

The highest level of phosphorus in the soil at this time, is observed for V8 - 282 kg P/ha to which $N_{150}P_{100}$ was applied; V7 - 243 kg P/ha and V6 - 207 kg P/ha, the soil in these variants being very well supplied with phosphorus.

At harvest, the phosphorus content is lower than the other two determinations, as expected, because the plants needed this element in their nutrition. It has values between 81 kg P/ha (where N_{60} is applied) and 236 kg P/ha (where $N_{150}P_{100}$ is applied). The phosphorus supply status of the soil is good for variants V5-V7, very good for V8 and medium supplied for V1-V4.

The dynamics of phosphorus in the soil for the 3 data analyzed highlights the fact that the chemical fertilizers used have an effect on its mobilization. Phosphorus mobilization as a result of the applied treatments was different depending on the administration of phosphorus as fertilizer.

Analyzing the data dynamics, it can be observed that the soil is medium and well supplied with phosphorus, providing the plants with the necessary phosphorus for their growth and development.

The potassium in the soil in easily assimilable forms has a regime during the vegetation period in which the fluctuations characteristics of nitrogen are absent (Figure 5). Due to the chemical and biological processes in the soil, it passes from the mineral form to the soil solution.

Analyzing the obtained data, it can be observed that the soil is medium supplied with potassium, its content oscillating between 143 kg K /ha (V1) on 14.08. and 296 kg K/ha (V8) on 05.05.

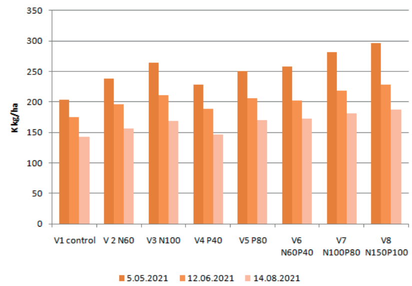

Figure 5. Potassium content in the soil during the vegetation period

From the data contained in Figure 5, it appears that the soil is medium supplied with potassium, its content varying between 203 and 296 kg K/ha at the first determination date (5.05.2021), regardless of the treatments applied, the soil providing potassium necessary for the growth and development of plants. The application of fertilizers with both nitrogen and phosphorus lead to an increase in the potassium content of the soil as a result of the nitrogen - phosphorus - potassium interaction, reaching 282-296 kg K/ha.

At heading stage, a weak supply of soil in potassium is recorded for V1-V2-V4 and medium supply for the other variants, the content oscillating between 202 kg K/ha (V6) and 228 kg K/ha (V8).

At harvest potassium content registers values between 143 kg K/ha (V1) and 188 kg K/ha (V8) which indicates a poor supply of soil in potassium for all 8 variants. This low content is due to consumption by the wheat plants during the vegetation period.

The content of nitrogen, phosphorus and potassium determined in all variants over the course of the vegetation period shows a variation of these elements. The dynamics of the main nutritional elements (N, P, K) in wheat plants is presented in the Figure 6.

The total nitrogen content of plants changes to a rather large extent under the influence of fertilizers.
Along with the growth of the plants, the nitrogen, phosphorus and potassium content decreases, eventually reaching the lowest content in the straw at harvest, due to the increase in the vegetative mass of the plants.

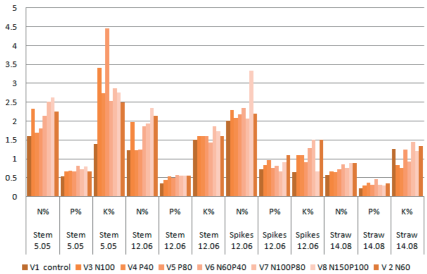

Figure 6. Dynamics of N, P, K accumulation in wheat plants

The maximum consumption of nitrogen is recorded at heading stage, after which a movement of a considerable part of nitrogenous substances is observed from the respective organs (stems, spikes) to the grain, where most of it is represented by protein compounds.
Based on the determinations made on the plants, it can be seen that before heading stage (5.05) the nitrogen content of the plants varies between 1.605% (control) and 2.631% (V8).
At heading stage, analyzes were done separately for spikes and analysis for stem + leaves (plant without spikes).
A higher content of total nitrogen is observed in the spikes than for stem + leaves, which indicates that the nitrogen has started to migrate into the spikes.
For stem + leaves, the total nitrogen varies between 1.221 % (control variant) and 2.34% (V8), while in spikes the nitrogen content varies between 2.013 % (control) and 3.341% (V8).
At harvest, the nitrogen content is very low compared to the content recorded for the plants before and after heading stage. At harvest, the nitrogen content varies between 0.564% (control) and 0.889% (V8). A higher value of the nitrogen content in the plants is observed in the variants where fertilization was done with nitrogen and phosphorus, the increase being due to the interaction between nitrogen and phosphorus.
The phosphorus content of the wheat plants decreases with the growth of the plants, leaving the phosphorus to be assimilated more intensively in the second part of the vegetation.
The application of nitrogen fertilizers increases the phosphorus content in plants. The phosphorus content drops significantly for straws, because it moves towards the grain to participate in the synthesis of protein substances, the desorption phenomenon being very little obvious for phosphorus.
Before heading stage, the phosphorus in the plants oscillates between 0.54% (control) and 0.81% (V6). It was observed that the phosphorus content was much higher in the spikes than in straws. For straws, the content varied between 0.35 (control) and 0.58 (V6). At harvest, the phosphorus content of the straws varied 0.21 at (control) and 0.46 (V6).
In terms of potassium dynamics, in the first phases of vegetation the largest amount accumulates at an accelerated and intense rate, observing an obvious decrease at the end of the vegetation period due to the desorption phenomenon.
The amount of potassium in the plants analyzed before heading stage oscillates between 1.4% (control)-4.45% (V5). At heading stage, the potassium content decreases both in straws and in spikes, straws having a higher content than spikes. For straws, the potassium content is between 1.5% (control) and 1.86% (V7), and in spikes between 0.65% (control) and 1.51% (V7).
At harvest, the potassium content of the straw is, in most variants, lower than the potassium content of the straw at the time of heading stage and higher than that of the spice at the time of heading stage.
In wheat grains, the N/P ratio registers higher values compared to plants, due to the fact that both elements are in large quantities compared to plants, and the N/K ratio has lower values in grains than in plants, because nitrogen has been accumulated in grains in increased quantities, while potassium was returned to the soil through the desorption phenomenon (Figure 7).
It can be stated that the application of phosphorus fertilizers increases the degree of use of nitrogen from fertilizers and from the

soil, and thus contributes to achieving significant increases in production.
The nitrogen content of the grains has a direct influence on their quality, and is influenced by the amount of fertilizer applied to the variant.
The nitrogen content oscillates between 1.95% (control)-2.351% (V6) and the phosphorus content oscillates between 0.64% (control) and 1.02 % (V8).

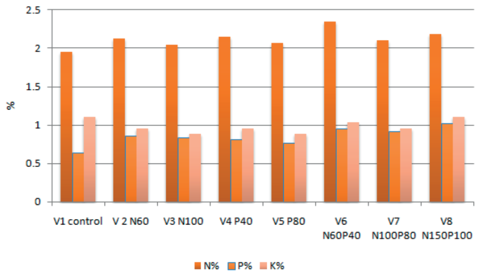

Figure 7. Accumulation of N, P, K in wheat grains

Regarding potassium, it oscillated between 0.89% (V3, V5) - 1.1% (V1, V8).
The potassium content in grains registers a normal decrease, being smaller than in straw, potassium having an auxiliary role in the formation and decomposition of the main substances in the grain, a good part of the potassium being returned to the soil.

Table 1. Grain wheat production (kg/ha)

Variant	Productions kg/ha	Difference compared to the average production	Significance	Difference from control	Significance	Yields kg a.s/kg
V1 control	5466	-1117	0	-	-	-
V2 N_{60}	6340	-243	0	+874	xxx	14.5
V3 N_{100}	6861	+278	0	+1395	xxx	13.9
V4 P_{40}	6121	-462	0	+655	xx	16.3
V5 P_{80}	6325	-258	0	+859	xxx	10.7
V6 $N_{60}P_{40}$	6525	-58	0	+1059	xxx	10.6
V7 $N_{100}P_{80}$	7181	+598	xx	+1715	xxx	9.5
V8 $N_{150}P_{100}$	7850	+1267	xxx	+2384	xxx	9.5

LSD 5% 404.7; LSD 1% 560.5; LSD 0.1% 773.3; Average production 6583 kg/ha

The productions obtained varied depending on the doses of fertilizers applied (Table 1). Thus, they varied between 5466 kg/ha for the unfertilized control and 7850 kg/ha when $N_{150}P_{100}$ was applied. The fairly high production obtained for control of 5466 kg/ha, is due to the previous years' fertilization of the soil and the previous crop - soybeans. The application of a moderate dose of nitrogen N_{60} leads to an increase in production to 6340 kg/ha, and a high dose of N_{100} leads to productions of 6861 kg/ha.
The moderate doses of phosphorus P_{40}, P_{60} also lead to higher production compared to the control 6121-6325 kg/ha, with a yield of 655 kg/ha and 859 kg/ha, due to the direct action of the applied phosphorus, but also due to the interaction nitrogen-phosphorus from the soil.
The highest productions are obtained after the use of doses of $N_{100}P_{80}$-7181 kg/ha and $N_{150}P_{100}$-7850 kg/ha.
Analyzing the productions obtained compared to the average productions, only the variants in which the above doses were applied are significant ($N_{100}P_{80}$) and distinctly significant when $N_{150}P_{100}$ is applied, with an increase in production compared to the average of 598 kg/ha and 1267 kg/ha. The application of NP fertilizers leads to higher wheat productions, these results are similar to Fana et al. (2012) and Kashif et al. (2023).
Compared to the control, all 7 variants in which chemical fertilizers were used in different doses are distinctly significant and only the variant in which the dose of 40 kg P/ha was applied is statistically significant.
The application of fertilizers leads to important increases in production per kg of active fertilizing substances. Nitrogen doses N_{60} and N_{100} lead to yields of 14.5 and 13.9 kg/ha a.s.
Overall, the use of organic and inorganic fertili-zers in combination plays a crucial role in enhancing the growth and yield of crops by providing essential nutrients, due to the efficient transfer of carbon synthesis products and the storage of carbohydrates and proteins in grains (Ramadhan, 2022).

CONCLUSIONS

Analyzing the dynamics of the contents of $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, P and K in the soil during the research carried out, it is generally found that the soil is well supplied with these elements.
Along with the growth of the plants, the nitrogen, phosphorus and potassium content decreases, eventually reaching the lowest content in the straw at harvest, due to the increase in the vegetative mass of the plants.
Following the dynamics of the accumulation of N, P, K in wheat plants, a decrease in the percentage content of main macroelements was observed, the younger the plants, the richer in N, P and K, and as the vegetative growth phase

ends, the content decreases. This decrease is slower at N and P and faster at K.

At the end of vegetative growth, along with the heading stage, the content of N, P and K in wheat plants is relatively constant until maturity.

Chemical fertilizers with nitrogen and phosphorus administered alone or together led to increases in production, the most significant increases in production were recorded when the fertilizers were administered together (V7 and V8).

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THE INFLUENCE OF SOWING DATE ON DEVELOPMENT OF MAIZE IN THE CONDITIONS OF CENTRAL ROMANIA

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Abstract

Maize, one of our country's most widespread field crops, is also the most exposed to atmospheric and soil drought, which is pronounced in the summer months. The following experimental variants were initiated and tested in this context: early sowing, sowing during the optimal period, and after the optimal period. The evolution of precipitation fluctuated over months and years. The studies focused on the influence of the sowing season on the duration of the growing cycle of maize grains in the area of the Research and Development Station for Cattle Breeding Tg. Mures (R.D.S.C.B. Tg. Mures). According to the data recorded in the 2018-2021 agricultural years, the studied hybrids responded well to early sowing, and each delay reduced the duration of the growing cycle in sowing. Early sowings increased the period from sowing to plant emergence but did not reduce the germination and the population density of the crop. Alternatively, late sowings reduced the number of days to physiological maturity producing higher humidity content in grain at harvest and taller plants.

Key words: *maize, sowing date, germination, growing cycle, plant height.*

INTRODUCTION

Maize (*Zea mays* L.) holds a significant place in Romanian agriculture, by the large area it occupies (on average 30% of arable land), the yields achieved, and the multiple uses of maize grain: food for people, industry, and feed (Ionescu et al., 2020). Therefore, the production and economic efficiency of maize crops are matters of national interest (Sarca et al., 2007).

Many cropping practices have been recommended as sound adaptations to the warming, such as adjusting the sowing date and cropping pattern (Mendelsohn et al., 1994; Rosenzweig and Hillel, 1998; Winters et al., 1998), adopting higher-yielding varieties with heat resistant, and improving management processes (Butt et al., 2005; Njie et al., 2006; Ogden and Innes, 2008).

Plant species have a unique requirement for the core temperature, which is the temperature at which they start growing. Maize, for instance, needs a temperature of 10ºC (Rao Prasada, 2008). Maize seeds germinate 4-5 days after sowing in conditions of heat and humidity, and when the temperature is lower than optimal, 14 to 16 days may be required to emergence (Hussen et al., 2013).

Plants from sowings made in March or early April were not significantly earlier or more productive than plants from sowings made in late April. For grain production, however, any further delay in sowing time usually led to lower yields, even with the earliest varieties tested. In contrast, the final production of dry material from the shoot was higher, on average, from mid-May sowings (Bunting, 1968).

The early sowing of maize is a strategy that farmers can employ to potentially stabilize maize yields, not only in regards to changing climatic conditions but also to avoid adverse conditions such as high temperatures and drought conditions during the grain filling period, phenomena that also occur in the Maize

Belt of the USA (Lauer et al., 1999). Breeding programs have facilitated germination of maize at colder temperatures (Sanghera et al., 2011). Bruns and Abbas (2006) reported technological improvements in maize hybrids such as better early season vigor and tolerance to germination in cool wet soils, better seed treatments to guard against damping off diseases and seedling insect pests, or the advent of herbicides. These factors have contributed to planting maize earlier than it was 30 years ago (Abendroth et al., 2017).

However, early sowing can be conflicted by low spring soil temperatures, which can reduce seed germination, plantar sunrise rate, and final stability (Hayhoe et al., 1996).

The use of the optimal planting window will play a critical role in the future productivity of maize, especially in the context of climate change (Seifert et al., 2017).

The effects of the warming of the Earth's atmosphere are diverse and include the increase in the number and intensity of extreme events such as storms and hurricanes, extreme droughts, floods. Meteorological data show that the last 20 years have been the warmest, and the amplitude between very rainy and very dry years has become much larger (Mircea et al., 2023).

The impact of climate change could be reduced through several technological measures, including the use of biological materials adaptable to less favorable climate and soil conditions, as well as efficient input management.

In this paper, results are presented on the influence of sowing age and climatic conditions on the development of maize crops.

MATERIALS AND METHODS

A four-year experiment (2018-2021) was conducted at the Research and Development Station for Cattle Breeding Tg. Mures (R.D.S.C.B. Tg. Mureș) experimental station at Sângeorgiu de Mureș, Romania, according to the method of subdivided plots, the first factor the sowing date, with 3 gradations: the first sowing date, early sowing was realized when 6ºC were recorded in the soil; the 2nd sowing date, an optimal sowing date when 10ºC was achieved in the soil and the 3rd sowing date, two weeks after the 2nd.

Factor 2 is represented by the 9 tested maize hybrids. Two hybrids from the Agricultural Research and Development Station Turda (A.R.D.S. Turda): Turda 248 (FAO 300), Turda 332 (FAO 380), two hybrids from the company Corteva, P 9900 (FAO 360), P9903 (FAO 360) and two hybrids from the company KWS Semences, KWS 2370 (FAO 290), KWS 4484 (FAO 380).

Was used for sowing a density of 65,000 harvestable plants per ha, with the distance between rows at 70 cm. Fieldwork was done by harrowing in August, deep plowing in the autumn, leveling discs in the spring, and a work with the cultivator to prepare the germinative bed. The application of the whole dose of fertilizers was done after the disk, and their incorporation into the soil was done with the combinator. The amount and type of fertilizer used were $N_{16}P_{16}K_{16}$ 500 kg/ha, and every three years 4 t amendments per ha were applied. The soil on which the experiment was located, was a brown forest soil, weakly podzolic, clay loam texture, with a humus content of 1.9, pH of 5.8, P_2O_2 supply is 17.4 mg/100 g soil, K_2O 21.7 mg/100 g soil and an N index of 1.4. The predecessor plant was soybean. The results of the studies were analyzed according to the climatic conditions of the area, recorded at the weather station in Sângeorgiu de Mureș and presented in Table 1.

The temperature evolution during the vegetation period of the crop, in the four years of the study, did not show significant differences. From the recorded data, it is highlighted that the average monthly temperatures during the vegetation period of the crop (April-October), during the studied years (2018-2021), exceeded the multi-year average. The average temperature during the 2018-2021 vegetation period was 17.1ºC, exceeding the multiannual average for the respective period by 2.2°C. The evolution of precipitation fluctuated over months and years. The average precipitation during the growing season in the studied years was 458 mm, 10 mm below the multiannual average. The wettest months were June with 187.2 mm in 2018 and May 134.45 mm in 2019. The driest month was in August 2018 when only 8.7 mm was recorded.

The height of 10 plants of the central rows was measured about one week after silking in each plot from the base of the crop to the top. The plant density was estimated before harvest,

counting the total plants of the two central rows in each experimental plot.
The grain harvest took place during September or October manually, after the plants had reached physiological maturity (Table 3). Grain yield was measured by harvesting two central rows from each plot. A mixed-effects analysis of variance (ANOVA) was carried out to assess the responses to sowing data (SD), with years evaluated as repeated measurements.

Table 1. Mean monthly (T_m) air temperatures and total monthly rainfall at Sangeorgiu de Mures, during the experiment (from 2018 to 2021). Long-term (60 years) mean annual temperature and rainfall values at Sangeorgiu de Mures are 14.9°C and 468 mm, respectively

Month	2018		2019		2020		2021	
	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
April	15.6	12.7	12.3	30.3	10.4	21.1	8.6	93.9
May	18.7	53.8	15.3	134.45	14.3	61.4	14.9	95.0
June	20.1	187.2	22.0	49.8	19.6	120.9	19.9	70.4
July	20.7	58.5	21.5	59.9	21.8	99.1	23.0	98.2
August	22.9	8.7	22.1	78.6	22.0	40.9	20.6	77.4
September	16.2	58.8	17.0	50.1	18.3	62.2	17.1	59.8
October	12.5	32.2	12.0	31.6	12.5	74.2	8.7	12.5

RESULTS AND DISCUSSIONS

The number of days when soil temperatures were below 10 degrees and the number of sowing-sowing days fluctuated based on the sowing season and year. Thus, in the early sowing season, there were an average of 14 days with temperatures below 10°C and the number of days sown-rising was 19, in the optimal sowing season there were on average 7 days with temperatures below 10°C, and the number of days sown-rising was 16, the number of days, in the late sowing season there were 2 days with temperatures below 10 degrees C and the number of days sown-rising was 11.
The climatic conditions of 2018-2021 were different, signaling quite long periods, especially in April, with soil temperatures below the minimum germination threshold. The most unfavorable year was 2021 when the number of days with temperatures below the germination threshold reached 20 in the early sowing season, resulting in the extension of the maize growth up to 23 days.
The number of days from sowing to emergence can be influenced by the climatic conditions, the technology applied but also the biological material used, as it resulted from the research carried out by Horablaga et al. (2023) a number of 10-12 days from sowing to emergence of corn depending on the studied germplasm.
So as the number of days with temperatures below 10ºC decreased, the number of days from sowing to emergence also decreased.
In the conditions of the Transylvanian Plateau, during the April-May period, some climatic phenomena such as late frosts (Simon et al., 2023) are quite common, such as late bumblebees or very high-temperature differences between day and night.
Based on previous research (Jones and Thornton, 2003; Lobell and Asner, 2003), it hypothesized that crop development is affected not only by input from factors of production but also by climate change. Therefore, the increase in maize results from the combined action of hours of sunlight, temperature, precipitation, and other environmental factors.
Nielsen et al. (2002) and Parker et al. (2016) pointed out that planting maize too early is associated with potentially suboptimal planting conditions in soil and climate, while planting it too late exposes plants to a short growing season. Tollenaar and Bruulsema (1998), and Shrestha et al. (2018) found that low temperatures prolonged the physiological maturity of maize during late planting. Differences in climate and the length of the growing season can affect the optimal sowing date of maize in different areas (Bruns and Abbas, 2006).
However, the greatest effect of sowing delay was observed in the number of days from sowing to silting, which decreased significantly from 104 days during mid-March sowing to 81 days (22% discount) and to 69 days (33% discount) during mid-April and mid-May sowing (Maresma et al., 2019).

The analysis of % of plants sprouted according to the sowing season highlights the fact that between the optimal sowing season and the early sowing season, there is a significantly positive difference, and compared to the late sowing season there were no significant differences, so we can say that early sowing at temperatures below the minimum germination threshold (10ºC) did not adversely affect germination.

Research conducted by Maresma et al. (2019), showed that early and late sowing has negative effect on plant density, with a number of plants/ha of about 71000 and 75000, respectively compared to normal sowing where the plant density was 78000 plants/ha.

From the statistical analysis on the influence of the years it is found that only in 2021 there was a significantly negative difference due to the non-favorable conditions of April. In the other 3 years the differences were not statistically assured.

Table 2. Soil temperature at sowing depth at 6 a.m. and number of days with suboptimal germination temperatures below 10°C at R.D.S.C.B. Tg.Mures

Sowing date	Year	Date of		No. days btw. sowing-emergence	Emergence plants %	Avg. temp. of soil at 6 a.m.	No. days with suboptimal Temperatures (>10°C)
		sowing	emergence				
Early	2018	April 10	April 21	12	98	8	1
	2019	April 4	April 24	21	98	8.3	17
	2020	April 7	April 24	18	98	7.6	17
	2021	April 10	May 21	23	97	7	20
	Avg.			19	98		14
Optimal	2018	April 24	May 3	10	95	13.5	0
	2019	April 18	May 8	21	99	11	7
	2020	April 21	May 10	20	98	9.6	12
	2021	April 24	May 6	13	97	9.2	8
	Avg.			16	97		7
Late	2018	May 7	May 15	9	95	15	0
	2019	May 5	May 18	14	97	12.4	2
	2020	May 8	May 17	10	98	12	2
	2021	May 7	May 15	9	98	10.2	3
	Avg.			11	97		2

From the analysis of the sowing age, on germplasm sources, it was found that compared to the average of hybrids there was only one hybrid (KWS 4484), at which % the plant emergence was above the average of the hybrids, making a significant positive difference. At the other sources of germplasm, the differences did not have a statistical coverage.

The high maintenance of % of germinated plants and in the years with colder springs we believe is due to the biological value of the seeds and the quality of the phytosanitary products used for treatment.

Another element studied was the plant size which can be influenced by both climatic factors (temperature, precipitation) and applied technologies, previous research has shown that plant size correlates greatly with biomass and production, so it is very often used in production estimation (Yin et al., 2011).

From the analysis of variance, it is found that the date of sowing at temperatures below the minimum threshold of germination had a negative effect on the plant's waist. The difference was very significantly negative in the early sowing season from the optimal sowing season, but this decrease we cannot put entirely on account of sowing below the minimum threshold of germination, but on accumulation of climatic conditions in the sown-flowering interval. Research conducted by Abdel-Rahman et al. (2001) showed that early sowing had a significant effect on the plant's waistline, which is, that the plants with the highest waist are obtained from sowing corn earlier compared to late sowing.

An analysis of the influence of the years on the plant's waist highlights the fact that there are very significant positive differences in 2018 and 2020, but also very significant negative differences in 2019.

From the analysis of variance on germplasm type, it was found that only the T 248 hybrid recorded a significantly negative difference.

The height of the plant is an important growth character directly related to the productive potential of the plant, is very often used in the evaluation of yield (Omotosho and Shittu, 2007; Bendig et al., 2015). Maize is situated in the category of plants with high-temperature claims. In conditions with optimal temperatures, plants can grow between 7 and 14 cm in 24 hours. The hybrid cultivation is made according to the thermal needs of the hybrids for the growth and development processes.

Research by Schitea and Motcă (2013) showed that drought and heat led to a shortening of the growing season, forcing late genotypes to mature.

Table 3. Sowing date (SD) effect on the dates of emergence, silk and black layer appearance, the days happened between sowing and silk and black layer appearance and plant height. Average 2018-2021

Sowing Date (SD)	Emergence plants	Emergence	Silk	Black layer	Plant height
	%	(days)	(days)	(days)	(cm)
Early	97.44	19	92	138	266
2018	97.06	13	75	130	270
2019	97.50	21	94	147	255
2020	97.56	18	101	146	271
2021	97.67	23	98	130	267
Optimal	96.40	16	85	133	278
2018	94.56	10	76	125	288
2019	98.56	20	86	138	263
2020	97.39	20	94	142	284
2021	95.11	13	85	125	279
Late	96.57	10	76	122	288
2018	97.89	9	72	116	303
2019	96.22	14	71	126	269
2020	97.39	9	83	130	295
2021	94.78	9	76	116	284
		ANOVA			
Year (Y)	**	**	**	**	**
Error					
Sowing Date (SD)	*	**	**	**	*
Y*SD	**	**	**	**	**
Error					
Hybrid (H)	**	**	**	**	**
SD*H	**	**	ns	**	**
Y*H	**	**	**	**	**
Y*SD*H	**	**	ns	**	**

Significant at p-value < 0.05; ** Significant at p-value < 0.01; ns = not significant.

The results of measurements on the plant height, by years, depending on the type of germplasm, highlight the fact that the most drastic plant height reductions were recorded in the American germplasm hybrids, 18 And 16 cm, followed by Romanian hybrids with 9 and 10 cm, then with German germplasm of 4 and 14.5 cm, depending on the hybrid.

Analysis of the variance representing the number of days from sowing to silk, in the early sowing season compared to the optimal sowing season, shows that there is a very significant positive difference.

The analysis of the year is highlights a very significant negative difference in 2018, and in 2020 there was a very significant positive difference.

Regarding the germplasm type, there was a distinctly significant difference in the T248 hybrid, and in the other hybrids, the differences did not have statistical assurance.

Most days between sowing and silk, in advance of the optimal era, were achieved in 2018 (15 days), 2019 (5 days), 2020 (6 days) and 2021 (4 days). The average per year and on hybrids is 8 days.

This is in line with the results achieved by Mederski and Jones (1963), who reported a decrease in the number of days from sowing to silting as the soil temperature rises.
The low amount of precipitation correlated with high temperatures makes plants consume more energy to achieve significant yields, with climatic conditions still being the most important factor that determines the yield of a crop (Popa et al., 2021).
Taller plants contribute to obtaining a larger LAI (Leaf Area Index) and intercepting solar radiation better than low-waisted plants.
After analyzing the amount of active temperatures achieved in different years and sowing seasons, it was found that the sum of active temperatures per sowing season was consistent in 2019, while there was a significant variation in the rest of the years due to different climatic conditions. The highest difference in the sum of temperatures was observed in the year 2020. Early sowing has the advantage that it outruns the onset of silkiness and removes the release phase of pollen from the influence of high temperatures that lead to pollen sterility. Post-emergence environmental factors have a significant impact on the yield of a plant (Kimmelshue et al., 2022).

Table 4. The number of days between sowing-silk in the three sowing seasons.
The number of days between the sowing season in the early in addition to the optimal sowing season and 'active temperatures achieved in years and sowing seasons

Year	Hybrid	No. days sowing-silk			Days no. in addition to Sowing season II	∑ active temperatures		
		Sowing season I	Sowing season II	Sowing season III		Sowing season I	Sowing season II	Sowing season III
2018	KWS 2370	73	72	70	14	648	653.4	634
	KWS 4484	80	78	73	12	697	711.4	675.8
	PIONEER 9900	75	72	72	15	661	703	665.6
	PIONEER 9903	76	78	73	16	666	711.4	675.8
	TURDA 332	75	77	72	15	661	703	665.6
	TURDA 248	73	76	71	16	648	694	654.8
	Average	75	76	72	15	663.6	696.0	661.9
2019	KWS 2370	93	83	71	3	688	684	662.7
	KWS 4484	97	88	77	5	724	715.1	724.1
	PIONEER 9900	93	85	76	6	688	696.2	711.5
	PIONEER 9903	92	87	76	8	676	707.9	711.5
	TURDA 332	95	85	75	3	709	696.2	700
	TURDA 248	95	84	73	3	709	690.8	679.8
	Average	94	85	75	5	698.9	698.4	698.3
2020	KWS 2370	97	90	79	6	600	632.2	671.4
	KWS 4484	103	96	86	5	654	694.4	759.9
	PIONEER 9900	104	97	84	6	663	703.7	733.2
	PIONEER 9903	102	93	79	4	644	660.8	671.4
	TURDA 332	98	93	83	8	609	660.8	718.9
	TURDA 248	100	95	86	8	625	682.5	759.9
	Average	101	94	83	6	632.5	672.4	719.1
2021	KWS 2370	98	86	76	4	676	700.8	697.3
	KWS 4484	97	83	74	5	661	654.2	678.5
	PIONEER 9900	99	86	76	5	691	700.8	697.3
	PIONEER 9903	101	88	83	5	721	724.6	785.5
	TURDA 332	98	86	77	4	676	700.8	708
	TURDA 248	94	81	72	4	614	621.8	649.1
	Average	98	85	76	5	672.9	683.8	702.6
	Average 2018-2021	92	85	76.5	8	667.0	687.6	695.5

Another element studied was the variability of the number of days from sowing to physiological maturity. From the statistical analysis of the data, it follows that between the early and optimal sowing seasons, there are very significant positive differences. The analysis of the variance on the influence of the years revealed a very significant negative difference between 2018 and 2021 and a very significant positive difference between 2019 and 2020.

An analysis of germplasm sources shows that there was a very significant negative difference for the KWS 2370 hybrid and very significant positive and distinctively significant positive differences in the American germplasm hybrids. In Romanian hybrids, the results did not have a statistical coverage.

Of particular importance of early sowing is the date of physiological maturity. From the table presented it follows that the number of days in advance at physiological maturity is between 9 and 3 days. From the analysis by year it follows that the highest advance was made in 2018 (8 days) and the lowest in 2019 (3 days). Early sowing leads to an advance maturation of hybrids, economically desirable goal, eliminating the drying stage, shattering the berries and favoring the filling of the berries and the growth of the MMB.

Table 5. Number of days between sowing-physiological maturity in the three sowing seasons. The number of days between sowing-physiological maturity in the early in addition of the optimal sowing seasons and ∑ of the active temperatures achieved in sowing years and sowing seasons

Year	Hybrid	No. days sowing-black layer			Days no. in addition to Sowing season II	∑ active temperatures		
		Sowing season I	Sowing season II	Sowing season III		Sowing season I	Sowing season II	Sowing season III
2018	KWS 2370	126	121	114	10	1230.9	1255.3	1209.5
	KWS 4484	133	126	115	7	1320.9	1319.9	1221.4
	PIONEER 9900	133	124	116	7	1320.9	1294.4	1232.4
	PIONEER 9903	134	128	117	7	1334.5	1343.5	1244.1
	TURDA 332	132	127	119	9	1307.9	1331.6	1269.6
	TURDA 248	127	122	115	8	1245.1	1268.5	1221.4
	Average	131	125	116	8	1293.3	1302.2	1233.1
2019	KWS 2370	143	135	120	5	1242.1	1275.2	1256.6
	KWS 4484	148	138	125	3	1308.2	1327.2	1308
	PIONEER 9900	146	136	127	3	1281.7	1288.3	1329.9
	PIONEER 9903	147	137	129	4	1294.6	1313.8	1352.4
	TURDA 332	150	140	128	3	1347.6	1348.1	1342.2
	TURDA 248	151	141	126	3	1360.2	1356.8	1318.2
	Average	148	138	126	4	1305.7	1318.2	1317.9
2020	KWS 2370	140	136	122	9	1094.9	1176.2	1177.7
	KWS 4484	142	138	130	9	1113.7	1192.4	1256.8
	PIONEER 9900	151	125	144	6	1214.5	1329.7	1364.5
	PIONEER 9903	149	140	126	4	1198.3	1210	1214.9
	TURDA 332	144	138	126	7	1133.5	1192.4	1214.9
	TURDA 248	151	144	130	6	1214.5	1247.2	1256.8
	Average	146	137	130	7	1161.6	1224.7	1247.6
2021	KWS 2370	138	134	134	9	1158.7	1210.2	1328.2
	KWS 4484	139	131	131	7	1165.5	1194.5	1285.1
	PIONEER 9900	142	137	128	5	1182.8	1234.8	1246.6
	PIONEER 9903	149	139	133	7	1222.9	1258.2	1318.3
	TURDA 332	146	135	131	3	1206.2	1223.2	1285.1
	TURDA 248	137	124	126	3	1151.3	1152.4	1222.4
	Average	142	133	131	6	1181.2	1212.2	1281.0
	Average 2018-2021	92	85	76.5	8	667.0	687.6	695.5

CONCLUSIONS

There were situations when the temperature remained below the germination threshold for several days, the studied hybrids germinated after 20-23 days, but without affecting the germination. There were no differences between germplasm sources, but there were differences between the years studied. The coldest year was 2021 when the plants emergence after 22-24 days without affecting the germination.

Early sowing did not adversely affect % of the plants sprouted compared to the optimal sowing season.

The date of the silk appearance was advanced by up to 17 days compared to the optimal sowing season. The differences were different depending on the year and the hybrid. The highest reduction was achieved in 2018, and depending on the germplasm, the biggest advance was made in hybrids with American germplasm. Early sowing has the advantage of eliminating the overlap of the flowering and silky phase appearance over high temperatures which can lead to poor fertilization.

Early sowing also outpaced physiological maturity by 3-9 days, positively influencing the filling of the berries.

Early sowing led to a reduction in the plant's waist by up to 13 cm, the difference is very significantly negative.

From the obtained results we consider that sowing of maize at temperatures below the minimum germination threshold can be a process of removing critical phases of the plant from the stressful influence of climate change during the summer, provided that the seed has superior quality indices.

Sowing at temperatures below the minimum germination threshold remains a solution until genomic biotypes with superior characteristics on climate change are developed.

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THE STATISTICAL ANALYSIS OF THE PRODUCTIVITY AND THE BENEFIT OF PESTICIDES APPLICATION IN THE SOUTH-EAST REGION, ROMANIA

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Abstract

Without doubts, a balance between the application of pesticides, productivity and soil health control is essential for assuring sustainable agricultural performance. Nevertheless, pesticides proceed to be a critical tools for worldwide food guarantee, but their negative consequences should not be ignored precisely when sustainable agriculture is the worldwide target. The purpose of the present article is to present a statistical analysis using a parametric and a nonparametric correlations coefficient in order to study the effect of using three categories of pesticides: insecticides, fungicides, herbicides from 1990 to 2022, in the South-East region of Romania and the productivity of: barley, rape and soybeans. During the studied period, the quantity of pesticides per hectare is the following: insecticides 30.67 kilograms of active substance/ha; fungicides 103.81 kilograms of active substance/ha and herbicides 45.16 kilograms of active substance/ha. The statistical analysis shows that the highest correlation between insecticides and herbicides is that with barley of 0.816 and 0.860, while for fungicides is with rape - 0.683.

***Key words**: pesticides, statistical analysis, SE Region, Romania.*

INTRODUCTION

Agricultural chemical products as fertilizers and pesticides have turn into a significant factor of contemporary agricultural systems within the recent period to provide a substantial increase in crop harvests (Alexandratos and Bruinsma, 2012). It has been valuated that approximately 35-45% harvest production is lost as a result of insufficient crop protection actions contra pest invasion of insects, weeds and diseases (Larsen et al., 2019). During the last fifty years, significant increase in the global economy containning both technical and farming fields generated the gradual grow in the production and application of agriculture-based chemicals compounds that frequently cause devastating repercussions on the environment (Balog et al., 2017).

Inappropriate utilize of pesticides and other chemical pollutants in agricultural lands have devasting subsequent impact (Lampridi et al., 2019). In recent years attempts have been made to find alternatives accessible to manage crop damage attributable to pest attack that might contain the use of several biopesticides (Tudi et al., 2021). However, the utilisation of synthetic pesticide is still chosen comparing with the majority over all other options to protect harvest from efficiency damage. Currently, over the world about 2 million tonnes of pesticides are used, among them 47.5% are herbicides, 29.5% are insecticides, 17.5% are fungicides and 5.5% are different types of pesticides (De et al., 2014). According to Worldometers in 2020 the top ten pesticide consuming countries in the world are China, the USA, Brazil, Argentina, Canada, Ukraine, France, Malaysia, Australia and Spain (Worldometers, 2020).

Pesticides applied in agriculture has synthetic origin and are assimilated in the ground throughout surface drainage from processed plants. The type of organic mixture, cropping procedures, watering facilities and climatic causes influence the dissipation of pesticides in ground (Kniss, 2017; Möhring et al., 2021; van der Sluijs, 2020).

The remnants of these organochloride compounds moreover contaminate the subsoil water through percolating and successively influencing the characteristic of agricultural harvests (Martin et al., 2013). Pesticides are collected in soils immediately by its utilization in agriculture and household purposes or incidentally by deposition of aerial pollutants formerly originating from distinct locations or regions (Rossi et al., 2012). Soil has the role of storage segments attributable to larger predilection of biological chemicals with soil (Shattuck et al., 2023). The deposition of natural chemicals or pesticides in ground immediately exposes soil bacteria and in addition rises the hazard for diverse organisms through nutriment and may seriously impact ground habitat, aquatic areas, vegetation and human condition (Sharma et al., 2019; Möhring et al., 2020; Sabzevari et al., 2022).

MATERIALS AND METHODS

With the purpose of analysing the quantities of pesticides applied in the South-East region of Romania, during the period 1990-2022 a series of indicators were used: pesticides (insecticides, fungicides, herbicides) applied per hectare.

The data, collected by the National Institute of Statistics, have been statistically calculated and defined, presenting a clear image of the studied period. During the analysed period, data collection was based on the survey method, with the principal target of obtaining information related to the principal characteristics of agricultural resources. The surveys focus on agricultural holdings that carried out farming activities, either as a main activity or as a secondary activity.

The current study includes an analysis of the main categories of pesticides: insecticides, fungicides and herbicides applied in the South-Eastern region of Romania, more precisely the counties of: Braila, Buzau, Constanta, Galati, Tulcea and Vrancea. From the analysis of the surface of the lands on which pesticides were applied and the quantity of pesticides applied in agriculture, the amount per hectare was calculated in kilograms of active substance/ha.

The amount of pesticides/hectares used in agriculture, South-East region, Romania, is represented in Figure 1, the annual values being obtained by reporting the amounts of pesticides per hectare, calculating the total amounts of the three types of pesticides (Insecticides, Fungicides, Herbicides) that were applied in the six counties in the South-East region of the country.

If we discuss Figures 2-4, they contain two types of graphic representations: the amount of pesticides applied in agriculture, by county, South-East region and the surface of the land on which pesticides were applied and for both types of maps, s - they used the total values of the quantities of pesticides applied and of the surfaces on which pesticides were applied in the counties of Braila, Buzau, Constanta, Galati, Tulcea and Vrancea in the period 1990-2022

The production of Barley, Rapeseed and Soy beans in the South-East region of Romania compared to the pesticides applied is represented graphically in Figure 5, where the total values of the three categories of pesticides are represented (kilograms of active substance/hectares) and productivity of barley, rape and soy beans/hectares for the period 1990-2022.

At the same time, by using the SPSS 26 software, the correlation coefficients were calculated analysing the amounts of pesticides per hectare and three types of barley, rape and soy beans crops.

RESULTS AND DISCUSSIONS

Intense long-term utilization of pesticides on cultivated area causes substantial environmental and health concerns. As a pollutant, when applied on ground, pesticides are able to disturb the soil properties because of its contaminant effect and harmful impact on biodiversity. Over entire pesticides utilized for crops, it has been stated that an extremely small percent reaches the target organism whereas the residues of it pollute the surrounding field (air, water, and ground). All these aspects require a permanent monitoring in order to avoid a series of imminent dangers.

In the South-East region of Romania, in the period 1990-2022, an average amount of 179.65 kilograms of active substance/ha was applied.

Among these, if we analyse each category, the situation is as follows: insecticides 30.67 kilograms of active substance/ha; fungicides 103.81 kilograms of active substance/ha and herbicides 45.16 kilograms of active substance/ha.

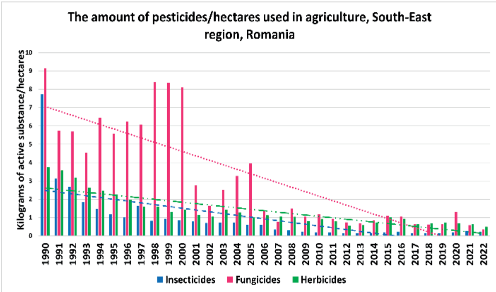

Figure 1. The quantity of pesticides/hectares used in agriculture, South-East region, Romania, 1990-2022

It is obvious that for all the types of pesticides: insecticides, fungicides and herbicides analysed in this study, the quantities applied per hectare decreased. Fungicides represent the pesticide category that decreased the most, from a quantity of 9.14 kilograms of active substance/ha in 1990 to 0.35 kilograms of active substance/ha in 2022. And insecticides registered a very large decrease, from 7.73 kilograms of active substance/ha in 1990 to 0.22 kilograms of active substance/ha in 2022.

Insecticides are crucial for modern agriculture to secure crop protecting and optimally harvests. Despite that, their disproportionate use increases difficulties concerning their negative consequences on agriculture and the ecosystem. Exaggerated insecticide utilization may conduct to the evolution of resistance in focus insects, requiring larger concentrations or stronger chemical substances, being the cause of higher production expenses and perturbation of natural pest control systems. Additionally, non-target organisms, like as useful bugs and watery life, are affected by the accidental repercussions of insecticide application, resulting into environment disparities and potential food cycle pollution.

The current study presents the situation of the use of pesticides in the counties of South-East Romania, analysing distinctly each type of pesticide applied at the county level.

In the period 1990-2022, in the South-East region of Romania, the largest quantities of insecticides applied were in Constanta County 3231535 kilograms of active substance, followed by Braila County 2447605 kilograms of active substance and Vrancea County 2274080 kilograms of active substance. The smallest quantities are those in Tulcea County 1482936 kilograms of active substance and Buzau County 1882068 kilograms of active substance. If we analyse from the point of view of the surfaces on which insecticide-type pesticides were applied, the first place is Constanta County 5702810 hectares, followed by Braila County 2936991 hectares, and the smallest quantities were applied in Vrancea County 990680 hectares and Buzau County 1165877 hectares.

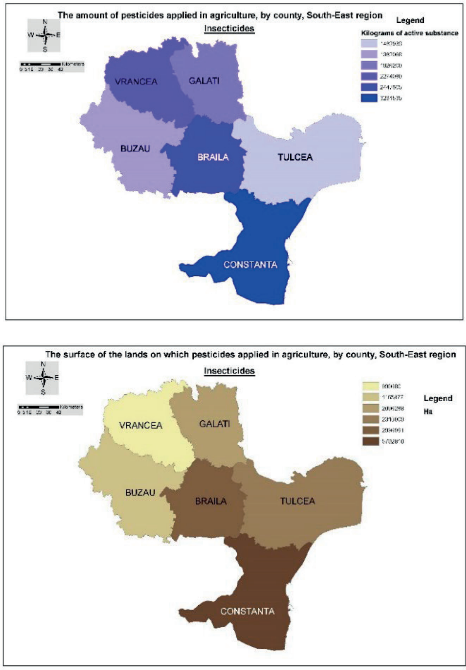

Figure 2. a. The quantity of insecticides used in agriculture, South-East region, Romania
b. The surface of the lands on which insecticides were applied, South-East region, Romania

Fungal diseases are well-known to impact yield property on majority crops and to generate serious economic global damage. The application of fungicides has been for a long period the principal method to manage various fungal pathogens. During the past few decades, higher public interes on food safety and ecological effect of farming activities has conducted authorities to assume extremely rigorous standards on the appraisal and license of plant protection goods, particularly for toxicological and ecosystems adverse side consequences associated to their apply.

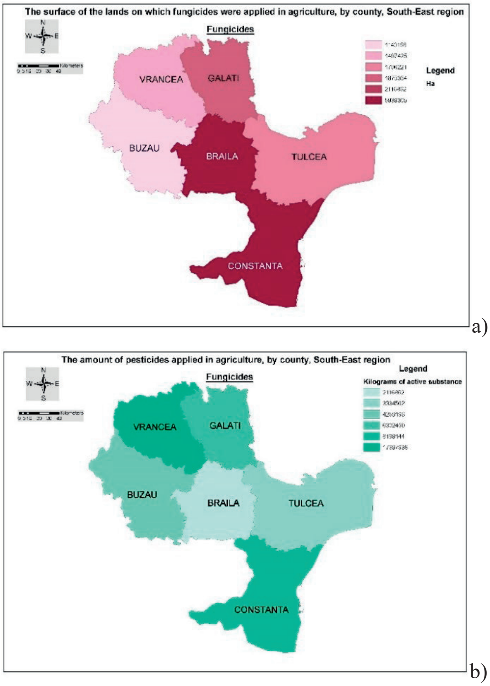

Figure 3. a. The quantity of fungicides used in agriculture, South-East region, Romania
b. The surface of the lands on which fungicides were applied, South-East region, Romania

Fungicides are used both on seeds or directly on harvest. Most of the seed-treated agro-chemical have integral action and could be absorbed into plant tissues protecting contra pests and microorganism equivalent to their insecticidal counterparts.

From the study of the quantities of fungicides applied in the counties of South-Eastern Romania, it is obvious that in the counties of Vrancea 17397938 kilograms of active substance Constanta 8199144 kilograms of active substance and Galati 6302450 kilograms of active substance the highest quantities were applied, while in the counties of Braila 2116862 kilograms of active substance and Tulcea 3334562 kilograms of active substance the lowest were applied quantities.

The areas in the South-East region on which the largest amounts of fungicides were applied between 1990 and 2022 are those in the counties of Constanta 5939305 hectares and Braila 3308517 hectares, and the least areas are those recorded in the counties of Buzau 1140169 hectares and Vrancea 1487425 hectares.

Herbicides are synthetics applied in order to manage or control undesired plants. Herbicide utilization appear mostly regularly in cultivated crop farming, where they are used formerly or within planting to maximize crop productivity by minimizing other vegetation. They also may be applied to crops in the fall, to improve harvesting herbicides applied with the major goal of maximising productivity and economic returns potentially act at the expense of ecosystem functions. Although not immediately, but these ecosystem services also contribute to crop health by supporting crop stubble yield, pathogen inhibition, nutrient cycling and conservation of soil structure.

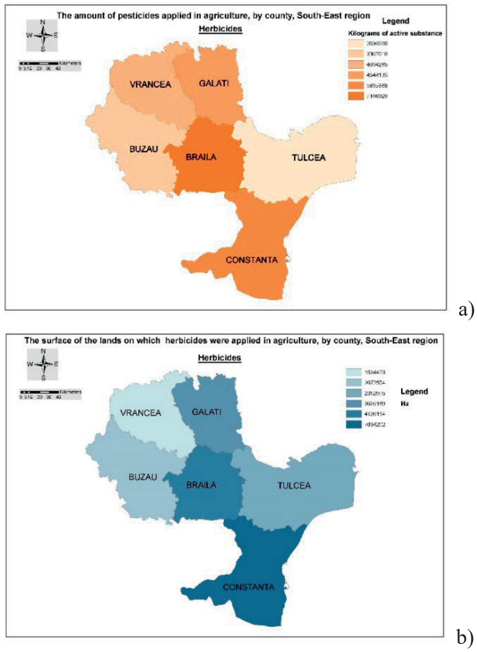

Figure 4. a. The quantity of herbicides used in agriculture, South-East region, Romania
b. The surface of the lands on which herbicides were applied, South-East region, Romania

A detailed analysis of both the quantities and the surfaces on which herbicides were applied is necessary for the purpose of tracking the production per hectare, therefore the resulting advantages, but also to observe the potential disadvantages arising especially in soil pollution and not only.

Over the analysed period, 1990-2022, the largest amounts of herbicides were applied in the counties of Braila 7166929 kilograms of active substance, Constanta 5885388 kilograms of active substance, and the smallest amounts in Tulcea County 2636698 kilograms of active substance and Buzau County 3362610 kilograms of active substance.

If we consider the areas on which herbicides were used, the first place is Constanta County 7034202 hectares, the second place is Braila County 4126194 hectares and with a significant difference on the last places are Vrancea County 1534478 hectares and Buzau County 2072554 hectares.

Considering that in the South-Eastern region of Romania the surfaces are cultivated with the main crops: barley, rapeseed and soy beans, in the present article we proposed to study if there is a correlation between these types of crops and the pesticides applied in the form of insecticides, fungicides and herbicides in the period 1990-2022.

The graphic representation shows that there is no interdependence between the productivity per hectare and the quantity of pesticides applied in the analysed period.

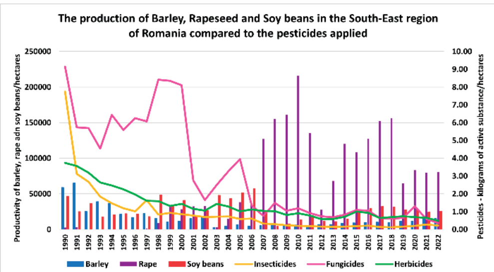

Figure 5. The production of Barley, Rapeseed and Soy beans in the South-East region of Romania compared to the pesticides applied, South-East region, Romania

It is obvious that the largest amounts of pesticides were applied until 2007, the decrease of fungicides from 9.15 kilograms of active substance/hectares in 1990 to 0.76 kilograms of active substance/hectares in 2007 is essential.

The statistical analysis of parametric correlation indicated that there are insignificant or moderately significant correlations in most of the studied situations.

Table 1 indicates the Pearson correlation coefficient for Insecticides and production for the 3 types of surfaces cultivated with barley, rape and soy beans.

Table 1. Pearson correlation coefficient for Insecticides and production for the 3 types of surfaces cultivated with barley, rape and soy beans

		Insecticides	Barley	Rape	Soy beans
Insecticides	Pearson Correlation	1	**0.816**	-0.481	0.281
	Sig. (2-tailed)		0.000	0.004	0.108
	N	34	34	34	34
Barley	Pearson Correlation	0.816	1	-0.486	0.024
	Sig. (2-tailed)	0.000		0.004	0.892
	N	34	34	34	34
Rape	Pearson Correlation	-0.481	-0.486	1	-0.395
	Sig. (2-tailed)	0.004	0.004		0.021
	N	34	34	34	34
Soy beans	Pearson Correlation	0.281	0.024	-0.395	1
	Sig. (2-tailed)	0.108	0.892	0.021	
	N	34	34	34	34

According to the statistical analysis, the most important correlation is that between insecticides and barley, of 0.816, and the lowest is the correlation coefficient between insecticides and soybeans of 0.281.

In Table 2 is presented the Pearson correlation coefficient for fungicides and production for the 3 types of surfaces cultivated with barley, rape and soy beans.

Table 2. Pearson correlation coefficient for Fungicides and production for the 3 types of surfaces cultivated with barley, rape and soy beans

		Fungicides	Barley	Rape	Soybeans
Fungicides	Pearson Correlation	1	**0.565**	**-0.683**	0.359
	Sig. (2-tailed)		0.001	0.000	0.037
	N	34	34	34	34
Barley	Pearson Correlation	0.565	1	-0.486	0.024
	Sig. (2-tailed)	0.001		0.004	0.892
	N	34	34	34	34
Rape	Pearson Correlation	-0.683	-0.486	1	-0.395
	Sig. (2-tailed)	0.000	0.004		0.021
	N	34	34	34	34
Soy beans	Pearson Correlation	0.359	0.024	-0.395	1
	Sig. (2-tailed)	0.037	0.892	0.021	
	N	34	34	34	34

There are two moderately significant correlations between fungicides and barley 0.565, respectively rape -0.683, which indicates that the application of this type of pesticide does not significantly influence production per hectare.

Table 3 indicates the Pearson correlation coefficient for herbicides and production for the 3 types of surfaces cultivated with barley, rape and soy beans.

Table 3. Pearson correlation coefficient for Herbicides and production for the 3 types of surfaces cultivated with barley, rape and soy beans

		Herbicides	Barley	Rape	Soy beans
Herbicides	Pearson Correlation	1	**0.860**	**-0.617**	0.211
	Sig. (2-tailed)		0.000	0.000	0.231
	N	34	34	34	34
Barley	Pearson Correlation	0.860	1	-0.486	0.024
	Sig. (2-tailed)	0.000		0.004	0.892
	N	34	34	34	34
Rape	Pearson Correlation	-0.617	-0.486	1	-0.395
	Sig. (2-tailed)	0.000	0.004		0.021
	N	34	34	34	34
Soy beans	Pearson Correlation	0.211	0.024	-0.395	1
	Sig. (2-tailed)	0.231	0.892	0.021	
	N	34	34	34	34

The third statistical correlation analysis indicates the highest degree of interdependence among the three types of pesticides analysed, namely the Pearson coefficient of 0.860 between herbicides and barley, and in the case of herbicides and turnip of -0.617, being a negative correlation.

CONCLUSIONS

The application of pesticide is one of the fundamental actions of modern agricultural practices in protecting the harvests from various pests. Fertilizers and in particular insecticides are particularly essential inputs and integral constituent of crop production system. Simultaneously these inputs are highly significant in linking the yield gap that exists among the feasible production and the yield obtained at the farm level.

In the period 1990-2022, the most used types of pesticides in the South-Eastern region of Romania, in terms of total quantity, were fungicides 41610152 kilograms of active substance, and if we discuss from the point of view of surfaces, then herbicides were applied on a surface of 20725592 hectares. The trend of pesticide application is a decreasing one, with significant variations from 20.62 kilograms of active substance/hectares in 1990 to only 1.07 kilograms of active substance/hectares in 2022.

In the 34 years in which the study was carried out, in the South-Eastern region of Romania, the largest quantities of insecticides were used in Constanta County 3231535 kilograms of active substance, and there is also the largest total area on which this was applied type of pesticides around 5702810 hectares.

Fungicides were applied predominantly in Vrancea county 17397938 kilograms of active substance and in Constanta County they were applied on the largest surfaces of 5939305 hectares, as the amount in the analysed period.

If we refer to the quantity, the third category of pesticides, herbicides were applied in Braila County 7166929 kilograms of active substance and Constanta County 7034202 hectares is in first place in terms of the surfaces on which herbicides were applied.

The study of the interdependence between the quantity of pesticides kilograms of active substance/hectares and production of barley, rape and soy beans indicate that there are no significant correlations, but in general the Pearson correlation coefficient varied from 0.565 between fungicides and barley and of 0.860 between herbicides and barley.

Among the three types of production analysed, in the case of barley, it has the most important influence resulting from the application of pesticides.

Therefore, for the purpose of controlling pesticide utilisation, innovative approaches and methods are needed in evaluating the impact of widespread use of pesticides on ecosystem and different actions should be done to ensure consciousness between community to reduce the utilization of damaging pesticides. To take advantage of biopesticides should be promoted over chemical pesticides

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THE CONSEQUENCES OF THE USE OF FERTILIZERS ON THE PRODUCTION OF THE MAIN CULTIVATED PLANTS, IN THE SOUTH-EAST REGION, ROMANIA

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Abstract

In the recent past in order to increase the harvest, the production per hectare and its protection against pests and diseases, began to be used, in high doses, chemical and natural fertilizers. The aim of this paper is to analyse the impact of using five categories of fertilizers: chemical, nitrogen, phosphate, potassium and natural fertilizers from 1990 to 2022, in the South-East region of Romania, a predominantly agricultural area on vegetable agricultural production taking into account the three main types of crops: grains, maize, sunflower. In the 33 years, in the South-East region of Romania, the following amounts of chemical fertilizers 2.72 tons of 100% active substance/hectares; nitrogen fertilizers 2.22 tons of 100% active substance/hectares; phosphate fertilizers 1.79 tons of 100% active substance/hectares; potassium fertilizers 1.37 tons of 100% active substance/hectares and natural fertilizers 651.83 tons of 100% active substance/hectares. The analysis of the impact of fertilizers grains production varies from 2808398 tons in 1990 to 3387102 tons in 2022.

Key words*: chemical and natural fertilizers, agricultural production, main types of crops.*

INTRODUCTION

The large use of fertilizers in the course of the last decennaries had as a consequence an enormous expand in the worldwide potential of food production. According to the UN Population prospects, the world-wide population is foreseen to increase approximately 35% in the coming 40 years (UN, 2022). Agricultural production will ought to grow considerably to adapt the increasing population. A considerable percent of the expand (in agricultural productivity) is estimated to consist in producing a large quantity of food on current farmland, despite the fact that some modern farmland will potentially be necessary (Penuelas et al., 2023). This increase and development could, anyhow, cause negative consequences on carbon stocks in soil and plants and on bio-diversity in the most productive arable lands at the global level (Alexandratos et al., 2012; Springmann et al., 2018).

Extension crop production and ending the gap among existent and achievable targets may be accomplished by performing multiple technologies and biotechnologies, e.g., the appropriate apply of fertilizers and systematic nutritional management may have an elemental roles for international farming and food production (Stewart et al., 2012).

Despite that, the fertilization enhancement of the recent decades intended to expand yields that has developed several recent ecological and geo-strategic issues, including nutrient disparity, (Lobell et al., 2009; Lu et al., 2017), percolation of nutrients from harvests to environment and the connected effects (Finér et al., 2011; Weihrauch et al., 2022), and raising price of fertilizers with severe bio-geographical and financial difficulties for the food preservation in poverty-stricken countries (MacDonald et al., 2011; Cordell et al., 2014). The growth of fertilizer demand at global proportions has risen at an exponential rate for nitrogen, phosphorus and potassium (White et al., 2008; Cleveland et al., 2011).

MATERIALS AND METHODS

For the purpose of studying the ratio between the quantity of fertilizers applied in the South-East Region (in the counties of Braila, Buzau, Constanta, Galati, Tulcea and Vrancea) in the

period 1990-2022 and the surface of the lands on which chemical and natural fertilizers were applied, we used a series of data sets collected by the National Institute of Statistics. The European legislation, regarding the statistics of agricultural items, provides for the organization and performing of public statistical surveys regarding the form of agricultural properties, respectively general agricultural inventories once every ten years and structural surveys in agriculture, based on statistical investigations.
The calculation of this paper allowed us to observe for each county and year the period of fertilizers applied to a certain surface and more than that we studied five types of fertilizers: chemical, nitrogen, phosphate, potassium and natural.
In this article, with the aim of obtaining a complete picture of the quantities of fertilizers used in the period 1990-2022, we added up these quantities for each type of fertilizers (chemical, nitrogen, phosphate, potassium and natural) and represented them graphically in the form of maps in Figures 2-6, on the six counties in the South-East region of Romania.
In Figure 1, tons of 100% active substance/hectares are represented, the calculations being made by dividing the quantities of fertilizers, for each type, on the respective surface.
Considering that the South-East region is a predominantly agricultural area, the three most important types of agricultural crops are: grains, maize and sunflower. Given the fact that these are the main types of agricultural vegetable crops, it is important to analyse the evolution of the quantity of fertilizers applied per hectare and their agricultural production.

RESULTS AND DISCUSSIONS

A permanent monitoring of the quantities of fertilizers applied, regardless of whether we are talking about chemical or natural fertilizers, is extremely important, especially in the context where, depending on the quantities used, a series of negative effects can occur. In Figure 1 chemical fertilizers, nitrogen fertilizers, phosphate fertilizers and potassium fertilizers are represented as column and natural fertilizers are represented as line (green) using the second axis.

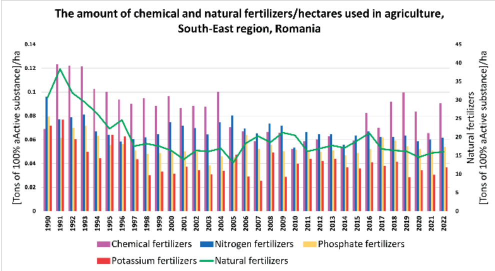


Figure 1. The amount of chemical and natural fertilizers/hectares used in agriculture, South-East region, Romania

In the period 1990-2022, the average annual quantities of fertilizers are as follows: chemical fertilizers 0.0825 tons of 100% active substance/hectares; nitrogen fertilizers 0.0674 tons of 100% active substance/hectares; phosphate fertilizers 0.0542 tons of 100% active substance/hectares; potassium fertilizers 0.0417 tons of 100% active substance/hectares; natural fertilizers 19.7524 tons of 100% active substance/hectares. More than that if we sum up the first four categories of fertilizers, we get a value of 8.1209 tons of 100% active substance/hectares comparable to the natural ones 651.8317 tons of 100% active substance/hectares. All five categories of applied fertilizers have a downward trend. As an evolution in time, it is obvious that in the years 1990-1996 much larger quantities were used compared to the last years.
For example, in the case of natural fertilizers in 1990, 30.7104 tons of 100% active substance/hectares were used compared to 2022 when only 16.0382 tons of 100% active substance/hectares were applied. During 1990-2022, in the period in the South-East region they were used cumulated 2,286,802 tons of 100% active substance of chemical fertilizers which were applied to a cumulated surface of 29,752,052 hectares.
Figure 2a shows the amount of chemical and natural fertilizers used in agriculture and Figure 2b shows the surface of the lands on which chemical and natural fertilizers were applied.
It is obvious that in the analysed period, 1990-2022, in the Southeast region, the county with the largest quantity of chemical fertilizers used was in Constanta County, 640407 tons of 100% active substance, in second place being Braila County with 567678 tons of 100% active

substance, and the smallest amounts of chemical fertilizers are in Buzau County 190786 tons of 100% active substance and Tulcea County 194591 tons of 100% active substance.

If we analyse the surface of the lands on which chemical fertilizers were applied, it can be seen that the largest surfaces on which fertilizers were applied are those in the counties of Constanta 8957995 hectares and Galati 6616642 hectares respectively, and the smallest surfaces are those in Buzau 2265453 hectares and Tulcea 2886812 hectares. Continuous monitoring in each county is important precisely for the purpose of preventing and combating some negative effects.

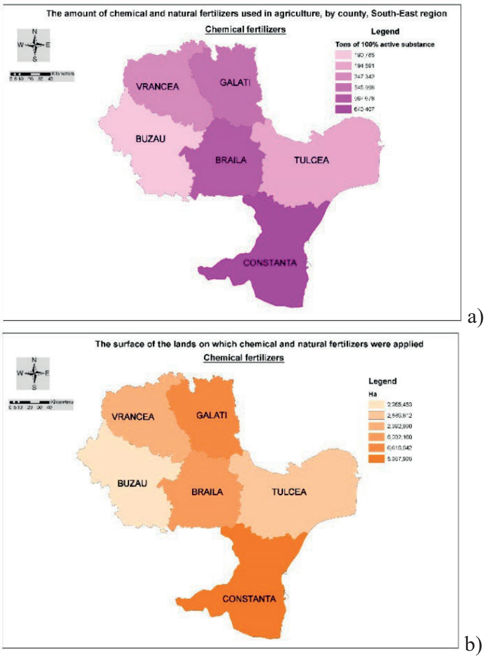


Figure 2. a. The quantity of chemical fertilizers used in agriculture, South-East region, Romania
b. The surface of the lands on which chemical fertilizers were applied, South-East region, Romania

From the analysis of the quantities of nitrogenous fertilizers applied in the analysed period, it appears that 1450183 tons of 100% active substance were used for 21587041 hectares in the South-East region of the country.

Nitrogenous fertilizers contain calcium, ammonium sulphate, sodium nitrate, urea and affects crop growth in several ways like stimulate the growth of foliage, confer a green colour to leaves and more than that in case of cereals, it tends to cause lumpiness in seeds and generate succulence or fragility in the plant. Constanta 396314 tons of 100% active substance and Braila 323189 tons of 100% active substance are the counties where the largest quantities of nitrogenous fertilizers were applied in the 33 years to which this study refers and on contrary on the last two places are situated the counties of Tulcea 125631 tons of 100% active substance and Buzau 121490 tons of 100% active substance are on the last places.

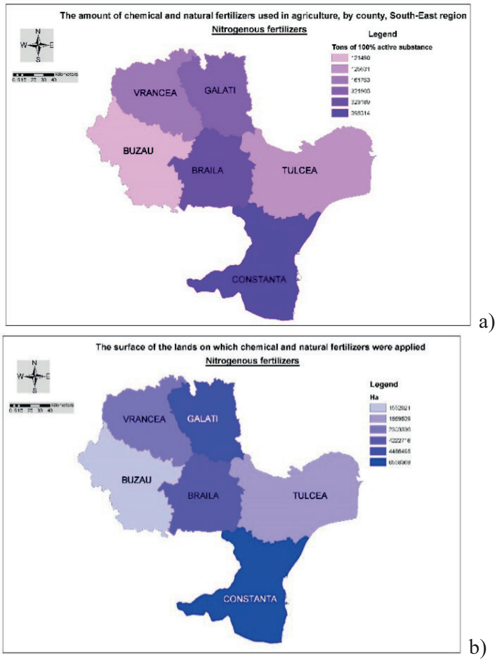


Figure 3. a. The nitrogenous quantity of fertilizers used in agriculture, South-East region, Romania
b. The surface of the lands on which nitrogenous fertilizers were applied, South-East region, Romania

According to the evaluation of the surfaces on which nitrogenous fertilizers were used, the counties of Tulcea 1869539 hectares and Buzau 1552021 hectares are on the last places and Constanta County 6558368 hectares and Galati County 4486495 hectares are on top.

Phosphate fertilisers are the second most significant fertilizers for numerous crops. In contrast to the nitrogen that is a mobile nutrient, phosphate ions have a minor change in the soil. Many of the soils are properly abundant in soil phosphorus, however just more than one percent or lower of the phosphorus is accessible for plant absorption without continuing additional transformation to a form that is more easily obtainable. Consequently, it is essential to use a soil analysis to establish the quantity of phosphorus that is usable to a crop, and then utilize the phosphorus fertilizers more efficiently to optimize economic crop profits.

Majority of the phosphate fertilizers used nowadays are produced from rock phosphate that is processed with acid. It is furthermore critical to understand that nearly all phosphorus fertilizers contain considerably water-soluble phosphorus. Hence, it is not as significant which phosphorus fertilizer material is chosen for application to an area. Liquid fertilizers generally do not have supplementary utility or agronomic advantages upon the dry fertilizers.

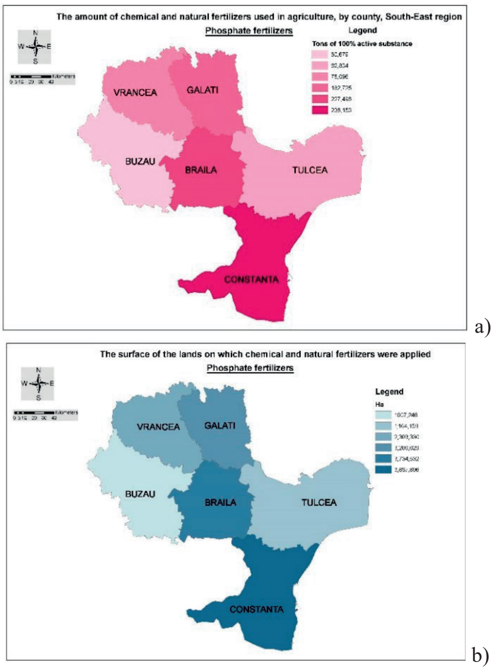


Figure 4. a. The phosphate quantity of fertilizers used in agriculture, South-East region, Romania
b. The surface of the lands on which phosphate fertilizers were applied, South-East region, Romania

The phosphate fertilizer, in the South-East region, are mainly used in Constanta County 236153 tons of 100% active substance and Braila County 227496 tons of 100% active substance, while Tulcea County 62834 tons of 100% active substance and Buzau County 62834 tons of 100% active substance use approximately three times less amount.

A careful analysis of the surfaces on which phosphorus-based fertilizers were applied in the period 1990-2022 indicates the following aspects: is at the top of the use of this type of fertilizers Constanta County 3867896 hectares and Braila County 3734532 hectares, and Tulcea County 164159 hectares and Buzau County 1007246 are the areas where the smallest quantities were used.

Potassium fertilizers are additional option for agriculture. Potassium fertilizers may without difficulty combine with granular complex fertilizers since their size is adaptable. This feature also permits device spreading. Most of them contain chloride, therefore their apply on sensitive harvests is not appropriate. Sulphate-containing potassium fertilizers are efficacious fertilizers but more overpriced as compared with nitrogen ones, so they are frequently used for major types of fruit and vegetable farming that enhance the crops quality.

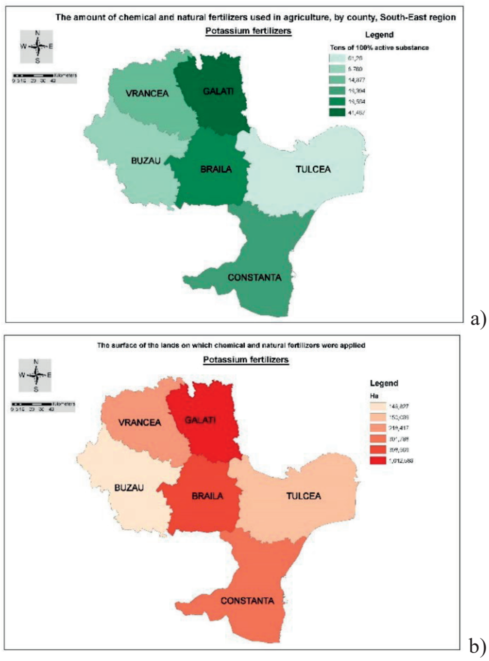
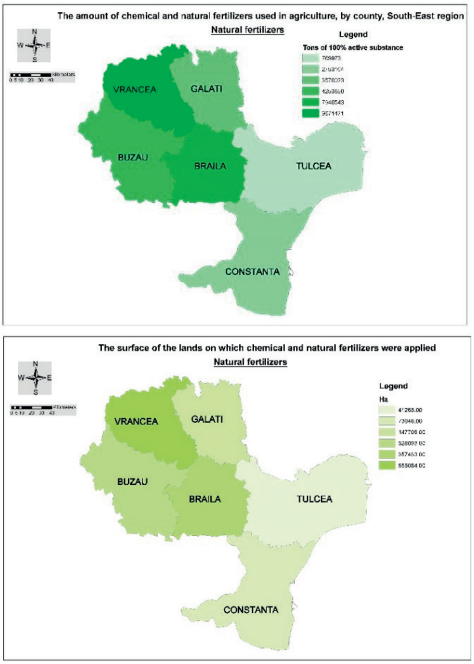


Figure 5. a. The potassium quantity of fertilizers used in agriculture, South-East region, Romania
b. The surface of the lands on which potassium fertilizers were applied, South-East region, Romania

Natural organic fertilizers are fundamentally available mineral sources that contain medium quantity of plant essential nutrients. Those ones are sufficient for reducing issues closely associated with synthetic composts. They diminish the necessity of regular request of synthetic fertilizers to maintain soil productivity. This type of fertilizers gradually releases nutrients into the soil solution and retain nutrient balance for beneficial rise of crop vegetation, being in the same time a substitute for an effective vitality source of soil bacteria that constantly increase soil system and crop growing.



a) b)

Figure 6. a. The natural quantity of fertilizers used in agriculture, South-East region, Romania
b. The surface of the lands on which natural fertilizers were applied, South-East region, Romania

From the analysis of the evolution of the amounts of fertilizers applied per hectare in the analysed period, it can be observed that in 1990 the quantity of fertilizers applied per hectare is 30.71 tons of 100% active substance/hectares compared to 16.04 tons of 100% active substance/hectares in 2022, when this amount practically halved.

In the South-East region, in the period 1990-2022, Vrancea county 9871471 tons of 100% active substance is at the top, in second place is Braila County 7948543 tons of 100% active substance, and in the last two places is Constanta 2753104 tons of 100% active substances and Tulcea 769973 tons of 100% active substances. From the analysis of the surfaces on which natural fertilizers were applied in the South-East region, Vrancea county is in first place with 555,084 hectares, Braila County is in second place with 357,463 hectares, and Constanta is also in the last two places. 73048 hectares and Tulcea 41266 hectares.

In order to analyse the influence of fertilizers compared to the vegetable agricultural production of grains, maize and sunflowers in the South-East region, for the period 1990-2022 we calculated the Pearson corelation coefficient considering the three categories of vegetable agricultural production and the fertilizers (summing up the five categories of fertilizers used in this study).

In Figure 7, is graphically represented the quantities of the three categories of production and natural and chemical fertilizers that were applied in the studied area.

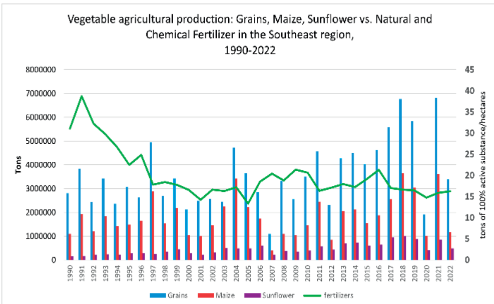


Figure 7. Vegetable agricultural production: Grains, Maize, Sunflower vs. Natural and Chemical Fertilizer in the South- East region, Romania 1990-2022

The Pearson correlation coefficient for grains and fertilizers is a negative one, indicating a relatively low influence of the two categories of variables - 0.207. There is also an insignificant negative correlation -0.236 between maize and fertilizers, while the strongest correlation is -0.522 between sunflower and fertilizers. If we analyse only from a graphical point of view the evolution over time of plant production compared to the quantity of fertilizers applied per hectare, we notice that the quantity of fertilizers has decreased over time, and production has increased, especially in the case of grains.

CONCLUSIONS

The request on agriculture to enhance production will persist to develop anyway for the next several decades. The majority of the states intend to expand production, thus requiring more intensive agriculture and greater crop yields. The main question for the world's farmers will thus be to increase production in a sustained mode that reduce environmental impact and simultaneously provides sufficiently, safe and nutritive products. From the analysis of the quantities of fertilizers applied per hectare in the period 1990-2022, it appears that natural fertilizers were applied in the largest amount 651.83 tons of 100% active substance/hectares and potassium fertilizers have the lowest use of only 1.37 tons of 100% active substance/hectares. From the point of view of evolution over time, it is obvious that in 1991 there was a maximum point of use of fertilizers 38.71 tons of 100% active substance/hectares, after which there was a sudden decrease in 1997 until 17.80 tons of 100% active substance/hectares, a value which, with small fluctuations in 2007-2011, was maintained in around this value, and in 2022 it reached 16.28 tons of 100% active substance/hectares.

Constanta county is in the top of the use of chemical fertilizers both in quantity 640407 tons of 100% active substance and in area 8957995 hectares, but also of nitrogen fertilizers with 396314 tons of 100% active substance and 6558368 hectares and Constanta County is in first place in the use of Phosphate fertilizers with 396314 tons of 100% active substance and 3867896 hectares. Potassium fertilizers were the most used in Galati County 41467 tons of 100% active substance and 1012589 hectares. In Vrancea County, the largest amounts of natural fertilizers 9871471 tons of 100% active substance were used on an area of 555084 hectares.

Recently, a series of recommendations have appeared whose main goal is the use of natural fertilizers, considering that these are safer alternatives to chemical fertilizers. Although there are a multitude of positive aspects and advantages of using fertilizers, I must not omit the fact that the inappropriate use of organic fertilizers causes overfertilization or nutrient deficiency in the agricultural land. Therefore, managed release of organic fertilizers is an efficient and progressive approach to overcome the negative consequences and support sustainable agriculture production.

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GRAIN YIELD STABILITY ANALYSIS USING PARAMETRIC AND NONPARAMETRIC STATISTICS OF BULGARIAN AND HUNGARIAN COMMON WINTER WHEAT GENOTYPES

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Abstract

The research was conducted in the period 2020-2023 on the experimental field at the IPGR "K. Malkov", town of Sadovo. 5 Hungarian varieties, 16 advanced lines and 4 Bulgarian varieties of common winter wheat were evaluated for grain yield and stability. The stability of the grain yield was determined by the variances of stability (σi^2 and Si^2), equivalency (Wi), the criterion of phenotypic stability (Ysi), regression coefficient b_i and general adaptability. The highest average yield for the study period was obtained from MV-Nemere 864.4 kg/da, followed by RU179/1400 and MV-Nador -766.6 and 766.5 kg/da. The yield formed by the Hungarian varieties is relatively high and they are of interest for selection improvement work. In terms of grain yield, the lines RU 129/3053 and RU 177/486 have a complex stability assessment. The variety Sadovo1 is defined as very stable in different environments and valuable for selection programs.

Key words: *common winter wheat, grain yield, variances of stability, equivalency, phenotypic stability.*

INTRODUCTION

The bread wheat (*Triticum aestivum* L.) is one of the most widespread and most consumed food crops worldwide. The 2 February 2024 report of the Food and Agriculture Organization of the United Nations (FAO) updates the forecast of the global cereal balance in 2023/24 (https://www.fao.org/worldfoodsituation/csdb/en). The global winter wheat crop is expected to decline moderately in 2024. One reason for this is lower grain prices. In the European Union, the occurrence of various extreme weather conditions (heavy rains and/or rainfall deficits in various regions, as well as changes in temperatures) are contributing to a small reduction in the total area of common winter wheat (Piepho, 2019; Bocci et al., 2020). The FAO's forecast for total wheat use in 2023/24 has been raised by 2.9 million tones since December and shows a 2.0% (15.4 million tons) increase from 2022/23. The revision reflects higher than previously expected feed use, particularly in the European Union, as well as in Australia and the United States (https://www.fao.org/worldfoodsituation/csdb/en). Amid global climate change, grain consumption is increasing. Globally, as temperatures rise for every 1°C increase, productivity is projected to decline and wheat yields are expected to fall by 6% (Asseng et al., 2015; Uhr et al., 2023; Guo et al., 2024).

In a changing climate, yield stability is becoming increasingly important for grain and livestock farmers. The creation and introduction of common wheat varieties with high genetic potential for productivity and grain quality is linked to the study of the ecological plasticity of individual varieties over the years, as well as the determination of the most appropriate varietal structure for each region, and micro-region of a region (Stoyanova et al., 2020; Hufford et al., 2019; Van Frank et al., 2020; Minoli et al., 2022). In this regard, it is imperative to thoroughly investigate the dependence between the variety and the specific meteorological conditions of different growing regions (Tsenov & Atanasova, 2015; Kucek et al., 2019; Gubatov et al., 2021). The results show that the influence of genotype and its interaction with the environment has been demonstrated in all observed traits (Reckling et al., 2021; Dimitrov et al., 2023; Dimitrov et al., 2023A). In a study by Uhr et al. (2023) the strength of environmental influence ranged from 61 to 82% in terms of grain yield. Yield stability analyses have become necessary in recent years, it is particularly important in research on the impacts of climate change. Declining and associated with its changes (Müller et al., 2018; Najafi et al., 2018; Ray et al., 2015; Tigchelaar et al., 2018; Lobell et al., 2011; Webber et al., 2020). According to Tsenov et al. (2022), it is advisable that an evaluation of varieties for yield and stability be easy and efficient is to do so through statistical programs in which the possibility of genotype x environment evaluation exists. Once the specific interactions between these are revealed, one can proceed to a correct assessment of yield stability. The terms "stability" or "phenotypic stability" are used in the literature to denote the phenotypic manifestation of a trait while a particular genotype, as such, remains relatively stable (Becker & Leon, 1988; Kang, 2020). Investigating this phenotypic stability is important for any breeding program where the effects of genotype and environment need to be studied and exploited (Kang, 2020; Akcura et al., 2006; Pour-Aboughadareh et al., 2022; Weedon & Finckh, 2019).

MATERIALS AND METHODS

The trial was carried out in the experimental field of IPGR - Sadovo in the period 2020-2023. The yield and its stability were studied in twenty-five genotypes of common winter wheat, originating from Bulgaria and Hungary. The varietal trials were conducted in a block design in three replications, with a trial plot size of 10 m^2, and the studied genotypes were compared with the country's complex standard variety Sadovo1. Of note is the fact that the hailstorm that fell in 2022 resulted in compromised yield. In order to present more reliable results, the mentioned year was excluded from the overall statistical treatment of the results. Yield data were processed by applying analysis of variance (Lidanski, 1988), where the strength of influence of the sources of variation - genotype, environment and their interaction - were estimated. Yield stability and adaptability of common winter wheat cultivars were evaluated by stability variance σ_i^2 and S_i^2 according to Shukla (1972), eco valence W_i according to Wricke, phenotypic stability criterion (Ysi) according to Kang (2020), regression coefficient b_i according to Finlay & Wilkinson (1963), general adaptability GA according to Eberhart & Russell (1966). Statistical and mathematical processing of the data was performed using Microsoft Excel and Stability soft software.

RESULTS AND DISCUSSIONS

Table 1 shows the mean values by year of the grain yield trait. In the first year, grain yield ranged from 658.6 kg/da to 1092.4 and the average yield was 863.6 kg/da. In the second year, the average yield was 800.48 kg/da, with a minimum of 638.87 and a maximum of 924.9 kg/da. In the third year, the average yield was 484.73 kg/da, the minimum was 321.8 and the maximum was 575.9 kg/da. The highest average yield of the genotypes was formed in the first year of testing.

Table 1. Mean Grain yield values by year

Genotype/Year	2020	2021	2023
1.MX 270/ 28	960.475	705.375	540.5
2.MX 270/ 50	1016.525	800.125	547.25
3.PY 129/3053	930.15	863.875	543.575
4.PY 33/3244	821.425	769.05	506.75
5.MX 270/ 3461	874.425	720.825	448.375
6.MX 285/1058	959.7	794.95	502.125
7.PY 48/2553	768.925	775.45	517.8
8.MX 286/1759	767.35	749.125	416.925
9.MX 286/1777	846.75	708.5	464.45
10.Avenue	777.975	828.975	375.075
11.Anapurna	797.85	829.1	321.8
12.Sadovo1	798.425	771.7	449.85
13.Enola	695.825	791.475	474.675
14.MX 272/3872	658.6	857.425	451.425
15.MX 215/3	832.35	638.875	534.85
16.PY134/1343	802.975	789.6	476.325
17.PY177/486	934.8	803.975	525.45
18.PY135/1456	834.475	730.975	520.3
19.PY179/1400	990.5	794.4	514.925
20.PY134/1370	869.975	866.9	547.325
21.MV-Nador	934.775	856.85	507.95
22.MV-Nemere	1092.375	924.925	575.95
23.MV-MENROT	854.6	902.125	395.3
24.MV-MENTE	838.525	883.3	456.575
25.MV-KAPLAR	931.25	854.275	502.825
Mean	863.64	800.48	484.73
Standard error of mean	20.09	13.67	12.11
Min.	658.6	638.87	321.8
Max.	1092.4	924.9	575.95
CV%	11.63	8.54	12.49

The analysis of variance presented (Table 2), shows that the trait grain yield was highly significantly influenced by genotypes, environments and genotype-environment interaction. Most of the phenotypic variation in grain yield trait was due to environment (79.7%). Genotypes and genotype-environment interaction have equal proportion of influence. For the trait grain yield, there is a very well established genotype-environment interaction. This allows the stability analysis of the studied materials. The genotype-environment influence found indicates that genotypes respond differently when changing environments.

Table 2. Grain yield variance analysis

Source	SS	df	MS	Sign.	η^2, %
Genotype	659218.8	24	27467.5	***	8.5
Environment	6181437.8	2	3090718.9	***	79.7
Interaction G x E	668391.3	48	13924.8	***	8.6
Error	250000.0	150	1666.7		3.2

***Significant at $p< 0.001$

Table 3 shows the reported mean yield as well as the calculated parametric and non-parametric estimates of the stability of the test samples with respect to the grain yield trait. For seven wheat lines and varieties (MX 270/ 28, MX 270/ 50, RU 129/3053, MX 285/1058, RU 177/486, RU 179/1400, RU 134/1370, MV-Nador, MV-Nemere, MV-KAPLAR), the reported average yield for the study period was above 750 kg/day. The yield formed is relatively high and these genotypes are of interest for breeding improvement work. The Hungarian variety MV-Nemere has the highest yield on average over the three years. The average yield of the genotypes was 716.3 kg/da. Eleven genotypes (MX 270/28, MX 270/50, RU 129/3053, MX 285/1058, RU 177/486, RU 179/1400, RU 134/1370, MV-Nador, MV-Nemere, MV-MENTE, MV-KAPLAR) had yields above the average of all genotypes in total for the three years.

The grain yield trait increasing is a major objective of breeding programs. The stability is not left behind and its importance is essential for newly developed varieties. Low values for the nonparametric estimates $S^{(1)}$ and $S^{(2)}$ determine the stability of genotypes. Eight genotypes RU 129/3053, MX 286/1751, Sadovo1, RU 134/1343, RU 177/486, MV-Nador, MV-Nemere and MV-KAPLAR showed low values for $S^{(1)}$, while four genotypes MX 286/1759, Sadovo1, RU 134/1343 and MV-Nemere showed low values for $S^{(2)}$. Low values for the $NP^{(1)}$ and $NP^{(2)}$ indices determined a higher stability of the genotypes. Two genotypes (RU 129/3053 and Sadovo 1) have low values for $NP^{(1)}$ and seven (MX 270/50, RU 177/486, RU 179/1400, RU 134/1370, MV-Nador, MV-Nemere and MV-KAPLAR) for $NP^{(2)}$. Notably, the MV-Nemere genotype has three low nonparametric stabilities and realizes the highest yield of all genotypes studied. The cultivar Sadovo1 also possesses three non-parametric stabilities and an average grain yield slightly below the mean.

The Wricke equilibrium (Wi^2) measures the contribution of genotype to the genotype x environment interaction. A W_i^2 value of zero or close to zero is an indicator of stability and conversely high Wi^2 values are an indicator of instability. A genotype with a low value for Eco valance is considered ideal in terms of grain yield stability. Low Wi^2 suggests that this genotype is stable given its weak contribution to the interaction. On the quantitative assessment of Wricke's Wi^2 parameter, two genotypes (RU 129/3053 and Sadovo 1) had a low stability value and on Shukla's $\sigma^2 i$ parameter, five (RU 129/3053, Sadovo1, RU 134/1343, MV-Nador and MV-KAPLAR).

The genotypes RU 129/3053 and Sadovo1 have low values for both parameters and possess high stability. Five genotypes (RU 129/3053, RU 33/3244, Sadovo1, MV-Nador and MV-KAPLAR) have low values for s^2d_i and are defined as stable according to this parameter. The bi parameter is one of the most commonly used to assess stability. In terms of yield, genotypes with values equal to one should be noted as having agronomic or dynamic stability and those with values greater than one as being responsive to specific conditions of favorable environments. The genotypes RU 129/3053, MX 270/3461, MX 286/175, Sadovo1 and RU 177/486 have values around or equal to one and possess agronomic stability. They are extremely valuable in this growing area. The genotypes MH 285/1058, Anapurna, RU 179/1400, MV-Nador, MV-Nemere, MV-MENROT, MV-MENTE and MV-KAPLAR have values above one and they are responsive to the specific conditions of favorable environments. These genotypes are valuable in terms of the regression coefficient bi for the grain yield trait.

The genotypes RU 129/3053 and RU 177/486 realized high yields and a regression coefficient b_i approximately equal to 1.00, respectively. In terms of coefficient of variation (CV%) with values below ten are stable, on the other hand, ten to twenty indicate higher grain yield under better conditions. All the genotypes in the study had coefficient of variation above 20. The Kang parameter combines yield and stability simultaneously and genotypes with low ranks have high stability. Four genotypes are considered stable: RU 129/3053, RU 177/48633, MV-Nador and MV-KAPLAR. These genotypes are very valuable in terms of complex evaluation against stability and grain yield. They should be included in breeding improvement work. Genotype RU 129/3053 and RU177/4863, have the lowest stability and grain yield parameters above the overall average. They appear to be extremely valuable for selection in terms of grain yield. Variety Sadovo1 has seven stability scores, although not high yielding and defined by the Kang score, its value is strongly emphasized.

Table 3. Parametric and non-parametric stabilities for grain yield

N	Mean Y	$S^{(1)}$	$S^{(2)}$	NP^1	NP^2	W_i^2	σ^2_i	s^2d_i	b_i	CV_i	Ys_i
1.	735.4	13.3	127	8.33	0.5	20431.3	10902.1	2842.0	0.91	28.7	33
2.	787.9	6.7	30.3	12.3	0.2	11868.1	6248.2	1583.9	1.09	29.8	21
3.	779.2	3.3	7	1.7	0.3	29.7	185.6	0.3	1.01	26.5	4
4.	699.6	4.7	13	7.7	0.8	2366.3	1084.2	0.0004	0.83	24.1	22
5.	681.2	8	44.3	7	0.7	4092.7	2022.5	565.8	1.04	31.6	28
6.	752.2	6	24.3	8.3	0.3	5678.7	2884.5	649.4	1.11	30.8	22
7.	687.4	8.7	43	10.7	0.8	8186.3	4247.3	194.2	0.71	21.3	31
8.	644.5	2	2.3	4.3	2.3	1033.4	359.84	133.9	0.96	30.6	28
9.	673.2	6.6	25.3	4.3	0.7	3597.7	1753.3	460.8	0.93	28.7	29
10.	660.7	9.3	54.3	11	0.9	10897.2	5720.6	1157.9	1.18	37.6	39
11.	649.6	10.7	67	10.7	0.9	18346.6	9769.1	1014.6	1.36	43.7	45
12.	673.3	1.3	1	2.3	1.1	761.4	212.00	81.6	0.95	28.8	20
13.	653.9	6	24.3	7.3	1.3	16702.2	8875.5	1456.5	0.71	24.8	43
14.	655.8	12.6	94.3	10	0.8	35423.2	19049.9	4517.0	0.78	30.9	47
15.	668.7	12.6	90.3	11	0.7	22812.9	12196.5	1717.4	0.63	22.5	44
16.	689.6	2	2.3	3.7	0.7	1738.2	742.9	139.9	0.90	26.8	21
17.	754.7	3.3	7	5.3	0.1	2297.3	1046.7	325.0	1.01	27.7	16
18.	695.2	8.6	42.3	9.3	0.6	5619.7	2852.4	216.9	0.77	23.0	26
19.	766.6	7.3	31	10.7	0.2	9445.2	4931.4	1132.2	1.13	31.1	20
20.	761.4	6	22.3	6	0.2	2262.9	1028.0	213.2	0.90	24.3	14
21.	766.5	2.6	5.3	3.7	0.1	1204.4	452.7	3.8	1.11	29.6	9
22.	864.4	0	0	11.7	0.04	10297.6	5394.7	549.3	1.27	30.4	18
23.	717.3	14	110	11.7	0.4	18407.3	9802.2	1277.0	1.33	39.0	34
24.	726.1	10	60.3	10.7	0.3	7990.9	4141.1	975.5	1.11	32.2	25
25.	762.8	3.3	8.3	4.3	0.1	1305.8	507.9	2.6	1.12	29.9	11

CONCLUSIONS

The grain yield is most strongly influenced by environment and less strongly by genotype and genotype-environment interaction. For this trait, the genotypes with complex stability scores were RU 129/3053 and RU 177/486. These are of great importance for breeding and improvement work.

Variety Sadovo1 has a large number of low parametric and non-parametric stabilities. This defines it as very stable in different environments and valuable for breeding programs.

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YIELD AND GRAIN QUALITY OF WINTER WHEAT UNDER SHORT-TERM ORGANIC AND MINERAL FERTILIZATION IN A SYLVOSTEPPE AREA FROM ROMANIA

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Abstract

Common wheat (Triticum aestivum L.) is one of the most widespread cereal crops in Romania. Various factors, including the fertilization regime, climatic conditions, etc. can influence wheat yield and quality. It is commonly known that fertilization has a fundamental position in obtaining high yields and high-quality grains. Given the current trend towards the use of organic matter for fertilization and the reduction of synthetic fertilizers to mitigate the climate effects, our study aimed to assess the effects of short-term organic and mineral fertilization on wheat grain yield and quality. The field trial was set up at the Research and Development Station for Agronomy (RDSA) of Moara Domnească belonging to USAMV of Bucharest, Romania, on a preluvosoil-type soil. Three doses of manure compost (15 t/ha; 30 t/ha and 60 t/ha) were applied in the autumn of 2021 either alone or in combination with NPK complex fertilizers 20:20:0. The yield components, yield, and grain quality of winter wheat were positively affected by the application of organic and mineral fertilizers, with significant differences being observed between variants and compared to the control (soil).

***Key words**: winter wheat, organic amendments, mineral fertilization, wheat yield, grain quality.*

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is among the most widely grown cereal crops in Romania, with a cultivation area of 2,311,095 ha in 2023 and an average yield of 4,154 kg/ha (INS, 2024). The chemical composition of wheat grains, which includes carbohydrates, protein, dietary fibers, lipids, minerals, and vitamins (Mitura et al., 2023), makes it a valuable food source and plays an important role in the production of high-quality grain products (Hospodarenko & Liubych, 2022). For example, protein content significantly influences the quality, technological properties, and nutritional value of flour, making it an important factor for farmers when selecting wheat cultivars and establishing agronomic management strategies (Lachuta et al., 2024).

Globally, wheat production has more than doubled since 1960. However, the frequency of dry seasons has risen over the past century, becoming a significant stress factor that negatively impacts wheat productivity and quality (Hernandez-Ochoa et al., 2023). High and constant yields are essential in wheat production systems and are gaining importance for agronomists (Macholdt et al., 2019). Wheat yield and quality are influenced by several factors, including cultivation practices, soil and climate conditions, crop rotation or cultivar selection (Tudor et al., 2023). Additionally, nutrient supply is a critical factor for enhancing and stabilizing wheat yields (Macholdt et al., 2019).

It is well established that adequate nutrient provision plays an important role in producing high-quality grains. Among these nutrients, nitrogen is particularly crucial for plant nutrition, as it often determines both the yield level and the baking quality of wheat due to its impact on grain protein content (Chiriță et al., 2023). Hlisnikovský and Kunzová (2014) point out that nitrogen promotes optimal conditions for wheat growth and increases seed protein content, but it has a negative effect on starch content. As it is one of the most mobile nutrients in the soil, nitrogen requires careful

management. High rates of nitrogen fertilizer rates must be evaluated, as improper applications can result in increased nitrate leaching, which in turn contributes to surface water eutrophication (Litke et al., 2018).
The concentration of nitrogen and other macronutrients in the upper soil layer can be influenced by the application of both organic and mineral fertilizers (Holik et al., 2018). Organic manures, for instance, have both direct and indirect impacts on the soil. Directly, they contribute to nutrient availability through the gradual release of nutrients via mineralization. Indirectly, they improve soil structure, which can further increase nutrient availability (Holik et al., 2018).
Using organic manures in combination with mineral fertilizers can reduce the need for chemical fertilizers while enhancing soil and crop quality, offering a sustainable technological solution (Chang et al., 2024). This combined approach also plays an important role in optimizing the soil nutrient pool, increasing crop yields, and improving water use efficiency (Zhang et al., 2016).
Given the growing emphasis on using organic matter for fertilization and reducing chemical fertilizers to address climate effects, our study aimed to evaluate the effects of short-term organic and mineral fertilization on wheat grain yield and quality.
The specific objective was to determine how different combinations of mineral and organic fertilizers influence the grain yield of winter wheat and to assess the grain quality.

MATERIALS AND METHODS

Experimental design

The field experiment was carried out during the 2021-2022 period at the Research and Development Station for Agronomy (RDSA) in Moara Domnească, part of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV). RDSA Moara Domnească is located in the Romanian Plain, within a sylvosteppe area (Figure 1).
The experiment was organized using a randomized block design with four blocks, featuring eight different fertilization treatments and four replicates (Table 1). Each plot was 15 m² in size (5 m in length × 3 m in width).

Figure 1. Winter wheat experimental field, 2021-2022 growing season, RDSA Moara Domnească

Table 1. Treatment variants and doses of mineral fertilizers applied for winter wheat during the 2021-2022 vegetation period

Treatment	Winter wheat	
	Doses of mineral fertilizers	
	Fraction (kg/ha)	
	1	2
	24.03.2022	15.04.2022
V1 - soil (Control)	-	-
V2 - NPK	57 N; 57 P_2O_5; 0 K_2O	28 N; 28 P_2O_5; 0 K_2O
V3 - 15 t/ha MC*	-	-
V4 - 15 t/ha MC + NPK	42 N; 42 P_2O_5; 0 K_2O	21 N; 21 P_2O_5; 0 K_2O
V5 - 30 t/ha MC	-	-
V6 - 30 t/ha MC + NPK	27 N; 27 P_2O_5; 0 K_2O	13 N; 13 P_2O_5; 0 K_2O
V7 - 60 t/ha MC	-	-
V8 - 60 t/ha MC + NPK	According to dose calculation, in V_8, the amount of MC should have ensured the nutrient requirements (NPK) and it was decided not to supplement it with chemical fertilizers	

*MC - manure compost

The fertilization treatment included three doses of manure compost - 15 t/ha, 30 t/ha, and 60 t/ha. These were applied at the end of September 2021, either alone or in combination with NPK complex fertilizers (20:20:0).
The NPK fertilizer was applied in fractions based on the nutrient requirements of the wheat crop (Figure 2).

Figure 2. Different doses of manure compost applied on the Moara Domnească experimental field in 2021

Climatic conditions

From September 2021 to October 2022, the climatic conditions in the Moara Domnească area influenced the crop growth.

During the growing season, the average rainfall was 30.29 mm. The monthly maximum and minimum temperatures are detailed in Figure 3.

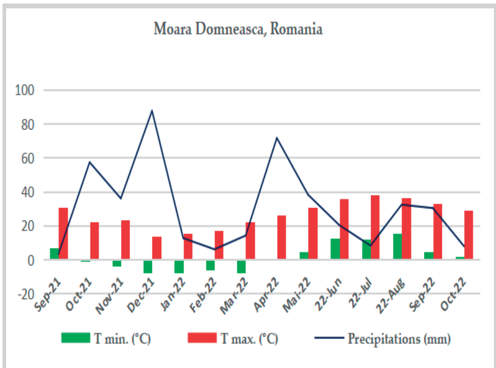

Figure 3. Rainfall (mm) and monthly temperatures (°C) in the Moara Domnească area between September 2021 and October 2022

Soil conditions

The soil in Moara Domnească is classified as red preluvosoil. Samples were collected from six points at a depth of 0-20 cm in order to assess its chemical characteristics prior to land preparation and winter wheat sowing. The samples were air-dried, sifted, and various chemical properties were analysed (Table 2).

Table 2. Agrochemical characteristics of red preluvosoil from Moara Domnească, before winter wheat sowing

Soil characteristics	Mean values ± SD*
pH	6.01 ± 0.154
C_org. (%)	1.94 ± 0.088
N_{total} (%)	0.31 ± 0.011
N-NO_3 (mg/kg d.m.)	9.63 ± 2.498
N-NH_4 (mg/kg d.m.)	10.11 ± 1.055
P_{AL} (mg/kg d.m.)	73.6 ± 10.561
K_{AL} (mg/kg d.m.)	243.3± 30.411

*SD – standard deviation

The soil was scarified twice to a depth of 40 cm due to autumn drought conditions, which prevented ploughing. Two passes with a cultivator were performed to prepare the seedbed. Alfalfa was the preceding crop. The compost used in the experiment was derived from cattle manure and straw collected at the RDSA Moara Domnească farm.

Winter wheat (Jaguar variety from ITC Seeds) was sown on October 22, 2021, and harvested on June 24, 2022.

Yield components, grain yield and quality determinations methods

Grain yield (kg/ha) and the yield components of winter wheat were assessed, including spike length (cm), number of spikelets per spike, number of grains per spike, grain weight per spike (g), and thousand grain weight (TGW) (g). To evaluate the quality of winter wheat yield, the following characteristics were analysed: moisture content (%), hectolitre mass (HLM) (kg/hl), protein content (%), and starch content (%).

For the TGW determination, 100 g of grains were weighed and counted once per replicate. The spike length, number of spikelets per spike, number of grains per spike, and grain weight per spike (g) were determined from samples of 10 randomly selected spikes.

During wheat harvesting, a representative 1 m x 1 m plot was selected from each experimental plot. The spikes were threshed to separate the grains, which were then weighed to determine the yield.

To asses winter wheat grain quality, a Hectolitre Measuring System - Chondrometer with a 0.5-liter capacity was used to determine hectolitre mass (HLM). Moisture, protein, and starch content were measured using a NIR Inframatic

9200 Product Instalab-Analizer (Ionescu et al., 2021).

Statistical analysis

A statistical analysis was performed using *Analyse-it* software for Microsoft Excel. A one-way analysis of variance (ANOVA) was employed to test all parameters and identify statistically significant differences between treatments ($p<0.05$). Additionally, the least significant differences (LSD) were calculated using Microsoft Excel.

RESULTS AND DISCUSSIONS

Winter wheat yield components

Data analysis revealed low differences in spike length between variants. The highest spike length was observed in V3, measuring 6.87 cm, followed by V6, V7 and V8 (Table 3).

Table 3. Winter wheat yield components in the 2021-2022 growing season, RDSA Moara Domnească experimental field

Variant/ Yield components	Spike length (cm)	No. of spikelets/ spike	No. of grains/ spike	Grain weight/ spike (g)	TGW (g)
V1	6.20^{c}	17.30^{c}	25.35^{c}	1.01^{b}	33.04^{c}
V2	6.23^{c}	17.42^{bc}	26.80^{bc}	1.02^{b}	33.31^{c}
V3	6.87^{a}	18.50^{a}	34.30^{a}	1.20^{a}	37.12^{a}
V4	6.29^{bc}	17.55^{bc}	23.90^{d}	0.98^{c}	32.94^{d}
V5	6.41^{b}	17.82^{b}	28.43^{b}	1.05^{b}	35.56 ^{b}
V6	6.75^{ab}	18.10^{ab}	29.73^{b}	1.13^{ab}	36.40 ^{ab}
V7	6.70^{ab}	18.02^{ab}	28.50^{b}	1.11^{ab}	36.24^{ab}
V8	6.67^{ab}	17.90^{b}	27.96^{bc}	1.03^{b}	34.26 ^{bc}
The similar letters show that there is no significant difference according to Duncan's test at the level of 5% probability					

For the number of spikelets per spike, no significant differences were observed among the variants, with values ranging from 17.3 (Control) to 18.5 (V3).

The number of grains per spike, which depends on the number of spikelets, varied across treatments. The highest number of grains per spike was found in V3 (15 t/ha MC), with 34.30 grains. This was followed by V6 (30 t/ha MC + NPK) with 29.73 grains per spike, and V7 (30 t/ha MC) with 28.50 grains per spike. The lowest number of grains per spike was noted in V4 (30 t/ha MC+ NPK), with 23.90 grains.

The grain weight per spike, which reflects the efficient use of nutrients by plants and their translocation to generative parts, plays an important role in winter wheat yield formation (Protić et al., 2013). The grain weight per spike ranged from 0.98 g (V4) to 1.20 g in V3 (15 t/ha MC), though these differences were not significant.

The dry climate of 2022 strongly impacted the thousand-grain weight, a key yield component examined in this study. TGW was highest in V3 (37.12 g), with 15 t/ha manure compost, and lowest in V4 (32.94 g) (Table 3).

The yield components were less influenced by the different fertilizer treatments, possibly due to the slow-release effect of organic fertilizers throughout the growing season.

Winter wheat yield

Grain yield in winter wheat varied between treatments. While most mineral and organic fertilized variants produced in most cases slightly higher yields, statistically significant differences compared to the control were observed only in V6 and V7 (Figure 4).

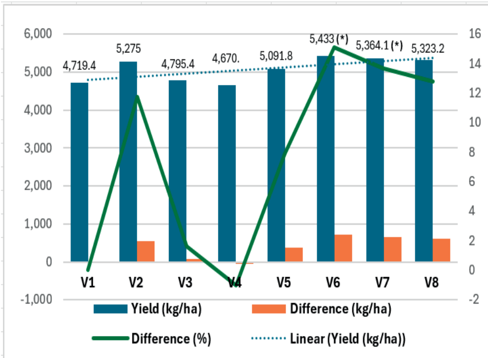

Figure 4. Winter wheat yield in the 2021-2022 growing season, RDSA Moara Domnească experimental field

The lowest yield was registered in V4 (4,670.6 kg/ha), which was 48.8 kg/ha less than the Control. Conversely, the highest yield was determined in V6 (5,433 kg/ha), where 30 t/ha of compost + NPK were applied. In V6, this represented a 15.12% increase compared to the Control (Figure 4 and Table 4).

Table 4. Winter wheat grain yield, 2021-2022 growing season, Moara Domneasca experimental field

Variant	Yield (kg/ha) - 14% moisture	Differences from the Control		Significance
		kg/ha	%	
V1	4,719.4	-	-	-
V2	5,275.0	555.6	11.77	ns
V3	4,795.4	76.0	1.61	ns
V4	4,670.6	-48.8	-1.03	ns
V5	5,091.8	372.4	7.89	ns
V6	5,433.0	713.6	15.12	*
V7	5,364.1	644.7	13.66	*
V8	5,323.2	603.8	12.79	ns

LSD 5% - 610.41 kg/ha *ns – not significant*
LSD 1% - 830.51 kg/ha
LSD 0.1% - 1121.17 kg/ha

Winter wheat grain quality

After harvesting, several winter wheat grain quality parameters were assessed to evaluate the impact of mineral or organic fertilization, or their combination.

The grain moisture content ranged from 11.75% in V4 to 11.95% in V8 and Control (V1), with no significant differences between treatments (Table 5).

Table 5. Winter wheat grain quality after harvesting, 2021-2022 growing season, RDSA Moara Domnească experimental field

Variant	Grain quality parameters			
	Moisture (%)	HLM (kg/hl)	Protein (%)	Starch (%)
	Mean values and SD*			
V1	11.95±0.07	74.35±0.21	11.30±0.28	66.35±0.64
V2	11.90±0.14	73.65±3.6	14.15±0.35	64.00±0.57
V3	11.90±0.01	73.00±0.14	11.50±0.14	65.45±0.35
V4	11.75±0.07	70.50±0.70	13.15±0.07	64.75±0.07
V5	11.85±0.07	73.80±0.56	11.60±0.99	66.50±0.42
V6	11.90± 0.01	74.20±0.91	13.50±0.57	64.70±0.14
V7	11.90±0.01	74.45±0.84	11.35±0.35	66.45±0.07
V8	11.95±0.07	74.20±0.01	11.95±0.07	65.95±0.07
SD	± 0.6	± 1.30	± 1.12	± 0.95

Hectolitre mass (HLM) can be influenced by factors such as soil and climatic conditions, crop management practices, pest attack, and impurity level (Dumbravă et al., 2019).

In this study, the lowest HLM value was registered in variant V4 (70.5 kg/hl), where 15 t/ha MC were applied combined with NPK. The highest HLM value was observed in V7 (74.45 kg/hl), with the application of 60 t/ha of manure compost (Figure 5).

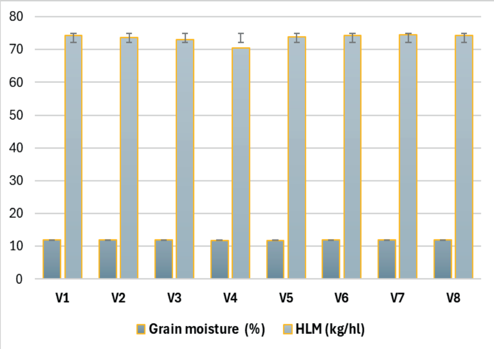

Figure 5. Moisture and hectolitre mass (HLM) for winter wheat grains, 2021-2022 growing season, RDSA Moara Domnească experimental field. The bars represent the standard deviation

The grain protein and starch contents varied with different fertilizer rates (Figure 6). Compared to the Control (V1), the grain protein contents were slightly higher in V7 (11.35%), V3 (11.50%), V5 (11.60%), and V8 (11.95%) with even higher values in V4 (13.15%) and V6 (13.50%). The highest protein content was registered in V2 (only NPK), at 14.15%.

Starch content in the wheat grains ranged from 64% in V2 (only NPK) to 66.50% in V5 (30 t/ha MC) (Figure 6).

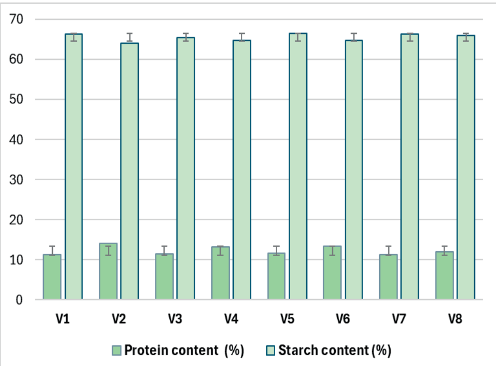

Figure 6. Protein and starch contents (%) for winter wheat grains, 2021-2022 growing season, RDSA Moara Domnească experimental field. The bars represent the standard deviation

The data from this study indicates a trend where increased grain protein content corresponds to decreased starch content, an aspect also reported by Balla et al. (2011). This could be attributed to the fact that higher protein levels could reduce starch accumulation by decreasing starch synthase activity, leading to lower starch levels. However, it is important to note that starch content does not consistently correlate with protein content (Yu et al., 2017).

CONCLUSIONS

The impact of different nutrient regimes on wheat yield components and production requires ongoing research. A significant challenge in this area is the scarcity of long-term field experiments that can reflect the complexity of factors affecting these characteristics.

The analysed data indicated that small doses of organic fertilizers positively affected yield components. For yield and yield quality, medium to high doses of organic fertilizers - either alone or in combination with mineral fertilizers - demonstrated beneficial effects. The environmental conditions during the growing season play a crucial role (Dumbravă et al., 2019). In our study, both TGW and protein content were also influenced by water stress and other environmental factors.

Wheat yield varied among experimental variants, with higher values observed in the treated variants. The highest yield was registered in V6, with the application of 30 t/ha of manure compost and NPK. Additionally, the thousand grain weight, protein content and starch content also varied between treatments. Notably, an increase in protein content was associated with a decrease in starch content in the grains.

Optimizing fertilization strategy is crucial for sustaining crop productivity, reducing nitrate residues (Liu et al, 2024) and maintaining grain quality. Implementing sustainable agricultural practices can enhance resource use efficiency and the overall sustainability of agroecosystems. The results from this study lay the foundations for further research into the impact of different fertilization practices on yield and grain quality in the short and long term in a preluvosoil within a sylvosteppe area of Romania.

ACKNOWLEDGEMENTS

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YIELD STABILITY AND SEED QUALITY UNDER DIFFERENT CLIMATIC CONDITIONS AT SOME EUROPEAN AND CHINESE SOYBEAN GENOTYPES

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Abstract

A field experiment organised in three different years (2021, 222, 2023) at Research and Development Station for Agriculture (RDSA) Turda studied the yield stability and seed quality of some soybean genotypes of European and Asian origin, cultivated in the conventional system. The 119 soybean genotypes were sown in the experimental field of the Soybean Breeding Laboratory and consisted of seven different maturity groups (MG), from 0000 MG to III MG, characterised by different growing season. Based on the experimental results, genotypes that originated in Europe performed better in terms of grain yield compared to Asian genetics. In years with high temperatures and water deficit during the growing season, such as 2022, it seems that better results were obtained in genotypes with purple flower compared to those with white flower; with grey pubescence compared to the brown one, and in terms of growing season, the extremes of the experiment, namely extra and very early soybean genotypes along with late and very late genotypes, overcame critical phases characterized by lack of water and high temperatures, achieving superior yields.

Key words*: Asian, European, genotypes, soybean, yield stability.*

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.), with its multiple uses, is one of the most important crop plants worldwide (Hahn and Medanier, 2013), especially for developing countries facing malnutrition and food insecurity (Yirga et al., 2022). As demand for plant foods increased, protein (≈40%) and oils (≈20%) (De Visser et al., 2014) of soybeans have gained importance in recent years (Singh, 2010).

Soy protein has a number of advantages over other types of natural proteins: increased biological value, lower price and relatively long shelf life due to stability (Deng et al., 2012), soybean oil being used and appreciated in various fields of gastronomy due to its versatility (De Maria et al., 2020).

With changing climate and environmental conditions, throughout the growing season, soybeans can often encounter several stressors (abiotic and biotic) that affect the reproductive structure and ultimately lead to lower yields (Kesar et al., 2023).

Identifying biological material with tolerance to climate stress and a good adaptability, as high as possible to various external disturbing factors, can be a starting point for creating new soybean varieties (Melnyk et al., 2022) that are stable in time and space for both productivity and quality.

A basic requirement for successful crop breeding is to secure and continuously use genetic diversity, in order to ensure the existence of traits for tolerance that will be led to adapt crops to new environmental conditions and climate change (Andrijanić et al., 2023).

Crop yields level is dependent on environmental conditions (Suciu et al., 2021), so the adaptability of varieties to environmental changes is important to ensure stability regardless of year or location (Habtegebriel and Abebe, 2023).

Some characteristics of soybeans, such as stem pubescence colour or flower colour, although not part of the main selection criteria for production and quality, are nevertheless correlated with tolerance to abiotic stress conditions (Xie, 2007). Authors such as Morrison and Voldeng (1994) point out that the colours of the

pubescence of the soybean stem and hilum have a direct or indirect influence on the growth, development and even yield of plants.
In the context of global climate change, Romania is also affected by an increase in air temperature and a lack of precipitation in critical phases for plants. Thus, the study of such a varied biological material allowed the identification of genotypes adapted to these changing conditions, capable of achieving high yields even in the presence of stressors.

MATERIALS AND METHODS

For evaluating the stability of the yield capacity and the quality of a biological material with origin in Europe and China, respectively, at RDSA Turda a field experiment was conducted in the climatic conditions of: 2021, 2022, 2023.
The 119 soybean genotypes (Figure 1) evaluated in the study consisted of:
- 65 European genotypes;
- 54 Asian genotypes.

Genotypes are classified into 7 different maturity groups, from extra-early to very late, as following:
- Maturity group of extra early (0000) and very early (000) soybean genotypes: 18 genotypes;
- Maturity group of early (00) soybean genotypes: 20 genotypes;
- Maturity group of semi-early (0) soybean genotypes: 38 genotypes;
- Maturity group of semi-late (I) soybean genotypes: 40 genotypes;
- Maturity group of late (II) and very late (III) soybean genotypes: 3 genotypes.

The varieties were sown mechanized, experiment being placed linearly, each cultivar being sown on:
- two rows with a length of 10 m;
- 50 cm distance between rows;
- sowing density was 55 germinate grains per m^2.

During the growing season, notations were made regarding: emergence date, flowering date, flower colour, maturity beginning date, maturity end date, pubescence colour. Based on the notations, the growing season of each genotype was calculated.
The harvesting was mechanized, using a Classic Wintersteiger Combine for experimental plots, the yield of each genotype being related to ha. The experimental data were statistically processed using Excel.

Figure 1. Aspects from experimental field (original)

RESULTS AND DISCUSSIONS

Of the three experimental years, the climatic conditions from 2022 were totally unfavourable for soybean crop. Based on the climatic conditions from 2021, respectively 2023, good yields were obtained, 2021 being the most favourable. Although of the three experimental years, the conditions from the first year allowed the studied genotypes to achieve the highest yields, late-maturing cultivars do not reach their genetic potential due to the limiting climatic factor. The drought in the last decade of June and in the first two decades of August affected the flowering stage of late genotypes, respectively on the phase of pod formation and grain filling for early genotypes (Figure 2).
The months of April and May, which are the timing of sowing and emergence periods, were not only dry but also cool. The summer months presented a general warming trend for almost the entire soybean growing season.

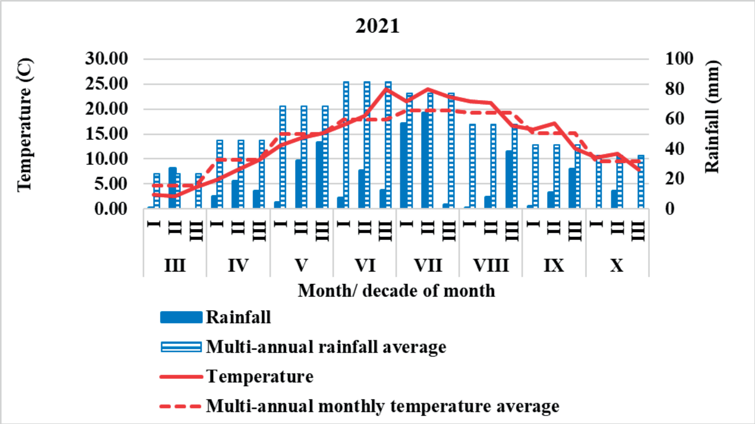

Figure 2. Mean air temperature and rainfall of each month during soybean growing season (Turda, 2021)

The analysis of the data on the thermal and pluviometry regime recorded in 2022 in Turda presented in Figure 3, highlights that 2022 can be characterized as a hot and excessively dry year, until August, September when we have an excess of precipitation.

The water reserve and the evolution of temperatures at the beginning of the year (January-April 2022), at RDSA Turda, were less favourable to sowing in optimal humidity and temperature conditions. Thus, the genotypes had a difficult start in vegetation, the emergence being noted at a time interval of two weeks after sowing.

Reproductive stage and development were greatly affected by climatic conditions in June and July, especially by the lack of rainfall, but also by the high temperatures during this period. Also, the beginning of maturity and the end of maturity were affected by the climatic conditions of this year, due to the rainfall recorded at the end of August and September, respectively, the harvesting of very early genotypes but also of those with a longer vegetation period was delayed. With the exception of very early genotypes harvested in the third decade of September, most genotypes were harvested in the first decade of October.

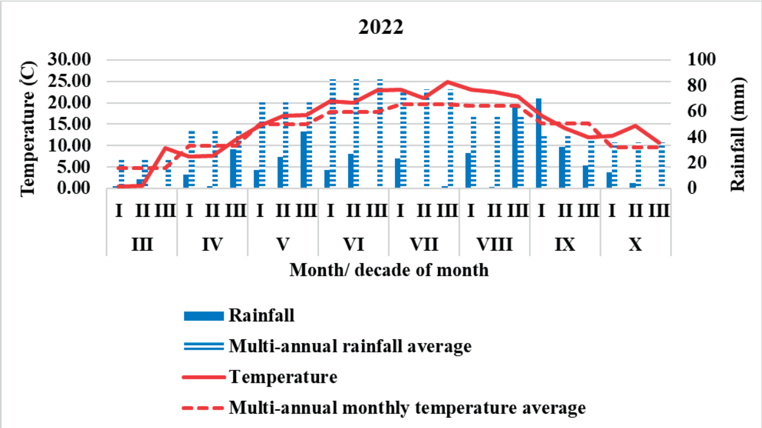

Figure 3. Mean air temperature and rainfall of each month during soybean growing season (Turda, 2022)

The analysis of the data on the thermal and pluviometric regime recorded in 2023 in Turda, presented in Figure 4, highlights the fact that 2023 can be characterized as a warm and excessively dry year in spring. However, in the summer months except July, and in the first month of autumn, due to significant rainfall, they were characterized as excessively rainy.

The water reserve and the evolution of temperatures pre-sowing (January-April 2023) at RDSA Turda were less favorable regarding optimal humidity and temperature conditions. Thus, genotypes had an uneven emergence, in staggered stages, at a time interval of three weeks after sowing. However, the main vegetative and generative stages were not affected to a significant extent by next climatic conditions. The harvesting of soybeans was slightly delayed due to rainfall in early September, but without having a negative influence on yield and quality.

With the exception of very early genotypes harvested in the third decade of September, most genotypes were harvested in the first decade of October.

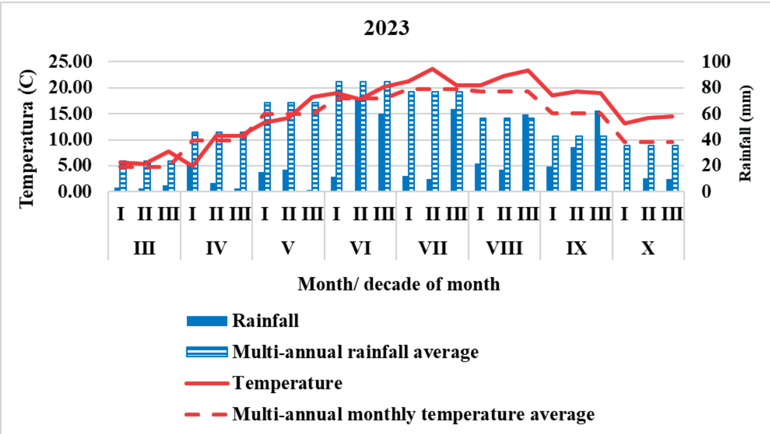

Figure 4. Mean air temperature and rainfall of each month during soybean growing season (Turda, 2023)

It is well known that a crop that has been developed to adapt to the climate of a particular country cannot be fully successfully grown in other countries (Jung et al., 2023), but through breeding processes a certain degree of adaptability can be achieved.

The ecological plasticity of a genotype, evidenced by the stability of yield capacity from one year to another and from one location to another, allows the choice of valuable cultivars that could capitalize, in the context of current climate change, the difficult climate and soil conditions by maximising grain production.

The analysis of stability of the studied genotypes (Figure 5) according to their origin indicates that the biological material coming from Europe seems to have a superior behaviour to the Asian one under the conditions of the three experimental years. Djanta et al., (2020) highlights that the lack of locally adapted soybean varieties is one of the major factors restricting crop production.

However, in 2022, a totally unfavourable year for soybean cultivation in the reference area, it would appear that both germplasm types were strongly affected by atypical climate conditions, with the average yield of China genotypes being at the same level as European ones.

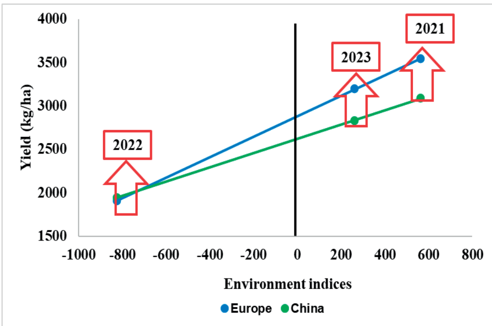

Figure 5. Stability of yield capacity depending on the origin of biological material

In 2019, Voss-Fels et al. point out that intensive research was needed to develop improved and highly stable soybean varieties that fit food standards worldwide.

The stability of the genotypes yield capacity in the three experimental years, at RDSA Turda, was also evaluated according to the maturity group analysed in the experiment (Figure 6).

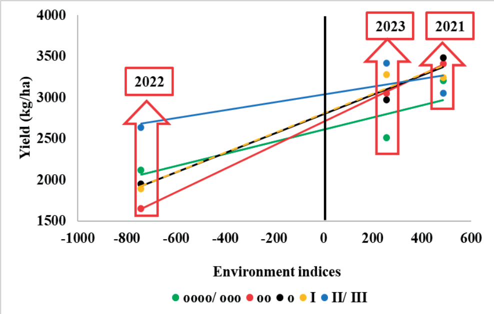

Figure 6. Stability of yield capacity depending on the maturity of biological material

In this case it is noted that out of the three experimental years, 2022 was very unfavourable for soybean cultivation. If in the favourable conditions of 2022, early and semi-early soybean genotypes had the highest yields, which were otherwise recommended to be grown in our growing area, in 2022, good yields were obtained in very early soybean genotypes and in late and very late ones.

Some authors consider that often the long period of the growing season is directly correlated with high yield potential (Mushoriwa et al., 2022), but even if it would seem that genotypes with a longer vegetation period are more productive, their cultivation in our growing area presents a high-risk factor in years with heavy rainfall. This could extend their growing season, delaying harvesting.

Chaudhary and Wu stated in 2012 that it is important to investigate the stability over time of soybean varieties in terms of quality parameters under current and future climate change.

Regarding the analysis of the stability of yield capacity according to the colour of the steam pubescence, it would seem that (Figure 7), genotypes with grey pubescence show better adaptability to adverse environmental conditions, while under normal conditions, brown genotypes achieve higher yields. The 2022 year was characterized by soybean yields below 2000 kg/ha. The best year for soybean cultivation was 2021, in which yields of 3500 kg/ha were obtained, on average, at cultivars with brown pubescence.

Research by Zhou et al. (2024) has shown that unlike soybean plants with grey pubescence, plants with brown pubescence possess higher yield capacity and improved stability in cold regions, and that this characteristic of soybeans also indicates cold-resistant ability, so it is recommended to specifically select soybean varieties with brown pubescence.

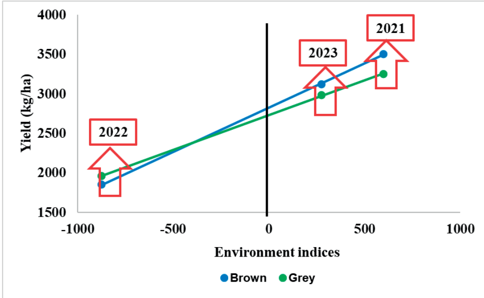

Figure 7. Stability of yield capacity depending on pubescence

Most cultivated soybean varieties are characterized by the purple colour of the flower, which is dominant. From the analysis of the stability of soybean yield capacity depending on the colour of the flower, on average, regardless of the environmental conditions encountered, purple flower genotypes have a consistently good behaviour and superior production to those with white flower (Figure 8).

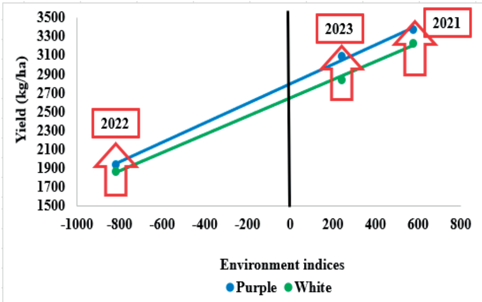

Figure 8. Stability of yield capacity depending on the colour of flowers

The experiment located at RDSA Turda during the three years allowed the study of Asian and European origin biological material and with different maturity.

In order for soybeans to be not only an important source of vegetable protein, but also a very good precursor for autumn grain crops, especially winter wheat, it must be harvested as early as possible.

That stated soybean genotypes characterized by growing season of less than 135 days and yield of more than 3 t/ha were highlighted. Grouping genotypes by maturity and production revealed a group of 13 cultivars, 10 of European origin and 3 of Asian origin, which could be extended into Transylvanian Plateau area of cultivation. Some of these are: Heinong 64, Heihe 39, Ns Kaca, Augusta, Alexa, Ancona, Regina, Heihe 5, Pannonia Kincse, TriaDa, Ns Hogar, and NS L-401156 (Figure 9).

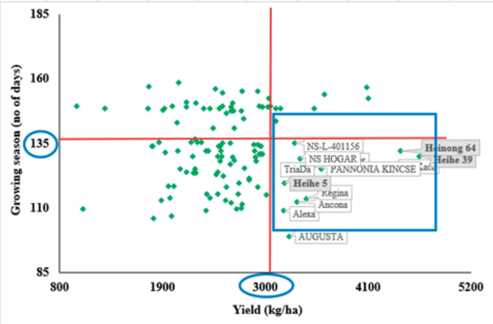

Figure 9. Genotypes with high yield and suitable growing season

For future research, there is an interest in obtaining high yielding genotypes with high values for thousand kernel weight to allow the use of soybeans in the food industry. In this sense, out of the 119 genotypes studied, 7 had yields greater than 2500 kg/ha and TKW over 160 g. Among them we mention the Obelix and Paco varieties, but also the Mengdou 30 variety, which had a thousand kernel weight of almost 180 g (Figure 10).

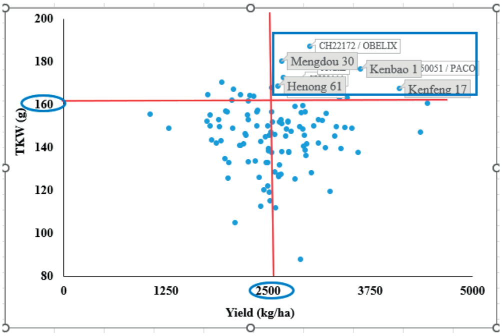

Figure 10. High-yielding, high-grain genotypes

There is known the negative relationship between protein and oil, and because of this is difficult to improve them simultaneously. Grouping the 119 genotypes according to the two quality indices (Figure 11), revealed a group of 15 genotypes that had a fat content of over 22 percent and a protein content of over 40 percent. Also noted was the Paco variety for the highest protein content and the Dongnong 50 variety which proved to be the oiliest.

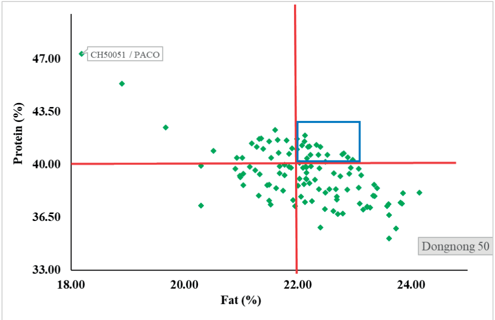

Figure 11. High protein and fat genotypes

Ray et al., (2008) stated that in the case of soybeans, characteristics such as protein and oil are influenced by environmental and genetic variables as well as their interactions, and the protein and oil content of soybeans correlate negatively (Hurburgh et al., 1987).
Yield response of the best soybean genotypes is highlighted in the following table (Table 1). More than 4 t/ha yield was observed at three European soybean varieties (Es Gladiator, Ns Kaća and Paco) and two Asian varieties (Beidou 30, Kenfeng 17).
The average of top ten best performing European genotypes in terms of yield (3,657 kg/ha) was 200 kg/ha higher than of Chinese genotypes.
Paco soybean variety is not only high yielding but also appreciated for its high values of TKW and protein content respectively.

Table 1. Rank of soybean genotypes depending on origin using yield performance

Rank	Europe		Asia	
	Genotype	**Yield (kg/ha)**	**Genotype**	**Yield (kg/ha)**
1.	Es Gladiator	4,647	Beidou 30	4,445
2.	Ns Kaća	4,367	Kenfeng 17	4,105
3.	Paco	4,085	Kenbao 1	3,633
4.	Castetis	3,523	Suinong 24	3,600
5.	Regina	3,439	Heihe 36	3,468
6.	Triada	3,396	Heihe 48	3,373
7.	Ancona	3,336	Dongnong 54	3,162
8.	Ananda	3,317	Kennong 36	3,112
9.	Augusta	3,258	Dongnong 51	2,974
10.	Es Tenor	3,206	Henong 60	2,971
	Average	**3,657**	**Average**	**3,486**

CONCLUSIONS

The study of the 119 soybean genotypes grown in conventional system at RDSA Turda, in three different years in terms of climatic conditions, allowed highlighting some valuable genotypes both in terms of yield, yield stability and harvest quality.

The results obtained in this research are of equal interest to researchers who can identify valuable sources of genitors that can be used in cross-breeding, as well as soybean growers who can identify genotypes adapted to various climatic conditions.

It would appear that, in the three experimental years, genotypes that originated in Europe performed better in terms of seed yield as compared to Asian genetics.

An average yield of 3543 kg/ha in 2021, 1907 kg/ha in 2022 and 3197 kg/ha was obtained by European cultivars.

Following yield were obtained by Asian cultivars: 3088 kg/ha, 1945 kg/ha, 2829 kg/ha.

Also, in normal crop conditions, following genotypes achieve higher yields:

- brown pubescence (3503 kg/ha, 3121 kg/ha), compared to the grey one (3255 kg/ha, 2986 kg/ha);
- purple flower (3370 kg/ha, 3089 kg/ha) compared to the white one (3232 kg/ha, 2845 kg/ha);
- early (3407 kg/ha, 3053 kg/ha) or semi-early maturity (3481 kg/ha, 2970 kg/ha), compared to other maturity groups.

In years with high temperatures and water deficiency during the growing season, such as in our case 2022, it would seem that better results were obtained at genotypes with:

- purple flower (1942 kg/ha) compared to those with white flower (1870 kg/ha);
- grey pubescence (1960 kg/ha), compared to brown one (1850 kg/ha).

In terms of the limits of growing season, namely extra and very early soybean genotypes (2120 kg/ha) along with late and very late genotypes (2633 kg/ha), overcame critical phases characterized by lack of water and high temperatures, achieving superior yields in unfavourable conditions.

Grouping genotypes by maturity and yield revealed 13 cultivars, 10 of European origin and 3 of Asian origin, which could be grown in the Transylvanian Plateau. Among them are: Heinong 64, Heihe 39, Ns Kaca, Augusta, Alexa, Ancona, Regina, Heihe 5, Pannonia Kincse, TriaDa, Ns Hogar, and NS L-401156.

In terms of quality, the Paco variety stood out for the highest protein content and the Dongnong 50 variety, which proved to be the oiliest.

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AGRO-CLIMATIC CONDITIONS FOR GROWING OF SUNFLOWER IN DIFFERENT CLIMATIC AREA IN BULGARIA

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Abstract

Assessment of agro-climatic resources of the agricultural regions relates to the biological requirements of the crops and the applied agricultural techniques. Agro meteorological conditions determine 65-80% of the potential productivity of agricultural crops. In the years after 2000, unfavorable tendencies in the values of meteorological elements, as well as an increase in the frequency of extreme weather events, are a fact. These features of the weather create risky conditions for the cultivation of sunflower in some areas of traditional producers of this crop. The aim of this study is the assessment of the agro-climatic conditions for the cultivation of sunflower in Bulgaria. A comparative analysis and evaluation of the agro-climatic conditions for growing sunflowers in three locations - Chirpan, Karnobat and G. Toshevo and their influence on the growth, development and productivity of sunflowers was made. The experimental fields are located in two climatic sub-regions of the European-continental climatic region - moderately continental (G. Toshevo) and transitionally continental (Chirpan and Karnobat) regions. The new climatic norms of the meteorological elements for the period 1991-2020 were used in the comparative analysis.

Key words*: sunflower, hydrothermal conditions, principal component analysis (PCA), phenology, yield.*

INTRODUCTION

Sunflower is the main oil-producing crop in our country and in most countries of Central and Western Europe. It is grown mainly for the production of sunflower oil, which is distinguished by varied unsaturated acids content (rich in linoleic acid and protein). According to the content of oil in the seeds, the sunflower occupies the second place after the soybean – 55% against 62.4%, among the oleaginous (oil-bearing) crops in our country. Sunflower seed and oil have been considered as a part of the healthiest diets as they could be a rich source of various phytochemicals, tocopherols, sterols or microelements (Rauf et al., 2020).

Because of these qualities, its production rapidly increased in the second half of the 20th century. The largest amount of sunflower is produced in Europe, and among the countries producing this crop in the EU, Romania ranks first with 3450 thousand tons, followed by Bulgaria 1940 thousand tons, Hungary 1830 thousand tons, France 1325 thousand tons, Spain 960 thousand tons and Italy 250 thousand tons. In the 70s of the 20th century, Bulgaria occupied the first place in the world in sunflower production per capita, averaging over 55 kg.

In conditions of climate change, and climate variability, especially during last decade at southern latitudes, temperature increases, precipitation decreases as well as increases in climatic inter-annual variability, and a higher frequency of extreme events are to be expected (IPCC, 2014). These combined changes will lead to a shorter growing season (especially grain filling phase), increased water deficit and heat stress, which will reduce yields, lead to higher yield variability, and probably reduce the agricultural area of this traditional crop in regions as Romania, and Bulgaria.

Over the last several years, the Bulgarian oilseeds industry has invested in expanding capacities, diversifying and adding value to sunflower crop. The country was a net exporter of sunflower seeds and during last several years has increased its crushing capacity. As a result of these developments, Bulgaria is on a trend to consume most of its sunflower crop and become a net exporter of processed products. The planting areas have increased because of higher profitability, low input requirements and better exporting possibilities. Nevertheless, some factors still limited the sunflower production (Valkova et al., 2016). Sunflower is considered as a moderately drought resistant plant. Its cultivation is dependent on global climate change marked by sudden enhanced temperatures, hailstorms, strong wind or erratic rainfall (Debaeke et al., 2020). High temperatures led to increase of leaves evapotranspiration, soil water extraction as well as the irrigation water demand. Sunflower crop is sensitive to drought and heat stresses from early flowering to grain filling stages due to inadequate availability of soil moisture. This conducted to significant reduction of yield and quality of seeds (Hussain et al., 2018). In sunflower, environmental factors have a great influence on the seed yield, as it is a polygenic character. Abiotic stresses especially supra-optimal temperature affect the viability of pollen, thus affecting the size and number of seeds produced by the plant (Razzaq et al., 2019; Mehmood et al., 2023). Significant results have been recently achieved in sunflower breeding program in DAI-General Toshevo. Some new sunflower hybrids, such as Krasela, Enigma and etc., with high productivity and resistance to abiotic and biotic stress factors were registered and distributed in Bulgaria (Georgiev et al., 2018; Nenova et al., 2019).

Climate change is causing changes in the conditions under which ecosystems grow and develop. This leads to changes in their growth, development and productivity. To the greatest extent, this applies to the cultivation of agricultural crops, as changes create conditions that lead to the reduction of plant productivity, mainly caused by biotic and abiotic stress factors.

The assessment of agrometeorological conditions in Bulgaria over the last 30 years and their changes compared to the previous period (Georgieva et al., 2022) show an increase in average monthly air temperatures in all regions of the country from January to September. The trend of advancing the beginning of the growing season compared to the reference period is also confirmed in the transition through 10°C. The period with temperatures above 5°C in the agricultural regions of the country lasts 235-300 days.

During the October-March period, an increase of more than 10% was found in the amount of precipitation in North-Eastern Bulgaria. A decrease in precipitation amounts is observed in Central North, East and South-West Bulgaria. The previous studies for the period 1971-2000 show that in the central part of the Danube Plain, full saturation is reached in the root zone. Second period, April-June, the deviations of the sums of precipitation are mostly negative, and reach 10-15% in Central and North-Eastern Bulgaria. The greatest decline is observed in the extreme north-western regions and in the extreme southern regions. The third period, June-August, is characterized by a significant decrease in precipitation in central southern Bulgaria and part of the Danube region. The deviation varies between 7 and 25%. An increase of more than 10% in the amount of precipitation is observed in individual stations in North-Eastern Bulgaria. The sunflower has a relatively long growing season. The hybrids that were created in our country or were introduced to our conditions belong, according to the duration of their vegetation period, to the group of mid-early mature with duration up to 130-145 days. For their full development, sunflower crops in our country need a sum of active temperatures of 2600-2850°C. According to the research of some authors, the best production results are obtained if average temperatures in July-August are 21-22°C and optimal humidity levels. In correspondence to the heat conditions, the sunflower maturity considered is occurs around August 10-15 in the Danube Plain and around August 15-20 in Dobrudja.

Regarding moisture conditions, it is considered that the need fluctuates in 9-13 l/m² during the vegetative development and reaches up to 50-60 l/m² during the formation of the yield and pouring of the grain. Because sunflower plants developing a powerful central root that reaches a depth of 2-3 m. Under such conditions yields of 2800-3900 kg/ha are considered very well.

Similar studies have been conducted in some European countries (Debaeke et al., 2017) in France, (Kalenska and Rizhenko 2020) in Ukraine, (Eloisa Aguera, 2021) in Spain. In these studies, the authors pay attention to climate changes and conditions for sunflower development in their countries.

There are also many other studies related to the growth and productivity of this culture, but they are more or less intertwined with other goals of their authors, such as monitoring methods (López-Granados et al., 2008; Buzna et al., 2023), different norms of fertilization, etc.

The aim of this paper is to assess hydrothermal conditions for sunflower cultivation and their impact on the yield in three experimental fields located in two climatic sub-regions.

MATERIALS AND METHODS

For this study, the data from the planned experiment carried out within the National Research Programme "Smart crop production" in three locations - Chirpan, Karnobat and G. Toshevo during the period 2020-2023. Data from standard meteorological and agrometeorological observations in the network of National Institute of Meteorology and Hydrology for the period 1991-2020 are used also. Observations include data on temperatures - average, maximum and minimum, total precipitation, relative humidity, wind speed, sowing dates, dates of occurrence of the main phonological stages, yield and water content in the soil. All observations and measurements were carried out in production areas. Observations include data on temperatures - average, maximum and minimum, total precipitation, relative humidity, wind speed, sowing dates, dates of occurrence of the main phenophases, yield and water content in the soil. All observations and measurements were carried out in production areas.

The regions of Karnobat and Chirpan are located in the Transitional-Continental climatic sub-region, the climatic region of Eastern middle Bulgaria, which is characterized by a mild climate - warm winters and hot summers.

General Toshevo is located in the Eastern climatic region of the Danube Plain, which is part of the Moderately Continental climatic sub-region. Cold winters and hot summers are characteristic of this region.

During the experiment from 2021 to 2023, the occurrence of the phenological phases was observed - second and fourth pair of leaves, inflorescence formation, flowering and technical maturity. In 2022 and 2023, the Krasela hybrid was grown in all three locations, and in 2021, P 64 HE 114 was grown in Chirpan, in Karnobat PL25, and in G. Toshevo - LE 25. During the experimental period biometric measurements was made. There were calculated some agrometeorological indices.

The main phenological phases were observed, the sowing dates and the interphase periods were recorded during the experiment. For comparison, the same data for the period 1991-2020 were used, Table 1. Meteorological conditions during the period 2021-2023 are characterized by average temperatures. Periods of severe and extreme drought over the years are at different times and of different duration and intensity, Table 2.

Table 1. Average multi annual dates of main phenological stages of Sunflower (*Heliantus annuus* L.) occurrence and duration of interphase periods in different regions of Bulgaria

Experimental fields	Sowing (date)	Inflores cence (date)	Flowering (date)	Maturity (date)	Sowing-Inflores cence (days)	Inflores cence-Flowering (days)	Flowering-Maturity (days)	Vegetation season
G. Toshevo	15.04	18.06	13.07	27.08	59	25	43	129
Karnobat	10.04	16.06	9.07	16.08	68	18	38	129
Chirpan	6.04	14.06	2.07	14.08	73	18	43	130

Table 2. Periods and duration with strong and extreme drought in 2021-2023

Station/ Year	2021	2022	2023
Chirpan	July, August and September ~90 days	May, July, August, October and November ~150 days	July, August, September, October and November ~150 days
Karnobat	July, August and September ~90 days	July, August, September, October and November ~150 days	July and August ~60 days
G. Toshevo	September ~30 days	July, August, October and November ~120 days	June, July, August and September ~120 days

RESULS AND DISCUSSIONS

The regions of Karnobat and Chirpan are located in the Transitional-Continental climatic sub-region, the climatic region of Eastern and Central Bulgaria, which is characterized by a milder climate - mild winters and warm summers, Figure 1. From the climatograms it can be seen that the average temperature during the winter months remains positive, and the highest and monthly values are in July. The amount of precipitation has a spring-summer maximum and a minimum in February and August.

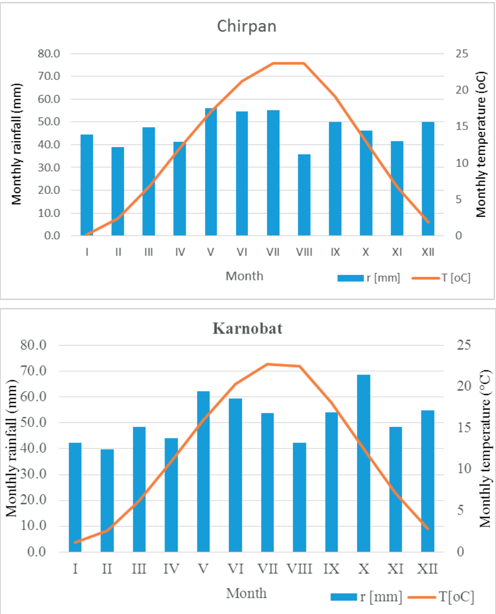

Figure 1. Climatograms for the regions of Chirpan and Karnobat (1991-2020)

General Toshevo is located in the Eastern climatic region of the Danube Plain, which is part of the Moderately Continental climatic sub region. This region is characterized by colder winters and hot and dry summers, Figure 2. The climatogram well shows these values characteristic of the region with minimum temperatures in January and maximum in July. The maximum of precipitation is in June and September, and the minimum in February and August, which is a prerequisite for prolonged periods with insufficient moisture.

A comparison was made of the conditions during the years of the experiment (2021-2023) compared to the period 1991-2020, the values of the average monthly temperatures were calculated, Figure 3 a-c and the rainfall totals for the three locations, Figure 4.

Deviations of air temperature in all three stations during the experiment, were positive. The largest positive deviations are in 2023. The deviations were positive in all three stations in January, February, July, August and November. During the growing season of the sunflower, the months of April, May and June are characterized by both positive and negative. The deviations in April 2021 and 2023 and in June 2021 in the three locations are negative. The Chirpan deviation in 2023 is negative. Figure 3 a-c.

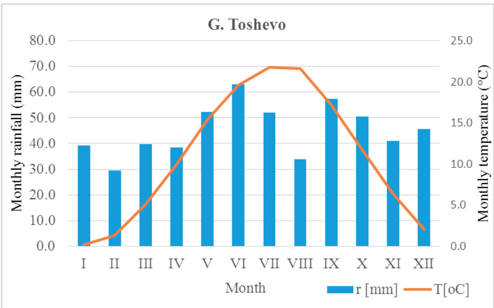

Figure 2. Climatogram for G. Toshevo in the period 1991-2020

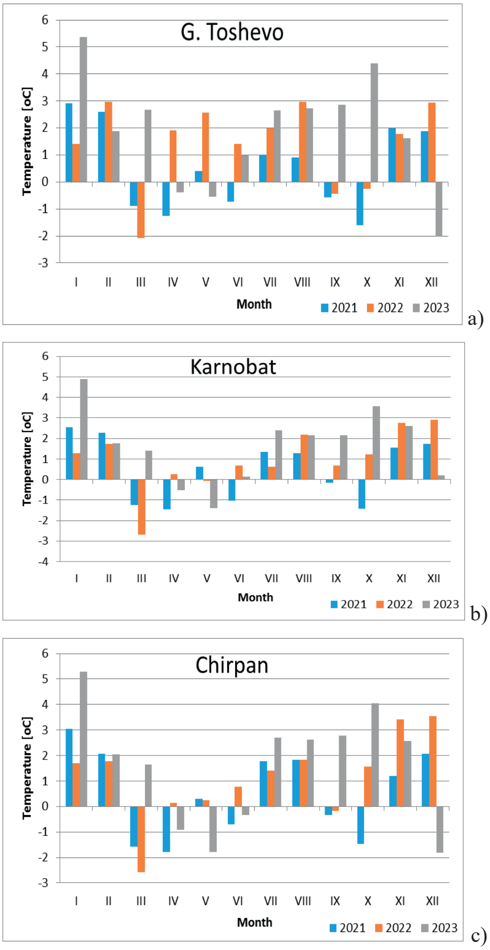

Figure 3. Deviations of average monthly air temperatures for the period 2021-2023 compared to the norm 1991-2020 in: a) G. Toshevo; b) Karnobat; c) Chirpan

The annual amount of precipitation had a positive deviation in 2021 in all three stations and a significant negative deviation in 2022. In 2023 in Karnobat and G. Toshevo deviations were also negative, but insignificant, while in Karnobat the precipitation was significantly below the climatic norm.

To study the influence of agrometeorological conditions, we conventionally divided the vegetation period into three sub-periods – from sowing to the formation of an inflorescence; from inflorescence formation to flowering and from flowering to ripening. During these periods, we tracked the dynamics of the sums of active temperatures and the sum of precipitation. The average duration of the growing season in the three locations is 129-130 days, Table 3.

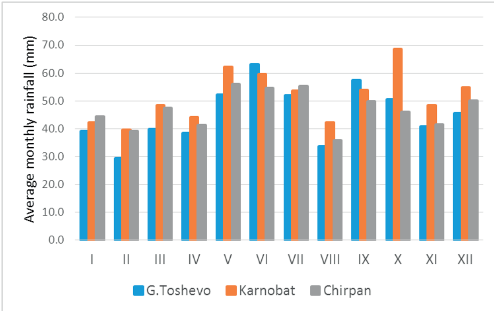

Figure 4. Monthly values of the amount of precipitation for the period 2021-2023

For the years of experiment the sum of active temperatures during the growing season, in Karnobat reaches 3445°C-4132ºC, in Toshevo this sums are 3197°C-4068°C, and in Chirpan 3960°C to 4230ºC. The amount of precipitation in Karnobat during the growing season is 216-375 mm, in Chirpan it reaches 278-352 mm, and in Toshevo this value is about 179-354 mm.

To characterize the humidification conditions, the precipitation amounts for the periods October-March and April-August in the three locations were determined (Figure5).

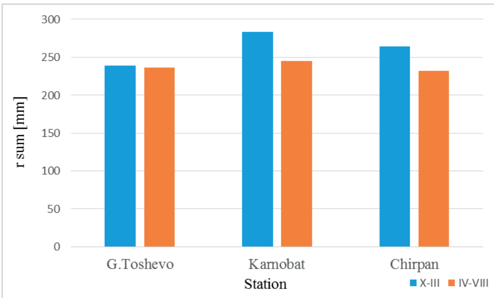

Figure 5. Multi annual rainfall sums during periods October-March and April-August for the periods 1991-2020

The amounts of precipitation during the period X-III in G. Toshevo are lower than those necessary to reach full saturation of the one-meter soil layer at the beginning of the growing season. In the rest two stations, the precipitation totals exceed 250 mm, which is a prerequisite for favorable humidification conditions at the start of the vegetation in the spring (Figure 5).

The sums of precipitation during the vegetation period for the years of the experiment are characterized by a positive deviation in 2021, negative deviations in 2022 and insignificant positive deviations in G. Toshevo and Chirpan and negative in Karnobat (Figure 6).

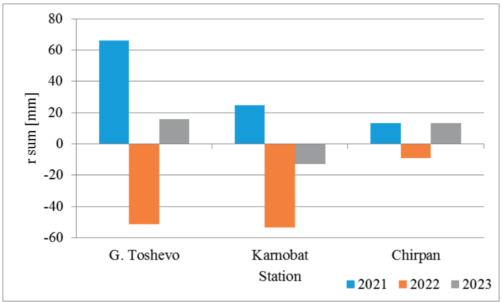

Figure 6. Rainfall sums deviations during sunflower vegetation period for experimental years and climatic norm (1991-2020)

The average yield of grain for the period 1991-2020 is respectively - in Karnobat 1.57 t/ha, in G. Toshevo 2.3 t/ha and in Chirpan 2.33 t/ha. The maximum yields are – Karnobat 2.7 t/ha, G. Toshevo 3.84 t/ha and Chirpan 3.6 t/ha.

The phenological development of the sunflower during the period of experiment was monitored in the following phases - sowing, inflorescence formation, flowering and maturity, Table 3. For assessment of development of sunflower development during the experiment was used the average dates of occurrence of phases and the duration of interphase periods, Table 3. The earliest sowing dates have been registered in Chirpan and Karnobat - during the first decade of April, except 2021 in Karnobat. In G. Toshevo the sowing date have been realised one month later, during the first decade of May. In relation to average dates significant deviation is finding in G. Toshevo. The base date here is 15 April. The earliest dates of maturity have been registered in Chirpan, during the second decade of August, after that in Karnobat, during the third decade of August and the latest in first decade on September in G. Toshevo.

To assess the relationship between yields and temperature-humidity characteristics of the regions, we applied multiple analysis using the method of principal components, where the components were the sums of effective temperatures for the periods from sowing to flowering - tef_sum1 and from flowering to ripening tef_sum2, as well as the sum of precipitation for these periods – r_sum1 and r_sum2 (Figure 7).

Table 3. Dates of main phenological stages of Sunflower (*Heliantus annuus* L.) occurrence and duration of interphase periods during the experiment (2021-2023) in the experimental sites

Location	Observed dates for phenol stages occurrence				Development periods duration			Vegetation Season Duration (days)
	Sowing (date)	Inflorescence (date)	Flowering (date)	Maturity (date)	Sowing-Inflorescence (days)	Inflorescence-Flowering (days)	Flowering-Maturity (days)	
G. Toshevo	6.05	1.07	30.07	9.09	86	29	41	126
G. Toshevo	10.05	5.07	28.07	8.09	57	22	42	121
G. Toshevo	8.05	20.06	21.07	13.09	73	31	54	128
Karnobat	16.04	30.06	11.07	25.08	75	11	45	131
Karnobat	10.04	8.07	18.07	28.08	89	10	41	140
Karnobat	5.04	30.06	15.07	25.08	86	15	41	142
Chirpan	8.04	17.06	30.06	15.08	70	13	46	129
Chirpan	6.04	15.06	29.06	11.08	73	14	43	127
Chirpan	4.04	16.06	3.07	15.08		17	42	132

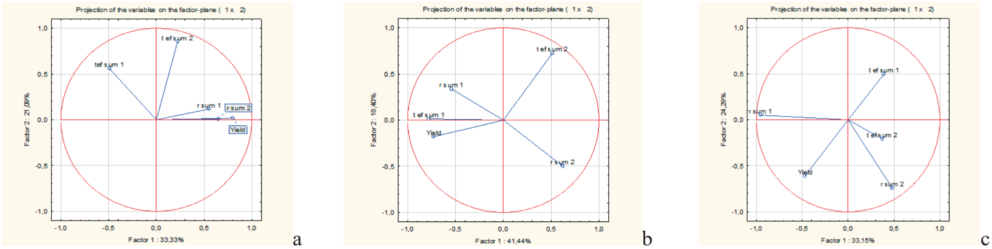

Figure 7. Principal Components Analysis for Karnobat a), G. Toshevo b) and Chirpan c)

The dates of phenological stages occurrence reported by us in comparison with the average dates of occurrence of the same stages for the last 30 years shows a deviation in the real vegetation period in Karnobat, but no more than 10%. Analysis of the duration of inter-stages periods shows deviations corresponding to differences in agrometeorological conditions. In G. Toshevo (2021 and 2023) and Karnobat (2022 and 2023) an extension of the leaves formation was observed.

The analysis of the results indicates that the yield of grain in Karnobat is most strongly influenced by the amount of precipitation during the entire vegetation period, as precipitation has a positive effect on the growth and development and productivity of the crop, and by the sum of the effective temperatures during the period from flowering to maturation. In General Toshevo, the impact of the sum of effective temperatures and the sum of precipitation on yields from sowing to flowering is decisive, their role is positive, and from flowering to maturity, there is a possibility that they have a negative effect during flowering. In Chirpan, as in Karnobat, yields depend on the amount of precipitation during the growing season, as the amount from sowing to flowering is decisive.

As a result of the conducted research and numerical experiments, we established the role and importance of temperatures, respectively the temperature sums of the effective temperatures and the sum of precipitation during the vegetative and reproductive periods of the development of sunflower crops in our country.

CONCLUSIONS

The temperature conditions in our country in all three locations are favorable for sowing and growing sunflower;

The mode of atmospheric humidification largely determines the productivity of sunflower crops, and the analysis indicates that in all three locations the amount of precipitation during the period from sowing to flowering is decisive;

In General Toshevo, the role of the sum of the effective temperatures from sowing to flowering is firstly decisive, and then the sum of precipitation during this period;

The differences of the main components in G. Toshevo compared to Karnobat and Chirpan are natural and are due to the fact that they are located in different climatic areas;

Due to the possibility of prolonged drought in Chirpan from July to September, and not infrequently until October-November, irrigation measures should be taken during the period of yield formation, and also a precise selection of hybrids should be made in view of their durability of drought.

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THE INFLUENCE OF SEED TREATMENT ON GRAIN YIELD AND THEIR QUALITY IN SOME VARIETIES AND LINES OF WINTER WHEAT (*Triticum aestivum* L.)

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Abstract

Wheat crops are affected by various pathogens and pests, which can cause considerable production losses, therefore it is essential to carry out seed treatments. In our study, the biological material was represented by the Trivale variety, being an older variety, resistant and adapted to specific soil conditions, and two new wheat lines. This paper presents results obtained during one year, regarding the effect of seed treatment and additional fertilization on the production and quality of the Trivale wheat variety and lines A4-10 and A44-13. The use of fertilizers is important because it causes qualitative and quantitative changes. The experimental factors studied, the genotype, the seed treatment and the fertilization, generated statistically assured interactions.

Key words*: quality, fertilization, wheat, production, seed, treatment.*

INTRODUCTION

The production capacity is a complex and fundamental property of any wheat variety, largely determined by the genetic material, the applied cropping technology, but also by the influence of climatic conditions (Leonte, 2011). One of the main problems encountered is related to the ability to adapt winter wheat genotypes to a complex of factors that are constantly changing (Shah et al., 2018).

In general, tillage, crop rotation, sowing date and nitrogen fertilization are considered very important tools in the control of crop plant diseases, and they must be optimized in relation to climatic conditions (Cotuna et al., 2018). Low soil fertility and increased incidence of diseases and pests pose a threat to food security (Mubeen et al., 2006). The wheat crop is affected by a large number of pathogens, which leads to huge production losses, up to 40% (Cardozo et al., 2024). Among these diseases, *Blumeria graminis* f. sp. *tritici* stands out, which is one of the most important and widespread diseases (Basandrai et al., 2022).

Wheat seeds need optimal moisture and positive soil temperatures to germinate and to develop during the early growth stages (Abendroth et al., 2017). Seeds chemical treatments is a technological sequence with great effectiveness against the attack of diseases and pests (Racz et al., 2016). Sowing at the optimal time leads to increased yield and helps to avoid unjustified delays, while if sowing is done later than the optimal time, low soil temperatures affect the ability of seed germination and root growth. The amount and availability of water in the soil and the appropriate temperatures during the sowing period are also the limiting factors that affect the optimal plant density and, last but not least, the yield of the wheat crop and the quality of the production (Donatelli et al., 2012).

Climate change, the multiplication and spread of invasive species, biotic and abiotic stress, have over time had an impact on natural systems and continue to represent increasing threats to agriculture, leading to a decrease in crop production (Cotuna et al., 2021). The health status of wheat crops is a permanent concern for research (Iosub et al., 2021). Hatfield and Beres in 2019, showed that thanks to technological advances in genetics and agronomic practices it was possible to increase the yield. The main limiting factor being the favorable conditions during the growing season. Addressing these challenges requires new solutions, both technological and genetic, that contribute to obtaining large and good quality productions (Săulescu et al., 2010).

Within the Agricultural Development Research Station (ARDS) Pitesti from Romania there is a continuous concern regarding the improvement of the production of wheat lines and varieties, as well as their characteristics related to resistance to pathogens. Considering the above, this paper presents a small part of the results obtained during one year regarding the effect of seed treatment and additional fertilization on the yield and production quality of wheat lines A4-10 and A44-13 compared to the Trivale variety in experimental field conditions.

MATERIALS AND METHODS

The research was carried out in the 2022-2023 agricultural year at ARDS Pitești on luvosol type soil, with a low content of nutrients available for plants. This type of soil is characterized by a high content of mobile aluminum ions (0.92-1.39 mg/exchangeable Al/100 g soil) in the arable layer, which causes blocking of mobile phosphorus. That is why calcium carbonate-based amendments were applied (Neutrosol, $CaCO_3$ > 95%; Holcim.ro), 9.2 t/ha. Chemical weed control was carried out at the end of wheat twining using the systemic product Axial One (45 g/l pinoxaden + 5 g/l + florasulam + 11.25 g/l cloquintocet - mexil; Syngenta.ro), 1 l/ha . The seed treatment (for the treated variants) was carried out with the insecto-fungicide Austral Plus (40 g/l tefluthrin + 10 g/l fludioxonil; Syngenta.ro), 5 l/t. The biological material used in the experiments: the Trivale wheat variety, line A4-10 and line A44-13, all belonging to ARDS Pitesti. The experiment was organized according to the method of randomized blocks, in 4 repetitions, the harvestable plot being 6 m^2. The protein content (P%) was determined using the Inframatic IM 9500 device. The obtained data were statistically processed using the PoliFact program.

RESULTS AND DISCUSSIONS

The 2022-2023 agricultural year began with the month of September, whose average temperature was lower than the multiannual average, with a negative thermal deviation of -0.4 °C. The following 5 months had positive thermal deviations: October +1.8°C; November +2.8°C; December +2.4°C; January +5.3°C; February +3.4°C; March 2.4°C. In the months of April and May, the temperatures were colder, the average temperature being lower than the multiannual average with negative thermal deviations, and in the month of July a positive thermal deviation of +0.5°C was registered. The average temperature of the period recorded a positive thermal deviation of 1.8°C, compared to the multiannual average of the period (Figure 1). The precipitation values recorded in the months of September-June, with the exception of January, were below the multi-year average. The month of January was very rainy, with an excess of 75.6 mm compared to the multiannual average of the month (Figure 2).

The period September 2022-June 2023 was characterized as a warm period, with a positive thermal deviation of 1.8°C compared to the 40-year multiannual average and a precipitation deficit of -71.3 mm. Wheat is a cereal rich in nutrients that are necessary for human food, carbohydrates, proteins, lipids, but also for animal feed (Boruga et al., 2016). In Table 1, it can be observed that regarding the influence of the variety on the wheat production, both for A4-10 line and A44-13 line, the differences are distinctly significantly positive compared to the Trivale control.

Sowing in colder soils delays wheat emergence, so seed treatment is really necessary (Mureșan et al., 2020). Our experimental results highlighted that the treatment of the seed had a very significant positive influence on wheat production compared to the untreated variant (Table 2).

The most widely used fertilizers, globally, are those with nitrogen, this nutrient being essential for the growth and development of plants (Datcu et al., 2019). In Table 3, it can be seen that additional fertilization brings an increase in wheat production in the pedoclimatic conditions of ARDS Pitesti. Thus, the production difference compared to the control is very significantly positive.

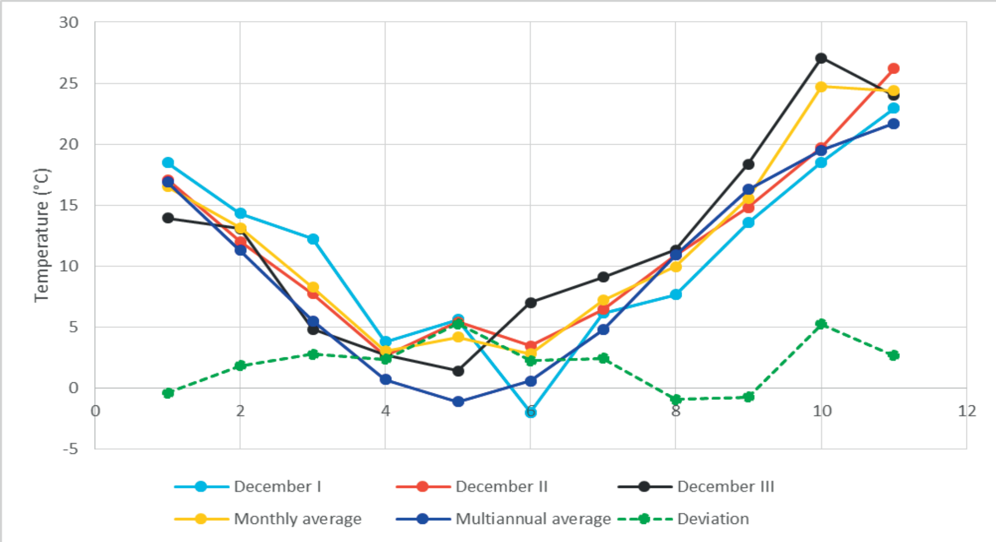

Figure 1. Average monthly temperatures recorded during September 2022-June 2023 at ARDS Pitesti

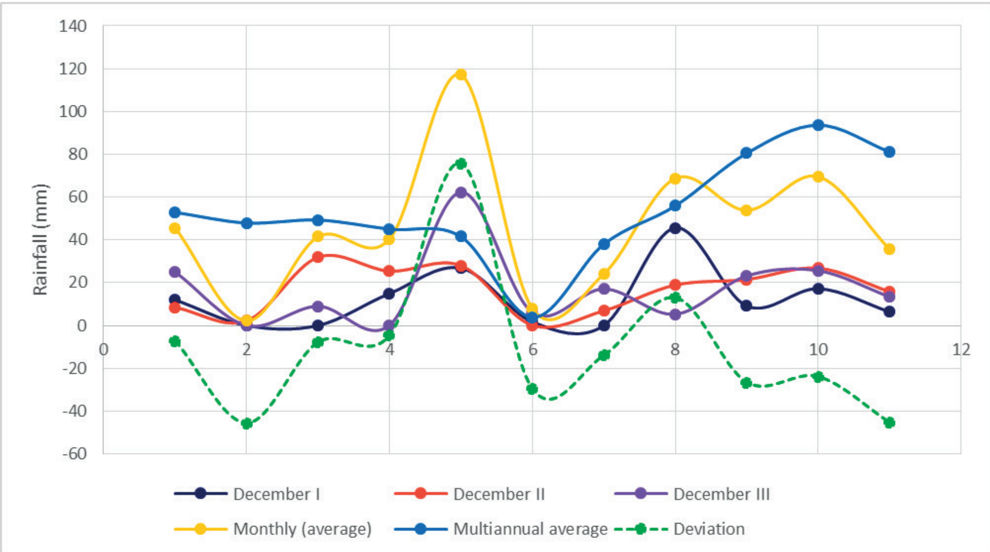

Figure 2. Average monthly rainfall recorded during September 2022-June 2023 at ARDS Pitesti

Table 1. The influence of genotype on wheat production

Genotype	Production (kg/ha)	%	Difference	Significance
Trivale	7440.13	100.0	0.00	Control
Line A4-10	8309.75	111.7	869.63	**
Line A44-13	8274.00	111.2	833.88	**
	DL (5%)		480.58	
	DL (1%)		727.73	
	DL (0.1%)		1169.08	

Table 2. The influence of seed chemical treatment on wheat production

Treatment	Production (kg/ha)	%	Difference	Significance
Untreated seed	7626.29	100.0	0.00	Control
Treated seed	8389.63	110.0	763.33	***
	DL (5%)	138.20		
	DL (1%)	198.74		
	DL (0.1%)	292.30		

Table 3. Influence of nitrogen doses on wheat production

Fertilizer rates	Production (kg/ha)	%	Difference	Significance
120 kg N/ha	7855.92	100.0	0.00	Control
160 kg N/ha	8160.00	103.9	304.08	***
	DL (p 5%)	159.24		
	DL (p 1%)	218.38		
	DL (p 0.1%)	297.24		

Regarding the influence of the genotype on the protein content, the A4-10 line recorded a significantly negative difference compared to the control (Trivale variety), while the A44-13 line recorded a highly significant positive difference (Table 4).

Table 4. Influence of variety on protein content

Genotype	Protein (%)	%	Difference	Significance
Trivale	14.15	100.0	0.00	Mt.
A4-10	14.18	98.1	-0.27	0
A44-13	15.11	104.5	0.66	***
	DL (5%)	0.25		
	DL (1%)	0.37		
	DL (0.1%)	0.60		

In the variants in which the seed was treated, an increase in protein was achieved in the grains, with a distinctly significant difference compared to the variants in which the seed was not treated (Table 5).

Table 5. Influence of seed treatment on protein content of wheat grains

Treatment	Protein (%)	%	Difference	Significance
Untreated	14.48	100.0	0.00	Mt.
Treatated	14.68	101.3	0.19	**
	DL (5%)	0.13		
	DL (1%)	0.19		
	DL (0.1%)	0.27		

Improving wheat productivity and grain quality remains a major concern because, in general, these traits are negatively correlated (Dobre et al., 2016). The protein content of wheat grains is influenced by the nitrogen content of the soil. Corresponding to the data presented in Table 6, due to the negative correlation between production and protein content, an insignificant difference is observed.

Powdery mildew is one of the main diseases of wheat, being present in all cultivated areas and producing quantitative and qualitative production losses by shrinking the grains (Cotuna et. al., 2015). In the case of additional fertilization, a higher frequency of the attack produced by the fungus is observed, the variants registering a very distinctly significant difference compared to the control (Table 7).

Table 6. Influence of nitrogen doses on wheat grain protein content

Fertilizer rates	Production (kg/ha)	%	Difference	Significance
120 kg N/ha	7855.92	100.0	0.00	Control
160 kg N/ha	8160.00	103.9	304.08	***
	DL (p 5%)	159.24		
	DL (p 1%)	218.38		
	DL (p 0.1%)	297.24		

Table 7. Interactions between fertilization rates and seed treatment on tillering

Fertilizers doses/Treatment	F (%)	%	Difference	Significance
120 kg N/ha / Untreated	124.67	100.0	0.00	Mt.
160 kg N/ha / Untreated	143.67	115.2	19.00	***
120 kg N/ha / Treated	83.67	100.0	0.00	Mt.
160 kg N/ha / Tratated	100.00	119.5	16.33	***
	DL (p 5%)	4.93		
	DL (p 1%)	6.77		
	DL (p 0.1%)	9.21		

CONCLUSIONS

Additional nitrogen fertilization and chemical seed treatment ensure superior expression of the production and quality potential of wheat genotypes. The production losses determined by the occurrence of powdery mildew vary from one year to another depending on the climatic conditions, as well as the nitrogen supply, the variant registering very significantly positive differences compared to the control dose.

The combined influence of genetic and technological factors, highlights a distinctly significant positive difference in yield for lines A4-10 and A44-13 and a highly significant positive difference in protein content of wheat grains in line A44-13.

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COMPARATIVE ANALYSIS OF VARIOUS WINTER WHEAT VARIETIES CULTIVATED UNDER THE CLIMATIC CONDITIONS OF ARDS BRAILA

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Abstract

In the context of ensuring better food security and in line with the objectives of the field crop research strategy, increasing wheat production capacity remains a priority. This has been achieved by studying a set of local and foreign winter wheat varieties to test their genetic potential in specific pedoclimatic conditions of the ARDS Braila area of influence, respectively, the North-East Baragan. During the agricultural years 2020-2021 and 2021-2022, a total of 14 varieties of autumn wheat were tested under conventional technology conditions. Yield levels under the conditions of an optimal rainfall agricultural year 2020-2021 were satisfactory for the wheat crop, and yields in the range of 5582-7994 kg/ha were obtained. In the agricultural year 2021-2022, characterized by very dry rainfall throughout the year, with a total deficit of 155.7 mm compared to the multi-year average, wheat yields were in the range of 5435-6746 kg/ha.

Key words: *winter wheat, pedoclimatic conditions, yield.*

INTRODUCTION

Wheat is the most widely grown crop globally, covering 220.76 million hectares, followed by maize at 205.87 million hectares, and rice at 165.25 million hectares. Wheat accounts for a third of total grain production, estimated at 770 million tons, ranking below rice at 787 million tons and maize at 1,210 million tons (FAOSTAT, 2021; Gulino et al., 2023). Wheat yield is a multifaceted genetic variable influenced by numerous genes that determine both the potential yield and how much of that potential is achieved when the crop faces specific environmental challenges. (Curtis & Halford, 2014).

Wheat with enhanced physiological characteristics is cultivated in high-rainfall and irrigated wheat-producing regions worldwide, potentially increasing global wheat production by 37%, as demonstrated in Argentina and Chile. Worldwide wheat production and yield might potentially double if limited solely by solar radiation, but achieving this is a challenging task on a worldwide level. A 37% increase in world wheat production would suffice to meet the lower estimate of the predicted future grain demand in 2050 without the need to extend the current agricultural cropping area (Guarin et al., 2022).

Choosing the appropriate variety and growth techniques according to the environmental conditions of the year is crucial for achieving consistent yields. Wheat's morphological traits are affected mainly by the variety and environmental conditions rather than the sowing density. Productivity factors are impacted by planting density, variety, and experimental conditions (Melucă et al., 2021).

High-yielding wheat genotypes that lack stability across different settings may be recommended for specific environments where they have shown good performance. This information would assist wheat producers and breeders in choosing suitable wheat cultivars that can thrive in less fertile soil conditions. (Ljubicic et al., 2021)

Developing high-yielding, environmentally adaptable winter wheat cultivars with strong adaptive capabilities is crucial for mitigating the adverse impact of climate change on crop output and guaranteeing food security for nations. One common way for determining the traits of high-yielding varieties is to compare and analyze the parameters of different varieties using certain indicators. Analyzing data can help pinpoint physiological trends linked to high yields (Morgun et al., 2008; Morgun et al. 2019)

The objective of this paper is to present the results obtained for 14 varieties of autumn wheat studied under field conditions in ARDS Braila during two agricultural years, 2020-2021 and 2021-2022, respectively, and to study the evolution of temperatures and rainfall compared to the multiannual average of the region.

MATERIALS AND METHODS

The study was carried out during the agricultural years 2020-2021 and 2021-2022 to evaluate the capacity of 14 genotypes of domestic and foreign autumn wheat to adapt to local conditions. Yield capacity and quality elements such as hectolitre mass HM (kg/hl) and thousand kernel weight TKW (g) were studied for all varieties tested.

The study and field experiments were organized in the EC Chiscani of ARSD Braila on a carbonate vermic chernozem soil with an apparent density ranging from 1.19 g/cm^3 in the worked horizon (Ap) to 1.44 g/cm^3 in the other soil horizons.

Regarding the chemical characteristics of the soil profile in the experimental perimeter of SCDA Brăila, the content of mobile phosphorus, with values ranging from 41 ppm to 62 ppm, the soil falls into the medium and is well supplied with phosphate. Mobile potassium supply is medium, with values ranging from 98 ppm to 108 ppm, and the humus content in the worked layer is 3.04% (Trifan D. et al., 2021).

In both years of experimentation, the technology applied was classical, the basic work being ploughing. Base fertilization was done with NPK 18:48:0 complex fertilizer at a rate of 200 kg/ha, and phase fertilization was done with slow-release urea 200 kg/ha in one application, having a prolonged availability for the plants.

Sowing was done on 7 October in the first year of experimentation and on 13 October in the second year at a density of 500 germinable grains per square metre.

The experimental variants were randomized within each block to eliminate data errors variants influence and effect of neighboring.

Analysis of variance (ANOVA) was performed to examine differences and a Fisher's protected least significant difference (LSD) test was used to determine the significance of the differences among the variants results and control (p-values 0.05, 0.01, and 0.001).

RESULTS AND DISCUSSIONS

Climatic aspects

Overall, the agricultural year **2020-2021** was optimal: precipitation totaled 589 mm with a positive deviation of 147 mm from the multi-year monthly average of 442 mm (Table 1, Figure 1).

Autumn was moderately supplied from precipitation, with the months analyzed, October and November, cumulating 52.1 mm, 11.9 mm less than the two-month average.

Winter well supplied with 116.6 mm, 25.6 mm above the multi-season average of 91 mm;

Spring was evenly and well supplied from precipitation, accumulating 160.6 mm precipitation, 51.6 mm above the multi-year average of 109 mm;

Summer was rich in precipitation, with 250.9 mm accumulated and a deviation of +103.9 mm from the multiannual average; June was excessively rainy, recording 173.8 mm and a deviation of +118 mm from the multiannual monthly average.

Table 1. The main climatic elements of agricultural year 2021-2022

Climatic elements		Month values												Total/ Average
		X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	
Precipitation (mm)	Agricultural year 2020-2021	26,6	24,5	68	41,2	7,4	31,4	53,3	75,9	173,8	40,4	36,7	10	589
	Normal	30	33	36	28	27	26	35	48	62	46	39	32	442
	Deviation ±	**-3,4**	**-8,5**	**32**	**13,2**	**-19,6**	**5,4**	**18,3**	**27,9**	**111,8**	**-5,6**	**-2,3**	-22	+147
Air Temperature (°C)	Agricultural year 2020-2021	15,1	5,7	4,7	2,2	2,4	4,7	9,4	16,7	20,2	23,9	23,4	16,9	12,1
	Normal	11,5	5,6	0,6	-2,1	-0,2	4,7	11,2	16,7	20,9	22,9	22,1	17,3	10,9
	Deviation ±	**3,6**	**0,1**	**4,1**	**4,3**	**2,6**	**0**	**-1,8**	**0**	**-0,7**	**1**	**1,3**	-0,4	+1,2

Source: Meteorological Station of Braila

Analyzing the thermal regime for the agricultural year 2020-2021, it was found that the annual average air temperature of 12.1°C exceeded the multi-year average of 10.9°C by 1.2°C, which characterizes the agricultural year as very hot (Table 1, Figure 1). In terms of temperature, autumn was warmer than normal for the area; winter was excessively warm by 3.6°C above the seasonal average; spring was cooler than normal by 0.6°C, and summer recorded a deviation of +0.5°C.

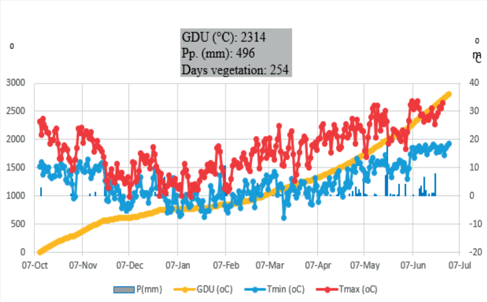

Figure 1. Presentation of climatic conditions and the sum of degrees of useful temperature during the vegetation period for wheat, at ARDS Braila, in 2022

In the 2020-2021 crop year the wheat crop completed 254 growing days in which 496 mm of precipitation accumulated and the sum of useful degrees was 2314°C.

The analysis of the rainfall regime for the 2021-2022 agricultural year allows us to specify the following features:

The autumn of 2021, through October and November, provided a rainfall of 60.2 mm, typical for this period, with a deficit of only 2.8 mm compared to the multiannual average for these months (63 mm).

Table 2. The main climatic elements of agricultural year 2021-2022

Climatic elements		Month values												Total/ Average
		X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	
Precipitation (mm)	Agricultural year 2021-2022	33.1	27.1	43.8	6.5	11.5	13.8	25.1	24.3	33.3	8.9	26.9	32	**286.3**
	Normal	30	33	36	28	27	26	35	48	62	46	39	32	**442**
	Deviation ±	**3.1**	**-5.9**	**7.8**	**-21.5**	**-15.9**	**-12.2**	**-9.9**	**-23.7**	**-28.7**	**-37.1**	**-12.1**	**0**	**-155.7**
Air Temperature (°C)	Agricultural year 2021-2022	10.2	8.1	2.5	1.3	4.1	3.8	11.9	18	22.7	24.8	24.9	17.9	**12.5**
	Normal	11,5	5,6	0,6	-2,1	-0,2	4,7	11,2	16,7	20,9	22.9	22.1	17.3	**10.9**
	Deviation ±	**-1.3**	**2.5**	**1.9**	**3.4**	**4.3**	**-0.8**	**0.7**	**1.3**	**1.8**	**1.9**	**2.8**	**0.6**	**+1.5**

Source: Meteorological Station of Braila

The analysis of the rainfall regime for the 2021-2022 agricultural year allows us to specify the following features (Table 2, Figure 2):

The autumn of 2021, through October and November, provided a rainfall of 60.2 mm, typical for this period, with a deficit of only 2.8 mm compared to the multiannual average for these months (63 mm).

Winter started with a wet December, with 43.8 mm of rainfall, 7.8 mm above the multiannual average. However, January and February recorded deficits of 21.5 mm and 15.9 mm, respectively. Overall, winter was also poorly supplied by rainfall, with a total deficit of 29.6 mm compared to the multiannual average of 91 mm.

The spring of 2022 brought a deficient water supply, totalling 63.2 mm, 45.8 mm below the multiannual average (the multiannual average is 110 mm). The months of March, April, and May were dry, with rainfall deficits ranging from 9.9 to 23.7 mm.

Summer 2022 started with June providing 33.3 mm of rainfall, 28.7 mm below the multi-year average of 62 mm. July recorded only 8.9 mm with a deficit of 37.1 mm, and August recorded 26.9 mm and a deficit of 12.1 mm. September was generally supplied with precipitation (32 mm)

The particularities of the thermal regime specific to the 2021-2022 agricultural year show that in autumn 2021, October was cooler than the multiannual monthly average by 1.3°C, and November was very warm, exceeding the multiannual monthly average by 2.5°C.

Winter was warm, recording a positive deviation of 3.2°C from the multiannual seasonal average. Spring 2022 as a whole, with an average temperature of 11.3°C, exceeding the spring multi-year (10.9°C) by 0.4°C, can be characterised as a near normal season. Summer 2022, with an average temperature of 24.1°C exceeded the multiannual seasonal average by 2.1°C being very warm. On the whole, the period analyzed can be characterized from a rainfall point of view as very dry throughout the agricultural year, with a total deficit of 155.7 mm compared to the multi-year average. From a thermal point of view, it is warm in winter and the first month of summer and close to normal in autumn and spring. Under the conditions of the very dry agricultural year 2021-2022 it was necessary to apply irrigation at an average rate of 400 m^3/ha.

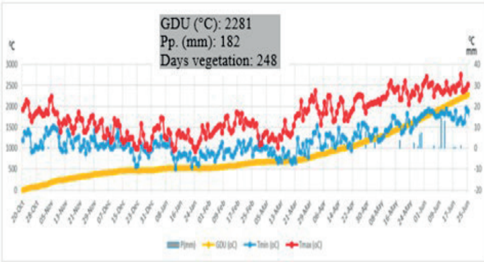

Figure 2. Presentation of climatic conditions and the sum of degrees of useful temperature during the vegetation period for wheat, at ARDS Braila, in 2022

In the 2021-2022 crop year the wheat crop completed 248 growing days in which 182 mm of precipitation accumulated and the sum of useful degrees was 2281°C.

Grain yield

Analysis of production potential for all genotypes of winter wheat studied (Table 3) showed higher values in the crop year 2020-2021 with an average yield of 6778 kg/ha under conditions of a well-supplied rainfall year, and in the crop year 2021-2022, yield levels were lower influenced by the drier crop year.

Table 3. Grain yield obtained in winter wheat genotypes, 2021-2022

Varieties	Yield 2021 kg/ha	Yield 2022 kg/ha	Yield Average kg/ha	(%)	Diff. by control	Significance
Glosa	6288	6048	6168	97.2	-175	-
Boema	5582	6121	5851	92.2	-492	°°°
Miranda	7125	6098	6611	104.2	268	-
Pitar	6540	5626	6083	95.9	-260	-
Potential	6364	6469	6416	101.2	73	-
Apache	7994	6746	7370	116.2	1027	***
Athlon	5687	5724	5706	89.9	-638	°°°
Andino	6311	6322	6317	99.6	-27	-
Altezza	7880	5467	6674	105.2	331	*
Forhand	6265	5521	5893	92.9	-450	°°
Genesi	6288	5746	6017	94.9	-326	°
Jaguar	7740	5549	6645	104.7	301	*
Iris	7036	5846	6441	101.5	98	-
Angelica	7795	5435	6615	104.3	271	*
Average (control)	**6778**	**5908**	**6343**	**Mt.**	**Mt.**	
LSD 5% = 268,31 kg/ha, LSD 1% = 357,56 kg/ha, LSD 0,1% = 465,53 kg/ha						

Yield values recorded in 2020-2021 ranged from 5582 kg/ha for the Boema variety to 7994 kg/ha for the Apache variety, and in the 2021-2022 crop year, yield values ranged from 5435 for the Angelica variety to 6746 kg/ha for the Apache variety.

The average yield recorded in the two test years varied between 5706 kg/ha for the variety and 7370 kg/ha for the variety Apache.

Compared to the average of 6343 kg/ha of the two years tests, a very significant positive result was recorded for Apache with a difference from the average of 1027 kg/ha, and significantly positive results for Altezza, Jaguar, and Angelica with differences from the average between 271 and 331 kg/ha. The varieties Boema and Athlon recorded very significant negative results compared to the average of the tests, the variety Forhand a distinctly significant negative result, and the variety Genesi a significant negative result.

Table 4. Thousand-kernel weight (TKW) results 2021-2022

Varieties	TKW 2021 (g)	TKW 2022 (g)	TKW Average (g)	(%)	Diff. by control	Significance
Glosa	44.77	44.73	44.75	109.4	3.83	***
Boema	44.10	41.50	42.80	104.6	1.88	**
Miranda	46.43	40.73	43.58	106.5	2.66	***
Pitar	44.36	41.37	42.87	104.8	1.95	**
Potential	37.86	36.23	37.05	90.5	-3.87	°°°
Apache	36.53	35.80	36.16	88.4	-4.75	°°°
Athlon	42.53	38.33	40.43	98.8	-0.49	-
Andino	47.07	41.60	44.34	108.4	3.42	***
Altezza	40.69	43.00	41.85	102.3	0.93	-
Forhand	44.33	42.33	43.33	105.9	2.41	***
Genesi	34.98	35.33	35.16	85.9	-5.76	°°°
Jaguar	39.26	38.81	39.04	95.4	-1.88	°°
Iris	38.29	37.67	37.98	92.8	-2.94	°°°
Angelica	43.86	43.23	43.55	106.4	2.63	***
Average (control)	**41.79**	**40.05**	**40.92**	**Mt.**	**Mt.**	
LSD 5% = 1.25 g, LSD 1% = 1.67 g, LSD 0,1% = 2.17 g						

Thousand kernel weight (TKW) values for the analyzed autumn wheat varieties (Table 4) ranged between 34.98 and 47.07 g in 2021, with the highest value recorded for the variety Andino. In 2022, TKW values ranged between 35.33 and 44.73 g, with the best result recorded for the variety Glosa.

The average TKW values over the two years of testing ranged from 35.16 g for Genesi to 44.75 g for Glosa, a Romanian variety grown in large areas in the country and with superior stability and quality.

The average of 2021 and 2022 TKW was 40.92 g, and very significant positive results compared to the average were recorded for Glosa, Miranda, Andino, Forehand, and Angelica, and distinctly significant positive results for Boema and Pitar. Highly significant negative values were also recorded for Potential, Apache, Genesi, and Iris, and a distinctly significant negative result was recorded for Jaguar.

In 2021, **the hectolitre mass (HM)** values for the autumn wheat varieties analyzed (Table 5) ranged from 69.23 to 76.73 kg/hl, with the highest value recorded for the variety Glosa. In 2022, the HM values ranged from 79.10 to 84.43 kg/hl, with the best result recorded for the variety Boema.

Table 5. Hectolitre mass results 2021-2022

Varieties	HM 2021 (kg/hl)	HM 2022 (kg/hl)	HM Average (kg/hl)	(%)	Diff. by control	Significance
Glosa	76.73	83.87	80.30	104	3.07	***
Boema	76.60	84.43	80.52	104.2	3.28	***
Miranda	74.90	81.67	78.28	101.4	1.05	*
Pitar	76.23	83.53	79.88	103.4	2.65	***
Potential	73.13	79.10	76.12	98.6	-1.12	°°
Apache	72.87	79.37	76.12	98.6	-1.12	°°
Athlon	71.87	80.37	76.12	98.6	-1.12	°°
Andino	72.90	81.63	77.27	100	0.03	-
Altezza	74.40	81.27	77.83	100.8	0.60	-
Forhand	74.20	81.40	77.80	100.7	0.57	-
Genesi	69.23	78.43	73.83	95.6	-3.40	°°°
Jaguar	70.00	80.20	75.10	97.2	-2.13	°°°
Iris	72.10	80.27	76.18	98.6	-1.05	°
Angelica	72.00	79.87	75.93	98.3	-1.30	°°
Average (control)	**73.37**	**81.10**	**77.23**	**Mt.**	**Mt.**	
LSD 5% = 0.86 kg/hl, LSD 1% = 1.11 kg/hl, LSD 0,1% = 1.45 kg/hl						

Average HM values over the two years of testing ranged from 73.83 g in Genesi to 80.52 g in Boema.

The average HM in 2021 and 2022 was 77.23 g, and there were very significant positive results compared to the average in Glosa, Boema, and Pitar and significant in Miranda. There were also very significant negative values compared to the average for Genesi and Jaguar, distinctly significant negative values for Potential, Apache, Athlon, and Angelica, and a significant negative result for Iris.

CONCLUSIONS

The crop year 2020-2021, characterized by well-supplied rainfall, has exhibited higher grain yield than the drier crop year 2021-2022. This highlights the significant influence of environmental factors, particularly rainfall, on wheat yield.

Varieties such as Apache consistently showed higher yields across both years, while others like Boema and Angelica exhibited lower yields. This suggests genetic variability in yield potential among the studied wheat varieties.

Apache showed a very significant positive deviation from the average yield of the two test years, indicating its superior performance. Conversely, varieties like Boema, Athlon, and Forhand demonstrated significant negative deviations from the average, indicating poorer performance.

TKW values varied among different varieties and years, with some varieties consistently exhibiting higher TKW, such as Glosa and Andino, while others, like Genesi and Jaguar, had lower TKW. This indicates varietal differences in seed size and density.

Similar to TKW, HM values varied over many years. Glosa and Boema consistently showed higher HM values, while Genesi and Jaguar had lower values. This suggests differences in kernel density and grain quality among varieties.

Glosa consistently exhibited superior performance in terms of both TKW and HM, indicating its stability and quality across different environmental conditions. This suggests that Glosa could be a promising variety for cultivation due to its consistent performance.

The variations observed in grain yield, TKW, and HM among different varieties underscore the importance of genetic factors and environmental adaptation in determining wheat productivity and quality.

In summary, the study highlights the significant influence of genotype and environmental factors on wheat yield and quality, with certain varieties showing superior performance across multiple metrics. These findings could be valuable for wheat breeding programs and agricultural practices to improve productivity and resilience in varying environmental conditions.

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SOME AGROBIOLOGICAL PECULIARITIES AND QUALITY INDICES OF BIOMASS OF *Macleaya cordata* 'MIHAELA'

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Abstract

Plume poppy – Macleaya cordata (Willd.) R.Br., Papaveraceae family is a perennial herbaceous species with medicinal, melliferous, ornamental and energy biomass utility. The harvested stem biomass from Macleaya cordata can be used as feedstock for the production of solid fuel – briquettes and pellets. The objective of this research was to evaluate some agrobiological peculiarities and quality indices of biomass of Macleaya cordata 'Mihaela' grown in experimental field of the NBGI MSU Chişinău, Republic of Moldova. At the end of the flowering period, Macleaya cordata shoots reach 303-320 cm in height, with 35.8-38.2% leaf share. The content of alkaloids in Macleaya cordata leaves was: 7.36-8.12 mg/g sanguinarine, 5.91-6.82 mg/g chelerythrine and 0.659-0.757 mg/g fagaridine. It has been determined that the stem dry matter productivity of Macleaya cordata harvested in August was 1.19-1.40 kg/m^2, but – of plants harvested in November – 1.67-1.79 kg/m^2. The comparative analysis of cell wall components revealed that Macleaya cordata substrate contained 406-503g/kg cellulose, 212-243 g/kg hemicellulose and 91-104 g/kg acid detergent lignin, the estimated theoretical ethanol yield from cell wall carbohydrates averaged 448.7-541.8 L/t in Macleaya cordata substrates.

Key words: *agrobiological peculiarities, alkaloids, biomass, gross calorific value, Macleaya cordata 'Mihaela' theoretical ethanol potential.*

INTRODUCTION

The introduction and adaptation of new plant species would help meet the actual demands for food, forage, fibers, fuel, pharmaceuticals, chemicals and other important raw materials, and also could stimulate economic growth.

M*acleaya cordata* (Willd.) R. Br. is a species in the family Papaveraceae, which includes 44 genera and 825 species, most of them are herbaceous plants, distributed mostly in the temperate regions of the northern hemisphere.

This botanical family includes many cultivated oilseed crops, others are very important as medicinal and ornamental species.

The genus *Macleaya* consists of 2 species: M*acleaya cordata* (Willd.) R. Br. and *Macleaya macrocarpa* (Maxhn.) Fedde, besides, there is the hybrid *Macleaya cordata x Macleaya macrocarpa*, also mentioned in the specialized literature as *Macleaya x kevensis* Turrill. (Abizov, 2004; Bykova et al., 2023).

Macleaya cordata (Willd.) R. Br. = *syn. Bocconia cordata Willd.* syn. *Bocconia cordata* Willdenow, *Bocconia japonica* J.N. Haage & E. Schmidt; *Marzaria cordata* (Willd.) Raf., *Marzaria cordata* (Willd.) Raf., fam. Papaveraceae, is a perennial species with laticifers, native to South-East Asia, namely, to China and Japan. It is an herbaceous plant with cylindrical stem, erect, glaucous, slightly branched in the upper part, growing 230-356 cm tall, green, covered with a gray waxy layer, at the base – strongly lignified, bluish-orange in color.

The leaves are alternate, with a 1-12 cm long petiole, cordate, with 5-7 lobes, those growing at the base of the shoot are 25-35 cm long and 13-20 cm wide, those at the top are much smaller, 12-15 cm long and 6-9 cm wide, the upper part of the leaf blade is glabrous, brownish-green to gray-green, and the underside is pubescent, gray or gray-yellow.

The flowers are small, 6-7 mm, greyish yellow, with cream or orange shades, grouped in erect airy panicles, up to 40 cm long, located at the top of the stems. The flowers are hermaphrodite, actinomorphic, with 2 deciduous sepals and 4 petals. *M. cordata* blooms in July-August and produces fruits in September. The fruit is a flat obovate capsule, up to 8 mm long and up to 4 mm wide, glabrous, attenuate at the base and rounded or obtuse at the apex, with 4-6 ovoid seeds, 1.5-2.0 mm long. The weight of 1000 seeds is 0.6-0.8 g. The root system is a taproot, the plant has rhizomes and numerous dark orange adventitious roots. The rhizome of the plant is vertical, located at a depth of 10-13 cm, short, with numerous buds. The lateral roots are branching and grow mostly horizontally, reaching down to 50 cm deep in the soil. Most of the roots are found in the arable soil layer. One-year-old roots and rhizomes are flexible, dark orange with numerous adventitious roots. Starting in the autumn of the first year of life, adventitious buds are formed on the lateral roots, located in groups, from which root shoots emerge. The younger the rhizome, the smaller and denser the buds are on it. *Macleaya* reproduces by seeds, seedlings and pieces of rhizomes. It is a thermophilic species, so, young shoots can be affected by spring frosts of -3...-4°C. In spring, the plants come out of dormancy at an average temperature of 10°C. Under a snow layer, the plants can withstand temperatures of -20…-25°C. In autumn, when temperatures below 0°C are recorded, the leaves fall off completely. It is a mesophilic plant and makes very good use of the water accumulated in the soil during the dormancy period and from the rains that fall during the growing season. *Macleaya cordata* plants hardly tolerate high temperatures (over +30°C), particularly in the bud formation and flowering periods. Generally, the plants are characterized by low seed productivity, associated with low pollen viability and severe fruit shedding during ripening. Therefore, vegetative propagation of the crop is mostly used for industrial cultivation. It is not demanding to soil, thanks to its deep root system and has high capacity of solubilization and absorption of nutrients (Ţîţei & Roşca, 2021). According to the specialized literature, thanks to this fact, it is able to grow well even on lands with low fertility, with pH = 6.5-8.5, it can be cultivated on degraded soils, fertilized with organic fertilizers and sewage sludge, the plants react to the application of growth stimulators by increasing productivity and the concentration of alkaloids (Sidelnikov, 2014; Lin et al., 2018; Yakhtanigova et al., 2022; Bykova et al., 2023).

Macleaya cordata contains many biologically active compounds. Scientific studies have identified 147 alkaloids, most of these compounds are isoquinoline alkaloids, including sanguinarine, chelerythrine, protopine and allocryptopine (Lin et al., 2018). It is widely used in traditional Chinese medicine for the treatment of injuries, arthritis, rheumatic arthralgia and trichomonas vaginalis. In North America and Europe, *Macleaya cordata* is also considered as a traditional medicinal plant used as a remedy for insect bites and ringworm infection. Extracts from *Macleaya cordata* and their components have many biological properties, such as anti-microbial, anti-fungal, pesticidal and anticancer properties (Satou et al., 2002; Abizov, 2004; Frolova, 2005; Stiborová et al., 2008; Li, 2012; Wang et al., 2012; Baek et al., 2013; Liu et al., 2013; Li et al., 2015; Lin et al., 2018; Liu et al., 2023).

Current studies have shown that *Macleaya cordata* stimulates the growth of animals and poultry, as a feed additive, it can replace antibiotics and is an all-natural feed additive (Köroğlu & Kocabağli, 2019; Buyarov et al., 2020; Toprak, 2020; Manaa et al., 2022; Wang et al., 2022; Chen et al., 2023; Krzykawski et al., 2023; Ling et al., 2023).

The objective of this research was to evaluate some agrobiological peculiarities and quality indices of biomass of *Macleaya cordata* 'Mihaela'

MATERIALS AND METHODS

The local cultivar 'Mihaela' of *Macleaya cordata*, commonly known as plume poppy, created at the "Alexandru Ciubotaru" National Botanical Garden Institute (NBGI) of Moldova State University (MSU) and grown in the experimental collection of the, Chisinau, Republic of Moldova, served as research subject.

The experiments with *Macleaya cordata* started on the experimental field, in late autumn, by planting the rhizomes at a depth of 7-10 cm. The biological peculiarities were studied in the

4^{th} and 5^{th} growing season. The leaf/stem ratio was determined of the end flowering period by separating the leaves and inflorescences from the stem, weighing them apart and establishing the ratios for these quantities (leaves/stems). The dry matter content was detected by drying samples to constant weight at 105°C. For phytochemical analyses, the plant leaves samples were protected from the impact of sunlight and dried in an air oven at 25-30°C. Then, they were milled in a beater mill equipped with a sieve with a mesh size of 1 mm and some assessments of the bioactive compounds, such as sanguinarine, chelerythrine and fagaridine, were made according to standard procedures reported by Casian et al. (2017; 2019).

As energy biomass, the *Macleaya cordata* stems were collected of the end the flowering period, in August, and also at the end of the growing season, when temperatures below 0°C were recorded, in November, apple tree pruning residues were used as control variant. The harvested stems were chopped and disintegrated in a knife mill with a sieve with the mesh size of 1 mm. To perform the analyses, the biomass samples were dried in an oven at 85°C. After that, the total carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) amounts were determined by dry combustion in a Vario Macro CHNS analyzer. The content of ash was determined at 550°C in a muffle furnace HT40AL according to SM EN ISO 18122; the automatic calorimeter LAGET MS10A with accessories was used to determine the calorific value, according to SM EN ISO 18125. The content of cell walls was evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200. Theoretical Ethanol Potential (TEP) was estimated according to methodology, based on the conversion of hexose and pentose sugars into ethanol (Goff et al., 2010).

RESULTS AND DISCUSSIONS

As a result of the study of the biological peculiarities of *Macleaya cordata*, we observed that, by the end of April, in the first year of vegetation, from the planted rhizomes, at the soil surface the young plants emerged, which, in the first days of June, developed already erect stems that, by the end of the growing season, grew 160-180 cm tall and 3-5 mm thick at the base, with 8-12 leaves. Some plants also produced inflorescences. In the following years, in spring, when the air temperature exceeded 8-10°C, *Macleaya cordata* 'Mihaela' started the growth and development of the new plants from the dormant buds formed on the rhizomes, and then, the plants went through all stages of ontogenetic development. It was observed that in 4th year of vegetation (2022), the plants came out of dormancy in the middle of April, and in 5th year of vegetation (2023) in the first days of April. It has been established that the number of shoots per plants may vary depending on the climatic conditions and the age of plants. Thus, in the 4th year of vegetation there were 5-6 shoots/plant and in the 5th year of vegetation 8-9 shoots/plant. We found that, in May, the growth rate of the stems was 33-45 mm/day, and in June the growth and development intensified even more, reaching values of 56-64 mm/day. In the second half of June, the *Macleaya cordata* plants formed inflorescences and, at the end of June, the flowering stage started. The flowering stage lasted about 27-34 days.

Some biological peculiarities and the structure of the phytomass harvested from *Macleaya cordata* 'Mihaela' at the end of the flowering period are presented in Table 1.

In the 4th year of vegetation, the mass of a shoot reached 737.1 g of fresh matter or 235.3 g of dry matter, the share of leaves being 38.2%, and in the 5th year of vegetation, the mass of a shoot reached 542.2 g of fresh matter, 161.9 g of dry matter, respectively, the leaves representing 35.8%.

Natural products and phytochemicals have always been of great importance in the treatment of various diseases in humans and animals. The accurate determination of the bioactive compounds in the plant mass is very important for the more comprehensive quality control of the raw materials for the pharmaceutical industry. It is generally known that the leaves have a higher and more diverse content of bioactive compounds as compared to the stems. Among the multi-target natural products, alkaloids have demonstrated a variety of pharmacological properties as anti-inflammatory, anticancer, cardio protective and neuroprotective, which support their potential in the treatment of chronic multifactorial diseases (Abizov, 2004; Frolova, 2005; Liu et al., 2013; Liu et al., 2022).

The results of the study on the content of some isoquinoline alkaloids in the leaves of *Macleaya cordata* are presented in Table 2. The content of alkaloids in *Macleaya cordata* leaf blade was: 8.12 mg/g sanguinarine, 6.82 mg/g chelerythrine and 0.757 mg/g fagaridine, but in leaf petiole 1.88 mg/g sanguinarine, 1.63 mg/g chelerythrine and 0.108 mg/g fagaridine.

Table 1. Some biological peculiarities and the structure of the harvested phytomass at the end flowering period of *Macleaya cordata* 'Mihaela'

Growing season	Plant height, cm	Stems, g		Leaves, g		Inflorescences, g	
		green mass	dry matter	green mass	dry matter	green mass	dry matter
4th year of vegetation	320	358.3	112.1	281.5	90.0	97.6	33.2
5th year of vegetation	303	273.2	85.2	218.9	58.0	50.1	18.7

Table 2. The alkaloid content in *Macleaya cordata* leaves, 5th year of vegetation

Alkaloids	Leaves	Petioles	Leaves with petioles
Sanguinarine, mg/g	8.12	1.88	7.36
Chelerythrine, mg/g	6.82	1.63	5.91
Fagaridine, mg/g	0.757	0.108	0.659

Several literature sources described the pytochemical composition of *Macleaya* species. According to Abizov (2004) in the flowering period the sum of chelerythrine and sanguinarine alkaloids in *Macleaya cordata* reached 1.18 % in leaves, 0.20% in stem mass and 3.21% in rhizome parts; in *Macleaya macrocarpa* – 1.75% in leaves, 0.29% in stems mass and 4.74% in rhizome parts; in *Macleaya x kevensis* – 1.15% in leaves, 0.22% in stem mass and 3.16% in rhizomes. Frolova (2005) stated that the highest content of isoquinoline, chelerythrine and sanguinarine, in above-ground parts of *Macleaya* plants was recorded in the budding phase – 0.84%, but in the roots and rhizomes 0.96 % in the dormancy period. Kosina et al. (2010) found that *Macleaya cordata* aerial part contained 4.51 mg/g sanguinarine and 2.88 mg/g chelerythrine; seeds contained 0.07 mg/g sanguinarine, 0.02 mg/g chelerythrine, but capsules – 32.08 mg/g sanguinarine, 7.36 mg/g chelerythrine. Pěnčíková et al. (2011) revealed that aerial parts of *Macleaya macrocarpa*, depending on the age of plants, contained 2.43-4.37 mg/g sanguinarine, 2.93-5.75 mg/g chelerythrine, but the underground part 1.68-3.11 mg/g sanguinarine, 2.60-4.98 mg/g chelerythrine. Sidelnikov (2014) revealed that sanguinarine levels in samples of *Macleaya cordata* from Belgorod region and Krasnodar region reached 12.1 and 12.4 mg/g dry matter, and in the Moscow region they were lower, 4.2 mg/g, and chelerythrine levels were 5.9-6.1 mg/g dry matter in Belgorod region and Krasnodar region, and 2.2 mg/g in Moscow region, Russian Federation. Casian et al. (2017) found that *Macleaya microcarpa* leaves, in the budding stage, contained 9.70 mg/g sanguinarine, 8.91 mg/g chelerythrine and 0.377 mg/g fagaridine, but the leaves collected in the flowering stage of the plant contained 8.94 mg/g sanguinarine, 7.06 mg/g chelerythrine and 0.308 mg/g fagaridine. Tuzimski et al. (2022) reported that the content of isoquinoline alkaloids in the aerial part dry matter was 2.41-4.09 mg/g sanguinarine and 3.26-5.36 mg/g chelerythrine, but in root part 1.23-1.78 mg/g sanguinarine and 0.88-1.54 mg/g chelerythrine. Bykova et al. (2023) mentioned that the content of alkaloids in *Macleaya cordata* was 0.162-0.164%, as compared to 0.98-1.04% in *Macleaya x kevensis*. Misiurek et al. (2023) remarked that the content of alkaloids in dry plant material samples of *Macleaya cordata* decreases as follows was: in leaves 0.051 mg/g sanguinarine, 0.046 mg/g chelerythrine; in stems 0.027 mg/g sanguinarine, 0.024 mg/g chelerythrine; in root mass of 0.026 mg/g sanguinarine, 0.019 mg/g chelerythrine.

The use of phytomass and crop residues for energy production includes the production of gaseous, liquid and solid fuels. It has been determined that stem productivity of *Macleaya cordata* harvested in August was 1.19 kg/m^2 dry matter in 4th year of vegetation and 1.40 kg/m^2 dry matter in the 5th year of vegetation, but plants harvested in November had a productivity of 1.67 kg/m^2 dry matter in the 4th year of vegetation and 1.79 kg/m^2 dry matter in the 5th year of vegetation. The quality indices of energy stem substrates from *Macleaya cordata* 'Mihaela' are shown in Table 3. It has been determined that *Macleaya cordata* stem substrates contained 406-503 g/kg cellulose, 212-243 g/kg hemicellulose and 91-104 g/kg acid detergent lignin, the estimated theoretical ethanol yield from cell wall carbohydrates averaged 448.7- 541.8 L/t.

Table 3. The quality indices of stem energy substrates from *Macleaya cordata* 'Mihaela'

Indices	4th year of vegetation		5th year of vegetation		Apple tree pruning residues
	August	November	August	November	
Minerals, g/kg DM	56	32	49	24	59
Acid detergent fibre, g/kg DM	497	568	528	607	547
Neutral detergent fibre, g/kg DM	709	802	727	850	766
Acid detergent lignin, g/kg DM	91	99	79	104	110
Cellulose, g/kg	406	469	444	503	437
Hemicellulose, g/kg	212	234	283	243	219
Hexose sugars, g/kg	72.73	83.30	80.15	89.87	78.17
Pentose sugars, g/kg	34.87	38.49	46.55	39.97	36.02
Theoretical ethanol potential, L/t	448.7	507.8	528.3	541.8	476.3
Gross calorific value, MJ/kg	18.35	18.98	18.45	19.28	18.90

The concentrations of structural carbohydrates were much higher in *Macleaya cordata* stem substrates collected in the 5th year of vegetation, with positive impact on the ethanol yield in comparison with the control – apple tree pruning residue substrate. The *Macleaya cordata* stem biomass collected in August had high content of minerals and lower content of structural carbohydrates and acid detergent lignin than stem biomass collected in November. The gross calorific value was higher in stem biomass collected in November.

CONCLUSIONS

The *Macleaya cordata* 'Mihaela' leaf biomass is a valuable resource for the pharmaceutical industry. Besides, due to its optimal cellulose and hemicellulose content *Macleaya cordata* stem biomass makes an attractive material for the production of bioethanol and densified solid biofuel.

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FORMATION OF YIELD AND BIOCHEMICAL PARAMETERS OF WINTER WHEAT GRAIN DEPENDING ON AGRONOMIC PRACTICES OF CULTIVATION

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Abstract

In the Northern Steppe of Ukraine, a significant effect of weather conditions, predecessors, fertilisation and sowing dates on the formation of yield and biochemical parameters of winter wheat grain was established. It was found that the cultivation of winter wheat on black fallow and after spring barley resulted in higher yields at the optimal sowing date (20-25 September) compared to early (5-10 September) and late (5-10 October) sowing dates. Pre-sowing application of complete mineral fertiliser and nitrogen feeding led to an improvement in both yield and grain quality of winter wheat. Moreover, more significant increases in winter wheat yield were recorded after the non-fallow predecessor. The durum winter wheat of Burshtyn variety produced a lower yield compared to the soft wheat varieties, but it exceeded these varieties in terms of protein content. The sedimentation values of durum wheat flour were very low (in the range of 12-20 ml), which indicates the specificity of its uses. At the same time, the sedimentation values of flour of soft wheat varieties used for baking purposes varied between 35-50 ml, depending on the agronomic practices of cultivation.

Key words: *biochemical parameters of grain, fertilisation, predecessor, sowing date, winter wheat, yield.*

INTRODUCTION

Wheat is the main food crop for many countries around the world. Increasing the grain yield and quality is a priority for humanity in the modern world (Shelepov et al., 2004; Golomsha & Dziadykevich, 2017). Soft wheat (*Triticum aestivum* L.) grain is used for flour production and bread baking, while durum wheat (*Triticum durum* Desf.) grain is used for making groats and pasta (Hlynka et al., 1964). In Ukraine, according to the current DSTU 3768-2019, soft wheat grain is graded into four classes depending on its quality characteristics, and durum wheat grain into five classes. Grain with low quality characteristics is used for fodder and for alcohol production.

The main grain quality indicators regulated by the standard include moisture content, grain volume weight, vitreousness, protein content, crude gluten content, falling number and others. At the request of the customer, such important indicators as flour strength and sedimentation value are also determined (Kirpa et al., 2019). Higher quality wheat grain has a higher nutritional value and is therefore in greater demand among customers.

The biochemical quality indicators of wheat grain, which characterise its nutritional value, include the content of protein, carbohydrates, fats, enzymes, vitamins and other compounds. Protein is one of the essential components of wheat grain. Amino acids are the main structural elements of proteins, and wheat grain contains 20 amino acids. Eight essential amino acids are especially significant because they are not synthesised in the human body (Sisakian & Markosian, 1959).

The protein content in grain of winter wheat, which cultivation prevails over spring wheat in Ukraine, rages from 7-8% to 19-20%. The average protein content in grain of this crop is about 10-14% (Cherenkov et al., 2021). Winter wheat grain of modern high-yielding varieties has lower protein content than of the extensive varieties that were common in the past

(Nikolaev, 1991). Grain with the highest protein content is usually produced in the south-eastern regions of the country (Netis, 2011).

As mentioned earlier, high-quality soft winter wheat grain is primarily used in the flour production. The properties of flour determine the volume of bakery products, their appearance, colour, porosity and elasticity of the breadcrumb, and other indicators. Direct methods for assessing grain and flour quality and the rheological properties of dough include determining the gluten content and quality in grain and flour, flour strength with an alveograph, water absorption capacity of flour, stability and mixing tolerance index with a farinograph, etc. However, the most objective assessment of flour can be obtained by means of laboratory or trial baking of bread (Strelnikova, 1971).

At the same time, there are methods for indirectly assessing the baking properties of wheat flour, which are distinguished by their expressiveness and high productivity, in contrast to cumbersome and time-consuming direct methods. The main requirement for these methods is their maximum correlation with the assessment by an alveograph, mixograph, farinograph, depending on the nature of the measurements. Among these methods, the sedimentation method is widespread. The method is based on mixing a small amount of flour with weak solutions of organic acids. After settling for five minutes, the volume of sediment formed by the swelling of gluten and starch is measured. A higher volume of sediment means better quality wheat (Zeleny, 1962). According to these studies, the sedimentation value of flour is an indicator of gluten quality, but other scientists have found a strong correlation between the sedimentation value and the protein content (Gospodarenko et al., 2020).

The formation of yield, as well as physical and biochemical parameters of grain quality depends on a number of factors that affect winter wheat during its growth and development. These include hydrothermal conditions during the growing season, and such components of agricultural technology as variety, predecessor, fertilisation, sowing date and other (Zhemela & Musatov, 1989; Cherenkov et al., 2015).

As we know, winter wheat is one of the crops that requires a significant amount of nutrients for harvest formation and responds well to mineral fertilisation, especially nitrogen fertilisers (Marchuk, 2009; Chirita et al., 2023). At the same time, an excessive dosage of nitrogen fertilisers does not ensure further increase in yield and grain quality of winter wheat (Stefanova-Dobreva, 2022). Some researchers estimate that the increase in yield dependent on nitrogen by 50-55%, and the increase in protein content in grain by 70% (Gospodarenko et al., 2020), and the effect of nitrogen prevail over weather conditions and other factors. However, the impact of different factors on grain yield and quality may vary from year to year (Netis, 2011).

Experimental results reveal that the increase in yield from fertilisers in dry growing conditions is 25-30% lower than in years with favourable moisture conditions. In such years, phosphorus fertilisers have a positive effect, while high doses of nitrogen fertilisers have a negative effect on plant development (Krut & Tararyka, 2000; Netis, 2008).

It is known that one of the critical steps in growing winter wheat is the optimal sowing date. Sowing dates have a major effect on the winter wheat growth and development, overwintering of plants, yield and grain quality. Sowing dates differ for various soil and climatic zones and should be adjusted considering the particularities of the weather conditions of the year, predecessors, soil moisture content, etc. It has been established that a shift in sowing dates from the optimal ones, both towards early and late sowing, leads to a sharp decrease in yield (Cherenkov et al., 2015). In wet, warm and long autumns, winter wheat provides the highest yields when sown later than the average long-term dates, and in years with less favourable autumn conditions - at earlier dates (Netis, 2011).

There are different points of view on the effect of sowing date on the grain quality of winter wheat. According to the opinion of numerous scientists, the lowest protein content is formed at early sowing dates. Sowing at optimal and late dates, when plants develop a smaller vegetative mass, increases the protein content in winter wheat grain (Korkhova, 2014; Partal et al., 2023). According to other researchers,

such dependencies were not observed in all cases (Solodushko et al., 2016).
Given the considerable experimental data obtained in different soil and climatic zones, the integrated effect of weather conditions and main agricultural practices on the formation of grain yield and biochemical parameters of modern soft winter wheat varieties compared to durum wheat varieties under cultivation in the Northern Steppe of Ukraine has not been sufficiently studied.

MATERIALS AND METHODS

The field trials with winter wheat were laid out on black fallow and after spring barley fields at the State Enterprise Institute of Grain Crops of the National Academy of Agrarian Sciences of Ukraine. The soil of the experimental plots is low humus full-profile ordinary chernozem. The mechanical composition of the soil is medium loamy. The nitrification capacity of the topsoil is 17-20 mg per 1 kg of absolutely dry soil. The climate of the zone is temperate continental with insufficient and unstable moisture content.
The most widespread winter wheat varieties in the region were selected for the research. Sowing and fertilisation of wheat crops was carried out by the experimental design in accordance with generally accepted methods (Tsikov & Pikush, 1983; Dospekhov, 1985). Sowing was carried out using a mounted seeder CH-16. Complete fertiliser $N_{0\text{-}30}P_{60}K_{30}$ was introduced into the pre-sowing cultivation on black fallow, and $N_{60}P_{60}K_{30}$ was applied after the stubble predecessor. Nitrogen feedings were carried out on frozen-thawed soil in the spring and locally at the end of the tillering stage of plants, using ammonium nitrate fertiliser. A Sampo-500 combine harvester was used to harvest the wheat, and the grain yield was recalculated for 14 % moisture content. Obligatory sampling of grain from different experimental variants was carried out during the research to determine its quality indicators. The protein content in the grain was determined by infrared spectroscopy using a NEOTEC 4256 device. For calibration of the device, the values of total nitrogen obtained by the Kjeldahl method were used (DSTU ISO 20483:2016). The sedimentation value was evaluated by the micromethod in a 2% acetic acid solution using a 3.2 g flour sample and a 100 ml calibrated cylinder (Pumpyansky, 1971; Sozinov et al., 1977).

RESULTS AND DISCUSSIONS

Weather conditions during the growing season of winter grain crops have a significant impact on the plant growth, development in autumn, their overwintering, root system development and growth of aboveground mass in spring, formation of generative organs, yield and grain quality. During the research, significant variations were observed in such indicators as air temperature and precipitation at different stages of plant development. The last 15 years of research have been characterised by mostly satisfactory and favourable weather conditions for winter wheat. However, the 2011/12 growing season should be singled out, when a combination of unfavourable meteorological factors had a negative impact on plant productivity. The dry autumn growing season, occasional sharp temperature drops in winter and acute moisture deficit during critical stages of plant development in spring resulted in low wheat productivity, especially after the fallow predecessors.
After the stubble predecessor, the average yield of winter wheat for the studied soft winter wheat varieties in 2012 was 2.30 t/ha, while in the more favourable years (2013 and 2014) the values of this indicator were 4.45 and 5.05 t/ha, respectively. On black fallow, the yield in 2012 was also lower (4.08 t/ha) compared to 2013 and 2014, when it was 7.03 and 7.05 t/ha, respectively. Despite the low yields in 2012, the protein content in grain was the highest compared to other years of research. Thus, the protein content of winter wheat after spring barley was 14.9% and 14.6% on black fallow. After the stubble predecessor, these indicators correlated with the indicators of flour sedimentation. At the same time, no such correlation was observed for black fallow (Table 1).
In the relatively favourable 2017-2019 years for winter wheat, a significant advantage of the black fallow over the stubble predecessor for this crop in yield formation was also revealed.
Depending on the variety, sowing date and fertilisation option, the wheat yield on black

fallow varied from 4.36 to 7.16 t/ha, and after spring barley, this indicator varied from 2.55 to 6.05 t/ha (Table 2). It should be noted that after both predecessors, the yield of soft winter wheat varieties Lastivka odeska and Holubka odeska was higher than that of Burshtyn variety of durum wheat. At the sowing date of 20-25 September, the yields of the varieties were higher compared to the early sowing date (5-10 September) and the late sowing date (5-10 October). At the same time, the Holubka odeska variety was more plastic in terms of sowing dates than the varieties Lastivka odeska and Burshtyn.

Table 1. Winter wheat yield and biochemical parameters depending on the predecessor under various weather conditions of the seasons under study

Predecessor	Years of study	Yield,t/ha	Protein content, %	Sedimentation value, ml
Black fallow (background $P_{60}K_{30}$)	2012	4.08	14.6	59.2
	2013	7.03	13.2	63.4
	2014	7.05	12.3	64.6
Spring barley (background $N_{60}P_{60}K_{30}$)	2012	2.30	14.9	58.4
	2013	4.45	11.8	47.8
	2014	5.05	12.1	49.4

Table 2. The effect of sowing time and fertilization on the winter wheat yield, t/ha, 2017-2019

Variety	Sowing time	Fertilizer option		
		without fertilizer (control lot)	pre-sowing application of complete fertilizer	fertilizing system*
Predecessor – black fallow ($N_{30}P_{60}K_{30}$)				
Lastivka Odeska (soft wheat)	5-10 September	5.34	5.86 (+0,52)	6.13 (+0,27)
	20-25 September	6.04	6.85 (+0,81)	7.10 (+0,25)
	5-10 October	5.23	5.79 (+0,56)	6.08 (+0,29)
Holubka Odeska (soft wheat)	5-10 September	6.14	6.69 (+0,55)	7.04 (+0,35)
	20-25 September	6.18	6.76 (+0,58)	7.16 (+0,40)
	5-10 October	6.01	6.52 (+0,51)	6.89 (+0,37)
Burshtyn (durum wheat)	5-10 September	4.87	5.44 (+0,57)	5.97 (+0,53)
	20-25 September	5.22	5.85 (+0,63)	6.40 (+0,55)
	5-10 October	4.36	4.98 (+0,62)	5.49 (+0,51)
Predecessor – spring barley ($N_{60}P_{60}K_{30}$)				
Lastivka Odeska (soft wheat)	5-10 September	3.27	4.35 (+1,08)	5.47 (+1,12)
	20-25 September	3.39	4.77 (+1,38)	5.75 (+0,98)
	5-10 October	3.13	4.46 (+1,33)	5.60 (+1,14)
Holubka Odeska (soft wheat)	5-10 September	3.74	5.05 (+1,31)	5.91 (+0,86)
	20-25 September	3.82	5.26 (+1,44)	6.05 (+0,79)
	5-10 October	3.44	4.81 (+1,37)	5.67 (+0,86)
Burshtyn (durum wheat)	5-10 September	2.76	4.13 (+1,37)	4.99 (+0,86)
	20-25 September	3.11	4.63 (+1,52)	5.53 (+0,90)
	5-10 October	2.55	3.94 (+1,39)	4.73 (+0,79)

Notes: *On the background of pre-sowing fertilizer on black fallow - N_{30} at the end of plants tillering locally; after spring barley - N_{30} was applied on frozen-thawed soil + N_{30} locally. Ammonium nitrate was used for nitrogen feeding.

The analysis of all sowing dates showed that the lower yield of winter wheat was formed on the unfertilised background, pre-sowing application of complete fertiliser $N_{30}P_{60}K_{30}$ on black fallow contributed to an increase in yield compared to the control by 0.51-0.81 t/ha, and after the stubble predecessor, the application of $N_{60}P_{60}K_{30}$ before sowing led to an increase in grain yield by 1.08-1.52 t/ha. Further feeding winter wheat on black fallow at the end of tillering stage with a local application of N_{30} contributed to a further increase in yield by 0.25-0.55 t/ha, and after barley spring, feeding with N_{30} in two steps (on frozen-thawed soil and locally) resulted in an additional increase in yield, which varied within 0.79-1.14 t/ha.

The application of mineral fertilisers had a positive effect on the protein content of winter wheat grain. The highest values after both predecessors were formed against the background of pre-sowing fertilisation followed by nitrogen feedings. The protein content of durum wheat grain was generally higher in comparison with soft wheat (Figures 1 and 2).

In the cultivation on black fallow, the protein content in the grain of Burshtyn variety varied from 12.9 to 14.6% depending on the sowing date and fertilisation, in Lastivka odeska variety - 12.0 to 13.6%, and in Holubka odeska variety it was the lowest and varied from 11.0 to 12.6%. After the stubble predecessor, the protein content in durum wheat varied within 11.9-14.5%, in soft wheat varieties Lastivka odeska and Holubka odeska - 11.5-13.4% and 10.7-12.4%, respectively.

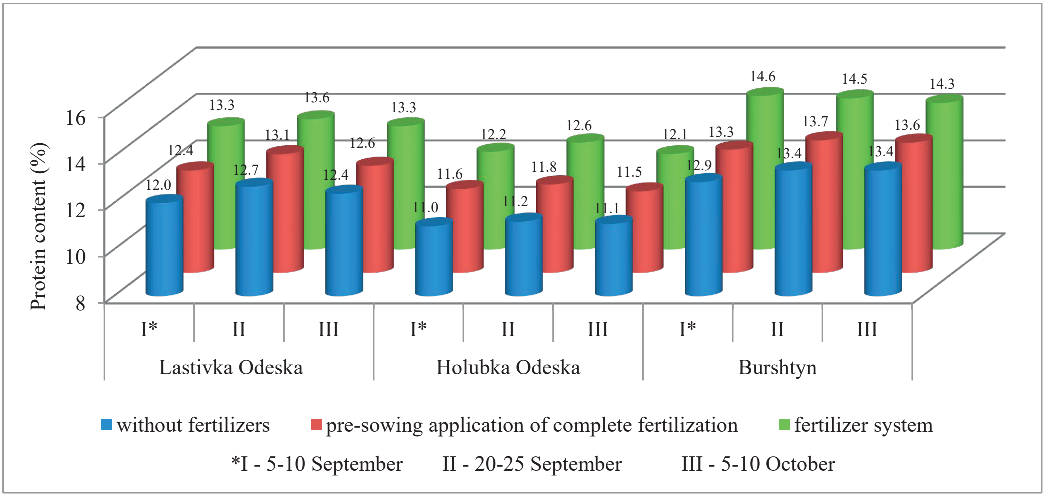

Figure 1. The effect of sowing time and fertilization on the protein content of winter wheat grain after black fallow, 2017-2019

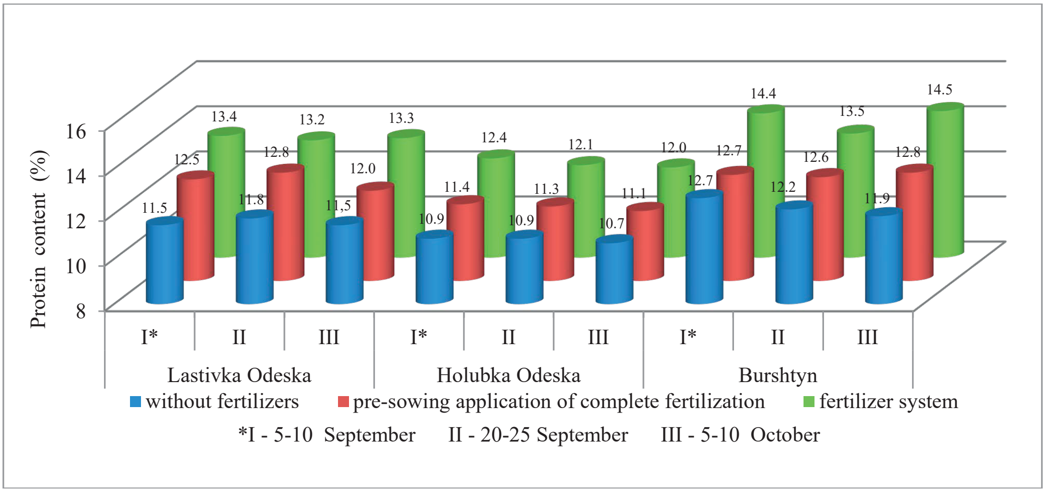

Figure 2. The effect of sowing time and fertilization on the protein content of winter wheat grain after barley spring, 2017-2019

The sedimentation value of flour in all varietie was the highest at the late sowing date (5-10 October), and the lowest at the early sowing date (5-10 September). However, the value of this indicator was significantly higher in the soft wheat varieties compared to the durum wheat variety and, depending on the experimental variant, amounted to 37-50 ml for black fallow, and 35-45 ml after spring barley. The sedimentation value of flour in the durum wheat of Burshtyn variety varied between 12 and 20 ml depending on the predecessor, fertilisation and sowing date (Figures 3 and 4). These data once again confirm the different purposes of different wheat types. Soft wheat flour should be used for bread baking, because it is characterised by higher flour sedimentation, which is closely related to the flour strength and rheological properties of the dough.

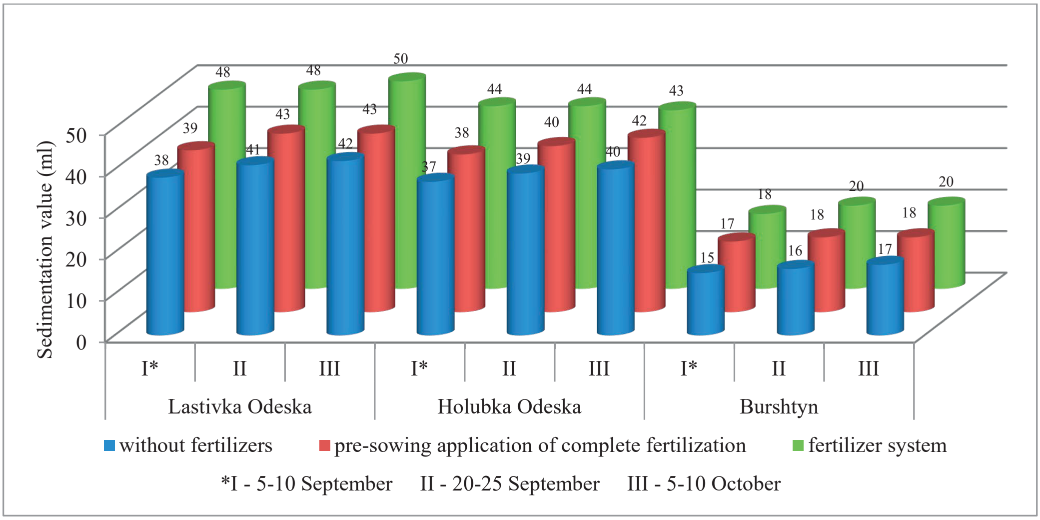

Figure 3. The effect of sowing time and fertilization on the sedimentation value of winter wheat flour after black fallow, 2017-2019

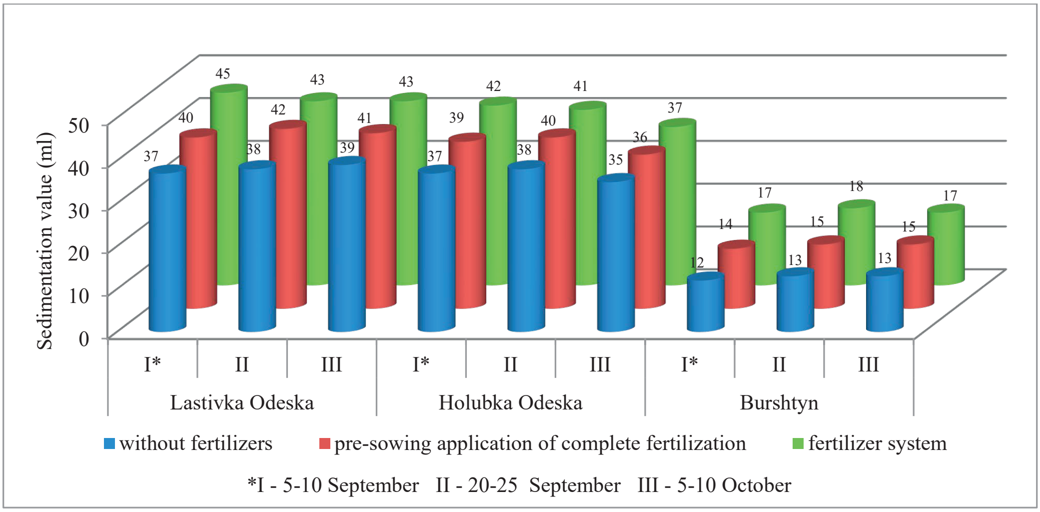

Figure 4. The effect of sowing time and fertilization on the sedimentation value of winter wheat flour after barley spring, 2017-2019

CONCLUSIONS

Based on the studies conducted in the Northern Steppe of Ukraine, the peculiarities of the effect of weather conditions, predecessors, sowing dates and mineral fertilisation of crops on the formation of yield, protein content in grain and flour sedimentation of different varieties of winter wheat were revealed. It was found that higher yield was recorded during the cultivation of winter wheat on black fallow compared to the stubble predecessor, at the optimal sowing date (20-25 September), and with the fertilisation system, which includes nitrogen feeding against the background of pre-sowing application of complete fertiliser. Higher increases in yield as a result of fertilisation were observed when winter wheat was grown after spring barley. In the case of shifting the sowing date from the optimal one towards early or late sowing, the lowest decrease in yield was observed in the soft wheat of Holubka odeska variety compared to the varieties Lastivka odeska and Burshtyn.

The biochemical properties of grain depended mostly on the type of winter wheat (soft or durum) and fertilisation of crops. In the durum wheat of Burshtyn variety, the protein content in the grain under different experimental variants was mostly higher than in the soft wheat varieties (Lastivka odeska and Holubka odeska). At the same time, the sedimentation value of flour, which is closely related to baking properties, was significantly lower and ranged from 12-20 ml, depending on the technology components. For soft wheat varieties, the values of this indicator varied between 35-50 ml.

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RESEARCH REGARDING THE INFLUENCE OF INTERMEDIATE CROPS ON POTATO HARVEST AND QUALITY FOR CONSUMPTION

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Abstract

The research was carried out in the Bozovici Depression located in southwest Romania. From which it follows that the area falls under the moderate temperate continental climate. The soil type is semicarbonate eutric alluvial soil. The experiments were bifactorial in which factor A was the preceding plant and factor B was the number of nests/hectare. The choice of the bean (Vicea faba L) used as an intermediate plant is motivated by its ecological flexibility and the amount of green mass it produces. The synthesis of the harvest results obtained in the experimental cycle 2021-2023 for potatoes showed that the highest harvest was obtained in the variant autumn barley + grain of 32,376 kilograms/hectare followed by the variant of autumn wheat + grain of 31,287 kilograms/hectare, and the lowest harvest was obtained in the variant in which the potato followed after grain corn of 26,492 kilograms/hectare. Among the plots tested, the variant with 65,000 nests/hectare stood out.

Key words: *potato, intermediate crops, yield impact.*

INTRODUCTION

The research was carried out on the potato crop, currently spread in 140 countries of the world and represents after FAO, the fourth source of food energy, after rice, wheat and maize. In Romania, the potato ranks 4th in terms of area, after corn, wheat and sunflower (Hînda et al., 2023). Currently, in the transformation of intensive, ecological agriculture, of particular attention are the research on intermediate crops, respectively in rotations, after a main crop, with early harvest (wheat, barley, rapeseed, etc.) to follow an intermediate crop (successive) with soil ameliorating characteristics, from which biomass used as green fertilizer is obtained (Si J et al., 2024). Frequently, intermediate plants are used in agricultural practice in intermediate crops, to obtain biomass for animal feed or even for grains (Hînda et al., 2021). In this regard, Berca, 2011, mentions that through intermediate crops the functions of feed production, green fertilizer, reduction of CO2 in the soil and soil protection function are fulfilled. By permanently covering the soil with vegetation, a permanent accumulation of biomass is obtained, which ensures the enrichment of the soil with nitrogen and carbon and thus contributes to its reconstruction and development. (CKM system). In the soil reconstruction function, protection against erosion, weeding, soil structuring, increased biological activity should also be estimated (Niedziński et al., 2024).

Results on the importance of using legumes in successive crops, on the economic and ameliorative effect are also present works (Muntean, 2003; Borcean et al., 2004; Imbrea; 2019; Hînda, 2021).

MATERIALS AND METHODS

The research was carried out between 2021 and 2023 in the Bozovici Depression, located in southwest Romania. The area falls within the temperate continental climate. The geographical position, the relief, as well as other local factors give the area its own climatic dynamic, in which Mediterranean and oceanic influences are felt. The perennial average rainfall varies between 670 - 750 mm. The average annual temperature is 9.3ºC. The soil type is semicarbonate eutric alluvial. The experiments were bifactorial type with 3 repetitions, in which factor A was the intermediate crops used, and factor B the number of potato nests, with the following graduations of factors: Factor A - a1 winter barley in succession with grain; a2 winter wheat, in sequence with grain; and a3 grain maize. Factor B number of potato nests, with graduations: b1 - 55000 nests/ha; b2 - 60000 nests/ha, b3 -65000 nests/ha and b4- 70000 nests/ha. The choice of grain (*Vicea faba* L) as a successive plant after barley and wheat respectively was made because it is a species adapted to areas with moderate humidity, with good ecological flexibility, which ensures good crops of vegetative mass with an improving effect on the soil. The potato variety was Productive, from the semi-late maturity group, from the quality class A/B, with a production capacity of 52 t/ha. It is worth mentioning that in vegetation develops a rich leaf bush with semi-erect port. Of the cereals, preference was given to barley, since it leaves the field early in June, and wheat, which leaves the field in the first half of July. Preparing the land after harvesting the grain for the grain carried out a work with the gruber, associated with the tusk harrow. The harvesting of the grain was carried out in autumn, after the arrival of frosts by rolling it, after which it was plowed at 23-25 cm. Fertilization for the potato was done with $N_{120}P_{80}K_{80}$. In the spring the field was worked with the combiner in aggregate with fields of tusked harrows. Potato planting was carried out in the first half of March, at the distance between rows of 70 cm and a depth of 6-7 cm. Weed control was carried out by herbicide. During the vegetation, two rebellions and 3 treatments against diseases and pests were carried out. The harvesting of potatoes was done on the date of drying of the stems and the ripeness of the tubers.

RESULTS AND DISCUSSIONS

A summary of the potato crop results is given in the following (Table 1).

Table 1. Summary of potato yield

Number of nests/ha					Factor A means			
Factor A	55000	60000	65000	70000	Yield kg/h	%	Diff kg/ha	Signif.
Barley + grain	28927	31033	34856	34688	32376	100		
Grain + grain	28189	30210	33827	32922	31287	97	-1089	XX
Corn	24579	25163	28556	27672	26492	82	-5884	XXX

DL 5% = 1256 kg/ha DL 1% = 2079 kg/ha DL 0.1% = 3891 kg/ha

Factor B averages

Nest/ha	55000	60000	65000	70000
Yield kg/ha	27232	28802	32413	31760
%	100	106	119	117
Diff. kg/ha		1570	5181	4528
Signif.		X	XXX	XXX

DL 5% = 2264 kg/ha DL 1% = 3106 kg/ha DL 0.1 = 4227 kg/ha

From the data presented it results that the largest potato harvest, on average on the 4 planting densities of 32376 kg/ha was obtained in the autumn barley rotation and grain in successive crop, followed by the variant in which the potato followed wheat and grain in successive crop of 31,887 kg/ha. The lowest average harvest was obtained in the variant in which the potato followed grain corn, of 26,492 kg/ha. The difference in harvest between the variants in which barley harvested 10 days earlier than wheat and grain sowing gained 10 days during the growing season, the green mass production of the grain crop was higher, which benefited the potato that followed the following year. The smallest potato harvest was in the corn grain rotation with 18%, respectively with 5884 kg/ha statistically assured as very significantly negative. Among the densities used for the potato, the variant with 65,000 nests/ha was noted, in which the harvest was higher by 19%, compared to the control variant with the density of 55000 nests/ha, respectively there was a very significant difference of 5181 kg/ha. Increasing the density to 70000 nests/ha is not motivated, the harvest increase was 17% compared to the control variant.

The results of the quality analyses recorded on the potato in the experimental cycle 2021-2023 include: annual analyses of each variant on the percentage of starch and data on the average starch production of the variants taken in the study.

The results of the average quality analyze show close values between the variants of the barley + grain precursors (17.6%) and the wheat + grain variant (17.7%). The highest content of 18.2% was determined in the variant in which the preceding plant was grain corn (Figure 1). The results show that between the barley + grain and barley + wheat precursors the values were significantly equal, and after maize the content was higher because the nitrogen content in the soil was lower than in the variants in which the favorable effect of the nitrogen content left in the soil was felt. It follows that the higher nitrogen content in the soil negatively influenced the accumulation of starch.

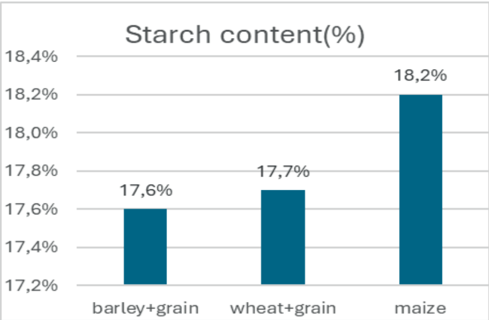

Figure 1. Average starch content depending on the walkers

The average starch content depending on the number of nests and the 3 preceding plants varied between the limits of 17.7% in the version with 60000 nests/ha and 18.0% in the version with 70000 nests/ha (Figure 2). The data represent average summary values from the experimental cycle mentioned, the annual data being influenced by the annual climatic conditions and are dimmed on the average.

On the basis of tuber production and starch content, starch production/ha was determined. Data are presented in Tables 2 and 3.

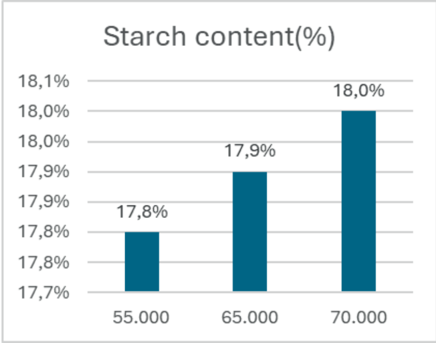

Figure 2. Average starch content as a function of density (number of nests/ha) of potato crop

Table 2. Starch production depending on the preceding plant

Pre-culture	Barley + grain	Grain + grain	Grain maize
Potato harvest kg/ha	32376	31287	26492
Amount of starch kg/ha	5698	5537	4821
%	100	97	87
Difference kg/ha		-161	-877

Table 2 shows that the largest amount of starch was obtained in the variant when barley and grain were pre-potato in successive crop from the variant with wheat and grain rotation in successive crop, the amount of starch decreased by 3%, respectively by 161 kg/ha. The smallest amount of starch was obtained in the potato rotation after grain maize, with 13% and 877 kg/ha respectively. It follows that the beneficial effect of grain grown in successive cereal crops was also felt on starch production.

Table 3. Starch production by crop density

Density Nests/ha	55000	60000	65000	70000
Potato harvest kg/ha	27232	28802	32413	31760
Amount of starch kg/ha	4683	5897	5801	5716
%	100	109	124	122
Difference kg/ha		414	1118	1033

The Table 3 shows that, depending on the density of the culture, the amount of starch compared to the control variant increased by 9% in the variant with 60000 nests/ha, by 24% in the variant with 65000 nests/ha and showed a downward trend in the variant with 70000 nests/ha to 22%.

It follows that by increasing the number of nests per hectare, compared to the variant with 55000 nests/ha, the amount of starch increased by 414 kg/ha in the version with 60000 nests/ha, by 1118 kg/ha in the version with 65000 nests /ha and by 1033 kg/ ha in the version with 70000 nests/ha.

CONCLUSIONS

The research was carried out in the Bozovici Depression, Patas territory, located in southwest Romania.

The research carried out between 2021 and 2023 on the introduction of intermediate crops in the rotation for potato cultivation, respectively after the straw cereal crops (barley and wheat), the introduction of grain (L) for green mass, used as green fertilizer, led to obtaining a harvest increase in potato for consumption of 18%, compared to the potato grain corn rotation practiced in the area.

After harvesting grain barley in the second half of June, the use of grain as green fertilizer, harvested before frost came, increased the potato harvest by 18%, compared to potato rotation after corn.

After the wheat crop, harvested in the first decade of July, the use of the grain as green fertilizer increased the potato harvest by 11.8%.

The optimum potato density, where the highest harvest was obtained, was 65000 nests/ha of 34856 kg/ha after barley + grain, and after wheat and grain was 33827 kg/ha, compared to the corn grain potato variant, of 28356 kg/ha.

On average, after the three variants of previous crops, the harvest increase was 19% in the variant with 65000 nests/ha, compared to the control variant with 55000 nests/ha.

Increasing the density to 70,000 nests/ha is not justified, the harvest increase being only 17%.

The amount of starch obtained for potatoes in the rotation after barley and grain was 5698 kg/ ha, after wheat and grain was 5537 kg/ha compared to potato grown after corn where the amount of starch was 4821 kg/ha.

In the version with a density of 65000 nests/ha, the largest amount of starch was obtained, of 5801 kg/ha, compared to 4683 kg/ha in the version with a density of 55000 nests/ha.

In conclusion, intermediate crops are of particular importance in the stage of transformation of agriculture to an intensive one, but to ensure, besides increasing crops, soil protection, by enriching with humus and improving physical and biological characteristics.

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EFFECTS OF DIFFERENT COMPLEX FERTILIZERS AND THEIR APPLICATION METHODS AT SUNFLOWER

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Abstract

Sustainable agriculture is correlated with the rational use of fertilizers, mineral nutrition being one of the main production factors that directly influence the achievement of safe, constant, efficient and quality agricultural production. The use of modern, intensive, but equally environmentally friendly technologies can ensure the production objectives of farmers, ensuring the profitability of activities carried out in compliance with production and environmental protection norms. The aim of this paper is to present the obtained results regarding the effects of different complex fertilizers and their application methods at sunflower under the specific growing conditions of South-Est Romania. In this respect, field experiments were performed in Dobrogea region from South-East Romania, under rainfed conditions in the years 2022 and 2023. The experimental factors were the following: Factor A – Application methods, with 2 graduations: a1 = Surface broadcast + incorporation; a2 = Banded with sowing; Factor B – Complex fertilizer product, with 3 graduations: b1 = NPK 20-20-0; b2 = Duofertil Eurocereal 34; b3 = DAP (18-46-0). The complex fertilizer Duofertil Eurocereal 34 proved to assure the highest grain yields especially when it was broadcasted and incorporated at the seedbed preparation, but the average differences between the experimented complex fertilizers are not statistically significant. Surface broadcast of the experimented fertilizers followed by their incorporation through the seedbed preparation proved to be more efficient then the banded fertilizer application with sowing regardless of the climatic conditions of the experimented year.

Key words: *sunflower, fertilization, complex fertilizer, application method, yield.*

INTRODUCTION

Being one of the most important oil plants on the globe and the most important one in Romania, sunflower has been a subject of study for many researchers internationally and nationally. The carried out research has aimed the elucidation of many theoretical and applied aspects related to this important cultivated plant, but still there are a lot of issues to be discovered, better understood, or clarified.

The use of modern, intensive, but in the same time environmentally friendly technologies can ensure the profitability of farmers' activities carried out in compliance with production and environmental protection rules. Therefore, in recent years, more and more attention is being paid to assessing the effect of fertilization considering global warming to ensure sustainable soil fertility management (Tănase et al., 2022), efficiency and sustainability of farmers' activities. Undoubtedly, fertilization is one of the major factors that could increase sunflower yield (Shoghi-Kalkhoran et al., 2013). Nutrient management is one of the main factors that influence sunflower achene yield, achene oil, and fatty acid contents (Mahmood, 2021). But, the need for nutrients for yield formation depends both on the specific soils and climatic conditions and on several other factors (Gerassimova et al., 2023). Practically, fertilization is truly effective if it is in compliance with the nutritional requirements of the plants and the soil properties.

To attain maximum yield, the sunflower crop requires fertilizers (Abubaker et al., 2020). Sunflower reacts well to nitrogen fertilization and nitrogen excess lowers the seed oil content and plant resistance to diseases, while balanced phosphorus and potassium fertilization increase yield and oil content in seeds (Gerassimova et al., 2023). Nitrogen levels significantly affect the total number of grains head^{-1}, grain setting efficiency, grain yield, 1000-grains weight, and oil yield of sunflower (Modanlo et al., 2021). Phosphorus fertilization increases the yield of achenes and yield of oil (Soares et al., 2020).

Generally, the imbalance use of fertilizer has been responsible for low fertilizer response (Babu et al., 2014), this being one of the major causes of low yield of sunflower (Tahir et al., 2017).

Band placement of the fertilizer at the seed level resulted in the largest and most efficient use of applied phosphate as compared to placement below the seed or mixed with the soil (Warder & Vijayalakshmi, 1974).

The use of new types of fertilizers implies, as in the case of any new technology, higher costs. As such, naturally these elements defined as being innovative are adopted by farmers initially within the crop technologies of some species that bring a higher added value. One of the best candidates in this regard is the sunflower crop for the researched area, where even increases in the yield of hundreds of kg per hectare can prove to be significant from an economic point of view.

The aim of this paper is to present the obtained results regarding the effects of different complex fertilizers and their application methods at sunflower under the specific growing conditions of South-Est Romania.

MATERIALS AND METHODS

The research was carried out in Dobrogea region from South-East Romania, respectively in Cerna commune from Tulcea county, and consisted in field experiments performed in rainfed conditions in the years 2022 and 2023.

The field experiments were organized as subdivided plots with 3 replications being of type 2 x 3 with the following experimental factors:

- Factor A – application methods, with 2 graduations:
  - a1 = Surface broadcast + incorporation;
  - a2 = Banded with sowing.
- Factor B – complex fertilizer product, with 3 graduations:
  - b1 = NPK 20-20-0;
  - b2 = Duofertil Eurocereal 34;
  - b3 = DAP (18-46-0).

In the variants of surface broadcast, the fertilizers were broadcasted before seedbed preparation and were incorporated by this tillage.

In the banded variants, the fertilizers were applied with sowing in furrows 5 cm from and 5 cm deeper than the seeds.

NPK 20-20-0 is produced by Azomureș company in Romania and it is a classic fertilizer with a content of 20% nitrogen (11.3% as NH_4^+ and 8.7% as NO_3^-) and 20% phosphorus (63% water soluble).

Duofertil Eurocereal 34 (NPK Eurocereal 10-24-0) is part of the Duofertil fertilizers created by the research department of Timac Agro. It contains the MPPA DUO specificity, which is an association between the MPPA complex (European patent 945000107) and the physiological agent, acting as a phytoregulatory precursor XCK [European patent EP11447706(A2)]. MPPA is a natural complex based on extracts of organic origin containing solubilized polyphenolic molecules of different molecular mass. Thanks to the molecules in MPPA, phosphorus is protected against any type of degradation in a similar way to chelation. XCK stimulates the production of amino acids forcing the roots to produce exudates (of the type of amino acids) called phytoregulatory precursors with a role in cell division at the root level. Duofertil Eurocereal 34 contains 10% nitrogen as ammonium (NH_4^+), 24% P_2O_5 (10.1% water-soluble), 20% SO_3 (water soluble sulfur anhydride), 0.1% Bor, and 0.1% Zinc.

DAP (18-46-0), respectively Diammonium Phosphate [$(NH_4)_2HPO_4$] is the world's most widely used phosphorus fertilizer. It is produced in Morocco and contains 18% nitrogen as ammonium (NH_4^+) and 46% P_2O_5 (min. 43% water soluble).

All the experimental variants were based on a total of 70 kg/ha of nitrogen and 70 kg/ha of P_2O_5. So, the experimented fertilizers were calculated to cover the necessary rate of 70 kg/ha of P_2O_5. According to each complex fertilizer product, respectively to its nitrogen content, the difference of the nitrogen up to 70 kg/ha was applied as ammonium nitrate (34.4% nitrogen content) through surface broadcasting before seedbed preparation.

Each experimental variant had 67.2 m^2 in size which consisted of 12 rows of plants with 70 cm between rows resulting in a width of 8.4 m and 8 m of length along the rows.

The preceding crop was maize. Tillage in the experimental field consisted in a harrowing work performed after harvesting the preceding crop, and in October there was performed the ploughing at 20 cm depth. The ploughing was followed in Autumn by a harrowing work and in Spring there was performed the seedbed preparation before sowing, this being made with a combinatory.

Sowing was performed on the 9th of April in 2022 and on the 3rd of May in 2023. The assured plant density was 64,000 plants per hectare.

The sunflower hybrid used in the research was SY Onestar CLP, a hybrid with an exceptional production potential, adaptable and stable in different growing conditions.

The weed control was performed by applying the herbicide Pulsar 40 (Imazamox 40 g/l) in a rate of 1.2 l/ha in the growing stage of 6 leaves of sunflower plants.

For controlling the sunflower diseases, there was applied the fungicide Pictor Active (Boscalid 150 g/l and Pyraclostrobin 250 g/l) in a rate of 1 l/ha in the growing stage of 10 leaves of sunflower plants.

In the growing stage of 8 leaves of sunflower plants, there was used the foliar fertilizer Lebosol Nutrifos, which contains 3% N, 30% P_2O_5 and 7% Zn, in a rate of 4 l/ha.

The performed determinations whose results are presented in the present paper were the plant height and the grain yield (kg/ha) reported at 9% moisture content.

The geographical coordinates of the research location determine its inclusion in the temperate continental climate zone. So, the climate is characterized by high temperatures in summer and sometimes very low in winter. The average annual temperature is around 10.7ºC.

Regarding the temperatures registered during the vegetation period of the sunflower plants, compared to the year 2022, in the year 2023 the months March, July and August were warmer, while the months April, May and June were colder (Table 1). As average values for the period March-August, the year 2023 with 17.2ºC was warmer that the year 2022 with 16.8ºC.

Regarding the rainfall registered during the vegetation period of the sunflower plants, the year 2022 with 162 mm was droughter than the year 2023 with 250 mm. In the year 2023, after a drought month March with only 8 mm rainfall, there followed a rainy month April with 125 mm. As a consequence, in 2023 the sowing was significantly delayed compared to usual sowing period for the studied area, which is between end of March and beginning of April. Thus, in 2023 the sowing was performed on the 3rd of May. In both experimental years, the months July but especially August were characterized by small amounts of rainfall.

In the studied area, the specific soil is carbonate chernozem with 2.5% humus content and pH of 7.8-8.1.

Table 1. Climatic conditions during sunflower plant's vegetative period at Cerna, Tulcea county, Romania

Month	Temperature (ºC)		Rainfall (mm)	
	2022	2023	2022	2023
March	3.4	7.8	32	8
April	11.1	10.2	15	125
May	16.8	15.5	49	36
June	21.8	19.8	34	41
July	23.5	24.7	20	25
August	24.2	25.4	12	15
Average/Sum	***16.8***	***17.2***	***162***	***250***

RESULTS AND DISCUSSIONS

Due to the climate changes, in general but especially when it comes to South-East Romania, which is one of the driest area in the country, there is necessary to adapt and find the best and most efficient fertilization options.

In the research carried out, the complex fertilizer Duofertil Eurocereal 34 broadcasted and incorporated at the seedbed preparation assured the highest yields in both experimental years, respectively 2574 kg/ha in 2022 and 2768 kg/ha in 2023, with significant differences (+37% in 2022 and +33% in 2023) compared to the average yield of all the experimental variants. In fact, the complex fertilizer Duofertil Eurocereal 34 determined the highest yields at both application methods (Table 2).

The average yield obtained in 2023 is higher than in 2022, respectively 2081 kg/ha in 2023 and 1873 kg/ha in 2022, this being the result of the higher rainfall in 2023 during the vegetation period of the sunflower plants, which totaled 250 mm, compared with 162 mm in 2022. In fact, all the average yields either reported to the application method of the fertilizers or the complex fertilizer products are higher in 2023 than in 2022 (Figure 1). Practically, the better water supply of sunflower plants gives them the possibility to use in more efficient way the available nutrients.

Among the two application methods of the experimented fertilizers, the surface broadcast followed by the incorporation of the fertilizers through the seedbed preparation proved to be more efficient regardless of the climatic conditions of the experimented year (Figure 1).

Among the three complex fertilizer products, Duofertil Eurocereal 34 assured the highest yields in both experimental years, this being followed by NPK 20:20:0 and DAP (18-46-0). It has to be mentioned that even there are differences between the three fertilizer products, the differences are not statistically significant.

Table 2. Sunflower grain yields at different conditions of application and complex fertilizer products under different climatic conditions in South-East Romania

Experimental factors		Yields obtained in 2022			Yields obtained in 2023		
Application method	Complex fertilizer product	Yield (kg/ha)	Differences to control		Yield (kg/ha)	Differences to control	
			kg/ha	%		kg/ha	%
Surface broadcast + incorporation	NPK 20-20-0	1777	-96	-5	2014	-67	-3.2
	Duofertil Eurocereal 34	2574	701 *	37	2768	687 *	33.0
	DAP (18-46-0)	1583	-290	-15	1779	-302	-14.5
Banded with sowing	NPK 20-20-0	1610	-263	-14	1807	-274	-13.2
	Duofertil Eurocereal 34	2033	160	9	2284	203	9.8
	DAP (18-46-0)	1661	-212	-11	1836	-245	-11.8
Average		*1873*	*Control*	-	*2081*	*Control*	-

$LSD_{5\%}$ = 697.76 kg/ha
$LSD_{1\%}$ = 978.27 kg/ha
$LSD_{0.1\%}$ = 1382.71 kg/ha

$LSD_{5\%}$ = 676.62 kg/ha
$LSD_{1\%}$ = 948.63 kg/ha
$LSD_{0.1\%}$ = 1340.82 kg/ha

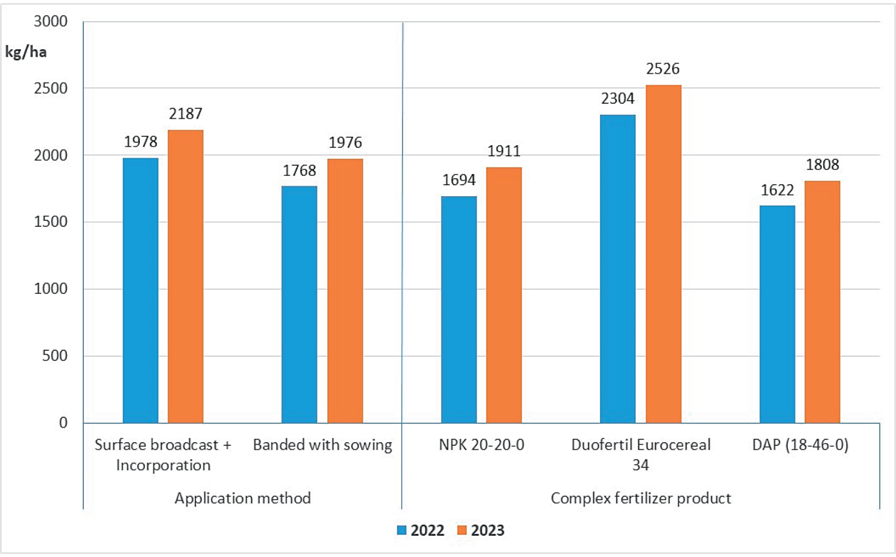

Figure 1. Yield average values at different conditions of application and complex fertilizer products under different climatic conditions in South-East Romania

Regarding the sunflower plant height, the highest values (137.5 cm in 2022 and 147.0 cm in 2023) were determined by the complex fertilizer NPK 20-20-0 broadcasted and incorporated at the seedbed preparation, with a difference statistically significant in 2023 compared to the average value of all the experimental variants (Table 3). If in the case of the application method of surface broadcast with incorporation at seedbed preparation the highest plant heights were determined by the complex fertilizer NPK 20-20-0 in both experimental years, in the case of application method of banded with sowing the highest plant heights were determined by the complex fertilizer Duofertil Eurocereal 34.

The smallest plant height values were registered in both experimental years in the case of complex fertilizer DAP (18-46-0) of the application method of surface broadcast with incorporation at seedbed preparation.

The better water supply of the sunflower plants through the high rainfall determined higher values of the plant height in 2023 (135.6 cm in average) compared to 2022 (131.8 cm in average) (Table 3). In fact, all the average values of the plant height reported to the application method of the fertilizers or the complex fertilizer products are higher in 2023 than in 2022 (Figure 2).

Table 3. Sunflower plant height at different conditions of application and complex fertilizer products under different climatic conditions in South-East Romania

Experimental factors		Plant height in 2022			Plant height in 2023		
Application method	Complex fertilizer product	Plant height (cm)	Differences to control		Plant height (cm)	Differences to control	
			cm	%		cm	%
Surface broadcast + incorporation	NPK 20-20-0	137.5	5.7	4.3	147.0	11.4 *	8.4
	Duofertil Eurocereal 34	132.9	1.1	0.8	135.8	0.2	0.1
	DAP (18-46-0)	125.7	-6.1	-4.6	128.5	-7.1	-5.2
Banded with sowing	NPK 20-20-0	128.7	-3.1	-2.4	130.6	-5	-3.7
	Duofertil Eurocereal 34	133.7	1.9	1.4	136.7	1.1	0.8
	DAP (18-46-0)	132.1	0.3	0.2	135.1	-0.5	-0.4
Average		*131.8*	*Control*	-	*135.6*	*Control*	-

$LSD_{5\%}$ = 8.01 cm
$LSD_{1\%}$ = 11.22 cm
$LSD_{0.1\%}$ = 15.86 cm

$LSD_{5\%}$ = 9.37 cm
$LSD_{1\%}$ = 13.15 cm
$LSD_{0.1\%}$ = 18.58 cm

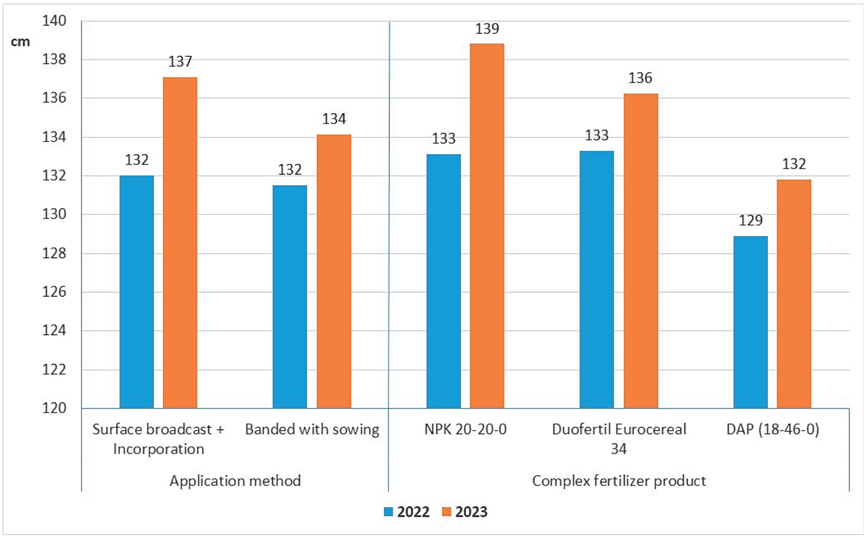

Figure 2. Plant height average values at different conditions of application and complex fertilizer products under different climatic conditions in South-East Romania

Regarding the two application methods of the experimented fertilizers, if in 2022 there are no differences of the average values of the plant height, in 2023 the average value of the plant height is higher in the case of the application method of surface broadcast with incorporation at seedbed preparation.

Regarding the three complex fertilizer products, the highest average plant height was register in 2022 in the case of complex fertilizers NPK 20-20-0 and Duofertil Eurocereal 34 (133 cm), but in 2023 the complex fertilizers NPK 20-20-0 with a value of 139 cm of the plant height proved to be the best.

CONCLUSIONS

Following the research carried out in South-Est Romania in 2022 and 2023, the complex fertilizer Duofertil Eurocereal 34 proved to assure the highest grain yields especially when it was broadcasted and incorporated at the seedbed preparation. Even there are differences between the three experimented fertilizer products, the differences are not statistically significant.

Surface broadcast of the experimented fertilizers followed by their incorporation through the seedbed preparation proved to be more efficient then the banded fertilizer application with sowing regardless of the climatic conditions of the experimented year.

The better water supply of sunflower plants gives them the possibility to use in more efficient way the available nutrients, this resulting in higher grain yields and plant heights.

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TESTING NEW DURUM WHEAT VARIETIES FOR PRODUCTIVITY

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Abstract

In the Educational, Experimental and Implementation Base of the Agrucultural University of Plovdiv, a field trial was conducted in 2018-2021, in which new varieties of durum wheat (Triticum durum Desf.) were tested, including: Viomi, Saya, Railidur and Helix. The varieties were compared with the Predel variety, which is the productivity standard in Bulgaria. From the field trial it was proved that the productivity of durum wheat variety Helix was the highest 4.732 t/ha which is 0.511 t/ha (12.1%) higher grain yield compared to the standard variety Predel. The yields of the other durum wheat varieties studied were as follows: variety Viomi 4.572 t/ha with 0.351 t/ha (8.3%); variety Saya 4.421 t/ha with 0.200 t/ha (4.7%) and variety Railidur 4.379 t/ha with 0.158 t/ha (3.7%) more grain compared to variety Predel.

***Key words**: durum wheat varieties, grain yield.*

INTRODUCTION

The productivity and grain quality of durum wheat are influenced by a number of factors including variety (Delchev et al., 2000; Yanev & Kolev, 2008), soil and climatic conditions (Gómez et al., 2000; Ivanova et al., 2010;) and cultivation technology (Dzhugalov, 2010; Pleshkutsa, 2018; Arduini et al., 2006; Bilgin et al., 2011). Optimization of these factors leads to obtaining high results in the cultivation of durum wheat (Kolev et al., 2020).

Over the last few years, there has been significant success in durum wheat breeding in Bulgaria (Dragov et al., 2019). As a result of the combination of intratype hybridization and traditional breeding scheme, new durum wheat varieties have been developed at the Institute of Field Crops, Sofia, Bulgaria. (Dragov et al., 2017; Dragov et al., 2020).

To realize the genetic potential in terms of productivity and grain quality, an important role is played by growing a variety in the most suitable region of the country under the interaction of relevant environmental factors (Sabella et al., 2020; Asseng et al., 2014) and optimal cultivation technology (Yu et al, 2021).

Durum wheat is grown globally on 13.5 million ha in 2020/2021, accounting for 6.2% of wheat area (Martínez-Moreno et al., 2022). Durum wheat in Europe in 2021 occupies an area of 2348000 ha with an average yield of 3230.0 kg/ha and a resulting production of 7581000 t and in 2022 occupies an area of 2388000 ha with an average yield of 3000.0 kg/ha and a resulting production of 7161000 t (Coceral Crop Forecast, December 2022).

In order to solve the grain problem, an increase in crop production is needed, including the production of durum wheat (*Triticum durum* Desf.). Creation and introduction of new varieties in production contributes to solve the grain problem (Alvaro et al., 2008; Parvaneh et al., 2014; Sabella et al., 2020).

Soil and climatic conditions in Bulgaria are very heterogeneous, so the selection of the appropriate variety composition for the respective ecological region is essential to obtain high and stable yields and good income from durum wheat. This is necessitated by the global changes that have occurred in climatic conditions in recent years and the adaptability of varieties to these conditions.

In conducting the field experiment, we aimed to study the productive potential of new durum wheat varieties under the soil and climatic conditions of the Plovdiv region.

MATERIALS AND METHODS

The experiment was carried out in the Educational, Experimental and Implementation Base of the Agrucultural University of Plovdiv in 2018-2021. The field trial was laid out using the block method, in four replications with a harvest plot size of 15 m^2. New varieties of durum wheat (*Triticum durum* Desf.) were studied, including Viomi, Saya, Railidur, Helix, being a selection of the Institute of Field Crops in Chirpan. The varieties were compared with the productivity standard Predel variety.

The field trial was conducted on Molic Fluvisols (FAO-UNESCO, 1990), a carbonate alluvial-fluvial soil with a medium sandy loam mechanical composition and a humus content of 1-2%. The soil is characterised by a slightly alkaline pH reaction (7.2-7.7), the presence of carbonates (4.3-7.4%) and the absence of salts. The content of the main nutrients in the 0-20 cm soil layer is as follows: N - 15.1 mg/1000 g; P O_{25} - 30 mg/100 g; K_2O - 45 mg/100 g. (Popova & Sevov, 2010). The soil is characterized by good physical and mechanical properties, loose texture, weak plasticity and stickiness with good moisture and filtration capacity (Tahsin & Popova, 2005).

The durum wheat was grown in accordance with the established technology in Bulgaria (Bozhanova et al., 2018). The field trial was sown after a rapeseed precursor at the optimum time from 20.10. to 5.11. with a sowing rate of 500 germinating seeds/m^2 and mineral fertilization of 80 kg/ha P_2O_5 and 140 kg/ha N in active substance. Mineral fertilizers were applied to the soil according to the following scheme: the whole amount of phosphorus fertilizer and 1/3 of nitrogen fertilizer before the main tillage, and in early spring as feeding the remaining part of nitrogen fertilizer.

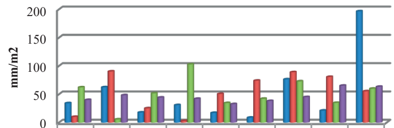
The biometric data of durum wheat varieties were recorded: productive maturity, ear length (cm), number of grains per ear, and grain mass per ear (g). Grain yield (t/ha) was reported by variants and replications.

The following physical properties were taken into account for grading the grain of the tested durum wheat varieties: mass per 1000 grains according to BDS ISO 520:2003; hectolitre mass according to BDS ISO 7971-2:2000; vitreousness of the grain according to BDS EN 15585:2008. The analysis of the grain according to the listed indicators was carried out at the Accredited Laboratory Testing Complex at the Agricultural University of Plovdiv. The field trial was harvested at full grain maturity by direct harvesting with a small Wintersteiger seedmaster universal combine.

The statistical processing of the data obtained for the studied indicators was performed with the BIOSTAT software (Penchev, 1998).

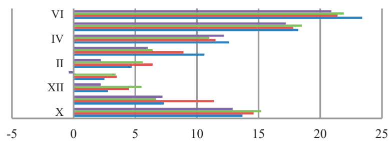
RESULTS AND DISCUSSIONS

In implemented field experiments, a number of authors (Spaldan et al., 1984) have demonstrated the influence on durum wheat grain productivity and quality of rainfall and its distribution over critical developmental phenophases and air temperatures during the growing season. Rainfall availability and average monthly temperatures compared to the climatic norm for the present experiment are presented in Figures 1 and 2.



	X	XI	XII	I	II	III	IV	V	VI
2018-2019	34,3	62,5	17,9	30,9	17,2	8,8	76,5	21,3	196,7
2019-2020	10,2	90,4	25,3	3	50,7	74,1	89,1	80,7	55,4
2020-2021	62,3	5,7	51,8	103	34,6	42,2	72,9	34,9	60
Climatic norm	40,1	48,4	44,3	42,1	32,7	38,2	45,1	65,3	63,4

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



	X	XI	XII	I	II	III	IV	V	VI
Climatic norm	12,9	7,2	2,2	-0,4	2,2	6	12,2	17,2	20,9
2020-2021	15,2	6,7	5,5	3,4	5,6	6,4	11	18,5	21,9
2019-2020	14,6	11,4	4,5	3,5	6,4	8,9	11,5	17,8	21,4
2018-2019	13,7	7,3	2,8	2,5	4,7	10,6	12,6	18,2	23,4

Figure 2. Monthly temperatures (average)

During the durum wheat growing season, rainfall quantity in all three years of the field trial (2018-2021) exceeded those for the multi-year time period. In the first year 2018-2019 the amount of rainfall was 466.1 mm, in the second year 2019-2020 it was 478.9 mm and in the third year 2020-2021 it was 467.4 mm against 419.6 mm for the climatic norm (Figure 1). During the durum wheat growing season, higher average monthly temperatures were observed compared to the climatic norm Figure

2. In terms of rainfall availability during the critical phenological phases of plant development determining the yield quantity and grain quality, the 2020-2021 crop year was more favourable for durum wheat growth. In the spring of 2020, there was more rainfall during flowering in the months of April and May by 44 mm/m^2 and 15.4 mm/m^2, respectively, compared to a multi-year period, which negatively affected pollination and flower fertilisation and this also led to the formation of fewer grains and lower grain yield in 2020 (Figures 1 and 2).

Biometric data of the durum wheat varieties studied

Height of plants

Plant height is a relatively constant value and primarily a varietal trait (Boudersa et al., 2021), but soil and climatic conditions as well as cultivation technology have a significant influence on the values of this indicator. Plant height is significantly influenced by the amount of rainfall from the tillering phenologicaal phase to the end of the ear formation phenological phase (Table 1[a]). In the conducting of this field experiment, it was found that the plants with the tallest stem were those of Railidur variety with an average of 88.1 cm for the study period with 2.2 cm or 2.6% more than Predel variety (Table 1[a]).

Table 1[a]. Biometric data (average 2018-2021)

Variety	Height of the plants, cm	Productive tillering, number	Lenght of the spike, cm
Viomi	87.3	1.58	8.46
Saya	86.7	1.51	8.21
Railidur	88.1	1.46	8.16
Helix	87.8	1.67	8.39
Predel	85.9	1.42	7.87
GD 5%	1.38	0.15	0.42

The varieties Helix (by 1.9 cm), Viomi (by 1.4 cm) and Saya (by 0.8 cm) were taller than the standard variety Predel.

Productive tillering

Tillering depends mainly on durum wheat variety, but soil and climatic conditions, sowing period, sowing depth, sowing rate, seed size, nutrient availability, light, etc. have a significant influence (Spaldan et al., 1984). A number of authors have shown that tillering is highly correlated with yield formation (Saman & Rashidi, 2012; Elhani, 2007; Delchev et al., 2000; Yani et al., 2012). The number of ears formed per unit area relative to the number of maximum tillers formed determines the productive tillering.

The highest productive tillering was observed in the varieties Helix - 1.67 tillers and variety Viomi - 1.58 pcs, respectively by 0.25 tillers and 0.16 tillers more than the standard variety Perdel. Followed by the varieties Saya - 1.51pcs and Railidur - 1.46 pcs which is 0.09 tillers and 0.04 tillers more than the standard, see Table 1[a]).

Ear length

Ear length is a relatively constant quantity because it is a genetically determined trait in each individual durum wheat variety (Yanev et al., 2000).

The longest ears on average during the study period were formed by plants of variety Viomi 8.46 cm (7.5%), followed by variety Helix 8.39 cm (6.6%), Saya 8.21 cm (4.3%), Railidur 8.16 cm (3.7%) compared to standard variety Predel (Table 1[a]).

Number of spikes in an ear

The length of the ear shoot and the number of spikelets per unit length determine the ear density. The formation of a higher number of spikes per ear is influenced by climatic conditions and the application of appropriate cultivation technology for durum wheat.

In the experiment we found that variety Helix had the highest number of spikes in the ear 27.9 pcs followed by variety Viomi by 27.1 pcs, Saya by 26.3 pcs and Railidur by 25.8 pcs, which is 15.3%, 12.0%, 8.7% and 6.6% more spikes than the standard respectively (Table 1[b]).

Table 1[b]. Biometric data (average 2018-2021)

Variety	Number of the spikelets per spike	Number of the grains per spike	Mass of the grain per spike
Viomi	27.1	52.5	2.39
Saya	26.3	51.9	2.27
Railidur	25.8	51.1	2.23
Helix	27.9	53.2	2.47
Predel	24.2	47.9	2.19
GD 5 %	2.76	4.24	0.18

Number of grains per ear

The number of grains per ear is one of the important elements of productivity in cereal crops. Increasing the number of grains is achieved by applying optimal technological measures, which leads to an increase in grain yield per hectare. Precipitation during the tillering phase until the end of the flowering phase has a significant effect on the number of grains. The amount of rainfall in the months of April and May of the third year of the field experiment was 44 mm/m^2 and 15.4 mm/m^2 more, respectively, compared to the multi-year period. All durum wheat varieties tested in the spring of 2020 formed fewer grains per ear due to more precipitation falling during flowering (Figure 1). This rainfall prevented normal pollination and flower fertilization resulting in lower grain yields.

Hard wheat variety Helix formed the highest number of grains in the ear 53.2 pcs (11.1%) on average over the three years of the experiment. The second place was taken by variety Viomi by 52.5 pcs (9.6%), variety Saya by 51.9 pcs (8.4%) and variety Railidur by 51.1 pcs (6.7%) compared to standard variety Predel (Table 1[b]).

Grain mass per ear

The productivity of durum wheat depends very much on the mass of the grains in the ear. The climatic conditions during the period of grain formation, the optimum conditions for plant development until the end of the growing season, the cultivation technology and the genetic make-up of a variety are all essential for forming the value of this component. During the three-year experiment in field conditions, it was found that, of the durum wheat varieties tested, the highest grain mass per ear was formed by plants of the variety Helix 2.47 g (12.8%) (Table 1[b]). This was followed by the varieties Viomi by 2.39 g (9.1%), Saya by 2.27 g (3.7%), Railidur by 2.23 g (1.8%).

Grain yield

Soil and climatic conditions in the area of durum wheat production and the variety studied have a significant influence on the yield and quality of the grain obtained. The tested new varieties of durum wheat selected at the Institute of Field Crops, Chirpan, exceeded the productivity standard of the Predel variety (Table 2).

The productivity of the tested durum wheat varieties was highest in the climatically favourable year 2021 for plant growth and development, followed by that in 2019. The harvested grain of the durum wheat varieties included in the experiment was lowest in 2020 due to the higher rainfall in spring with 95.7 mm/m^2 more than the climatic norm. The unfavourable climatic conditions during flowering prevented normal pollination and fertilisation of the flowers, and hence to the formation of a small number of grains in the ear with low grain mass and lower productivity of the durum wheat varieties.

Table 2. Grain yield

Variety	2018-2019 t/ha	2019-2020 t/ha	2020-2021 t/ha	Average	
				kg/ha	%
Viomi	4.751	3.547	5.418	4.572	108.3
Saya	4.642	3.434	5.186	4.421	104.7
Railidur	4.601	3.388	5.147	4.379	103.7
Helix	4.960	3.671	5.564	4.732	112.1
Predel	4.425	3.318	4.921	4.221	100.0
GD 5 %	0.294	0.210	0.438		

From the data presented in Table 2, it is evident that both by year and on average for the study period, the highest grain yield was obtained from the Helix variety - 4.732 t/ha (12.1%), compared to 4.221 t/ha for the standard Predel variety. By year, Helix variety yielded 4.960 t/ha (12.1%) in 2019, 3.671 t/ha (10.6%) in 2020 and 5.564 t/ha (13.1%) in 2021. The increase in grain yield by year is 0.535 t/ha in the first year, 0.353 t/ha in the second year and 0.643 t/ha in the third year, or an average by 0.511 t/ha more than the productvity standard Predel variety. The results are unidirectional and mathematically proven. The durum wheat varieties Viomi and Saya yielded an average of 4.572 t/ha (8.3%) and 4.421 t/ha (4.7%) respectively over the study period, which is 0.351 t/ha and 0.2 t/ha more than the variety Predel. The higher productivity of the Helix variety was mathematically proven over the three years of the experiment, while the Viomi variety was proven to have higher yield only in 2021. The increase in grain yield in the Railidur variety is mathematically unproven.

Physical properties of durum wheat varieties

Mass per 1000 grains

Mass per 1000 grains is a varietal trait that is strongly influenced by soil and climate conditions and cultivation technology. This indicator characterises grain well-nourishness and serves as an indication of yield. When larger grains are used as seed, yields have been shown to be up to 15-20% higher (Dekov et al., 1989).

The variety Helix stands out with the highest mass per 1000 grains of the tested durum wheat varieties (Table 3). On average over the study period, the mass per 1000 grains of this variety was 49.9 g., followed by variety Viomi with 48.3 g., variety Saya 47.5 g. and variety Railidur 47.1 g.

Table 3. Physical properties of durum wheat varieties (average 2018-2021)

Variety	Mass of 1000 grains, g	Hectoliter mass, kg	Vitreousness, %
Viomi	48.3	79.3	96.9
Saya	47.5	77.8	96.1
Railidur	47.1	78.3	95.8
Helix	49.9	80.5	97.4
Predel	46.5	77.1	95.3
GD 5%	3.25	2.14	1.52

Hectolitre mass

Hectolitre mass is an important physical indicator used to determine the quality of grain. This indicator gives an idea of uniformity, surface area and density. The hectolitre mass depends on the type of impurities, weed seeds, unthreshed ears and the consistency of the grain. With higher hectolitre grain mass of the studied durum wheat varieties were Helix 80.5 kg and Viomi 79.3 kg compared to the variety Predel (Table 3).

Vitreousness

Grain vitreousness in durum wheat is strongly influenced by many factors, but the decisive ones are the variety with its genetic make-up, the soil and climatic conditions at the time of grain formation and ripening. Vitreousness is an important physical property and its high values result in high yielding flours.

Durum wheat is characterised by a high vitreous grain. It can be seen from Table 3 that, on average over the three-year study period, vitreousness was very high in all durum wheat varieties tested. In the case of the Helix variety it was 97.4%, followed by Viomy with 96.9%, Saya with 96.1%, Reilidur with 95.8% and in the case of the Predel standard it was 95.3%.

CONCLUSIONS

Under the soil and climatic conditions of the Plovdiv region, the highest grain yield was harvested from the Helix variety with an average of 4.732 t/ha (12.1%) compared to 4.221 t/ha for the standard Predel variety. By year, Helix variety yielded 4.960 t/ha (12.1%) in 2019, 3.671 t/ha (10.6%) in 2020 and 5.564 t/ha (13.1%) in 2021. The increase in grain yield by year is 0.535 t/ha in the first year, 0.353 t/ha in the second year and 0.643 t/ha in the third year, or an average of 0.511 t/ha more than the productivity standard Predel variety.

The yields of the other durum wheat varieties studied were as follows: variety Viomi 4.572 t/ha with 0.351 t/ha (8.3%); variety Saya 4.421 t/ha with 0.200 t/ha (4.7%) and variety Railidur 4.379 t/ha with 0.158 t/ha (3.7%) more grain compared to variety Predel.

The productivity of the tested durum wheat varieties was higher than the standard variety Predel, which was the result of the higher productive tillering, the longer and riper ear and the higher grain mass in the plant ear.

Durum wheat variety Helix had the highest mass per 1000 grains with an average for the experimental period - of 49.9 g., followed by variety Viomi with 48.3 g. Next follow the variety Saya with 47.5 g. and variety Railidur with 47.1 g.

The hectolitre grain mass of the studied durum wheat varieties was higher in the varieties Helix 80.5 kg and Viomi 79.3 kg compared to the variety Predel.

Vitreousness of the new durum wheat varieties tested was very high on average over the three-year study period. It was 97.4% for the Helix variety, followed by Viomi with 96.9%, Saya with 96.1%, Railidur with 95.8%, while - 95.3% for the Predel standard.

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RESEARCH ON CYTOGENETIC EFFECTS INDUCED BY TREATMENTS WITH DIFFERENT MUTAGENIC SUBSTANCES IN *Arachis hypogaea* L. (ARAHIDS)

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Abstract

The mitotic division, from a genetic point of view, ensures the copying of the genetic message in duplicate, transferring it to the descending cells and transmitting the genetic message from one cell to another within an individual (organism), maintaining the constants, number, shape and size of the chromosomes. In mitosis, the processes that take place are irreversible. As a result of mitosis, each of the two daughter cells has a diploid number of chromosomes (2n) in which DNA is included which gives the identity of the genetic information with the mother cell Mitosis interests in a special way, due to the wide possibilities offered by the karyotype study, a basic component in the identification of species. The mitotic index is one of the parameters successfully used in cytogenetic studies of mitosis. The main purpose of the research was to capture the cytogenetic effects induced by the mutagenic treatments in arahids species (Arachis hypogaea L.). For cytogenetic investigations, radicular meristems obtained from the germination of peanut seeds were used, which were later used to obtain microscopic preparations by the method developed by Feulgen. Following the research carried out it was established that the most pronounced mutagenic effect of mitotic indecision reduction was obtained after sodium azide treatments. In the case of ethyl metalsulfonate and dimethyl sulfate, there were noted increases in mitotic indecin at concentrations of 0.2% and 0.4%.

Key words: *peanuts seeds, ethyl metansulfonate (EMS), dimethyl sulfate (DMS), sodium azide (SA), mitosis.*

INTRODUCTION

The number of somatic chromosomes in *Arachis hypogaea* L. is equal to 40 (2n = 40) (Gregory et al, 1973; Resslar, 1980; Stalker, 1991; Dhesi and Stalker, 1994; Krapovickas and Gregory, 1994; Lavia Graciela Inés, 1996; Lavia Graciela Inés and Fernandez, 2004, Neiva et al, 2001; Peñaloza and Valls, 2005; Valls and Simpson, 2005; Silvestri et al, 2015; Ortiz et al, 2017, Dima et al, 2023).
One of the most important early indicators of mutagenic action of any physical, chemical or biological mutagenic agent is the frequency of chromosomal aberrations in mitosis (Kumar and Sing, 2003; Bhat et al, 2007; Kumar and Yadav, 2010; Jafri et al., 2013).
Important mutant lines have been developed in production after treatment with ethyl metansulfonate on peanut seeds. Previous attempts to induce mutations using chemicals have reported alternations on morphological characteristics, but rarely mentioned whether there were changes on the quality characteristics of peanuts (Jung et al., 2000; Wang et al., 2006). Mutant plants obtained by treatment with sodium azide are able to survive in different environmental conditions and have improved harvests, increased tolerance to stress, and longer life compared to untreated plants (Quarainy and Khan, 2009).
The successful use of sodium azide to generate variability in plant breeding has been discussed in barley (Kleinhofs and Sander, 1975) and other

culture species (Avila and Murty, 1983; Micke, 1988; Routaray et al., 1995). The dose with the most pronounced effect for inducing morphological mutations was established at 0.03% sodium azide. The main advantage of improvement using mutations is the ability to improve one or two characteristics without altering the rest of the genotype (Mensah and Obadony, 2007; Seijo et al., 2017).

Dimethyl sulfate is a colourless or slightly yellowish liquid with a slight onion odour. The substance is toxic, corrosive and mutagenic (Rippey and Stallwood, 2005; Pohanish, 2008).

Dimethyl sulfate may affect specific cleavage in DNA by breaking the imidazole rings present in guanine (Cartwright and Kelly, 1991; Rippey and Stallwood, 2005).

Sodium azide is considered to be a very potent chemical mutagen in culture plants. Mutant plants resulting from sodium azide treatment are able to survive in different environmental conditions, they have a better yield and are stress tolerant compared to normal plants (Fahad and Salim, 2009; Alka Ansari et al, 2012; Kumar and Srivastava, 2013).

The mutagenic effect results from the production of an inorganic sodium azide metabolite. This metabolite enters the nucleus, interacts with DNA and creates point mutations in the plant genome (Owais and Kleinhofs, 1988).

Ethyl metansulfonate causes the introduction into the nucleotide structure of some alkyl groups: at the atom level of the 7th position of the guanidine structure (most often), at the 3rd position of adenine, the, very rare in 1st position of adenine (Kumar and Rai, 2005; Srivastava and Kapoor, 2008; Srivastava et al., 2011; Girjesh Kumar and Kshama Dwivedi, 2013). Also, the presence of alkyl group in guanidine weakens the ß-glucoside binding and causes the elimination of guanine (depuration), with the formation of breaches in the DNA structure (Kumar and Dwivedi, 2013).

Pyrimidine nitrogen bases are more resistant to alkylating agents, although ethyl metansulfonate can sometimes cause cytosine alkylation. Alkylation is accompanied by DNA distortion or cross-linking or transverse nucleotides, which prevents normal mating. Ethyl metansulfonate is highly effective in modifying genetic material of both prokaryotic and eukaryotic cells (Roychowdhury et al., 2012).

The main purpose of the research carried out during 2013-2015 period was to capture the cytogenetic effects induced by the mutagenic treatments in two peanut genotypes.

MATERIALS AND METHODS

The biological material used for research was represented by *Arachis hypogaea* L. seeds, varieties Tâmbureşti and Jelud, varieties created at the University of Craiova, Romania.

The mutagenic substances used to induce variability were ethyl metansulfonate (EMS), dimethyl sulfate (DMS) and sodium azide (SA). Ethyl metansulfonate and dimethyl sulfate were in concentrations of 0.2%, 0.4%, 0.6% and 0.8%, and sodium azide was in concentrations of 0.02%, 0.04%, 0.06% and 0.08%, with each concentration having an action time of 6 hours. The control variant was represented by each variety, to which no mutagenic treatments were applied.

For the identification of chromosomal aberrations and their frequencies, the classical method was used which involves the following stages (Ţîrdea and Leonte, 2003): fixing, hydrolysis, coloring, performing microscopic preparations, examining preparations under a microscope.

Fixation was done when the roots reached the length of 10-15 mm. These were placed in the Carnoy I fixator (absolute ethyl alcohol and glacial acetic acid at 3:1 ratio) at room temperature for 24 hours (Figure 1).

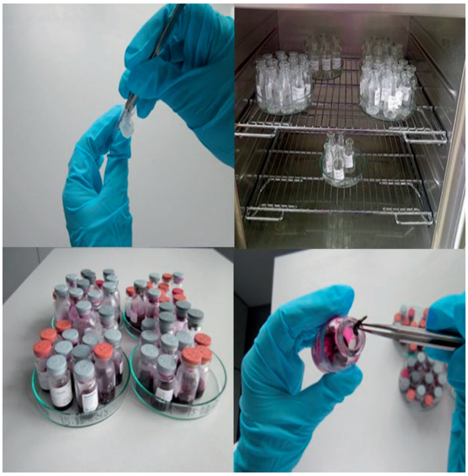

Figure 1. Fixing, hydrolysis and coloring of peanuts roots

Fixation is intended to kill the cells in the state they are in at that time, a process that takes place very quickly, almost instantly. By fixation, the fine structure of all cellular organelles must be preserved. In addition, the biocoloids of the cell coagulate and as a result, the cellular constituents can be colored with different dyes. Until the roots were processed for coloring, they were stored in the refrigerator, in ethyl alcohol 70%.

Hydrolysis aimed the maceration of tissues by partial dissolution of peptic substances between cells, which facilitates the process of staining and then display of cells between the blade and the slide.

The fixed roots were washed with distilled water, then introduced into HCl 1 N solution and left for 14 minutes at 60°C (Figure 1).

Coloring was done by keeping the roots in Carr solution in the refrigerator, until the tip of them became intensely colored (about 24 hours).

The area of the root apex has been colored faster and more intense because here most of the cells are in division, while the rest of the root remained visibly uncolored because the frequency of divisions was lower (the cells being large and elongated).

This dye caused the nuclei and chromosomes to color in red-violaceus, the cytoplasm being pale pink (Figure 1).

Making microscopic preparations, after the squash method was done on a clean, dry and degreased microscope blade, in a drop of Carr dye with the help of tweezers put the roots of a peanut grain.

Using a spatula needle or a shaving blade, 1-2 mm of the colored tip of the roots (of a single grain) were cut over which a clean and degreased. With the help of a filter paper, the blade was fixed so that excess Carr dye was absorbed and at the same time the slide of the blade onto the blade was avoided. With the help of a match stick hit the lemon (in the area where the root is located) at first weaker, then stronger, then stronger, to ensure perfect display of metaphasic cells and chromosomes.

After the display, between the blade and the slide, a very fine, barely perceptible layer of red-violaceus coloured material was observed with the naked eye. After the display was finished, a piece of filter paper was placed over the blade, gently pressing to absorb excess acetic acid, to complete the cell display, and, dispersion of chromosomes and so that the cells to cling to the blade, but especially to the slide and not fall when performing permanent preparations. And this operation was carried out with great care to avoid sliding the blade on the blade, from the initial site, which leads to cell rolling and partial or total compromise of the preparation.

The examination of the preparations under the microscope was carried out in bright light, at first with the 10x lens, with which the preparation is examined in the sea, and the reading of the preparation was done at the 40x objective. The data obtained were used to calculate the mitotic index and the frequency of cell types in mitotic division.

The photos were taken using the Cannon digital camera, adapted to the Hund - Wetzlar optical microscope at the 100x immersion lens.

Photographs were taken of the interphase, prophase, metaphase, anaphase and various types of chromosomal aberrations encountered.

Following the analysis of mitosis phases, the mitotic index was determined. This represents the percentage of cells in division, compared to the total number of cells analyzed (Tirdea and Leonte, 2003). The mitotic index will be calculated using the following relation:

$$I_m (\%) = (N_m \times 100) \cdot N_t^{-1}, \text{ where:}$$

I_m = mitotic index;
N_m = number of cells in division;
N_t = total number of cells analyzed.

RESULTS AND DISCUSSIONS

In the Tâmbureşti variety, the mitotic index in the case of the control variant had the value of 18.04%. Table 1 shows that the mitotic index decreased as the concentration of the mutagenic chemical increased.

In the case of ethyl metansulfonate at a concentration of 0.2%, the mitotic index was higher, with a distinctly significant difference (5.12%). In the 0.4% concentration of ethyl metansulfonate, the mean value of the mitotic index was 13.17% and the concentration of 0.8% was 13.55%, the differences from the control being negative, distinctly significant (4.87% and 7.44%, respectively).

Table 1. Effect of treatments with mutagenic agents on mitotic index (%) of the Tâmbureşti variety

Variant		Average (%)	Diff. (%)	Signif.
Control		18.04	Control	Control
EMS 6 h	0.2%	23.16	5.12	**
	0.4%	13.17	-4.87	oo
	0.6%	13.55	-4.49	o
	0.8%	10.60	-7.44	oo
	LSD 0.05 = 3.1; LSD 0.01 = 4.7; LSD 0.001 = 7.5			
DMS 6 h	0.2%	27.95	9.91	***
	0.4%	23.78	5.74	***
	0.6%	14.00	-4.04	oo
	0.8%	9.82	-8.22	ooo
	LSD 0.05 = 2.2; LSD 0.01 = 3.4; LSD 0.001 = 5.4			
SA 6 h	0.02%	16.60	-1.44	
	0.04%	13.07	-4.97	oo
	0.06%	10.35	-7.69	ooo
	0.08%	9.59	-8.45	ooo
	LSD 0.05 = 2.3; LSD 0.01 = 3.4; LSD 0.001 = 5.5			

The decrease in the percentage of cells in the division was also found in the treatment with dimethyl sulfate at a concentration of 0.6% (14.0%) and 0.8% (9.82%), the differences with the witness are distinctly significant and very significant.

All dimethyl sulfate in the concentration of 0.2% and 0.4% had a strong stimulating effect, the mitotic index being higher than that of the control variant.

Sodium azide treatments had a more pronounced effect, negatively influencing the mitotic index at all concentrations of the chemical agent mutagen. In the 0.02% concentration, the mean mitotic index was 16.6%, the difference from the control variant being insignificant (Table 1). At 0.04% sodium azide treatment the difference from the control was distinctly significant (4.97%). Concentrations of 0.06% (7.69%) and 0.08% (8.45%) showed a sharp decrease in the percentage of cells in the division, the differences being very significant compared to the untreated control variant (Table 1).

From the analysis of the mitotic index, at the Tâmbureşti variety, it can be seen that the most pronounced mutagenic effect of reducing this value was recorded at the sodium azide treatments followed by those with ethyl metansulfonate and finally those with dimethyl sulfate. An increase in the mitotic index was observed in the concentration of 0.2% and 0.4% of dimethyl sulfate and 0.2% of ethyl metansulfonate. These concentrations have a slight stimulating effect on cell division (Table 1).

For the Jelud variety the mean value of the untreated control variant was 17.68% cells in the division (Table 2).

Table 2. Effect of treatments with mutagenic agents on mitotic index (%) of the Jelud variety

Variant		Average (%)	Diff. (%)	Signif.
Control		17.68	Control	Control
EMS 6 h	0.2%	17.68		
	0.4%	23.00	5.32	***
	0.6%	20.50	2.82	**
	0.8%	13.17	-4.51	ooo
	LSD 0.05 = 1.4; LSD 0.01 = 2.2; LSD 0.001 = 3.5			
DMS 6 h	0.2%	24.50	6.82	***
	0.4%	16.12	-1.56	oo
	0.6%	12.26	-5.42	ooo
	0.8%	8.42	-9.26	ooo
	LSD 0.05 = 0.9; LSD 0.01 = 1.4; LSD 0.001 = 2.3			
SA 6 h	0.02%	15.63	-2.05	o
	0.04%	12.27	-5.41	ooo
	0.06%	10.09	-7.59	ooo
	0.08%	8.40	-9.28	ooo
	LSD 0.05 = 1.5; LSD 0.01 = 2.3; LSD 0.001 = 3.6			

In the Jelud variety, treatment of peanut seeds with ethyl metansulfonate influenced the number of dividing cells compared to the control sample as the concentration of the mutagen increases. At the concentration of 0.2% an increase in the mitotic index was observed, the difference from the control being very significant (5.32%). Conversely, the 0.6% and 0.8% following ethyl metansulfonate treatment have very significant negative differences (4.51% and 8.30%, respectively) compared to the untreated control sample (Table 2).

Dimethyl sulfate at a concentration of 0.2% had a strong stimulating effect, with the mitotic index being higher than that of the control variant. With the increase in concentration, the percentage of cells in the division of dimethyl sulfate treatments appears to be reduced at a concentration of 0.4% (1.56%), 0.6% (5.42%) and 0.8% (9.26%), the differences from the control variant being distinctly significant and very significant (Table 2).

All dimethyl sulfate in the concentration of 0.2% and 0.4% had a strong stimulating effect, the mitotic index being higher. In the case of sodium azide, the mitotic index decreased as the concentration of the mutagenic chemical increased (Table 2).

Thus, the mitotic index recorded lower values, the differences being negative, significant at the concentration of 0.02% (2.05%) and very significant at the concentrations of 0.04%

(5.41%), 0.06% (7.59%) and 0,08% (9.29%) of the untreated control variant (Table 2).

In the control variant of the Tâmbureşti variety, chromosomal aberrations were identified in a proportion of 0.84%. Treatments with mutagenic substances in different concentrations caused chromosomal aberrations to occur in a higher percentage, the differences from the control variant were very significant. Exception to this rule were treatments with dimethyl sulfate at a dose of 0.2% (5.31%), the difference being significant compared to the untreated control variant (Table 3).

Table 3. Effect of treatments on frequency of chromosome aberrations (%) in mitosis of Tâmbureşti variety

Variant		Average (%)	Diff. (%)	Signif.
Control		0.84	Control	Control
EMS 6 h	0.2%	6.12	5.28	***
	0.4%	10.14	9.30	***
	0.6%	11.27	10.43	***
	0.8%	14.53	13.69	***
	LSD 0.05 = 1.8; LSD 0.01 = 2.7; LSD 0.001 = 4.4			
DMS 6 h	0.2%	6.15	5.31	*
	0.4%	13.11	12.27	***
	0.6%	14.69	13.85	***
	0.8%	20.32	19.48	***
	LSD 0.05 = 4.3; LSD 0.01 = 6.6; LSD 0.001 = 10.5			
SA 6 h	0.02%	5.41	4.57	***
	0.04%	6.57	5.73	***
	0.06%	11.29	10.45	***
	0.08%	15.36	14.52	***
	LSD 0.05 = 1.6; LSD 0.01 = 2.4; LSD 0.001 = 3.9			

Treatment with sodium azide at 0.02% resulted in a distinctly significant difference from the untreated control variant (5.47%).

Chromosomal aberrations identified in metaphase, anaphase and telophase of mitosis were obtained and photographed in microscopic preparations, resulting in the total frequency of chromosomal aberrations (Figures 2-5).

Like the Tâmbureşti variety, in the Jelud variety, most of the treatments made at peanut seeds have determined positive differences, very significant, compared to the untreated control variant (Table 4).

Ethyl metansulfonate at a concentration of 0.2% resulted in an increase in the number of chromosomal aberrations, with significant differences from the control variant (7.04%). Dimethyl sulfate at a concentration of 0.4% (4.01) produced an increase in the number of chromosomal aberrations, with distinctly significant differences from the control variant (Table 4).

Table 4. Effect of treatments on frequency of chromosome aberrations (%) in mitosis of Jelud variety

Variant		Average (%)	Diff. (%)	Signif.
Control		0.82	Control	Control
EMS 6 h	0.2%	7.86	7.04	*
	0.4%	13.19	12.37	**
	0.6%	19.85	19.03	***
	0.8%	20.70	19.88	***
	LSD 0.05 = 6.0; LSD 0.01 = 9.1; LSD 0.001 = 14.6			
DMS 6 h	0.2%	2.41	1.59	
	0.4%	4.83	4.01	**
	0.6%	14.05	13.23	***
	0.8%	16.36	15.54	***
	LSD 0.05 = 2.4; LSD 0.01 = 3.7; LSD 0.001 = 5.9			
SA 6 h	0.02%	6.29	5.47	**
	0.04%	13.04	12.22	***
	0.06%	19.82	19.00	***
	0.08%	22.83	22.01	***
	LSD 0.05 = 3.5; LSD 0.01 = 5.3; LSD 0.001 = 8.5			

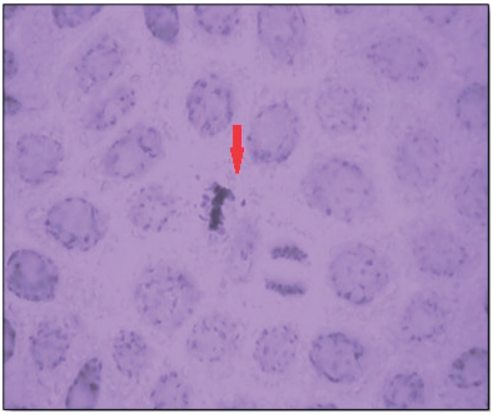

Figure 2. Metaphase, with the remaining chromosome and fragment

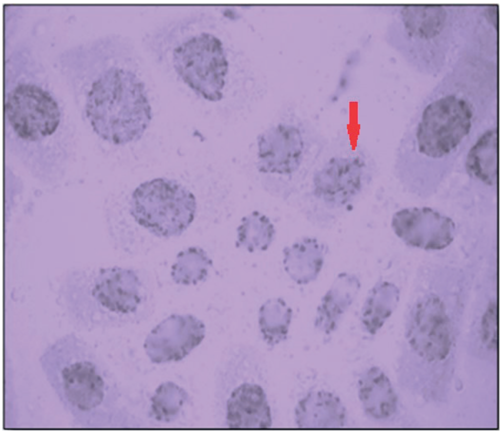

Figure 3. Telophase with micronucleus

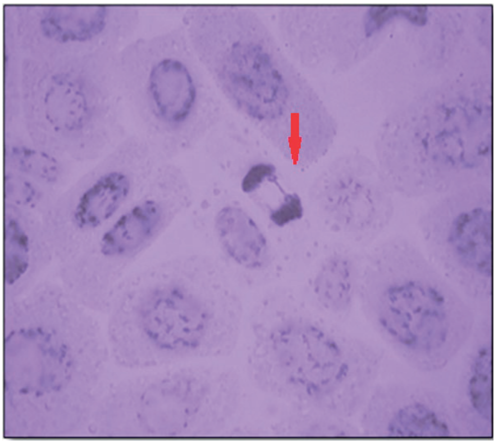

Figure 4. Anaphase with decks

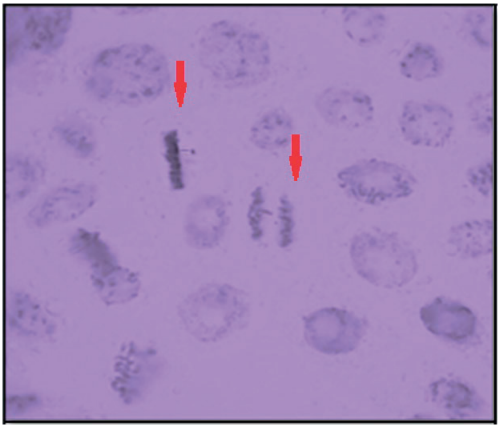

Figure 5. Metaphase with fragment and anaphase with bridge

CONCLUSIONS

As a result of the research carried out, the strongest mutagenic effect of mitotic index value reduction was recorded for sodium azide treatments followed by those with ethyl metansulfonate and dimethyl sulfate.

As the time of action of the mutagenic agent increases, a reduction in the mitotic index occurs. This is demonstrated by the presence of a smaller number of cell divisions, inversely proportional to the exposure time.

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PHENOLOGICAL DEVELOPMENT AND GRAIN YIELDS FOR TRITICALE VARIETIES IN CENTRAL SOUTHERN BULGARIA

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Abstract

The phenological development and grain yield of triticale varieties of different originated were studied in a three-year field experiment for the period 2019-2022. Based on the De Martonne Drought Index, the conditions of the growing seasons were characterized. The dates of the occurrence of the main stages of plant development were recorded, and the interphase periods were characterized based on the number of days with an active temperature above 5°C, the sum of the active temperature, the average temperature, and precipitation. Correlational dependences between grain yield and meteorological parameters during the interphase periods were found. The functional relationship between the amount of precipitation and grain yield was described using mathematical models thus, the grain yield can be predicted. The effects of the environment and the interaction between the varieties and the environment on grain yield were confirmed. The most productive varieties among the tested ones were shown.

Key words*: phenological development, grain yields, triticale.*

INTRODUCTION

Triticale (×*Triticosecale* Wittm.) is a crop with high potential for grain and biomass yield and high ecological plasticity. The triticale has demonstrated its ability to withstand harsh dryland conditions (Kankarla et al., 2020). Bulgarian triticale varieties have a high genetic potential for grain yield (Baychev & Stoyanov, 2019; Stoyanov, 2021; Stoyanov et al., 2022a; Stoyanov et al., 2022b).

Several studies have demonstrated a relationship between grain yield and specific phenological periods. Guo et al. (2018) have found a close relationship between grain yield and the length of the pre-flowering interphase period in which the ear forms. Uspenskaja et al. (2018) have confirmed that the most important traits associated with grain yield and grain fullness in winter triticale is the duration of the growing season, which consists of the duration of the interphase periods. In recent years, the acceleration of phenological phases and changes in their occurrence and duration have been reported (Kalbarczyk, 2009). Xiao et al. (2021) noted that changes in crop phenology are closely related to regional climate variability.

The biology of cereal crops is related to the particular requirements for temperature, light and moisture, during the growth phases and development stages (Chanev & Filchev, 2021). Because meteorological factors vary in geographical areas, cultivars have different levels of production. To achieve high grain production, the response of varieties to different agro-meteorological conditions should be considered, and cultivation should be localized in specific areas. One study showed a significant relationship between soil conditions and the growth, physiology, and yield of triticale (Habib-ur-Rahman et al., 2022).

Phenological studies of triticale in Bulgaria are scarce. Several studies have analyzed a small number of varieties in individual locations (Kirchev et al., 2010; Kirchev & Muhova, 2018; Krusheva, 2021).

The purpose of this study was to observe the phenological development of triticale varieties under the conditions of Central South Bulgaria and to evaluate grain yield.

MATERIALS AND METHODS

The experimental work was carried out in the period 2019-2022 at the Institute of Field Crops, Agriculture Academy (Bulgaria). The object of this study was eight Bulgarian varieties of triticale: Kolorit (standard), Akord, Borislav, Blagovest, Doni-52, Dobrudzhanets, Irnik, and Lovchanets, and two originating in Poland: Avocado and Casino. The field trial was carried out on Pelic Vertisols, under non-irrigated conditions without fertilization. A block method was used with an experimental plot size of 1.20/8.30 m and three repetitions of the variants. Seeds were sown at 550 germinating seeds/m^2 as follows: October 23, 2019, November 5, 2020, and November 15, 2021.

The phenological phases were registrered according to the BBCH scale (Zadoks et al., 1974), according to which the first number indicates the main stage of growth (0-9) and the second is the secondary growth stage (0-9) (Meier, 2018). The following codes were used to note phenological phases: sowing (00), emergence (09), 3^{th} leaf (13), tillering (21), stem elongation (31), spike emergence (49), first anthers visible (61), late milk (77), soft dough (85) and fully ripe (92). The beginning of the phase was recorded visually when it occurred at 10% of the plants. A destructive method was used to record the phase from germination to stem elongation. Ten plants for each variety were taken from two replicates, and the percentage at which the phase occurred was calculated as the total. The interphase period represents the time from the start of the previous phase until the start of the next phase. These periods are characterized by the following traits: number of days with active temperatures above 5°C (x_1); sum of active temperatures (Σ t $_{act.}$°C) for the period (x_2); mean temperature (t $_{aver.}$°C) calculated as the ratio of Σ t $_{act}$.°C and number of days with an active temperature above 5°C (x_3); amount of precipitation over the duration of a period (x_4).

Harvesting was performed using a small-calibre harvester in a fully ripe state and the grain yield in kg/ha was recalculated.

The average daily temperature and precipitation data were obtained from a weather station (National Institute of Meteorology and Hydrology) located in the experimental field of the institute (42°12'52", N 25°16'57" E).

Weather conditions during the growing season were evaluated using the De Martonne drought index (Faragó et al., 1989). The following formula was used:

$$I_{DMI} = \frac{P}{T + 10},$$

where: I_{DMI} - De Martonne drought Index; P - amount of precipitation for the growing season; T - average daily air temperature.

A statistical grouping of the data was performed. Mathematical methods were used for data evaluation and processing. The processing of the information was done with Statistica 13. The Biostat statistical program was used to perform an analysis of variance (Penchev et al., 1989-1991). The means were compared for significance using the least significance difference (LSD) test at p = 5.0, p =1.0, and p =0.1%. Correlation regression analysis was applied to investigate grain yield trends. This analysis helps determine changes over time in the dependent variable (y) using a linear regression equation:

$$y = a + b x,$$

where: y - grain yield; x - independent variable; a, b - parameters of the equation.

RESULTS AND DISCUSSIONS

The meteorological conditions during the study periods were compared with the long-term period of 1991-2021 (Figure 1). The three growing seasons are warmer and wetter. During the periods 2019-2020, 2020-2021 and 2021-2022, the temperature sums were higher, respectively by 339.0, 230.9 and 165.5°C, as well as the amount of precipitation, by 23.2, 51.8, and 74.8 mm. The De Martonne drought index values for 2019-2020, 2020-2021 and 2021-2022 are 22.3, 24.3 and 26.5, respectively. This index characterizes the climatic conditions in the first period as Mediterranean, and in the other two periods, the conditions were semi-humid. According to Royo et al. (1999), genotypic variability is primarily associated with plant phenology. The graphs represent the varietal characteristics of the meteorological parameter averages for the periods. The period from sowing to emergence did not differ according to the duration (Figure 2). This is determined by equal hydrothermal requirements for germination and emergence, and subsequently the development to the beginning of the light stage. Similar observations for triticale have been described by other authors (Kirchev et al., 2010; Kirchev & Muhova, 2018; Stefanova-Dobreva, 2021; Muhova, 2021).

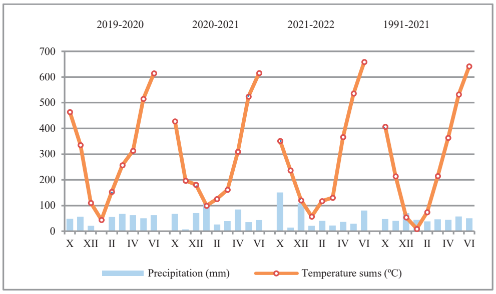


Figure 1. Weather conditions during the growing seasons in triticale and referent period

The Kolorit variety passed the tillering-stem elongation period for the shortest time (40 days). This has also been reported in another study (Stefanova-Dobreva, 2021). For the Avocado variety, the shortest period of spike emergence-first anthers visible was established (6.7 days) and longest stem elongation-spike emergence (22.7 days). For the Casino variety, the longest periods of tillering-stem elongation (45.6 days) and first anther visible-late milk (21 days) were characteristic. The Bulgarian variety Blagovest differed in the shortest duration of late milk-soft dough (14.7 days) and soft dough-fully ripe (7 days). The Casino variety reached full ripening for the longest period of ten days after the beginning of soft dough.

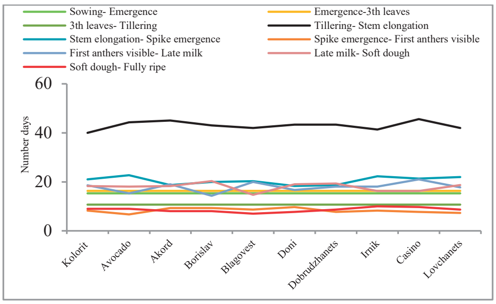


Figure 2. Duration of interphase periods on average for the varieties (2019-2022)

The temperatures were equal from sowing to tillering for all cultivars, as shown in Figure 3. This can be explained by the simultaneous occurrence of the phenological phases. Remarkable differences are visible in this parameter during the stages of spike emergence-first anthers visible, first anthers visible-late milk, and soft dough-fully ripe. For Kolorit and Dobrudzhanets, the lowest temperature sums during tillering-stem elongation and stem elongation-spike emergence were calculated to be 379.3°C and 259.0°C, respectively. The sum of the temperatures from stem elongation to spike emergence for the Avocado variety was the highest (308.5°C) and for Casino, it was the lowest (92.6°C).

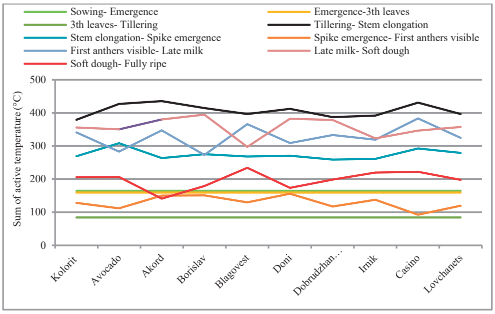


Figure 3. Temperature sum during interphase periods for the varieties (2019-2022)

The mean temperature showed a smooth dynamic within cultivars after phase of stem elongation to soft dough, as shown in Figure 4. The lowest average temperature during stem elongation-spike emergence (13.9°C) was determined for the Kolorit variety. Similarly, for Blagovest and Irnik varieties, the lowest average temperatures were recorded in four interphase periods, namely: for Blagovest during spike emergence-first anthers visible (15.2°C) and soft dough-fully ripe (23.0°C); for Irnik variety during stem elongation - spike emergence (13.9°C) and first anthers visible-late milk (17.5°C). The highest average temperatures respectively, 15.0 and 18.7°C were calculated for the Doni variety during stem elongation-spike emergence, and first anthers visible-late milk, respecively. The Casino variety passed through two periods with the highest average temperature - 17.3 and

21.1°C spike emergence-first anthers visible and late milk-soft dough, respectively.

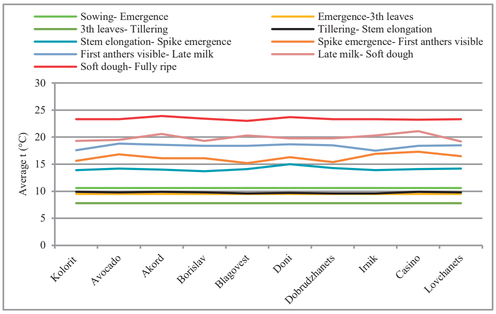


Figure 4. Average temperature during interphase periods for the varieties (2019-2022)

In the 3[th] growing season, different distributions of rainfall during the interphase periods were registered (Figure 5). During the critical period of spike emergence-first anthers visible, the least amount of precipitation was recorded for the Kolorit, Blagovest and Dobrudzhanets varieties. The highest amount of precipitation (13.6, 31.2 and 25.4 mm) was found for the Casino variety in three interphase periods: spike emergence-first anthers visible, first anthers visible-late milk and soft dough-fully ripe, respectively.

A characteristic of the average values of the meteorological parameters during interphase periods is shown in the following tables.

The average temperature and humidity of the soil layer are determinative factors for emergence. It is necessary for the seeds to absorb water of about 50-60% of their mass, to be able to imbibe and sprout. Zartash et al. (2020) have indicated an optimum temperature from germination to emergence in wheat of 24.0-28.0°C, referring to data from Porter & Gawith (1999).

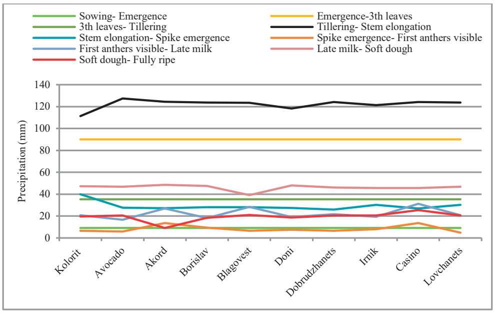


Figure 5. Precipitation during interphase periods for the varieties (2019-2022)

This first period of organogenesis is important because timely germination plays a key role in the formation of primary and secondary root systems and subsequently overwintering through tillering. The period from sowing to emergence had a duration 13-17 days under $t_{aver.}$ 8.3-14.5°C (Table 1). The conditions most favorable for rapid emergence were those in 2021-2022, when the amount of precipitation was 9.7 mm, $t_{aver.}$ 8.9°C. Variation on $\Sigma t_{act.}$, $t_{aver.}$ and precipitation was found for this period, according to the values of the coefficients of variation, 42.9, 32.1 and 59.1%, respectively.

Table 1. Characteristics of the meteorological traits during period of sowing - emergence average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	R	St. D.	CV%
x_1	17	16	13	13	17	15.3	4	2.1	13.7
x_2	245.8	132.7	115.8	115.8	245.8	164.8	130.0	70.7	42.9
x_3	14.5	8.3	8.9	8.3	14.5	10.6	6.2	3.4	32.1
x_4	14.1	3.4	9.7	3.4	14.1	9.1	10.7	5.4	59.1

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4-amount of precipitation.

The next interphase period of emergence-3[th] leaf has an average duration of 16.3 days, a sum of 159.5°C active temperature, $t_{aver.}$ 9.5°C and 90.0 mm of precipitation, according to Table 2. The shortest was the period in 2020-2021, when $\Sigma t_{act.}$ and $t_{aver.}$ are the lowest 91.4 and 7.0°C, respectively. Variations on $\Sigma t_{act.}$, and precipitation were found based on CV-39.8% and 37.0%, respectively.

Table 2. Characteristics of the meteorological parameters during period of emergence -3 th leaves average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV%
x_1	18	13	18	13	18	16.3	5	2.9	17.8
x_2	217.2	91.4	169.8	91.4	217.2	159.5	125.8	63.5	39.8
x_3	12.1	7.0	9.4	7.0	12.2	9.5	5.2	2.6	27.4
x_4	69	72.5	128.4	69.0	128.4	90.0	59.4	33.3	37.0

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4-amount of precipitation.

From Table 3, is obvious that the period of 3th leaf-tillering continued on average 10.7 days, Σ t $_{act.}$ are 84.2°C, a recorded precipitation of 35.3 mm. A wide variation in the duration of this period was found (CV=53.3%). This explains the variation on Σ t $_{act.}$ (CV = 55.5%) and precipitation (CV = 128.6%). The shortest period for the varieties was in 2021-2022, when the temperature sum was the lowest (44.7°C) and the precipitation was the least (0.4 mm). t $_{aver.}$ shows similar values across periods.

Table 3. Characteristics of the meteorological traits during period of 3 th leaves - tillering average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV%
x_1	9	17	6	6	17	10.7	11	5.7	53.3
x_2	72.2	135.8	44.7	44.7	125.8	84.2	91.1	46.7	55.5
x_3	8.0	8.0	7.5	7.5	8.0	7.8	0.5	0.3	3.8
x_4	19.0	86.6	0.4	0.4	86.4	35.3	86.2	45.4	128.6

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4- amount of precipitation.

During the period of tillering-stem elongation (43 days) the average recorded temperature sum was 410.0°C, the fallen prrcipitation was 22.2 mm, and t $_{aver.}$ Was 9.7°C (Table 4). The average duration for the varieties was the largest in 2020–2021 (52.1 days) and the recorded precipitation was the most (170.3 mm). In 2021-2022 the interphase period continued in the shortest time due to the lowest amount of precipitation (75.7 mm) and highest average temperature (11.5°C), which are premises for accelerating development. The most variable is precipitation (CV = 32.8%) (Figure 5). At the end of tillering, the light stage begins in cereal plants, during which the daily air temperature gradually increases under the influence of the lengthening of the day.

Table 4. Characteristics of the meteorological traits during period of tillering-stem elongation average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV%
x_1	43.2	52.1	33.7	32	56	43	24	8	18.6
x_2	339.0	465.7	385.1	379.0	513.2	410.0	194.2	48.3	11.8
x_3	8.8	8.9	11.5	8.7	12.0	9.7	3.3	1.3	13.2
x_4	120.6	170.3	75.7	75.4	172.1	122.2	96.7	40.1	32.8

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4-amount of precipitation.

According to Table 5, stem elongation-spike emergence is the second longest period, during which the meteorological parameters duration and precipitation are characterized by a large heterogeneity within cultivars, as described in Figure 3, Figure 6, and according to CV - 37.7% and 101.7%, respectively. They have reported similar observations for varietal differences in the duration of the period of emergence - flowering in 12 winter wheat cultivars (Rezaei et al., 2018). The period 2021-2022 was the shortest (15 days), and the lowest Σ $t_{act.}$ (218.0°C), but the highest $t_{aver.}$ (17.7°C) were recorded. The short interphase periods in 2020-2021 and 2021-2022 correspond to later sowing dates. Svystunova et al. (2020) have reported similar observations in triticale phenology, as well as differences in the duration of this period within varieties. CV values of 37.7% and 101.7% respectively, for the duration and the precipitation, determine strong variation from the mean.

Table 5. Characteristics of the meteorological traits during period of stem elongation-spike emergence average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV%
x_1	30.6	16	15	12	36	20.5	24	7.7	37.7
x_2	336.6	258.4	218.0	145.1	386	267.7	240.9	64.4	24.1
x_3	10.0	16.2	17.7	10.5	16.6	14.0	6.1	2.2	15.7
x_4	68.3	0.6	21.0	0.6	99.7	29.3	99.1	29.8	101.7

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4-amount of precipitation.

The shortest period of spike emergence-first anthers visible was in 2021-2022 (7.4 days), under the highest $t_{aver.}$ (18.2°C) and the least precipitation (2.6 mm), due to the accelerated growth rate under dry and warm weather (Table 6). The maximum and minimum duration over the years are 4 and 14 days, as shown in Table 6. Large deviations were established in values for Σ $t_{act.}$ (St. D = 28.7) and amount of precipitation (CV = 82.9%).

Table 6. Characteristics of the meteorological traits during period of spike emergence –first anthers visible average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV%
x_1	9	7.8	7.4	4	14	8.3	10	2.1	25.3
x_2	144.3	120.5	133.7	82.3	210.6	132.2	128.3	28.7	21.6
x_3	14.9	15.5	18.2	13.3	20.6	16.2	7.3	1.8	11.2
x_4	13.3	8.7	2.6	0	23.2	8.2	23.2	6.8	82.9

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4-amount of precipitation.

For the interphase periods of first anthers visible-late milk and late milk-soft dough, a duration of 17.9 days was found, similar values of Σ $t_{act.}$ (328.4 and 356.7°C) and $t_{aver.}$ (18.5 and 19.9°C), but contrasting precipitation conditions (Table 7, Table 8, and Figure 6). The optimal temperature range for flowering and grain filling is 18-22°C. This meteorological parameter is stable during the three study periods (Table 7 and Table 8). Precipitation in 2021-2022 (76.1 mm) increased air moisture and delayed the process of grain filling, resulting in extending the period. CV takes values 54.1 and 47.7% for the two periods.

Table 7. Characteristics of the meteorological parameters during period of first anthers visible-late milk average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV%
x_1	18.3	19.4	15.9	12	27	17.9	15	3.5	19.3
x_2	331.3	337.9	314.9	222.8	477.1	328.4	254.3	51.4	15.7
x_3	18.2	17.5	19.9	16.2	20.6	18.5	4.4	1.2	6.3
x_4	23.1	33.0	10.9	6.6	44.8	22.0	38.2	11.9	54.1

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4- amoun of precipitation.

Table 8. Characteristics of the meteorological traits during period of late milk - soft dough average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV%
x_1	15.8	18	20	11	25	17.9	14.0	3.3	18.4
x_2	288.3	356.1	465.7	222.6	540.0	356.7	317.4	78.1	21.9
x_3	18.4	20.1	21.2	16.9	21.9	19.9	5.0	1.5	7.5
x_4	29.8	32.2	76.1	12.1	80.5	46.0	68.4	22.0	47.7

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4- amount of precipitation.

A fluctuation in the amount of precipitation is also noticeable during soft dough-fully ripe, because over the years 0-50.2 mm, the fallen precipitation was reported respectively, and the CV value is 106.7%. A deviation is visible for duration (CV = 34.7%) (Table 9). The longest was the duration in 2019-2020, when the registered precipitation was 44.2 mm. The grain filling process is accelerating at a higher average temperature, as it is during 2020-2021 (25.5°C), and accordingly the maturation proceeded in the shortest time (5.9 days). Average temperatures over the years are within the optimal range for cereal crops (20-25°C) in moderate climates (Farooq et al., 2011). It is noticeable that precipitation during all interphase periods shows a strong variation.

The biological cycle of plants was shortest in 2021-2022, because maximum average temperature (14.6°C) and minimal precipitation (327.2 mm) were recorded for this period (Table 9). The cumulative active temperature is between 1,984.9 and 2,371.0°C. This result is in accordance with results of Soto et al. (2009) who found that common wheat and triticale require an active temperature sum range of 1,800°C - 2,400°C to complete their biological cycle. A one-year study by Akhlaq et al. (2015) recorded a 161 day growing season for triticale under the influence of fertilization, but it is not specified whether this period is calendar or based on active temperatures. In the study by Krusheva (2021) duration of 231-260 days, $\Sigma\ t_{act.}$ 1,995-2,936°C, $t_{aver.}$ 8.7-12.9°C and precipitation 341-580 mm, were indicated in triticale.

Table 9. Characteristics of the meteorological traits during period of soft dough –fully ripe average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV%
x_1	11.1	5.9	9	4	15	8.7	11	3	34.7
x_2	230.6	150.1	212.9	99.0	343.1	197.9	244.1	58.0	29.3
x_3	20.8	25.5	23.9	20.5	25.7	23.4	5.2	2.1	8.9
x_4	44.2	9.7	4.4	0	50.2	19.4	50.2	20.7	106.7

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4- amount of precipitation.

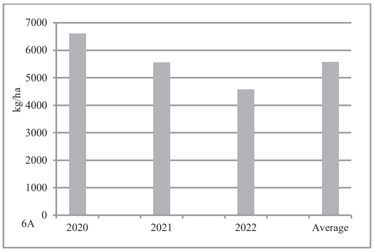
Results showed that in 2021 (5,560.7 kg/ha) and 2022 (4,568.3 kg/ha), the grain yields were significantly lower compared to 2020 (6,610.7 kg/ha) by 84.1 and 69.1%, respectively (Figure 2A). On Figure 2, is evidence that most varieties have a higher average yield compared to Kolorit variety (5,184.2 kg/ha), except for results, but no significance.

The highest average grain yield demonstrated Avocado (6,181.3 kg/ha), Casino (6,078.6 kg/ha), Dobrudzhanets (5,826.7 kg/ha) and Blagovest (5,728.7 kg/ha). These varieties exceed the control by 19.1, 17.3, 12.4, 10.5, and 9.8%. It was found that the conditions of the year have a significant effect on the yield (η=64.1%), followed by an effect of interaction between varieties and environment (η=17.2%) and variety (η=13.0%) (Table 10). These data show that the cultivars have different ranks over the years, and the average yields for the three periods studied are also different (Figure 6). It should be noted that average yields obtained were highest under Mediterranean conditions in 2020 and lower under semi-humid conditions in 2021 and 2022. Bekish et al. (2020) have also demonstrated a strong influence of year conditions (77.3%) and variety (19.9%) in triticale.

Table 10. Characteristics of meteorological traits during period of sowing-fully ripe average for the varieties and periods

Traits	Periods			Descriptive statistics					
	2019-2020	2020-2021	2021-2022	min.	max.	average	range	St. D.	CV %
x_1	155.2	148.7	138	138	178	158.5	40.0	15.3	9.7
x_2	2,249.7	2,049.0	2,020.4	1,984.9	2,371.0	2,106.5	368.1	115.9	5.5
x_3	13.4	12.4	14.6	12.2	14.7	13.4	2.5	1.0	7.5
x_4	401.4	410.6	327.2	323.1	474.5	379.7	151.4	41.0	10.8

x_1-number of days with active temperatures above 5°C; x_2-sum of active temperatures; x_3-mean temperature; x_4- amount of precipitation.



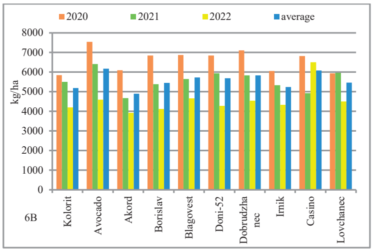


Figure 6. Triticale grain yields by year and average for the period

Correlative and regression dependences were analyzed. In the phase of tillering-stem elongation, the grain yield is strongly negatively correlated with $t_{aver.}$ This negative relationship is visible during all interphase periods, as well as during the period of sowing - fully ripe (Table 12). This can be explained by the conditions for taking the course of physiological processes related to growth, which are dependent on the temperature regime during the interphase periods. "A change in the optimal temperature is considered stress, and the greater the difference, the more the phases will be prolonged or accelerated"

Table 11. Analysis of variance of grain yield

Source	df	Sum of squares	η (%)	Mean square	F	p value
Total	89	9.766272E+07	100			
Block	2	64000	6.553166E-02	32000	0.3365206	0.72052
Variants	29	9.208346E+07	94.3	3175292***	33.39222	0.00000
Variety	9	1.271117E+07	13.0	1412352***	14.85267	0.00000
Year	2	6.25856E+07	64.1	3.12928E+07***	329.0835	0.00000
V×Y	18	1.678669E+07	17.2	932593.8***	9.807406	0.00000
Error	58	5515264	5.6	95090.76		

*** significance at p=0.1%.

(Wahid et al., 2007). This leads to a decrease in yields. Crops that are highly affected by stress attempt to survive and complete all the developmental stages within a shortened period of time (Hakim et al., 2012). Racz et al. (2017) have obtained conflicting results, namely that average temperature during germination and heading-flowering has a strong positive relationship with grain yield in triticale.

Moderate to high positive relationships of yield and precipitation were established for all the periods, except for late milk-soft dough. The quantity of precipitation is closely connected with soil moisture. During the period of tillering- stem elongation, the insufficiency of moisture retards tillers formation (r = 0.403). At the beginning of stem elongation, the reproductive organs are formed and this stage is crucial for future yields (r = 0.529). At the heading the insufficiency of moisture reduces pollination and fertilization, which results in a poorly grainy spike (r = 0.366). After flowering, the growth of the vegetative organs ceases and the need for water is less (r = 0.328).

During the grain filling period, from late milk to soft dough, it is known that the accumulation of dry matter becomes much more rapid at relatively high temperatures (Delogu et al., 2002). Precipitation and high humidity delay ripening and deteriorate grain quality. This is also associated with a decrease in temperature respectively, with the temperature sum. Therefore, a high negative correlation (r= -0.728), resulting in the yield correlates negatively with the other meteorological parameters.

During the two periods of tillering-stem elongation and stem elongation-spike emergence, moderate and significant dependences with positive character were confirmed: 0.430 and 0.678, respectively, of yield with duration. This can be explained by the theory of light stages, namely that for the life cycle of grain plants, it is necessary to extend the length of the day and, accordingly, the temperature.

The relationship between grain yield and precipitation during the period of soft dough-fully ripe is positive (0.640), because the grain is yellow-green and still moving, and deposition of nitrogenous substances and carbohydrates coming from the vegetative parts. The lack of rainfall and high temperatures lead to dehydration of the grain, shortening this period and yield reduction. A positive correlation was found with the grain filling period (r = 0.340), as was also reported by other authors (Wolde et al., 2016). There is data to reduce wheat grain yield from 31 to 35% as a result of drought stress during this period (Farooq et al., 2011). The duration correlated strongest with yield (r = 0.744). The results are in accordance with the findings of other scientists. Brzozowska et al. (2018) have found a significant positive correlation between spring triticale yields and the duration of a growing season and a negative with mean temperature. A study by Korkhova and Mykolaichuk (2022) showed a significant influence of the duration of interphases, vegetation periods, and the amount of precipitation on the grain yield of winter durum wheat.

Table 12. Pearson correlation coefficients between meteorological traits during interphase periods and grain yield

Parameters/Periods	21-31	31-49	49-61	61-77	77-85	85-92	00-92
Grain yield							
x_1	0.430^{**}	0.678^{***}	0.182^{ns}	0.245^{ns}	-0.522^{***}	0.340^{*}	0.744^{***}
x_2	-0.007^{ns}	0.638^{***}	-0.107^{ns}	0.096^{ns}	-0.688^{***}	0.226^{ns}	0.647^{***}
x_3	-0.722^{***}	-0.530^{***}	-0.484^{**}	-0.542^{**}	-0.682^{***}	-0.518^{***}	-0.550^{***}
x_4	0.403^{**}	0.529^{***}	0.366^{*}	0.328^{*}	-0.728^{***}	0.640^{***}	0.576^{***}

n = 30; Significant correlations are indicated by stars ($p < 0.01^{***} < p < 0.05^{**}$)

Based on correlational dependences a regression equations were constructed, that describe the functional connection between grain yield and precipitation. Thus, a grain yield can be predicted (Table 12). According to Table 12, the coefficients of determination (R^2) have the highest value during the periods 77-85 and 85-92 respectively, 0.530 and 0.410, or 53.0% and 41.1% the yield change can be explained by the change from precipitation. The mathematical models and coefficients a and b are adequate at significance level a = 0.05. According to the function, the grain yield will increase by 31.8 when the precipitation increases by 1.0 during the period 85-92. For interphase period 77-85, the grain yield is expected to decrease by 33.9 when precipitation increases by 1.0. Cetin et al. (2022) have established positive effects of rainfall (R^2 = 0.72) on grain yield in heading, anthesis and grain filling of durum wheat.

Table 13. Regression dependences for grain yield and precipitation during interphase periods

Periods	Model	R^2	F crit.	Sign. F	P value Intercept (a)	P value variable (b)
21-31	$Y = 4315.80+10.30968\ x_4$	0.162	5.426	0.027	2.83603E-08	0.027283472
31-49	$Y = 5041.66+18.2102\ x_4$	0.279	10.85	0.003	3.20522E-19	0.002677887
49-61	$Y = 5120.90+ 55.2480\ x_4$	0.134	4.330	0.046	4.73571E-17	0.046707963
61-77	$Y = 4955.89+28.1764\ x_4$	0.108	3.381	0.077	2.31049E-13	0.07655967
77-85	$Y = 7139.63–33.9927\ x_4$	0.530	31.60	5.08743E-06	7.74911E-20	5.08743E-06
85-92	$Y = 4958.40+31.7659\ x_4$	0.410	19.47	0.000	1.86868E-20	0.000137667

According to the R^2 of the remaining models, describe little change in yield, based on the change in precipitation. The equation describing the dependence of grain yield and precipitation during a period 31-49 explains 27.9% of yield values, or at that important period of triticale development when emergence is expected, it is possible to increase the yield by 18.2%, if the precipitation is higher by 1.0.

CONCLUSIONS

This work analyzed the phenological development and productivity of modern varieties of triticale for a three-year period, evaluated the effects of cultivar and location, and characterized the agrometeorological conditions during the growing seasons. Applied analyses found wide variation in precipitation, a negative correlation of yield with average temperature during all interphase periods, and negative dependences of grain yield with the duration of the period, the temperature sum, average temperature, and precipitation, during the period of late milk-soft dough. Functional dependencies between grain yield and rainfall during phenological periods were revealed. Mathematical models describing the relationship between grain yield and precipitation during the periods of late milk-soft dough and soft dough-fully ripe explain the yield change and present the best prognosis. They are defined climatic conditions of growing seasons in which varieties were grown-one in the Mediterranean and two periods under semi-humid conditions. The terms of the year have a large and significant impact on yield. The influence of cultivar-environment interaction as well as cultivar is significant but small. The average for the period with highest yielding varieties are indicated.

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THE IMPROVEMENT OF *Nardus stricta* L. PERMANENT MEADOW FROM THE DORNA DEPRESSION THROUGH MINERAL AND ORGANIC FERTILIZATION

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Abstract

In Romania, the grassland area dominated by Nardus stricta L. covers 200,000 hectares. Meadow degradation is caused by changes that occur in plant growing conditions and in the structure of vegetation. For long time, technological works and improvement measures were not applied on permanent meadows in Romania which led to a decrease in their production and to their degradation. The aim of this study was to evaluate the dynamics of productivity and quality of fodder, following the application of measures to stimulate and improve the production and quality of grasses on the permanent grasslands of Nardus stricta L. in the intramontane Depression of Vatra Dornei, from the North-Eastern Romanian Carpathians. At the same time, it was ensured that effects on the environment were minimal. The applied measures to Nardus stricta meadows, such as organic and mineral fertilization, led to a good plant growth and brought important changes in the chemical composition of the forage obtained (increase the content of CP and decrease the content of ADF and NDF). Thus, the quality and the digestibility of the feed improved significantly.

***Key words**: Nardus stricta L. meadows, mulching, organic and mineral fertilizers, crude protein, forage quality.*

INTRODUCTION

Grassland areas described as green oceans are important for the ecosystem services they may provide, such as: support, supply, cultural, and are the subject of several publications (Hopkins & Holz, 2005; Carlier et al., 2005; Lemaire et al., 2011; Boval & Dixon, 2012; O'Mara, 2012; Blair et al., 2014; Smit et al., 2015). The ability of grasslands to provide multiple ecosystem services largely depends on the intensity of the applied management (Galka et al., 2005; Hejcman et al., 2007a; Wang et al., 2014; Štýbnarová et al., 2010; Vîntu et al., 2011).

From Romania's almost 4,800,000 ha of meadows, 2,000,000 ha are grasslands of high natural value, which are generally located along the Carpathian Mountains. *Nardus stricta* L. meadows are Natura 2000 habitats and they hold a very important ecological paradox (Galvánek & Janák, 2008). The, *Nardus stricta* species is a widespread grass on plateaus and less inclined slopes, from the beech floor to the subalpine area, between 600 and 1800 m (Pușcaru-Soroceanu et al., 1963; Țucra et al., 1987; Chifu et al., 2014). It often develop monotonous meadows, almost monospecific or with a very small number of species (Vîntu et al., 2004), but the spread of *Nardus*-dominated meadows in the Carpathians is a phenomenon related to the massive expansion of sheep grazing in the mountains in the 20th century. The long period of grazing, the absence of fertilization, the severe climatic conditions led to the degradation of *Agrostis cappilaris* and *Festuca rubra* meadows, by infiltrating the phytocenosis structure of the species *Nardus stricta* L. with the formation of the sub-association *nardetosum strictae* (Marușca, 2021a). The productive potential of *Nardus stricta* meadows is low, and the forage obtained has a medium nutritional value. To improve the forage quality and productivity of these grasslands, ameliorative measures, especially fertilization, are of crucial importance (Hejcman et al., 2007b; Vîntu et al., 2015; Samuil et al., 2017; Blaj et al., 2019).

The main goal of the present study was to evaluate the effects of organic and mineral fertilization on the productivity of natural grasslands and the quality of forage. Also, identifying economically efficient solutions for their sustainable use and for the conservation of biodiversity.

MATERIALS AND METHODS

Soil and climatic conditions

The experiment was conducted during 2021-2022 period, on a permanent meadow derived of *Nardus stricta* L., in the region of the North-Eastern Carpathians (Dorna Depression) at 845 m above sea level. The type of the soil (Photo 1) within the experimental field is represented by hyposkeletal luvosoil (Stănilă & Dumitru, 2016).

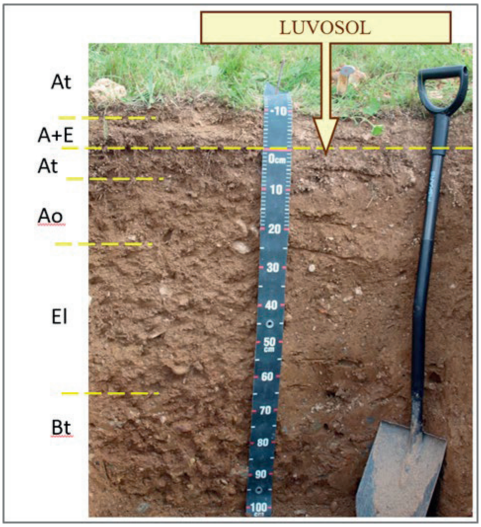


Photo 1. Soil profile of Luvosol within the field experimental

Within the luvosoil type, the well-developed sub-horizon with a thickness of 8-10 cm is noticeable (At), a horizon of accumulation of acid humus and lighter in color (Ao), the El horizon and the mineral horizon (Bt argic). The soil mountain-meadow is a luvosol hyposkeletal, belonging to the acid soils with pH of 4.2, and the land slope of 16º near a deciduous forest. The climatic conditions in the area are characterized by an average temperature of 6.3°C and 675 mm of total annual precipitation.

Experiment set-up

A monofactorial experiment was organized after the randomized block method, in three replicates with the following variants:

V_1 - unfertilized (control);
V_2 - abandon (unharvested or non-grazing);
V_3 - mulching (cut and leave the biomass on site);
V_4 - $N_{50}P_{50}K_{50}$ kg/ha anually;
V_5 - $N_{100}P_{100}K_{100}$ kg/ha anually;
V_6 - $N_{150}P_{150}K_{150}$ kg/ha anually;
V_7 - 10 t/ha cattle manure, annually;
V_8 - 20 t/ha cattle manure, annually;
V_9 - 20 t/ha cattle manure, at 2 years;
V_{10} - 30 t/ha cattle manure, annually;
V_{11} - 30 t/ha cattle manure, at 2 years.

Two types of fertilizers were used: an organic one (well fermented cattle manure, older than 2 years) and a mineral one (a complex fertilizer with $N_{20}P_{20}K_{20}$). The cattle manure had the following chemical composition: N - 0.445%, P_2O_5 - 0.212% and K_2O - 0.695%. Fertilizers were applied manually in early spring, before the start of active vegetation growth, respectively. The area of each (Table 1) variant was 12 m^2 (3 x 4 m), and the harvested area was 8.75 m^2 (2.5 x 3.5 m). The total experiment area was 396 m^2 (33 x 12 m).

Table 1. Experimental field parameters

Parameter	Dimension
The area of a plot (variant)	12 m^2
The harvestable area of a variant	8.75 m^2
Number of replicates	3
Number of variants	11 x 3 = 33
The total area of the experience	132 x 3 = 396 m^2

Measurements and analyses

Harvesting was done at earing-flowering stage of the dominant grasses. The production evaluation on each experimental variant was performed by weighing of the resulted green mass and was reported per hectare. After determining the dry matter (DM) content of the forage, the production was expressed in tonnes per hectare DM. The DM content was determined by drying the grass in an oven at 103°C for 3 hours; work device: Heat-adjustable oven - Venticell 111 I; standard - SR ISO 6496/2001. Forage analyses were performed by the Laboratory *AgroLab* of the "Ion Ionescu de la Brad" Iași University of Life Sciences, Romania.

The total nitrogen (Nt), was analysed using the Kjeldahl method standard - SR ISO 13325/1995, and the crude protein content (CP) was calculated by multiplying the total nitrogen amount with the conversion factor 6.25; the content of the forage in neutral detergent fiber

(NDF), represented by hemicellulose, cellulose and lignin, was determined using the Van Soest method, according to the standard - SR ISO 16475/2004; the acid detergent fiber (ADF) content, represented by cellulose and lignin, was determined using the Van Soest method, according to the standard - SR ISO 13906/2008; the content of the analyzed samples in cell walls (ADF and NDF) was determined using the FibreBag-System, 36-place (Gerhardt analytical systems). Relative Forage Quality (RFQ) was calculated using the Equation 1 (Ward & Ondarza, 2008; Linn & Martin, 2012).

$$RFQ = \frac{DMI \times TDN}{1.23}$$

where:
- DMI (Dry Matter Ingested) = 120 / NDF (%)
- TDN (Total Digestible Nutrients) = 4.898 + 89.796 x NEL (%)
- NEL (Net Energy Lactation) = 1.085 - 0.0124 x ADF (Mcal/kg).

Equation 1. Relative Forage Quality

Yields and forage quality data were processed using ANOVA, applying the Least Significant Difference (LSD) test. Also, correlations were calculated (quadratic regression significance) between the type of fertilization and production of dry matter.

RESULTS AND DISCUSSIONS

Researches carried out until today have demonstrated the positive effects of manure, as well as of mineral fertilizers applied reasonably on the meadows of *Nardus stricta* (Vîntu et al., 2011; Tarcău et al., 2012; Rotar et al., 2011). Yields were influenced by the rates and type of fertilizers applied. As, expected, the data on biomass yield, resulted from our study, indicated significant annual variation (Table 2). During the study period (2021-2022), the total biomass production responded very well to mineral and cattle manure application. Biomass production significantly increased with the addition of NPK or cattle manure in all treatments. We notice that the production achieved in 2021, in the unfertilized variant (control), was 1.16 t/ha DM, and the highest production in the same year, 4.38 t/ha DM, was recorded in the organically fertilized variant (30 t/ha cattle manure applied once every two years), with statistically ensured differences.

Meadows are important components of the Romanian ecosystems, being essential for their biodiversity. Despite the special value that biodiversity gives them, the meadows in the mountain area are threatened by degradation, including the risk of abandonment, due to the decline of traditional systems as a result of the decrease in livestock. Thus, large areas of meadows frequently remain ungrazed or unmowed, and the biomass left on the ground.

Table 2. The influence of organic and mineral fertilization on the dry matter production (DM), in 2021

Experimental variants	DM production t/ha	Difference t/ha	Difference %	Significance
V_1 - unfertilized (control)	1.16		100	Control
V_2 - abandon	-		-	
V_3 - mulching	1.22		105.2	
V_4 - $N_{50}P_{50}K_{50}$ kg/ha anually	2.04		175.9	*
V_5 - $N_{100}P_{100}K_{100}$ kg/ha anually	2.68		231.0	***
V_6 - $N_{150}P_{150}K_{150}$ kg/ha anually	2.93		252.6	***
V_7 - 10 t/ha cattle manure, annually	2.75		237.1	***
V_8 - 20 t/ha cattle manure, annually	3.13		269.8	***
V_9 - 20 t/ha cattle manure, at 2 years	3.51		302.6	***
V_{10} - 30 t/ha cattle manure, annually	3.96		341.4	***
V_{11} - 30 t/ha cattle manure, at 2 years	4.38		377.6	***
	LSD 5%	*0.75*		
	LSD 1%	*1.12*		
	LSD 0.1%	*1.39*		

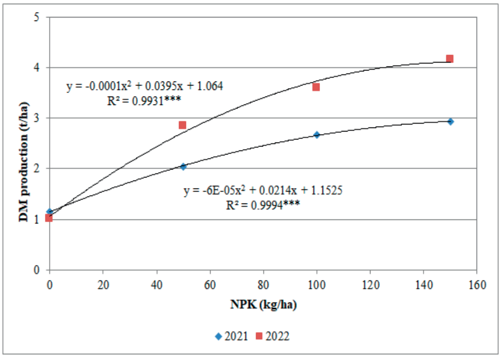
Considering these, it was proposed to study two variants (abandonment and mulch) in order to follow the changes that take place over time when no work is done on them. Therefore, in the study year 2021, in the variant with mulch (cut and leave the biomass on site), the production of dry matter recorded was of 1.22 t/ha DM, with an insignificant difference compared to the control, as resulted from Table 2. The data recorded in the second year of study (2022) shows a significant decrease of the dry matter production (Table 3) compared to the previous year due to the severe climatic conditions (high temperatures and low precipitation). Also, in 2022, the realized productions were differentiated according to the doses and type of fertilizer. The mineral fertilization applied in different doses generated in this second year of the study higher yields compared to the previous one, and the highest production of dry matter, 4.51 t/ha DM, was recorded in the mineral fertilized variant with the maximum dose of NPK, respectively $N_{150}P_{150}K_{150}$ kg/ha, with a 305.1% increase in production compared to the unfertilized variant.

Table 3. The influence of organic and mineral fertilization on the dry matter production (DM), in 2022

Experimental variants	DM production t/ha	Difference t/ha	Difference %	Significance
V_1 - unfertilized (control)	1.02	Control	100	Control
V_2 - abandon	-	-	-	-
V_3 - mulching	0.92	-0.10	90.1	-
V_4 - $N_{50}P_{50}K_{50}$ kg/ha anually	2.85	1.83	278.5	***
V_5 - $N_{100}P_{100}K_{100}$ kg/ha anually	3.60	2.58	351.8	***
V_6 - $N_{150}P_{150}K_{150}$ kg/ha anually	4.15	3.12	405.1	***
V_7 - 10 t/ha cattle manure, annually	2.01	0.99	196.3	***
V_8 - 20 t/ha cattle manure, annually	3.23	2.21	315.5	***
V_9 - 20 t/ha cattle manure, at 2 years	2.60	1.58	254.1	***
V_{10} - 30 t/ha cattle manure, annually	2.25	1.23	219.7	***
V_{11} - 30 t/ha cattle manure, at 2 years	2.69	1.66	262.5	***
	LSD 5%	*0.42*		
	LSD 1%	*0.58*		
	LSD 0.1%	*0.79*		

In the second year of the study (2022) in the variant with mulch (cut and leave biomass on site), a dry matter production of 0.92 t/ha DM was recorded with an insignificant difference compared to the control variant (Table 3). Long-term improvement of *Nardus stricta* L. subalpine meadows can be achieved by applying medium chemical and organic fertilization (Blaj et al., 2019). Also, previous reported researches (Smits et al., 2008; Păcurar et al., 2010; Vîntu et al., 2015; Samuil et al., 2017; Cirebea et al., 2020, Gaga et al., 2022; Zornić et al., 2023) showed that mineral fertilization had a positive influence on production depending on the applied doses, which confirm the results of our study. Regarding the organic fertilization and its impact on the productivity of these type of meadows, previous reserches showed that, by applying a minimum of 20 t/ha, important production increases are realized (Rotar et al., 2011). The importance of fertilization for these types of meadows that were the object of our study was also highlighted by Vîntu et al. (2010), who followed the evolution of *Nardus stricta* L. meadows located in the Dorna Depression and found that the production of these meadows can be doubled by fertilization.

The values of the regression coefficient (R^2), for the dry matter production, in both years in which the study was carried out, were significant for mineral fertilization based on NPK as well as for cattle manure fertilization. It was found that the values of the regression coefficient (R^2) in the period (2021-2022), were very significant in the mineral fertilized variants with the doses of NPK and significant in the case of amount of cattle manure application (Figure 1 a, b).



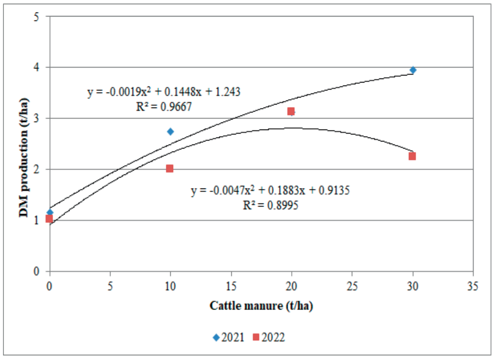


Figure 1 - a, b. Correlations between the type of fertilizer (doses of NPK and amount of cattle manure) applied and the dry matter production, in the 2021-2022 period

The influence of organic or mineral fertilization on forage quality is already known. Usually the forage's crude protein (CP) content varies depending on the plant species that are composing the crop or meadow and the fertilization. From a qualitative point of view, nitrogen fertilization contributes to increase the content of protein substances in feed, due to the nitrogen extracted in the protein composition (Dragomir, 2005). Therefore, during our study, the organic fertilization of *Nardus stricta* L. grassland using moderate amounts of 20 t/ha manure every year led to an increase in CP up to 11.39% in 2021 and up to 10.47% in 2022 compared to the control variant, unfertilized (Tables 4, 5).

At the same time, as it can be observed that there was a significant increase of 10.43% in 2021 and 10.31% in 2022 in V_{10} (30 t/ha cattle manure, annually). Tarcău et al. (2012), within an experiment carried out on the same type of meadow, with mineral fertilization in several doses ($N_{100}P_{100}$, $N_{140}P_{140}$ and $N_{200}P_{200}$ kg/ha) show that there is a significant increase in CP content with increasing amounts of fertilizers, 6.48% la 6.59-14.08%. Also, similar results were obtained by Vîntu et al. (2015); Cirebea et al. (2020); Mrázková et al. (2020); Simić et al. (2020); Iliev et al. (2022).

The NDF and ADF values can influence the quality of the forage obtained from the pasture, as well as their effect on the animal feeding. These parameters are used to determin the nutritional value of the feed because the components of the cell wall - cellulose, hemicellulose and lignin are completely separated (Stybnarova et al., 2010).

Table 4. The influence of applied management on forage quality obtained from the *Nardus stricta* (L.) permanent meadow, in 2021

Experimental variants	Quality parameters CP ($g \cdot 100\ g^{-1}$ DM)	NDF ($g \cdot 100\ g^{-1}$ DM)	ADF ($g \cdot 100\ g^{-1}$ DM)	RFQ
V_1 - unfertilized (control)	8.54^C	59.70^C	48.93^C	78.19^C
V_2 - abandon	-	-	-	-
V_3 - mulching	-	-	-	-
V_4 - $N_{50}P_{50}K_{50}$ kg/ha anually	8.72	57.57^{oo}	43.60^{ooo}	91.14**
V_5 - $N_{100}P_{100}K_{100}$ kg/ha anually	9.25*	54.38^{ooo}	40.17^{ooo}	103.34***
V_6 - $N_{150}P_{150}K_{150}$ kg/ha anually	10.29***	51.06^{ooo}	38.89^{ooo}	112.78***
V_7 - 10 t/ha cattle manure, annually	9.24*	58.59	45.14^{ooo}	86.70*
V_8 - 20 t/ha cattle manure, annually	11.39***	50.77^{ooo}	39.23^{ooo}	112.69***
V_9 - 20 t/ha cattle manure, at 2 years	10.16***	56.26^{ooo}	45.14^{ooo}	90.29**
V_{10} - 30 t/ha cattle manure, annually	10.43***	53.03^{ooo}	39.23^{ooo}	107.89***
V_{11} - 30 t/ha cattle manure, at 2 years	9.15	56.72^{oo}	44.23^{ooo}	91.30**
LSD 5%	*0.66*	*1.73*	*1.95*	*8.17*
LSD 1%	*0.93*	*2.38*	*2.68*	*11.26*
LSD 0.1%	*1.28*	*3.28*	*3.69*	*15.50*

The forage content in NDF decreased significantly from 59.70%, in the case of the unfertilized control variant, to 58.59% and 50.77%, depending on fertilization, in 2021. The content in ADF show also a significant decrease from 48.93% (unfertilized control) to 45.14% and 38.89% in the case of fertilized variants (Table 4).

Table 5. The influence of applied management on forage quality obtained from the *Nardus stricta* (L.) permanent meadow, in 2022

Experimental variants	Quality parameters			
	CP ($g \cdot 100\ g^{-1}$ DM)	NDF ($g \cdot 100\ g^{-1}$ DM)	ADF ($g \cdot 100\ g^{-1}$ DM)	RFQ
V_1 - unfertilized (control)	7.93^C	56.11^C	49.34^C	82.40^C
V_2 – abandon	-	-	-	-
V_3 – mulching	-	-	-	-
V_4 - $N_{50}P_{50}K_{50}$ kg/ha anually	9.05*	55.01	47.52	87.64*
V_5 - $N_{100}P_{100}K_{100}$ kg/ha anually	9.37**	53.70^{oo}	44.96^{ooo}	94.95***
V_6 - $N_{150}P_{150}K_{150}$ kg/ha anually	10.27***	51.85^{ooo}	43.10^{ooo}	102.24***
V_7 - 10 t/ha cattle manure, annually	9.86***	53.25^{oo}	48.60	88.33**
V_8 - 20 t/ha cattle manure, annually	10.47***	51.98^{ooo}	42.68^{ooo}	102.86***
V_9 - 20 t/ha cattle manure, at 2 years	9.94***	52.37^{ooo}	44.73^{ooo}	97.84***
V_{10} - 30 t/ha cattle manure, annually	10.31***	53.12^{oo}	43.70^{ooo}	98.57***
V_{11} - 30 t/ha cattle manure, at 2 years	10.07***	54.52^{oo}	45.27^{oo}	92.91***
LSD 5%	0.99	1.58	2.21	4.16
LSD 1%	1.36	2.18	3.05	5.72
LSD 0.1%	1.87	3.00	4.20	7.88

Similar results were obtained in 2022 with a significant decrease in content of NDF from 56.11% (unfertilized variant) to values between 55.01% and 51.85%, depending on fertilization and, also, the ADF values from 49.34% (unfertilized variant) to 48.60% and 42.68%, which were influenced by the rates and type of fertilizers applied. In 2021 we obtained a higher crude protein content and lower values of fiber content (ADF and NDF) compared to 2022, suggesting the presence of other factors that could influence the quality of forage, such as climatic conditions. As in our case, the results obtained by Vîntu et al. (2011), Tarcău et al. (2012), Samuil et al. (2018), have shown that the fertilization of meadows of *Nardus stricta* L. with 20-50 t/ha of manure led an improvement of content ADF and NDF.

The mineral and organic fertilizers positively influenced the RFQ of the fodder, all fertilized variants of our study achieving increases with statistically assured differences, compared to the control variant. As it can be seen in Tables 4 and 5, regardless of the type of fertilizer, forage quality increased along with the applied doses in both years of the study. Thus, the RFQ values were between 102.24 and 112.69, which characterize a good forage quality, in the variant fertilized with 20 t/ha of cattle manure (annually) compared to values between 78.19 and 82.40, which characterize a poor quality of the feed, in the case of the unfertilized variant.

CONCLUSION

The meadows of *Nardus stricta* L., being among the most degraded pastures in the Romanian mountains, they are the subject of a special concern regarding their economic value. Therefore, the identification of favorable solutions for their improvement is in the attention of researchers. Our research revealed the importance of organic and mineral fertilization for increasing the production of these meadows, as well as for increasing their yield and, at the same time, for improving the quality of the resulting fodder, a fact proven by the values of the chemical components and the parameters that characterize the fodder.

Thus, there was a significant increase in dry matter, crude protein, but also a decrease in the content of insoluble fibers and acid detergent fibers, respectively.

The values related to the relative quality of the fodder (RFQ) obtained in our study characterize a good quality of the fodder produced in the fertilized variants. The data obtained in this study could be useful in understanding the nutritional potential of forages, representing basic information in ruminant nutrition. Our study shows that the fertilization of *Nardus stricta* L. meadows is the most effective technological measure for their improvement, and a fertilization with 20-30 t/ha cattle manure annually or with $N_{100\text{-}150}P_{100\text{-}150}K_{100\text{-}150}$ is very relevant.

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ALLELOPATHIC EFFECT OF *Elettaria cardamomum* ESSENTIAL OIL VAPOURS ON THE WINTER SEED MYCOFLORA AND GERMINATION

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Abstract

Developing agroecosystems to reduce yield losses, effective use of water resources, and environmentally friendly innovative biotechnological interventions to stop using more chemical pesticides and fertilisers have been the focus of several sustainable strategies. Our research aimed to identify the allelopathic in vitro effect of Elettaria cardamomum essential oil (ECEO) on wheat seeds regarding the antifungal efficacy and germination stimulation. Surface-sterilized seeds (n = 100) were transferred in sterile Petri dishes (Ø 120 mm), and the ECEO doses were 5 µL, 25 µL, 50 µL and 100 µL. The Petri dishes were transferred to a growth chamber and kept at 25 °C in the dark for 5 days. Consequently, the microflora and germination rate was assessed on the PDA medium. The results suggest that the minimum concentration needed for ECEO to affect the seeds contamination index was at 25µL, while values of 50µL and 100µL inhibited the germination, resulting in germination rates at 86% and decreasing to 43%. ECEO oil exhibited promising antifungal activity and seems to have a potent fumigant activity against wheat mycoflora and could be used as possible future natural agent in agriculture.

Key words: *antifungal, Elettaria cardamomum, essential oil, germination, wheat seed.*

INTRODUCTION

Researchers are focusing on finding alternative methods to effectively fight and control microbial infections while being environmentally friendly and not compromising plant germination (Hulea at al., 2022; Alexa et al., 2020). These methods can have a significant impact on agriculture by reducing the use of harmful pesticides and promoting sustainable farming practices. In addition, using plant growth-promoting microorganisms has emerged as a promising approach for sustainable food production. By harnessing the beneficial properties of microorganisms, such as *Eleteria cardamomum* and plant growth-promoting microorganisms, researchers aim to develop eco-friendly solutions that can effectively combat microbial infections without negatively affecting plant germination. This approach aligns with the intention to promote stable grain production, green development in agriculture, and sustainable farming practices. *Elettaria cardamomum* is an aromatic, herbaceous, perennial plant belonging to the *Zingiberaceae* family and *Elettaria* genus. Its essential oil (ECEO), produced by various methods, contains a combination of various volatile lipophilic compounds, in some cases more than 100, that possess several biological activities such as antioxidant, anticarcinogenic, and antimicrobial effects (Ivanović et al. 2021; Al-Zereini et al., 2022; Castillo et al., 2023).

The antimicrobial effect of ECEO was demonstrated against *Penicillium* spp., *Pacelomyces* spp., *Mucor* spp., and *Aspergillus* spp. (*Aspergillus nigerxerophilic*, *Aspergillus ochraceus, Aspergillus flavus, Aspergillus parasiticus)* (Saleh et al., 2011; Naz et al., 2023; Ibrahim et al., 2017). Although the antifungal activity of the extract of *Elettaria cardamomum* against *Fusarium* spp. has been demonstrated (Aliaa et al., 2016), there is no data in the specialised literature regarding the antifungal activity of the essential oil from this plant against this mycotoxigenic fungus.

The study aimed to highlight the antifungal activity of ECEO, and the ability to stimulate germination of wheat seeds.

MATERIALS AND METHODS

Treatment of wheat seeds

The fumigation method used in the present study was described by Bota et al., 2022. Briefly, the wheat seeds were washed with 1:9 (*v*/*v*) sodium hypochlorite solution and then rinsed three times with sterile distilled water. To obtain a relative humidity of 14%, the seeds were dried in an oven at 100°C. Seeds (n = 100) were transferred in sterile Petri dishes (Ø 120 mm), and exposed to different concentrations of ECEO (doTERRA (Pleasant Grove, Utah, USA) vapours.

The concentrations tested, respectively 5 µL, 25 µL, 50 µL and 100 µL were added to a sterile filter paper and inserted into each Petri dish with wheat seeds. The dishes were kept in the dark at 25°C, for five days (Figure 1).

Figure 1. Sterile Petri dishes with wheat exposed to different concentrations of ECEO vapours

Triplicate samples were performed.

Analysis of Fungal Contamination

Direct plating technique on PDA (Potato Dextrose Agar, CM0139, Oxoid, Thermo Fisher Scientific Inc.) medium was performed to detect the fungal growth.

After exposure to vapours of different concentrations of ECEO, the wheat seeds were transferred to Petri plates containing PDA medium and were kept in the dark at 25°C.

Fungi were isolated for their identification on the 5th day.

The following formula was used to calculate the frequency of each fungal genus from the total fungal genera:

$$Fr\% = NG/TNF * 100$$

where:
NG - number of colonies from the genera;
TNF - total number of fungi.

The seed contamination index was calculated according to the formula:

$$SCI = NCS/TNS$$

where:
NCS - number of contaminated seeds;
TNS - total number of seeds in a petri dish.

Percentage of germination

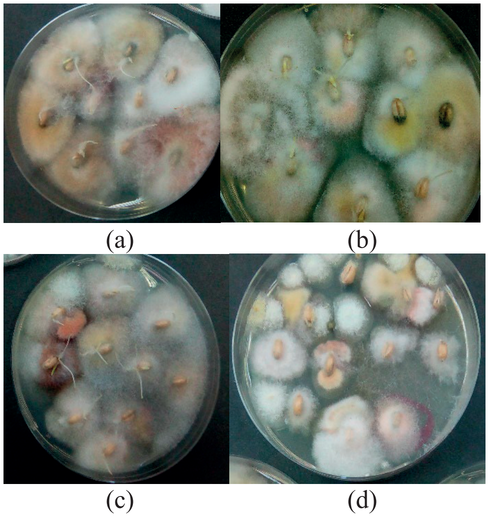
Germination was calculated on the 10th day according to the formula:

$$G\% = NGS/TNS$$

where:
NGS - number of germination seeds;
TNS - total number of seeds in a plate.

RESULTS AND DISCUSSIONS

The identified species presented in the control samples were represented by *Fusarium* spp. (60%), *Alternaria* spp. (10%), *Penicillium* spp. (10%), and others (10%).



(a) (b) (c) (d)

Figure 2. Petri dishes presenting the effect of different concentrations of ECEO on wheat seeds: (a) 5 µL; (b) 25 µL; (c) 50 µL; (d) 100 µL

The fungal contamination of wheat seeds after exposure to different concentrations of ECEO vapours is presented in Figures 2 and 3.

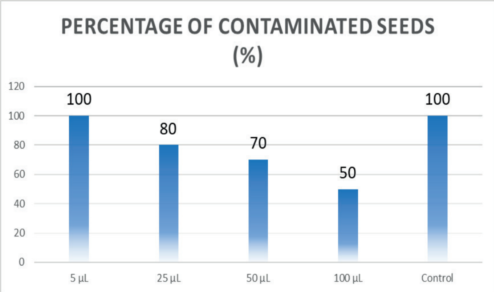


Figure 3. The percentage of contaminated seeds exposed to different concentrations of ECEO vapours

As is observed in the previous figure, the percentage of contaminated seeds in the presence of 5 µL of ECEO remains at the same value as the control samples, which means that this essential oil concentration doesn't have any antifungal activity. However, a concentration of 25 µL decreased the total number of contaminated seeds by 20%, while a concentration of 50 µL reduced the number of contaminated seeds by 30% compared with the control samples. The greatest reduction in the number of contaminated seeds was observed by exposure to a concentration of 100 µL, with the percentage reaching 50%.

Like the control sample, the dominant species was represented by *Fusarium* spp. followed by other species.

The germination percentage of the wheat seeds in the presence of the different concentrations of the ECEO is presented in Figure 4.

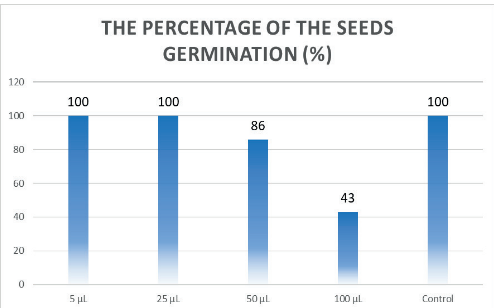


Figure 4. The percentage of seeds germination exposed to different concentrations of ECEO vapours

Regarding the germination rate, this had maximum values in the case of the control samples and those exposed to doses of 5 µL and 25 µL ECEO, respectively. The increase in the essential oil concentration determined a decrease in the germination percentage. Thus, 50 µL determined a germination percentage of 86%, and a concentration of 100 µL determined a reduction of the germination percentage by up to 43%.

The antifungal activity of essential oils has been demonstrated by numerous authors, who have shown that the effectiveness varies from one oil to another depending on its chemical composition and the fungal strain. Thus, Perczak et al., 2019, demonstrated that *Cinnamomum zeylanicum*, *Origanum vulgare, Cymbopogon martini, Thymus hiemalis, Mentha viridis, Foeniculum vulgare dulce*, and *Aniba rosaeodora* essential oil significantly inhibited the growth of *Fusarium graminearum* and *F. culmorum* (90.99-99.99%). In steed, orange essential oil inhibits the growth of *Fusarium* species in a smaller percentage, respectively of 68.33% (Perczak et al., 2019). Kedia et al., 2016, demonstrated that *Mentha spicata* essential oil produced 52.2% inhibition of *Alternaria flavus* at 1 µL/mL (Kedia et al., 2016). On the other hand, Li et al., 2016, demonstrated that *Litsea cubeba* essential oil determined 100% inhibition of *A. flavus* growth at 5 µL/g after 20 days (Li et al., 2016). *Rosmarinus officinalis* L. essential oil inhibited the growth of *A. flavus* at a percentage of 73.5% at 1.5 µL/mL (Prakash et al., 2015). The present study demonstrated that *Elletaria cardamom* essential oil had antifungal activity against *Fusarium* spp., *Penicillium* spp., *Alternaria* spp. All these prove that the antifungal effects of different essential oils vary depending on the chemical composition, which in turn depends on the geographical area and the climate in which the plant grew, as well as on the extraction method of the product.

The literature has shown that EO has antifungal activity against *Fusarium graminaerum* (Alexa et al., 2018). Furthermore, research has demonstrated that EO has an antifungal impact on *Aspergillius sp., Fusarium* sp., *Penicillium* sp., and *Verticillium dahliae* (Arslan et al., 2010; Kocic-Tanackov et al., 2013). EO has antifungal activity against *Fusarium graminaerum* (Alexa et al., 2018). Furthermore, research has demonstrated that EO has an antifungal impact on *Aspergillius* sp., *Fusarium* sp., *Penicillium* sp., and *Verticillium dahliae* (Arslan et al., 2010; Kocic-Tanackov et al., 2013). Also, studies show that the chemical composition of the essential oil can affect the germination rate, but most of them claim that an increase in oil concentration determines the decrease of this rate. Mirmostafaee et al., 2019 demonstrated that the germination rate of seeds exposed to *A. officinalis* essential oil varied, with an average of approximately 33% in control samples and slight modification, independent of seed age. The increase in essential oil concentration determined a decrease in the germination rate, indicating damage to seeds and embryos (Mirmostafaee et al., 2019). Post-ripening can improve seed germination rates and conditions. The germination rate and the conditions under which seeds germinate can increase during post-ripening (Stanisavljević et al., 2018). Terzić et al., 2023, demonstrated that treatment of seeds with *Althea officinalis* at a concentration of 0.02% showed full efficacy, the germination rate being increased by 13% in 3-year-old seeds. However, due to the high percentage of dormant seeds, the same treatment did not achieve full efficacy in 1- and 2-year-old seeds. (Terzić et al., 2023). The present study demonstrated that increased concentration of ECEO had as results a decreased of germination percentage.

CONCLUSIONS

Antifungal treatment of wheat seeds with *Eleteria cardamomum* has shown promising results in protecting the seeds from various fungal infections. The active compounds present in *Eleteria cardamomum* have demonstrated strong antifungal properties, making it a potential natural alternative to chemical fungicides.

Several studies have reported the efficacy of *Eleteria cardamomum* in inhibiting the growth of common fungi such as *Fusarium, Alternaria*, and *Aspergillus*, which are known to cause significant damage to wheat crops. The application of *Eleteria cardamomum* extract as a seed treatment has exhibited antifungal activity and the ability to enhance seed germination and promote healthy seedling development.

Moreover, using *Eleteria cardamomum* aligns with the increasing demand for sustainable and eco-friendly agricultural practices. By harnessing the natural antifungal properties of *Eleteria cardamomum*, farmers can reduce their reliance on synthetic fungicides, thereby minimising the environmental impact associated with chemical inputs.

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OPTIMIZING DRIP IRRIGATION YIELD IN GRAIN MAIZE CULTIVATION IN EASTERN ROMANIA

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Abstract

Maize cultivation stands as a vital pillar in Eastern Romania's agriculture, significantly contributing to food security and the local economy. In the face of climate change and increasing production demands, optimizing irrigation methods becomes crucial. This article explores the impact and advantages of drip irrigation on the yield of maize crops in the region. Modern technology allows for the customized configuration of drip irrigation systems, considering the specific needs of maize cultivation and local conditions. By providing water at the right time and in optimal quantities, drip irrigation significantly contributes to the increase in maize crop yield. Drip irrigation in maize cultivation in Eastern Romania represents an efficient and sustainable solution for optimizing agricultural yield. Through the adoption of these modern technologies, farmers can ensure increased and sustainable production, contributing to the prosperity of the region

Key words*: drip irrigation, uniform water distribution, crop efficiency, water provision, sustainable solution.*

INTRODUCTION

The growth of the global population, along with increased demands for food and energy, has led to a rising need for higher global production of maize (*Zea mays* L.). However, environmental factors such as high temperatures and drought have continued to reduce maize production in many regions over the past decades, and this decline is expected to worsen in the context of climate change (Meeks et al., 2013). The development of maize hybrids capable of performing efficiently under heat and drought conditions is a crucial objective for ensuring agricultural progress in the future. These traits include, but are not limited to, shortening the anthesis-silking interval (ASI), delaying leaf senescence, increasing root depth and density, osmotic adjustment, a high number of leaves, reduced plant height, performance under limited nitrogen conditions, seedling vigor, and the presence of epicuticular wax (Betran et al., 2003; Ludlow et al., 1990; Wan et al., 2000; Meeks et al., 2013). These objectives are a priority in breeding programs globally, including in Romania. Drought tolerance is recognized as a complex trait, which significantly complicates the development of effective methods for selection, breeding, and evaluation. Maize is an exceptionally valuable species due to its high production potential, wide diversity of uses as food and feed, as well as a raw material for industrial processes. Cultivated on large areas globally, including in Romania, maize plays a crucial role in the development of a modern and efficient agricultural market. Market and consumer demands drive research in maize breeding, directing it towards the creation of increasingly higher-performing hybrids in terms of production capacity (Sarca et al., 1996).

Maize (*Zea mays* L.) is a highly important forage plant due to its high dry matter yield and quality characteristics that support optimal animal production (Roth et al., 1995). The yield and quality of maize forage are determined by a complex interaction of environmental, agricultural, and genetic factors. It is well established that maize grain and forage yields decrease in the presence of drought and soil water deficit (Hajibabaei & Azizi, 2012). Maize (*Zea mays* L.) exhibits a high grain yield potential, primarily influenced by the genetics of the cultivated hybrid and environmental factors that affect plant development (Ion et al., 2014). The production capacity of the plants is determined by yield factors, which play a crucial role in its formation (Ion et al., 2013). Distinct reactions of the hybrids to water stress at various stages of crop development have been observed (Mandache et al., 2012). Research on water consumption related to maize irrigation is a major objective, considering the importance of this crop in providing plant material (Popa, 2021). Globally, drought and desertification affect approximately 47% of arid lands, with varying degrees of aridity. In recent years, there has been a trend of expanding drought-affected areas in most regions of the country, accompanied by a reduction in water resources available for irrigation (Huma, 2004). Given that Romania is a member of the European Union and is facing the challenges of a market economy and the global financial crisis, achieving high and stable yields, along with economic efficiency and environmental protection, has become an urgent necessity. For southeastern Romania, an average irrigation rate of 800-1500 m^3/ha is recommended. The lack of irrigation during the period of maximum water consumption by maize plants can have a significant impact on grain yield (Jinga I. & Cătălina, 2000). Drip irrigation plays a significant role in the proposed solutions for the water crisis. Average daily evapotranspiration will increase by approximately 6% if the average air temperature rises by 2°C and by about 15% at an average temperature above 5°C (Nitu et al., 2023). Drip irrigation "has the potential to double crop yields, including for most vegetables, cotton, sugarcane, and vineyards" (Postel, 2000). Drip irrigation, an advanced and water-efficient method, ensures that plant roots are maintained in optimal moisture conditions over extended periods, thus favoring both physiological activity and crop development (Yan et al., 2022). Other studies have indicated that drip irrigation helps reduce salt ion levels in the soil near the dripper, creating a favorable environment for plant growth in the root zone and mitigating the negative effects of soil salinization on crop development (Zhang et al., 2019). The objective of establishing an irrigation schedule is to accurately determine the volume and optimal timing of water application to crops (Sałata et al., 2022), based on at least one parameter from the soil-plant-atmosphere system (Kang et al., 2021). The selection of an appropriate irrigation scheduling strategy is crucial for supporting plant physiological processes and, consequently, for maximizing yield (Kumar Jha et al., 2019). Additionally, efficient irrigation scheduling contributes to reducing water and energy consumption (Souza & Rodrigues, 2022). On the other hand, over-irrigation or under-irrigation, resulting from an inadequate or poorly designed irrigation plan, has generally led to reduced grain production and decreased efficiency in the use of irrigation water (Irrigation Water Productivity), as well as issues such as land flooding, soil salinization, and elevated groundwater levels (Yohannes et al., 2019; Almeida et al., 2022; Quiloango-Chimarro et al., 2022).

MATERIALS AND METHODS

To achieve the objectives of the research, a bifactorial experiment of the 2 x 2 type with three replications was set up in Constanta County during the 2022-2023 period. Factor A was represented by two maize hybrids, a1 - P9889 and a2 - P0217, and factor B by the cultivation technology with the following levels: b1 - non-irrigated, b2 - irrigated at 50% I.U.A with ½ m = 200 m^3/ha $N_{90}P_{45}$, b3 - irrigated at 50% I.U.A with m = 400 m^3/ha $N_{180}P_{90}$ (Table 1).

Table 1. Experimental Variants

Factor A	Factor B		
a_1 - P9889	b_1 - non-irrigated	b_2 - irrigated 50% I.U.A at 0-40 cm with ½ m=200m^3/ha $N_{90}P_{45}$	b_3 - irrigated 50% I.U.A at 0-80 cm, with m=400 m^3/ha $N_{180}P_{90}$
a_2 - P0217			

The research was conducted in an area with flat microrelief, without groundwater contribution during the vegetation period.

Irrigation was applied at different stages of vegetation, with the objective of maintaining soil moisture above 50% of Pmin. IUA, regardless of the irrigation method used. In calculating the drip irrigation rate, it was essential to know the distance between emitters and the distance between drip lines in order to determine the percentage of wetted soil (P).

m brut = 1/ηc • H • Da • (CC-pmin) • P (m^3/ha)

m brut= drip irrigation norm;

H= irrigation depth;

Da= soil bulk density;

CC= field water capacity;

Pmin. = minimum soil moisture threshold;

P = percentage of moistened soil;

$$P = 100 \frac{Su}{dp * dc} \ \%$$

For calculating the irrigation rate, the weighted average values of the physical and hydro-physical indices of the soil from Constanta County were used, corresponding to the active layers of 0.8 m and 0.4 m. The irrigation rates were calculated for depths of 0.8 m and 0.4 m.

mbrut = 1/0.95 • 100 • 0.8 • 1.38 • (26.45 – 19.69) • 0.50 = 396 ≈ 400 m^3/ha

mbrut =1/0.95 • 100 • 0.4 • 1.38 • (26.9 – 19.75) • 0.50 = 209 ≈ 200 m^3/ha

Climatically, Constanta County is characterized by a transition between dry steppe and sub-humid forest climates. The year 2022 was marked by a significant warming in January and February. Starting from September, the average monthly temperatures approached the multiannual average. Therefore, from a thermal perspective, 2022 can be considered excessively warm, with an annual average temperature of 12.7°C, exceeding the multiannual average by 2.1°C. The lowest multi-year average temperature is recorded in January at -2.6°C, while the highest multi-year average temperature occurs in July, reaching 22.4°C (Figure 1). Regarding the annual average temperature, the year 2023 showed a minimal variation of just 0.2°C compared to the multi-year average of 10.6°C, thus it can be considered a drought year in terms of temperature (Figure 2). In 2022, the precipitation regime was deficient from the very beginning, with a significant deficit starting in the first month of the year. During the period from January to July 2022, precipitation levels were approximately 50% below normal, characterizing this period as extremely dry. During the warm season, precipitation decreased by approximately 32.5% compared to the multi-year average for this region. Thus, from the perspective of the precipitation regime, the year 2022 can be classified as a drought year, marked by prolonged extreme drought throughout almost the entire growing season of spring crops (Figure 3).

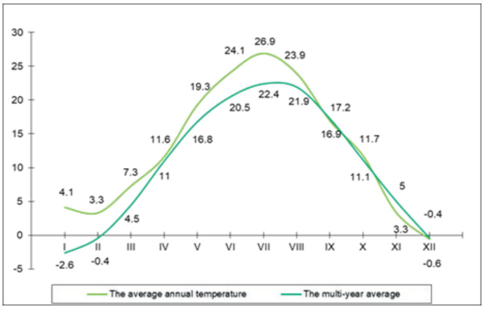

Figure 1. Monthly average air temperature recorded in the year 2022 and the multi-year average at the Constanta meteorological station

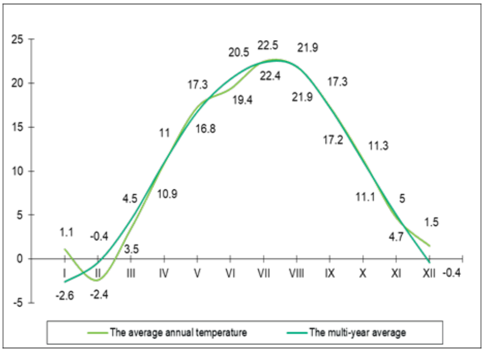

Figure 2. Monthly average air temperature recorded in the year 2023 and the multi-year average at the Constanta meteorological station

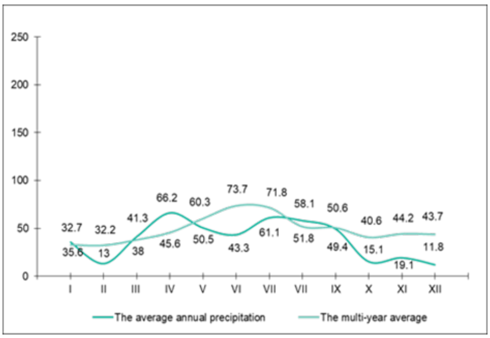

Figure 3. Monthly average precipitation recorded in the year 2022 and the multi-year average at the Constanta meteorological station

The year 2023 can be classified as a drought year, characterized by an extended drought period between May and August. During the cold season, precipitation decreased by 37% compared to the multi-year average for this period, while in the warm season, a reduction of 10% was observed (Figure 4).

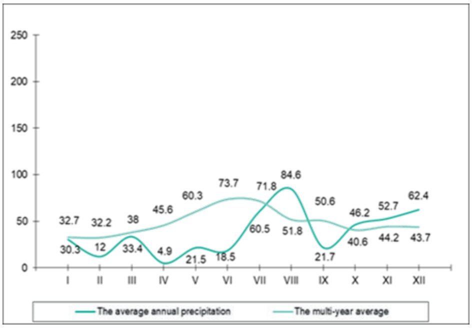

Figure 4. Monthly average precipitation recorded in the year 2023 and the multi-year average at the Constanta meteorological station

RESULTS AND DISCUSSIONS

The P0217 hybrid achieved a higher yield, with 76.17 q/ha, compared to the P9889 hybrid, which had a yield of 66.61 q/ha. The yield difference between the two hybrids is 9.56 q/ha, statistically ensured to be very significant (Table 2).

Table 2. The influence of maize hybrids on the average yield

Factor A	Production (q/ha)	Difference from Ct	Significance
a_1 - P9889	66.61	Ct	
a_2 - P0217	76.17	9.56	***

DL 5% 0.8
DL 1% 1.1
DL 0.1% 1.6

The yield obtained under non-irrigated conditions was 35.07 q/ha, serving as the reference level (Ct) for comparison. The introduction of irrigation combined with a fertilization regimen increased the yield to 69.70 q/ha, resulting in a production increase of 34.69 q/ha compared to the non-irrigated condition. Doubling the irrigation rate and intensifying fertilization further increased the yield to 109.33 q/ha, leading to a highly significant yield increase of 74.27 q/ha compared to the non-irrigated condition (Table 3).

Table 3. The effect of different cultivation technologies on the average yield of the two maize hybrids

Factor B	Production (q/ha)	Difference from Ct	Significance
b_1 - non-irrigated	35.07	Ct	
b_2 - irrigated 50% I.U.A at 0-40 cm with ½ m=200m³/ha $N_{90}P_{45}$	69.70	34.69	***
b_3- irrigated 50% I.U.A at 0-80 cm, with m=400 m³/ha $N_{180}P_{90}$	109.33	74.27	***

DL 5% 0.7
DL 1% 0.9
DL 0.1% 1.3

The interaction of factors reveals that irrigation and fertilization significantly increase yield compared to non-irrigated crops. The transition from non-irrigated (b1) to irrigated at 50% IUA at 0-80 cm depth with 400 m³/ha of water, along with the application of $N_{180}P_{90}$ fertilizers (b3), resulted in a highly significant yield increase of 69.92 q/ha. The application of irrigation at 50% IUA at 0-40 cm depth with a rate of 200 m³/ha, combined with a fertilization regime of $N_{90}P_{45}$ (b2), led to a statistically significant yield increase of 33.95 q/ha compared to the control variant. For the P0217 hybrid, the results showed a highly significant yield increase under the influence of irrigation and fertilization.

Compared to the non-irrigated variants, irrigation and fertilization according to the b3 technology led to a yield increase of 84.78 q/ha. This result shows that the P0217 hybrid has an increased capacity to respond to irrigation and fertilization strategies, indicating a better adaptation to the optimization of water and nutrient resources (Table 4).

Table 4. The impact of irrigation and fertilization on corn hybrids P9889 and P0217

Factor B	Factor A	Production (q/ha)	Difference from Ct	Significance
b_1 - non-irrigated	a_1 P9889	31.98	Ct	
b_2 - irrigated 50% I.U.A at 0-40 cm with ½ m=200 m³/ha $N_{90}P_{45}$	a_1 P9889	65.93	33.95	***
b_3 - irrigated 50% I.U.A at 0-80 cm, with m=400 m³/ha $N_{180}P_{90}$	a_1 P9889	101.90	69.92	***
b_1 - non-irrigated	a_2 P0217	38.15	6.17	**
b_2 - irrigated 50% I.U.A at 0-40 cm with ½ m=200 m³/ha $N_{90}P_{45}$	a_2 P0217	73.58	41.60	***
b_3 - irrigated 50% I.U.A at 0-80 cm, with m=400 m³/ha $N_{180}P_{90}$	a_2 P0217	116.77	84.78	***

DL 5% 2.8
DL 1% 3.9
DL 0.1% 5.3

In Constanta County, soils can often be deficient in nutrients due to erosion processes and prolonged drought conditions. Adequate fertilization thus becomes vital to ensure a sufficient supply of essential nutrients such as nitrogen (N) and phosphorus (P). Studies have shown that applying a fertilization regime with higher doses, such as N180P90 (variant b3), in combination with irrigation at 50% IUA at a depth of 0-80 cm and a water volume of 400 m³/ha, can lead to very significant yield increases, up to 69.92 q/ha for the P9889 hybrid and 84.78 q/ha for the P0217 hybrid.

The results obtained in variant b3 suggest that the P0217 hybrid has a superior capacity to adapt to irrigation and fertilization compared to P9889, making it an excellent option for east areas affected by drought. This variant has shown that a more intensive irrigation and fertilization strategy can compensate for water and nutrient deficiencies, maximizing yield even in unfavorable climatic conditions. Fertilization and irrigation of corn in the east region of the country are essential for ensuring high productivity, especially in the context of current climatic conditions characterized by drought and extreme temperatures. Experiments conducted on corn hybrids P9889 and P0217 have clearly demonstrated the importance of these agricultural practices.

CONCLUSIONS

Fertilization and irrigation of corn in the eastern region of the country are essential for maintaining and improving agricultural productivity, especially in the context of current climate changes, which bring frequent drought and extreme temperatures. In this region, which includes the counties of Moldova and Dobrogea, corn crops are exposed to climatic conditions that can significantly reduce yields if appropriate agricultural practices are not applied.

In the eastern part of Romania, soils are often subjected to erosion processes and can become nutrient-deficient due to torrential rains followed by periods of drought. This makes fertilization crucial to supplement the lack of essential nutrients, such as nitrogen (N) and phosphorus (P), which are necessary for the optimal development of corn.

Experiments conducted on corn hybrids, such as P9889 and P0217, have shown that applying a more intensive fertilization regime, such as $N_{180}P_{90}$ (variant b3), can lead to significant increases in production. Under current conditions, this fertilization, combined with appropriate irrigation practices, becomes even more important to ensure a constant supply of nutrients, thereby ensuring good crop development.

Irrigation is another essential factor in the eastern region of the country, where precipitation is often insufficient to meet the water needs of corn. Prolonged drought can cause water stress in plants, leading to a significant decrease in production. Experiments have shown that irrigation at 50% IUA (Active Moisture Index) at depths of 0-40 cm and 0-80 cm, together with a water volume of 200-400 m³/ha, can bring major improvements in crop yields.

In conclusion, for the eastern region of the country, characterized by frequent drought and soil erosion, fertilization and irrigation of corn are fundamental agricultural practices for obtaining high and stable yields. Experimental results suggest that the use of intensive fertilization strategies, combined with appropriate irrigation, can compensate for water and nutrient deficiencies, thus maximizing crop yields. The P0217 hybrid, in particular, has proven to be highly adaptable to these conditions, making it an excellent option for farmers in eastern Romania.

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MANAGEMENT OF FEW MAIN WHEAT DISEASES USING ALTERNATIVE ORGANIC PRODUCTS WITH FUNGICIDE EFFECT – A REVIEW

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Abstract

Worldwide the majority of fungal wheat diseases may be controlled chemically, but natural plant products have been shown to exert biological activity against wheat fungal pathogens in vitro and in vivo and can be used as bio-fungicidal products. Natural plant products have a specific mode of action and a confined target range. They are also often harmless to hostile microorganisms, have a shorter shelf life, and provide no residual traits. Frequently included in integrated pest management (IPM) schemes, they are generally less hazardous to humans and the environment than traditional synthetic chemical pesticides. Also, the use of chemical products is limited due to undesirable environmental effects and the emergence of resistant pathogens to fungicides. The major compounds that have been investigated to date include phenols, flavonoids, quinones, terpenes, tannins, alkaloids, lectins, polypeptides, saponins and sterols. These products may have fungicidal or fungistatic activity on plant pathogens or they can create conditions unfavourable for establishment and multiplication of pathogenic microorganisms on host plants. In this study, we have discussed the sensitivity of most important fungal pathogens of wheat against different natural extracts products and essential oils and their main components, together with their modes of action in controlling wheat diseases. The option of replacing fossil oil-based chemicals with plant product formulations fits well with food and agriculture policies directed to the future.

Key words: *wheat diseases, pathogens, natural products, plant extracts, essential oils.*

INTRODUCTION

In the last decades, organic crops are constantly expanding, and the demand for healthy food, with no chemical residual products, is increasing.

Cereals are one of the most important crops worldwide and they are vital for human consumption and animal feed (Neupane et al., 2022). To feed the estimated 9.8 billion people on the planet by 2050, the supply of cereal food must rise by 70-100% and 2-3% (annually) (Hawkesford et al., 2013; Ray et al., 2013; Tripathi et al., 2016). Maintaining access to safe, disease- and pest-free and affordable agricultural products and raw materials and ensuring sustainable agricultural production are challenges that must be faced in the context of climate changes, which impact both natural and agricultural ecosystems with severe economic consequences (Răduțoiu, 2022; Răduţoiu & Stan, 2022; Răduțoiu, 2023; Velea et al., 2021; Zală, 2021; Zală et al., 2023a). Also, there is serious concern that climate zones will move faster that it is possible for plant populations to track them, which is expected to determine disproportionate extinction of local endemic species (Răduţoiu & Băloniu, 2021; Răduțoiu et al., 2023).

One of the most significant impacts of climate change on cereals is the increase in temperature (Chakraborty & Newton, 2011; Chakraborty, 2013). According to previous studies, over the next 30 years, the earth's average surface temperature will rise at a rate of about 0.2 degrees Celsius every decade (Bernstein et al., 2008). Higher temperatures can lead to heat stress in plants, which can result in reduced yields and increased susceptibility to diseases (Coakley et al., 1999; Paraschivu et al., 2017; Paraschivu et al., 2019; Paraschivu et al., 2021; Paraschivu et al., 2023). For example, a 1°C increase in temperature during wheat cultivation could result in a 3-10% decrease in crop yields (Yao et al., 2012). This is because pathogens, such as fungi and bacteria, thrive in warm and moist conditions, and can infect plants more easily when they are stressed.

In order to control pathogens, the current problems related to the consequences caused by fungal and viral plant diseases along with those caused by agricultural pollution (Bonciu, 2023b; 2023c) require essential changes in plant cropping and breeding technologies (Bonciu, 2023a; Lipianu et al., 2023; Zălă et al., 2023b). One of the most modern such technologies is agricultural biotechnology and genomics (De Souza and Bonciu, 2022a; 2022b), which is able to ensure the creation of varieties and forms of plants with targeted performances: increased productivity and quality, resistance to diseases and tolerance to unfavorable climatic factors. Thus, in the context of increasingly obvious climate change, adapted technological measures are required in all crops in order to prevent the increasingly pronounced influence that weeds, pathogens and pests have on the quantity and quality of production (Matei et al., 2019; 2020; 2022).

Also, other practical way to reduce yield losses is to investigate alternative organic products with fungicide effect. This approach can result in the eco-friendly usage of natural products and other qualities that have minimal adverse effects.

For the past few decades, it is estimated that there are more than 250.000 higher plant species that can be evaluated for their antimicrobial bioactive chemical compounds, especially plant extracts and oils (EOs) (Tegegne and Pretorius, 2007; Shuping and Eloff, 2017; Zaker, 2016). Many plant extracts have demonstrated potent antifungal activity (with MIC values below 1.0 mg/mL) using in vitro assays, but only a few were tested in vivo (Drakopoulos et al., 2020; Seepe et al., 2020). The lack of resources and expertise needed to carry out pertinent in vivo experiments in the field or in a greenhouse remain one of the limiting issues.

Despite of this, medicinal and other plant extracts have attracted attention in the pesticide industry based on their antimicrobial properties due to spectrum of their constituent secondary metabolites such as phenols, polyphenols, flavonoids, glycosides, tannins, alkaloids and other compounds that may inhibit the development of resistance due to the presence of different constituent antimicrobial compounds and their synergisms (Calvo et al., 2011; Mahlo et al., 2010; Mdee et al., 2009; Rishi and Singh, 2003; Sultana et al., 2013). Also, the components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the plant pathogens, enhancing plant defence mechanisms against pathogenic microorganisms (Das et al., 2010).

Majority of the researches on bio-active plant extract and essential oils (EOs) emphasized on effect of plant extracts against plant pathogens or diseases. Essential oils contain a mixture of different compounds such as monoterpenes, diterpenes, sesquiterpenes, aliphatic and other aromatic compounds that are volatile in nature (Koul et al., 2008; Nuzhat and Vidyasagar, 2013).They are oily liquids obtained from medicinal plants, herbs, spices and aromatic plant species (parts including the flowers, leaves, barks, roots, seeds, fruits and whole plants) through fermentation, efleurage and steam distillation (Burt, 2004), whereas plant extracts, in contrast are obtained from dried plant products by filtration and evaporation using various solvents (Van de Braak and Leijten, 1999; Wang et al., 2004).

As part of Integrated Pest management (IPM), natural plant products have a narrow target range with specific mode of action, therefore are suitable for a specific target, mostly nontoxic for antagonistic microorganisms, show limited field persistence and have a shorter shelf life and no residual threats (Zaker, 2016).

Previous findings suggest that plant extracts can work by targeting the plasma membrane in the majority of infections, increasing the permeability of the fungal cell wall, and disrupting the normal actions of the topoisomerase enzymes (Kawakami et al., 2015), while essential oils (EOs) are associated with disruption of the fungal cell wall integrity through the inhibition of chitin and β-glucans synthesis, disruption of the cell membrane, such as by binding to or inhibiting ergosterol biosynthesis, mitochondria dysfunction arising from inhibition of electron transport and respiratory chain proton pumps, cell division inhibition via interference with microtubule polymerization, inhibition of ribonucleic acid, deoxyribonucleic acid or protein synthesis; and efflux pump inhibition (Lagrouh et al., 2017; Seepe et al., 2021). The underlying mechanisms are not clearly understood, but involvement of induced resistance is considered (Fokkema, 1993).

Different previous studies have demonstrated the excellent properties of plant extracts and essential oils for their antimicrobial and antioxidant activities (Celikel and Kavas, 2008; Tajkarimi et al., 2010).

Considering the findings above, a crucial first step toward sustainable crop production is reducing the use of traditional synthetic fungicides in the presence of efficient natural alternatives. Thus, the present paper emphasises in the following subsections, a review of some studies conducted in the past years on antifungal activity of plant extracts and essential oils isolated from plants against the main pathogens in wheat.

NATURAL PRODUCTS AGAINST WHEAT RUSTS

In many wheat-growing areas, one of the most significant constrainer to wheat productivity is *Puccinia triticina* which produces leaf rust. The fungus is able to cause severe yield losses reaching up to 50% (Draz et al., 2015). The severity of the disease depends upon the developmental stage of the plant and its susceptibility of the plant (Duplessis et al., 2021). Usually, the disease management is based on the use of resistant cultivars and application of chemical fungicides, but alternative control methods are required (Barro et al., 2017).

Hassan et al. (1992) reported that rust pustules on the wheat leaves can be reduced with leaf extracts of *Datura stramoniu*. In 2019 Draz et al. have tested five plant extract (i.e. henna, *Lawsonia inermis*; acalypha, *Acalypha wilkesiana*; chinaberry, *Melia azedarach*; pomegranate, *Punica granatum*; and lantana, *Lantana camara)* against leaf rust infection caused by *Puccinia triticina* showing that Infection Coefficient (IC) decreased for all tested variants with values that ranged between 7.5 to 20. The most effective one was the extract of *Lantana camara* with 88.88% efficiency which was very close to the fungicide "diniconazole" (efficiency = 89.92%).

The efficacy of eight plant extracts (garlic, clove, garden quinine, Brazilian pepper, anthi mandhaari, black cumin, white cedar and neem) in controlling leaf rust disease of wheat was investigated *in vitro* and *in vivo* (Shabana et al., 2017). *In vitro*, all treatments inhibited spore germination by more than 93%, while *in vivo* foliar spray application of wheat plants at mature stage with all plant extracts has significantly reduced the leaf rust infection.

The efficiency in the treatment of leaf rust of wheat was examined also for plant leaf extracts of Neem and Moringa at varied concentrations of 50, 100, and 150 ml correspondingly. All treatments decreased fungal growth *in vitro* by greater than 90% (Afzal et al., 2023).

The methanol extracts of henna, lantana, acalypha, chinaberry, and pomegranate exhibited a 100% - inhibition of *P. triticina* spores germination (Elkhwaga et al., 2018).

The aqueous leaf extracts of *Jacaranda mimosifolia* (Bignoniaceae), *Thevetia peruviana* (Apocynaceae) and *Calotropis procera* (Apocynaceae) inhibited *P. triticina* urediniospore germination *in vitro* by strongly stimulated defense-related gene expression and the subsequent accumulation of pathogenesis-related (PR) proteins in the apoplast of inoculated wheat leaves (Naz et al., 2014).

The plant oils could potentially be used alone in organic growing or in rotation with synthetic fungicides in an integrated pest management (IPM) program in the conventional wheat growing. Unlike many fungicides, resistance to plant oils has not been reported yet. In vivo results indicated that the use of plant oils could be also a valid treatment to control infections caused by *P. triticina*. Thus, the efficacy of eight plant oils (castor, corn, cottonseed, linseed, olive, peanut, soybean and sunflower seed oils) was evaluated as possible alternatives to synthetic fungicides for the control of *P. triticina* (Arslan, 2014). The percentage of inhibition in urediniospore germination of all tested plant oils ranged from 0 to 84.9% against *P. triticina*, linseed oil providing the best control at 2.5 % concentration *in vitro* and at 1% concentration *in vivo.*

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is one of the three main rusts attacking wheat plants worldwide.

Omara et al. (2020) showed that leaf extract of *Artemisia cina* inhibited spore germination and increased incubation and latent periods in wheat stem rust than other treatments. Also, garlic oil revealed the highest suppression of stem rust severity followed by cinnamon, thyme, onion and clove oils, respectively (Abdel-Kader et al., 2021). According to the report of Uwineza et al. (2018), pennyroyal (*Mentha pulegium*), Atlas cedar (*Cedrus atlantica*) and clove (*Eugenia aromatica*), are potentially effective plant sources against yellow rust caused by *P. striiformis* on wheat.

Wan et al. (2017) underlined that application of the EOs together with resistant cultivars may alleviate this constraint by preventing the emergence of new races.

NATURAL PRODUCTS AGAINST *Fusarium* species IN WHEAT

Fusarium fungal pathogens produce in wheat seedling and head blights leading to devastating economic yield loss in the field and result in a greater impact on food insecurity. Moreover, often in cereal grain samples analysed are found mycotoxins such as deoxyvalenol or vomitoxin (DON), toxin T-2, monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS) and nivalenol (NIV), which can produce food intoxications that are manifesting through sickness, vomiting, diarrhoea, headache, abdominal pains, fever, oesophageal cancer, carcinogenesis, mutagenicity and neural tube defects etc. in livestock (Reddy et al., 2010). Thus, beside conventional fungicides using alternative organic products with fungicide effect are required. Seepe et al. (2021) emphasized that he families with high frequencies of evaluated species against *Fusarium* pathogens were *Solanaceae*, followed by *Combretaceae* and *Fabaceae* and *Euphorbiaceae*. Leaf extracts from these plants demonstrated potent *in vitro* activities (minimum inhibitory concentrations <1.0 mg/mL) against nine *Fusarium* species. Often, in cereals, *Fusarium oxysporum* was the most frequently used pathogen, followed by *F. graminearum.*

Velluti et al. (2004) evaluated 37 essential oils (of which lemongrass, cinnamon, clove, palmarosa and oregano) and showed antifungal activity against *Fusarium* sp. Also, the antifungal effect of *Aloe Vera* (syn: *A. barbadensis*) as inhibitor of mycelium growth of *Fusarium oxysporum* was reported (Rodriguez et al., 2005). Kumar et al. (2007) reported *Chenopodium ambrosioides* can inhibit two aflatoxigenic strains of *F. oxysporum* and other fungi, while and essential oil of Peumusboldus was effective against *Fusarium* spp. (Souza et al., 2005). Cheng et al. (2008) investigated the antifungal activity of essential oil from *Calocedrus macrolepis* var. *formosana* and its constituents T-murolol and a-cadinol on the growth of plant pathogenic fungi which also inhibited the growth of *Fusarium oxysporum*. Also, Abo El-Seoud et al. (2005) evaluated essential oils of fennel, peppermint, caraway, eucalyptus, geranium and lemongrass for their antimicrobial activities against *F. oxysporum* and other fungi and found that essential oils of fennel, peppermint and caraway can be used as active ingredients for formulating biocides. The use of essential oils obtained from *Carum carvi*, *Cymbopogon nardus*, *Pelargonium roseum*, *Pimentadioica* and *Thymus vulgaris* was found as effective against mycelium growth *F. oxysporum* (Zabka et al., 2009). Also, Deba et al. (2008) tested the fungitoxic activities of the flower essential oils of *Bidens pilosa* against *Fusarium* spp., while in 2010, Naeini et al. observed anti-*Fusarium* properties of five EO of *Cuminum cyminum* and *Zataria multiflora*.

Different studies emphasized the antifungal activity against *Fusarium oxysporum* of essential oils obtained from plants used in traditional medicine, such as: *Aconitum laeve* Royle (Ranunculaceae) (MIC – Minimum Inhibitory Concentration - value of 300 μg/mL), *Artemisia sieberi* Besser. (Asteraceae) (MIC value of 60 μg/mL), *Bupleurum falcatum* L. (Apiaceae) (MIC of 2 μg/mL), *Cannabis sativa* L. (Cannabidaceae) (Inhibition of 93.58% at 1 μL/mL), *Cinnamomum camphora* (Lauraceae) (inhibition of 49% at 3000 μL/L), *Citrus aurantium* (Rutaceae) (inhibition of 57.75% at 1 μL/mL), *Citrus reticulata* L. (Rutaceae) (inhibition of 70% at 0.15 mL/100 mL), *Cuminum cyminum* (Apiaceae) (MIC value of 72 µg/mL), *Cymbopogon citratus*, Stapf. (Poaceae) (inhibition of 100% at 2500 µL/L), *Cymbopogon nardus* (L.) Rendle (Poaceae) (inhibition of 85.56% at 1 µL/mL), *Echinophora platyloba* DC. (Apiaceae) (inhibition of 51.8% at 1 µL/L), *Eucalyptus* sp. (Myrtaceae) (inhibition of 55.11% at 1500 µL/L), *Foeniculum vulgare* Mill. (Apiaceae) (MIC value of 72 µg/mL), *Helichrysum splendidum* (Thunb.) Less. (Asteraceae) (inhibition of 58% at 3000 µL/L), *Heracleum persicum* Desf. Ex Fischer. (Apiaceae) (MIC value of 530 µg/mL), *Lippia rehmannii* H. Pearson (Verbenaceae) (inhibition of 72% at 500 µL/L), *Matricaria recutita* (L.) syn. (Asteraceae) (inhibition of 56.0% at 62.5 µg/mL), *Melaleuca alternifolia* (Myrtaceae) (MIC value of 0.91 mg/mL), *Mentha spicata* L. (spearmint) (Lamiaceae) (inhibition of 79% at 2000 µL/L), *Nepeta cataria* L. (Lamiaceae) (inhibition of 97.86% at 1 µL/mL), *Ocimum basilicum* L. (Lamiaceae) (inhibition of 74.87% at 1 µL/mL), *Origanum heracleoticum* L. (Lamiaceae) (MIC value of 0.07 mg/mL), *Origanum majorana* L. (Lamiaceae) (inhibition of 59.36% at 1 µL/mL), *Origanum vulgare* L. (Lamiaceae) (MIC value of 50 µg/mL), *Pelargonium roseum* L. (Geraniaceae) (inhibition of 85.56% at 1 µL/mL), *Mentha piperita* L. (Lamiaceae) (MIC value of 1.50 µg/mL), *Pimenta dioica* (L.) Merr. (Myrtaceae) (inhibition of 100% at 1 µL/mL), *Pimpinella anisum* L. (Apiaceae) (MIC value of 120 µg/mL), *Rosa damascena* P. Mill. (Rosaceae) (MIC value of 0.29 mg/mL), *Rosmarinus officinalis* (rosemary) (Lamiaceae) (MIC value of 410 µg/mL), *Salvia sclarea* L. (Lamiaceae) (inhibition of 58.82% at 1 µL/mL), *Satureja hortensis* L. (Lamiaceae) (MIC value of 0.14 mg/mL), *Silene armeria* L. (Caryophyllaceae) (MIC value of 500 µg/mL), *Stachys pubescens* Ten. (Lamiaceae) (MIC value of 1 µg/mL), *Syzigium aromaticum* L. (Myrtaceae) (Inhibition of 83% at 250 µL/L), *Thymus daenensis* Celak. (Lamiaceae) (MIC value of 4 µg/mL), *Thymus kotschyanus* Boiss. & Hohen. (Lamiaceae) (MIC value of 0.5 µg/mL), *Thymus kotschyanus* Boiss. & Hohen. (Lamiaceae) (MIC value of 75 µg/mL), *Thymus vulgaris* L. (Lamiaceae) (MIC value of 0.14 mg/mL), *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae) (MIC value of 3000 ppm), Zataria multiflora Boiss. (Lamiaceae) (MIC value of 66 µg/mL) (Ahmad and Beg, 2001; Chutia et al., 2009; Da Cruz Cabral et al., 2013; Khosravi et al., 2020; Manganyi et al., 2015; Moghaddam et al., 2015; Mohammadi et al., 2024; Naeini et al., 2010; Stević et al., 2014; Tegang et al., 2018; Zabka et al., 2009).

In 2007 Singh et al. reported 100% effect of Cinnamon leaf oil against *Fusarium graminearum.* Also, other studies emphasized the antifungal effect of *Curcuma longa* L. (Zingiberaceae) (MIC value of 2450 µg/mL), *Eucalyptus* sp. (Myrtaceae) (inhibition of 55.11% at 1000 µL/L) and *Zhumeria majdae* Rech. f. & Wendelbo (Lamiaceae) (inhibition of 75.11% at 1000 µL/L) against *Fusarium graminearum* (Davari and Ezazi, 2017; Kumar et al., 2016).

The results also showed that the essential oils (Eos) of anise, cinnamon, spearmint and thyme have more effect in decreasing fungal growth and mycotoxins production in wheat grains. At 5% (v/v) concentration, sweet basil EO totally inhibited mycelial growth and prevented aflatoxin production, showing that the oil's minimum inhibitory concentration (MIC) is less than or equivalent to 5% (Atanda et al., 2007).

According to Ferreira et al. (2018), the EO of *Zingiber officinale* reduced biosynthesis of the mycotoxin dioxynivalenol (DON) and ergosterol at concentrations of 500 and 1000 g/ml, respectively. According to Kumar et al., 2016, the EO of *Curcuma longa* totally suppressed formation of zearalenone (ZEA) and reduced fungal biomass at concentrations of 3500 and 3000 mg/ml, respectively.

NATURAL PRODUCTS AGAINST SPOT BLOTCH IN WHEAT

Spot blotch (SB), a devastating disease of wheat in warmer growing areas of the world, is caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*). The pathogen is capable of producing toxins like helminthosporol and sorokinianin and can infect leaves, stems, roots, rachis, and seeds (Roy et al., 2023). Briquet et al. (1998) reported that helminthosporol affects membrane permeability, thereby inhibiting mitochondrial oxidative phosphorylation, the photophosphorylation in chloroplasts, and the proton pumping across the plasma membrane, as well as β-1,3-glucan synthase activity. Also, sorokinianin compound shows inhibitory activity on seed germination (Nakajima et al., 1998).

The susceptibility to the pathogen increases around Zadoks' growth stage DC 56 (three-quarters of the inflorescence emerged) and fungal infection accelerates leaf senescence at later growth stages (Dehne and Oerke, 1985).

On a worldwide scale, yield losses in wheat caused by *B. sorokiniana* (16-25%, whereas under severe epidemic condition it may reach up to 80%) indicate the need to search for alternative strategies for disease control (Joshi and Chand, 2002; Saari, 1998). One of the promising strategies is using alternative organic products with fungicide effect.

Plant extract from different species has been used to control spot blotch of wheat by encouraging lignification in host cell wall, reducing penetration of pathogen and enhancing wound healing in hosts (Hossain et al., 2016; Soylu, 2008).

Magar et al. (2020) reported that, *in vitro* experiment, different botanical extracts (neem (*Azadirachta indica*), garlic (*Allium sativum*), eucalyptus (*Eucalyptus globolus*), bojho (*Acorus calamus*) and asuro (*Justicia adhatoda*) in different concentrations (5%, 10% and 15%) inhibited the mycelial growth of fungus significantly. The mycelial growth inhibition of *Bipolaris sorokiniana* increased gradually with increased concentration of the extracts. The highest mycelial growth inhibition percentage was found by the application of garlic clove extract (52.85%) at 15% which was followed by bojho extract (52.48%) at 15% concentration. Similar results have been reported by several authors working with different botanical extracts (Yadav et al., 2015; Yasmin, 2016)

Different concentration of the oil of ginger, eucalyptus, clove, sesame and neem were evaluated *in vitro* for their antifungal effect against *B. sorokiniana.* The highest inhibition was observed by the application of clove oil, followed by garlic oil at 55.27% and 51.45% respectively, at a concentration of 3000 ppm (Debsharma et al., 2021). Yasmin (2016) reported that ginger extract inhibited the mycelial growth of *B. sorokiniana* up to 38.35% at 15% concentration, which suggested the antifungal activity of the extract. Similar findings were reported also by other authors (Hasan et al., 2005; Raveau, 2020). Other two essential oils were evaluated at a concentration of 0.31 µl/ml for *Origanum compactum* and 1.25 µl/ml for *Thymus satureioides* (Zahraoui et al., 2024). *Thymus satureioides* was found to be more effective than *Origanum compactum*, reducing the infection rate of B. *sorokiniana* by 52% and improving productivity by 50%. However, the results showed that both essential oils significantly reduced disease severity by 48% and increased grain yield by an average of 25% across all varieties used. The results confirm previous findings obtained by El Ajjouri et al. (2008), who have demonstrated that *Thymus satureioides* by his bioactive compound carvacrol or cymophenol (a phenolic monoterpenoid) is among the most active essential oils against fungi. Carvacrol can destabilize the cytoplasmic membrane and act as a proton exchanger, reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually leads to cell death (Ultee et al., 2002). It is also possible that the action of the carvacrol to be modulated by other minor molecules of other compounds, suck us P-cymene that makes carvacrol being more easily transported into the cells (Ultee et al., 2002).

Considering previous findings and the potential of plant extracts and essential oils to reduce infection rates caused by *B. sorokiniana*, they deserve further studies to explore their antifungal properties and evaluate the long-term effectiveness of the treatment.

NATURAL PRODUCTS AGAINST POWDERY MILDEW IN WHEAT

Powdery mildew in wheat caused by the obligate parasite *Blumeria graminis* f. s. *tritici* is one of the first recognized wheat diseases, widely distributed with significant global incidents over the last four decades, but more damaging in cool, wet climates and semi-arid areas (Morgounov et al., 2012). The disease is ranked sixth out of the ten most important fungal diseases of wheat and the 8th highest yield loss contributor of wheat globally (Dean et al., 2012; Savary et al., 2019). Grain yield reduction, which results from plant infection by pathogen, can reach 15-20%, and in the case of severe infection, can reach 50% or more (Jaczewska-Kalicka, 2006).

Use of chemical fungicides for controlling powdery mildew in wheat has led scientists to look for alternative antifungal substances such as plant extracts and essential oils that are safer than synthetic products, biodegradable in nature and non-polluting. However, little work has been done about the fungistatic effect of natural extracts on Powdery mildew in wheat.

Choi et al. (2004) reported that the extract of *Rumex acetosella* roots reduced development of powdery mildew. Even extracts prepared from oak bark (*Quercus robur* L.), *Reynoutria sacchaliensis* L., curcuma (*Curcuma longa* L.), and ginger (*Zingiber officinale* Roscoe) were effective against powdery mildew on the winter wheat (Vechet et al., 2005).

Also, Schuster et al. (2010) obtained good results using plant extract from *Glycyrrhiza glabra*. Natural extract from the roots of Chinese rhubarb (*Rheum officinale*) proved its high efficiency for controlling powdery mildew (Yang et al., 2008). Thus, according to a histological analysis, this substance inhibited the germination of conidia, accelerated appressorium deformation prior to the pathogen infecting wheat (*Triticum aestivum*) cells, and decreased the length and number of secondary haustoria following infection, among other effects on *Blumeria graminis in vivo*.

Uwineza et al. (2018) showed that Powdery mildew disease was completely controlled by 1.25 ml/L concentration of essential oils of *Mentha pulegium*, *Eugenia aromatica* and *Cedrus atlantica*.

These findings indicate that even natural compounds could provide long-term protection against powdery mildew in wheat.

CONCLUSIONS

Studies on the antifungal activity of botanical extracts to protect plants from diseases have received much attention lately. Thus, many reports approve the efficacy of natural products of plants in controlling fungal growth and mycotoxin production demonstrating that plant extracts and essential oils contain a high concentration of bioactive and antioxidant substances. Investigating the use of plant-based fungicides to reduce yield losses in cereals is a workable strategy that can also result in the environmentally eco-friendly usage of natural products and serve as a rich source of natural compounds with a variety of fungicidal and other qualities that have little negative effects. Furthermore, field studies under natural conditions would be necessary to thoroughly examine the effect of these plant extracts and essential oils in understanding the reaction of wheat pathogens and host-pathogen relationship.

In the near future, increased interest will be observed in alternative fungicides because of their safe status as they can be easily decomposed, nature friendly and nonphytotoxic. Thus, discovering sustainable, safe and effective control strategies for controlling crop diseases remains imperative towards achieving the second goal, amongst others, of the Sustainable Development Goals (SDGs), which is “to end hunger, achieve food security and improved nutrition and promote sustainable agriculture’’.

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EXPERIMENTAL RESULTS ON ALTERNATIVE WAYS OF INCREASING THE pH OF ACID SOILS, USING CARBONATION MUD (DEFECATION LIME), THE WASTE FROM SUGAR BEET INDUSTRY

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Abstract

The waste precipitate from sugar technology contains $CaCO_3$ and aggregated or adsorbed non-sugars. This work is an attempt to investigate the possibility of using sugar beet waste-carbonation lime residue (sludge) as an amendment to correct the pH of acid soils, embracing and implementing the concept of circular energy crop. Research were carried out during three agricultural years (2020-2022), in the experimental field of the National Institute of Research and Development for Potato and Sugar Beet Brașov, Romania. A total number of 96 experimental sugar beet plots were analyzed, divided into six variants (variables), V1 - control, V2 - $CaCO_3$ (classic), V3-V6 different amounts of carbonation lime sludge (Factor A), using two methods of incorporating amendments into the soil and different amounts of fertilizers (4 experiments) (Factor B). The use of defecation lime had an impact on soil pH, yield production (very significant differences compared to the control), sugar production (very significant differences compared to the control), and less on sugar biological concentration (significant differences compared to the control). For evaluating the results, analysis of variance (ANOVA) and Duncan multiple range test were used.

Key words: *amendment, carbonation sludge, sugar beet, pH.*

INTRODUCTION

The soil is a vital source for human food production. According to FAOSTAT, only 29% of the total global area is land, and out of this, only 6.4% is used as agricultural land. From this area, approximately 98% of food for the world's population is produced.

Currently, the state of soil fertility is continuously degrading. This degradation causes changes in the chemical properties of soils and includes nutrient loss, acidification, salinization, and alkalinization. Worldwide, in 62% of the area, soils have low or very low fertility, 27% have moderate fertility and only 11% have high fertility. Agricultural activity is the main cause of soil degradation on all continents (FAO, 2006).

In the context of the current efforts being made to protect the environment and maintain a natural biological and ecological balance in the soil favorable to the development of animal and plant life, the management of agricultural crops, through finding cost-effective alternative methods, acquires new values of maximum economic and scientific importance (Obaisi et. al., 2022).

Sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima)* is known in Romania and worldwide as one of the most valuable technical crops, from which the raw material needed for sugar factories and significant amounts of fodder for animal feed is mainly provided (Malmir et al., 2020).

Sugar beet is very demanding in terms of soil due to its deep root system, with a high absorption capacity and high consumption of nutrients and water. For this reason, it is not indicated to grow beets on compact soils that form a crust, because they prevent germination and normal development due to high physical-mechanical resistance, and the roots remain small and branch. Sugar beet prefers a neutral soil pH of 6.5-7. The highest root productions are obtained on lands with a deep arable layer, rich in nutrients, and well supplied with water throughout the growing season. Calcareous, too sandy, and heavy soils are not suitable for sugar beet cultivation. Also, compact, cold and impermeable soils, as well as too acidic or too

alkaline soils, are not suitable for sugar beet (Taus & Gherman, 2011).
Adjusting the pH by adding calcium is the first step if the soil is acidic or very acidic, because otherwise the plants cannot absorb the necessary nutrients and nutritional deficiencies can appear visible through the yellowing of the leaves or the lack of development of the plants.
The carbonation mud (technological sludge) is a residue resulting from the purification operations, by physico-chemical methods of the diffusion earth, in order to concentrate and crystallize the normal sucrose in the sugar factories (Gherman et al., 2022). The technological sludge is obtained through the process of classical purification in which calcium hydroxide and carbon dioxide are used for the removal of non-sugar in the following operations: pre-defecation and defecation, treatment with $Ca(OH)_2$ Ist saturation and treatment with CO_2 IInd saturation (Domșa, 1973).
Considering the large amount of $CaCO_3$ in the technological sludge (50-70%), it can be used with very good results to improve the acid reaction of soils. Before use, it must be dried in platforms, so that it has 10% moisture.
Technological sludge is a fast-acting amendment favoring production increases of 15-35% even in the first year after application. The ameliorative effect is combined with that of fertilizer because it contains 0.3-0.5% N; 0.8-1.5 P_2O; 0.15 K_2O and 10-15% organic substances (Rusu, 2005). The neutralizing power is 55-75%. The technological sludge can be used directly from the storage pits of the sugar factories or from the platforms where it is stored. Its physical condition is good after draining, being in the form of a semi-dry paste, easy to apply to the field in the following summer of the sugar processing campaign. From each sugar factory approximately 20-40 thousand tons of technological sludge result annually, depending on the amount of beet processed.
Currently, in our country, in the settling pits and storage platforms of the operating or closed sugar factories, there are very large amounts of technological sludge left over from the beet processing campaigns of recent years, which can be recovered free of charge in order to be used as an amendment (Gherman et al., 2022).
Land application of press sludge from cane sugar mills as an amendment has become a common practice in countries such as Pakistan and India.
Boeriu and Rusu (1972) used technological sludge (defecation foam) from the Luduș sugar factory as an amendment to correct the acidity of podzolic soils from Livada (Satu Mare county) in multi-year experiments on 11 crops. The average increase in production obtained on them was 36% compared to the control (unamended soil).
Lăpușan et al. (1980) tested at SCA Livada technological sludge as an amendment to correct soil acidity on a permanent meadow and a temporary meadow in a multi-year experience. The increase in green mass production obtained on average over 7 years on the permanent meadow was 111.6%, and on the temporary meadow, the increase in production was 129.4% compared to the unamended control.
In this context, this work aims to investigate the possibility of using sugar beet waste (carbonation lime residue -CLR) as an amendment to correct the pH of acid soils.

MATERIALS AND METHODS

The research was carried out during the 2020-2022 period in the experimental field of NIRDPSB Brașov on a cambic chernozimoid type soil with an average pH of 6.1 determined in the fall preceding the agricultural year. The main preceding crops were wheat (2020) and potato (2021, 2022).
Four experiences were established in the field:

- In the first two experiments, 800 kg/ha of complex NPK fertilizers (16:16:16) were applied in autumn before plowing.
- In the last two experiments, 1.000 kg/ha NPK complex fertilizers (20:20:0) were applied in autumn after plowing and incorporated into the soil with a tiller.

The experimental factors were the following:
Factor A - six variants with different doses of amendments ($CaCO_3$ and technological sludge)
V1 - Control (unamended soil);
V2 - 6 t/ha $CaCO_3$;
V3 - 7 t/ha/ technological sludge (TS);
V4 - 8 t/ha technological sludge;

V5 - 9 t/ha technological sludge;
V6 - 10 t/ha technological sludge.

Factor B - two methods of incorporating amendments into the soil:

- Under basic plowing
- Administration by plowing and incorporation into the soil with the tiller

The amendment doses in this study were calculated based on the formula developed by Borlan et al. (1982), incorporating indices of exchange capacity and neutralizing power of the amendment. Amulet, a monogerm beet hybrid was sown on March 26, 2020, and the sowing density was 1.2 GU/ha of pelleted seed treated with insecticides to safeguard young beet seedlings from diseases and pests during early vegetation stages (Figure 1). Precision seeding was carried out with a 6-row drill, with each repetition plot consisting of 6 rows, measuring 11.1 m in length and 2.7 m in width, totaling a surface area of 30 m^2.

Beet harvesting was carried out every year between October 15 - November 15. Manual harvesting involved knocking the beets to the ground, followed by scalping with a knife. The harvested beets were then counted and weighed to determine the production on each experimental plot.

Figure 1. Sugar beet (Amulet beet hybrid) during the growing season in the experimental field of NIRDPSB Brașov, 2021

For assessing beet technological quality, 20 beetroots from each of the 96 plots were taken for analysis. To calculate the yield, the roots of each variant with an area of 30 m^2 were weighed, after which it was reported to the area of one hectare.

After harvesting, soil samples were taken from each variant/repetition, and the pH of the soil samples was determined using the Test-Strip Quantofix Reader, with which the pH values before and after application could be compared amendments.

In the laboratory, the scalped beets were cleaned, and pasta samples were extracted separately using a milling cutter. The biological sugar content was determined with a polarimeter. The production of biological sugar (expressed in tons of sugar/ha) from each plot was calculated based on root production and beet sugar content.

For evaluating the results, analysis of variance and Duncan multiple range test were used, using the PoliFact program.

RESULTS AND DISCUSSIONS

Soil pH value

The initial pH soil value was 6.1, determined in the autumn of 2019.

The unamended variant (V1) kept its value of 6.1 after the 2020 harvest, while V2 (6 t/ha $CaCo_3$) improved its value, measuring 6.9 in experiences number 1 (E1), 2 (E2), 4 (E4), and 7.0, in the experience number 3 (E3). In Table 1 can be observed that variants V3, V4, V5, and V6, have improved their values, depending on the amount of amendment applied.

Table 1. Mean of pH values, determined on each experience 2020-2022

Year	Variant	E1	E2	E3	E4
2020	V1	6.1	6.1	6.1	6.1
	V2	6.9	6.9	7.0	6.9
	V3	6.6	6.6	6.6	6.7
	V4	6.8	6.7	6.7	6.8
	V5	6.9	6.8	6.9	6.9
	V6	7.0	6.9	7.0	6.9
2021	V1	6.0	6.1	6.0	5.9
	V2	6.9	7.0	6.8	6.9
	V3	6.6	6.7	6.5	6.6
	V4	6.8	6.7	6.7	6.7
	V5	6.8	6.8	6.8	6.8
	V6	6.9	6.9	6.8	6.8
2022	V1	5.9	5.9	5.9	5.9
	V2	6.8	6.7	6.8	6.7
	V3	6.6	6.5	6.5	6.5
	V4	6.7	6.7	6.7	6.6
	V5	6.7	6.7	6.7	6.7
	V6	6.8	6.7	6.8	6.7

In 2021 and 2022, the highest values were registered at V2 where 6 t of $CaCO_3$ were used (soil pH 6.8-7.0) and in V6, where 10 t/ha of technological sludge were used (soil pH 6.8-6.9) (Table1).

Root yield

In E1, where NPK (16.16.16) and amendments incorporated in the soil with rotary milling in autumn were applied, root yield (t/ha) varied between 79.66 t/ha (Control), and 92.26 t/ha (V6) (Table 2).

Table 2. Fertilization with NPK (16.16.16) and amendments incorporated in the soil with rotary milling in autumn - Root yield (t/ha) in the E1 experiment

Var	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	87.4	81.55	70.03	79.66	100	0	-	A
V2	98.55	92.09	85.45	92.03	115.5	12.37	***	B
V3	91.65	86.43	81.46	86.51	108.6	6.85	***	B
V4	93.30	87.83	82.12	87.75	110.2	8.09	***	C
V5	97.68	91.43	84.50	91.20	114.5	11.54	***	C
V6	98.85	92.35	8559	92.26	115.8	12.60	***	C

LSD (p 5%)= 2.54 t/ha
LSD (p 1%)= 3.60 t/ha
LSD (0.1%)= 5.22 t/ha

All variants had very significant differences, compared to the control.

In E2 where NPK (16.16.16) and amendments incorporated in the soil under plowing in autumn were applied, root yield (t/ha) varied between 77.39 t/ha (Control), and 89.76 t/ha (V6).

All variants had very significant differences, compared to the control (Table 3).

Table 3. Fertilization with NPK (16.16.16) and amendments incorporated in the soil under plowing in autumn - Root yield (t/ha) in E2

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	82.28	80.38	69.50	77.39	100	0	-	A
V2	95.35	89.90	82.58	89.28	115.4	11.89	***	B
V3	90.88	84.43	80.61	85.31	110.2	7.92	***	B
V4	91.63	85.62	81..46	86.24	111.4	8.85	***	BC
V5	92.40	88.70	82.33	87.81	113.5	10.42	***	C
V6	95.25	89.88	84.15	89.76	116	12.37	***	C

LSD (p 5%) = 2.48 t/ha
LSD (p 1%) = 3.53 t/ha
LSD (0.1%) = 5.11 t/ha

In E3 where NPK (20.20.0) amendments incorporated in the soil with the rotary milling in autumn were applied, the root yield (t/ha) varied between 78.80 t/ha (Control), and 89.50 t/ha (V6). The variants V2, V4, V5, and V6 had very significant differences, compared to the control (Table 4).

Table 4. Fertilization NPK (20.20.0) - amendments incorporated in the soil with the rotary milling in autumn - Root yield (t/ha) - E3

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	88.03	79.10	69.26	78.80	100	0	-	A
V2	96.08	87.70	81.86	88.55	112.4	9.75	***	B
V3	91.35	84.23	80.12	85.23	108.2	6.44	**	BC
V4	92.33	85.83	82.49	86.88	110.3	8.09	***	BC
V5	96.07	86.43	83.34	88.61	112.5	9.82	***	BC
V6	96.30	87.58	84.63	89.50	113.6	10.71	***	C

LSD (p 5%) = 3.19 t/ha
LSD (p 1%) = 4.53 t/ha
LSD (0.1%) = 6.57 t/ha

In E4 where NPK (20.20.0) - amendments incorporated under plowing in autumn where applied, the root yield (t/ha) varied between 78.15 t/ha (in which variant?) and 88.71 t/ha (V6) (Table 5).

Table 5. Fertilization with NPK (20.20.0) - amendments incorporated under plowing in autumn - Root yield (t/ha) in E4

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	87.48	78.45	68.52	78.15	100	0	-	A
V2	94.45	85.73	82.54	87.57	112.1	9.42	***	B
V3	90.18	82.60	79.57	84.12	107.6	5.97	**	BC
V4	92.35	84.08	82.60	86.34	110.5	8.19	***	BC
V5	94.40	85.15	84.07	87.87	112.4	9.72	***	BC
V6	94.50	85.60	86.04	88.71	113.5	10.56	***	C

LSD (p 5%) = 3.90 t/ha
LSD (p 1%) = 5.55 t/ha
LSD (0.1%) = 8.03 t/ha

Biological sugar content

Regarding the biological sugar content (°S), in E1, the values varied between 18.01°S (Control) and 18.70°S (V2). Variant V2 was followed by V6 with a sugar content of 18.66°S. The most significant results were obtained in V2 (0.69°S difference) and V6 (0.65°S difference) (Table 6).

Table 6. Biological sugar content (°S) in E1

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	16.25	18.90	18.88	18.01	100	0	-	A
V2	16.58	19.80	19.73	18.70	103.8	0.69	***	AB
V3	16.26	19.20	18.95	18.14	100.7	0.13	-	BC
V4	16.50	19.53	19.20	18.41	102.2	0.40	**	C
V5	16.65	19.68	19.15	18.49	102.7	0.48	**	C
V6	16.60	19.78	19.60	18.66	103.6	0.65	***	C

LSD (p 5%) = 0.27°S
LSD (p 1%) = 0.39°S
LSD (0.1%) = 0.56°S

In E2 the average biological sugar content (°S) varied between 17.71°S (Control) and 18.64 °S (V6). Significant results were found in V2 (0,75°S difference from the control) and V5

(0.72°S difference), V6 (0.93°S difference) (Table 7).

Table 7. Biological sugar content (°S) in E2

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	16.25	18.55	18.33	17.71	100	0	-	A
V2	16.48	19.53	19.38	18.46	104.3	0.75	**	AB
V3	16.30	19.10	18.40	17.93	101.3	0.22	-	ABC
V4	16.38	19.23	18.95	18.19	102.7	0.48	-	BC
V5	16.50	19.45	19.35	18.43	104.1	0.72	**	C
V6	16.53	19.50	19.88	18.64	105.5	0.93	**	C

LSD (5%) = 0.49°S
LSD (1%) = 0.70°S
LSD (0.1%) = 1.01°S

In E3 the average biological sugar content (°S) varied between 17.57°S (control) and 18.20°S (V2). The result obtained in V2 is very significant, 18.13°S (V6) - very significant (Table 8).

Table 8. Biological sugar content (°S) in E3

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	16.23	18.20	18.27	17.57	100	0	-	A
V2	16.43	19.03	19.13	18.20	103.6	0.63	***	B
V3	16.20	18.58	18.68	17.82	101.4	0.25	*	BC
V4	16.42	18.75	18.78	17.98	102.4	0.42	**	BC
V5	16.48	18.83	18.85	18.05	102.8	0.49	**	C
V6	16.40	18.95	19.05	18.13	103.2	0.57	***	C

LSD (p 5%) = 0.24°S
LSD (p 1%) = 0.35°S
LSD (0.1%) = 0.50°S

In E4 - Average biological sugar content (°S) varied between 17.47°S (control) and 18.10°S (V2) - very significant, 18.05°S (V5) 18.10°S (V6) - very significant. The application of carbonation mud improved significantly the biological sugar content in V5 (0.59°S difference) and V6 (0.64°S difference from the control) (Table 9).

Table 9. Biological sugar content (°S) in E4

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	16.08	18.10	18.22	17.47	100	0	-	A
V2	16.30	18.95	19.05	18.10	103.6	0.63	***	B
V3	16.23	18.55	18.58	17.79	101.8	0.32	*	BC
V4	16.35	18.68	18.68	17.90	102.5	0.44	**	C
V5	16.40	18.88	18.88	18.05	103.4	0.59	***	C
V6	16.33	19.00	18.98	18.10	103.6	0.64	***	C

LSD (p 5%) = 0.25°S
LSD (p 1%)= 0.36°S
LSD (0.1%)= 0.51°S

In E1, the average biological sugar yield (t/ha) varied between 14.28 t/ha (Control) and 17.14 t/ha (V2), 17.15 t/ha (V6).
All variants had very significant differences, compared to the control (Table 10).

Table 10. Biological sugar yield (t/ha) in E1

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	14.20	15.41	13.22	14.28	100	0	-	A
V2	16.33	18.23	16.87	17.14	120.1	2.87	***	B
V3	14.85	16.59	15.44	15.63	109.5	1.35	***	B
V4	15.39	17.15	15.77	16.10	112.8	1.83	***	C
V5	16.26	17.99	16.18	16.81	117.7	2.53	***	C
V6	16.41	18.26	16.78	17.15	120.1	2.87	***	C

LSD (p 5%) = 0.56 t/ha
LSD (p 1%) = 0.79 t/ha
LSD (0.1%) = 1.14 t/ha

In E2, the average biological sugar yield (t/ha) varied between 13.47 t/ha (Control) and 16.43 t/ha (V2), 16.66 t/ha (V6). All variants had very significant differences, compared to the control (Table 11)

Table 11. Biological sugar yield (t/ha) in E2

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	13.37	14.91	12.74	13.47	100	0	-	A
V2	15.71	17.55	16.02	16.43	120.1	2.75	***	B
V3	14.82	16.13	14.86	15.27	111.7	1.60	***	BC
V4	15.01	16.47	15.44	15.64	114.4	1.97	***	CD
V5	15.24	17.25	15.94	16.14	118.1	2.47	***	D
V6	15.74	17.52	16.71	16.66	121.8	2.98	***	D

LSD (p 5%) = 0.58 t/ha
LSD (p 1%) = 0.82 t/ha
LSD (0.1%) = 1.19 t/ha

In the Experience no. 3, the average biological sugar yield (t/ha) varied between 13,73 t/ha (Control) and 16.08 t/ha (V2), 16.26 t/ha (V6).
Variants V2, V4, V5, V6 v had very significant differences, compared to the control (Table 12).

Table 12. Biological sugar yield (t/ha) in E3

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	14.28	14.40	12.52	13.73	100	0	-	A
V2	15.78	16.68	15.79	16.08	117.1	2.35	***	B
V3	14.80	15.64	14.86	15.10	110.0	1.37	**	BC
V4	15.16	16.09	15.51	15.59	113.5	1.85	***	C
V5	15.82	16.27	15.85	15.98	116.4	2.25	***	C
V6	15.79	16.60	16.39	16.26	118.4	2.53	***	C

LSD (p 5%) = 0.80 t/ha
LSD (p 1%) = 1.13 t/ha
LSD (0.1%) = 1.64 t/ha

In the E4, the average biological sugar yield (t/ha) varied between 13.58 t/ha (Control) and 15.79 t/ha (V2), 16.01 t/ha (V6) (Table 13).

Table 13. Biological sugar yield (t/ha) in E4

Var.	2020	2021	2022	Av.	%	Diff.	Sign.	DT
V1	14.06	14.20	12.47	13.58	100	0	-	A
V2	15.40	16.25	15.79	15.79	116.3	2.21	***	B
V3	14.63	15.32	14.91	14.91	109.8	1.33	***	BC
V4	15.10	15.70	15.41	15.41	113.5	1.83	***	C
V5	15.48	16.07	15.81	15.81	116.4	2.23	***	C
V6	15.43	16.27	16.01	16.01	117.9	2.43	***	C

LSD (p 5%) = 0.81 t/ha
LSD (p 1%) = 1.16 t/ha
LSD (0.1%) = 1.67 t/ha

Variants V2, V4, V5 and V6 had very significant differences, compared to the control.

CONCLUSIONS

The replacement of calcareous amendments purchased from fertilizer factories and used to improve the acid reaction of soils with technological sludge obtained free of charge from sugar factories presents advantages such as the reduction of the purchase of calcareous amendments and the efficiency of the transport of the technological sludge, because the trucks intended for the transport of sugar beet to the factory make only one round trip.

Compared to the unimpaired control over the course of the three-year testing period, increases in sugar beet yield between 10 and 13 t/ha were obtained in the 2020–2022 experiments conducted at The National Institute of Research and Development for Potato and Sugar Beet Brașov using technological sludge as an amendment to correct the acidity of a soil with an average pH of 5.9 (unamended) (Gherman et al., 2022).

Yield values per hectare and average biological sugar yield (t/ha) values from all four experiments exceeded the control variant, highlighting the fact that technological sludge can be an alternative to $CaCo_3$ (V2) currently used for soil amendment.

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THE EFFECT OF HISTORICAL POLLUTION ON MICROBIAL FUNCTIONAL KINETICS IN BIOREMEDIATED SOILS FROM BAIA MARE

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Abstract

This industry is a key contributor to environmental disturbances, with residual impacts such as contaminated water sources and heavy metal presence affecting local ecosystems. Microorganisms exhibit a high susceptibility to contamination by heavy metals and play a crucial role in the recycling of materials and the energy dynamics within the ecosystem. The revised Biolog-Ecoplate approach involves employing soil contaminated with heavy metals from Baia Mare at five polluted locations - Craica, Romplumb, Colonia Topitorilor, Ferneziu, and Urbis. The kinetics has been noted, referring to the percentage growth rate from 24 hours to the next 24 hours. The overall microbial functional profile exhibits multiple fast changes within a time frame of 24 hours, leading to a specific site-microbial activity. The results offer insights into significant characteristics of soil microbial functional communities affected by the presence of heavy metal pollution in all analyzed locations.

***Key words**: functional microbial communities, biolog ecoplate, heavy metal toxicity, microbial community structure.*

INTRODUCTION

The progress in mining, metallurgy, industrialization, and urbanization has caused the introduction of heavy metals like lead (Pb), mercury (Hg), chromium (Cr), and cadmium (Cd), as well as metalloids such as arsenic (As) into the environment (Mishra et al., 2023). Many industrial byproducts that contain harmful heavy metals and metalloids can dissolve in water and combine with soil and water. As a result, the ecological environment is severely affected (Ahmed et al., 2022).

With rapid urbanization and industrialization in developing country, relatively and intensive short-term human activities will bring many organic pollutants (such as polycyclic aromatic hydrocarbons and phenols) and inorganic pollutants (such as heavy metals, arsenic compounds) into the urban environment (Li et al., 2018). Some heavy metals and metalloids (like As, Pb, Cd, and Hg) are not necessary for biological functions and can be harmful (Gall et al., 2015). These substances have been classified as dangerous by the United States Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2007; Khalid et al., 2017; Rai, 2018a). On the other hand, certain heavy metals like Cu, Fe, Zn, and even Cr (III) are essential for metabolic processes in living organisms (Marschner, 2012). The relationship between soil, food crops, and the environment illustrates the interaction between abiotic and biotic factors (Rai et al., 2019).

Heavy metals are recognized for their ability to decrease or inhibit the functioning of soil enzymes, disrupt the transformation of carbon, nitrogen, and organic matter, and diminish both the biodiversity and biomass of microorganisms in soil (Giller et al., 2009). Consequently, this can result in the presence of particular microorganisms that are tolerant to heavy metals in soil environments (Giller et al., 2009).

Over the past few years, global concern has risen regarding soil contamination by heavy metals (HMs) because of their elevated toxicity, resistance to biodegradation, and prolonged accumulation (Fajardo et al., 2019). These heavy metals not only impact soil fertility but also interfere with the bacterial community, resulting in a reduction in biodiversity (Pan et al., 2020). Heavy metal pollution-induced stress can alter the attributes of bacterial communities (Lin et al., 2016). Sensitive soil bacteria may experience a decline in both diversity and population, whereas resilient bacteria can easily adapt and proliferate, leading to the establishment of a distinct bacterial community structure. The presence of heavy metals continually influences both bacterial biomass and activity (Liu et al., 2020). In contrast to plants and animals, soil microorganisms are more sensitive to fluctuations in heavy metal levels due to their ability to react and adapt quickly to such stressors (Giller et al., 1998).

Heavy metals, soil, and bacteria have complex interactions. The presence of heavy metals significantly influences the microbial community structure, especially in soils with moderate to severe contamination (Li et al., 2017a). However, acidic wastewater from mining adds extra stress for microorganisms, as they have to cope with both heavy metals and acidity, further disrupting soil nutrient cycling (Pereira et al., 2014).

Hence, it is essential to comprehend how soil microbial diversity and composition changes under different degrees of heavy metal contamination (Azarbad et al., 2015).

Removing heavy metals from the environment is a significant challenge, as their decomposition, much like other pollutants, cannot be achieved through biological or chemical means (Sharma et al., 2023).

The purpose of this article was to investigate the kinetics and dynamics of microorganisms in soils polluted with heavy metals in Baia Mare, focusing on five pilot sites, utilizing the EcoPlate method to provide comprehensive insights into microbial activity and community structure.

MATERIALS AND METHODS

Soil samples were collected in 2023 from an ongoing experiment initiated in 2019 across five sites located inside the town of Baia Mare (47°39′ N 23°34′ E) in North-West Romania, covering a total area of 7.3 hectares of brownfields.

The locations exhibit varying degrees of soil heavy metal contamination due to human-caused pollution, primarily stemming from mining, metallurgical activities, and urban development.

For EcoPlate examinations, soil samples and protocols adhered to the approach outlined by (Weber et al., 2009), arranging all substrates according to their chemical resemblance. The solution introduced into EcoPlates underwent dilution up to 10^{-4}, and measurements were taken at 590 nm using a plate reader. The entire procedure spanned five days, reaching the plateau phase wherein no further increments were noted in the readings.

The EcoPlates functional guilds and groups are analyzed according to Stoian et al., 2022:

- CH - Carbohydrates; P - Polymers; CX - Carboxylic & acetic acids; AA - Amino acids; AM -Amines/amides;
- Water - W; Pyruvic acid methyl ester - CH1; Tween 40 - P1; Tween 80 - P2; α-Cyclodextrin - P3; Glycogen - P4; d-Cellobiose - CH2; α-d-Lactose - CH3; β-Methyl-d-glucoside - CH4; d-Xylose - CH5; i-Erythritol - CH6; d-Mannitol - CH7; N-Acetyl-d-glucosamine - CH8; d-Glucosaminic acid - CX1; Glucose-1-phosphate - CH9; d,l-α-Glycerol phosphate - CH10; d-Galactonic acid γ-lactone - CX2; d-Galacturonic acid - CX3; 2-Hydroxy benzoic acid - CX4; 4-Hydroxy benzoic acid - CX5; γ-Hydroxy butyric acid - CX6; Itaconic acid - CX7; α-Keto butyric acid - CX8; d-Malic acid - CX9; l-Arginine - AA1; l-Asparagine - AA2; l-Phenylalanine - AA3; l-Serine - AA4; l-Threonine - AA5; Glycyl-l-glutamic acid - AA6; Phenylethylamine - AM1; Putrescine - AM2.

The data analysis primarily adhered to traditional methods (Garland, 1997), calculating the Least Significant Difference (LSD), a statistical test used to determine if there are significant differences between the means±standard errors between in terms of various microbial parameters or characteristics affected by heavy metal pollution. Data analysis was performed in RStudio, version 2022.02.3 (RStudio Team. RStudio, 2019.), with packages “psych” (Revelle, 2019; Corcoz et al., 2022a) and “agricolae” (de Mendiburu, 2020; Corcoz et al., 2022b).

RESULTS AND DISCUSSIONS

Among the water group (WAT), it is observed that the highest level recorded is 107.51 in 3_CR. On the other hand, the lowest level is recorded in 3_FR, being 94.84, indicating a considerable difference between the two extremes. As for reading 4, there is a maximum level of 103.98 and a minimum of 87.91. These findings highlight significant differences between 4_ROMP and 4_CT. In relation to reading 5, the levels range between 101.99 and 99.14, with no significant difference between them (Table 1).

Table 1. Dynamics of basal and amines/amides functional groups in long-term contaminated sites

	WAT	AM1	AM2
3_CR	107.51±0.76a	102.87±3.36c	149.53±36.87ab
3_CT	103.01±3.25ab	144.76±18.14bc	179.93±14.29a
3_FR	94.84±2.71c	140.86±11.48bc	134.49±12.76abc
3_ROMP	104.28±1.5ab	108.88±9.69c	150.88±46.24ab
3_URB	99.56±0.9bc	104.96±0.34c	103.06±1.52bc
4_CR	100±0.41bc	102.53±1.92c	113.04±12.82bc
4_CT	87.91±1.57d	146.23±13.8bc	132.42±2.9abc
4_FR	100.91±3.54b	219.91±12.35a	183.36±23a
4_ROMP	103.98±1.79ab	164.34±61.98b	121.44±13.04bc
4_URB	101.21±0.18b	102.39±0.75c	108.55±3.37bc
5_CR	100.1±0.33bc	111.08±5.57c	107.85±4bc
5_CT	101.74±1.98b	104.12±7.29c	93.62±3.11c
5_FR	99.22±1.14bc	110.46±2.72c	95.88±4.48c
5_ROMP	99.14±2.97bc	116.36±6.13bc	105.11±3.1bc
5_URB	101.99±2.16ab	105.66±1.07c	111.53±2.32bc

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

In the amine group, phenyltinamine or AM1, it is highlighted that 3_CT records the highest value, i.e. 144.76. In contrast, the lowest value, i.e. 102.87, is observed for 3_CR, thus illustrating that there is no significant difference between 3_CT and 3_CR. Regarding reading 4, the values for 4_FR and 4_URB are significantly different. In the context of reading 5, the maximum value is 116.36 for 5_ROMP, while the minimum is recorded at 5_CT, where it is observed that there is no significant difference between them (Table 1). Among the putrescine or AM2 group, Within the putrescine or AM2 group, we observe that the highest value is recorded at 3_CT. On the other hand, the lowest value is identified at 3_URB, showing significant differences from the maximum value, but not from the other groups. Therefore, significant differences are found between 3_CT and 3_URB after 48 hours of incubation. For reading 4, the highest value is represented by 4_FR, while the lowest value is identified at 4_URB. In this context, it can be concluded that there are significant differences between these values after 72 hours of incubation. Concerning reading 5, the highest value is observed at 5_URB, while the lowest is recorded at 5_CT. Comparing these two values, it can be seen that there are no relevant differences (Table 1).

Within the pyruvic acid methyl ester family, CH1, 3_FR highlights with the highest value, marking a significant difference compared to the other groups. On the other hand, 3_URB has the lowest value, being clearly different from the groups with high values, but not from those with medium or low values. Therefore, 3_URB and 3_FR are significantly different, suggesting possible environmental variations or pollution influences. For 4_CR and 4_CT, there is a significant difference between them, with the values for 4_CT being considerably lower than those for 4_CR. In contrast, 4_FR, 4_ROMP and 4_URB do not show significant variations between them or from the other groups (4_CR and 4_CT). 5_ROMP stands out with the highest mean value, marking a significant difference from all other groups. On the other hand, 5_CT has the lowest mean value, differing from the high value groups, but similar to the medium value groups. In comparison, 5_ROMP and 5_CT are significantly different, with the values for 5_CT being much lower than those for 5_ROMP. Finally, 5_CR, 5_FR and 5_URB do not differ significantly from each other or from the other groups (Table 2).

Regarding the compound CH2 or D - cellobiose, it is noted that the maximum value is recorded at 3_CT, showing a significant difference compared to the other groups. In contrast, the lowest value is recorded at 3_URB, indicating significant differences from the maximum value, but not from the other groups. Thus, significant differences between 3_URB and 3_CT after 48 hours of incubation are evident. Regarding reading 4, the maximum value is represented by 4_CR, with significant differences from the other groups, while the lowest value is identified in 4_CT. Therefore, it can be concluded that there are significant differences between these values after 72 hours of incubation.

Table 2. Dynamics of carbohydrates functional guilds in long-term contaminated sites

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
3_CR	140.77±17.35abc	118.71±3.05de	103.67±1.07cd	136.41±30.23abcd	118.19±7.19abcd	111.28±3.38abcd	149.54±20.71bc	154.55±21.63bc	139.36±27.14a	110.96±2.79bc
3_CT	161.15±16.01a	212.49±33.17a	107.65±2.75cd	166.34±16.32a	127.15±6.46abc	111.18±4.41abcd	170.76±13.45b	186.82±16.83ab	126.92±8.3ab	112.2±3.46b
3_FR	161.46±14.53a	108.75±1.68e	95.86±1.74d	106.06±9.8cd	109.19±3.33abcd	101.79±0.58d	137.89±9.38bc	143.4±15.65bc	97.32±3.73c	134.92±5.43a
3_ROMP	124.37±21.27abc	106.02±1.6e	104.91±3.04cd	100.53±2.52d	102.88±1.14cd	107.46±1.76bcd	111.1±5.08c	107.6±1.13c	105.11±3.49bc	104.29±2.47bc
3_URB	103.7±0.96bc	102.28±1.33e	100.81±1.08cd	101.44±0.36cd	101.4±0.13d	104.91±0.54bcd	120.98±13.96c	105.52±1.87c	106.92±1.99bc	101.53±1bc
4_CR	155.15±4.48ab	201.5±55.43ab	103.18±0.24cd	112.32±7.44cd	133.97±26.12a	107.33±4.59bcd	225.09±16.24a	152.81±9.25bc	110.97±8.57bc	104.19±1.02bc
4_CT	96.48±2.13c	122.78±4.27de	176.08±19.8a	104.43±1.75cd	117.18±3.27abcd	146.33±8.94ab	113.32±2.91c	118.01±2.61c	126.82±9.69ab	107.59±4.87bc
4_FR	121.54±1.68abc	149.78±15.5bcde	114.78±15.57cd	154±32.22ab	128.86±7.05ab	139.54±1.29abcd	224.35±9.67a	222.37±50.81a	142.12±15.64a	107.65±10.72bc
4_ROMP	132.57±27abc	138.81±29.42cde	105.43±0.91cd	104.73±3.01cd	110.3±9.51abcd	144.21±40.58abc	150.37±31.03bc	108.27±1.67c	104.68±2.82bc	102.68±1.19bc
4_URB	115.95±10.84abc	134.75±9.8cde	105.7±2.54cd	115.23±10.53bcd	107.25±3.6bcd	104.31±1.62cd	130.03±25.39bc	113.4±7.26c	102.23±0.18bc	103.17±0.93bc
5_CR	132.3±23.68abc	177.82±14.46abcd	100.89±0.42cd	109.86±10.32cd	133.04±10.71a	125.76±22.17abcd	154.21±26.23bc	130.56±4.76c	101.12±0.75bc	103±1.41bc
5_CT	103.24±4.82bc	106.6±3.1e	149.55±14.35ab	97.47±0.93d	97.21±0.57d	127.56±3.99abcd	111.44±1.87c	104.74±2.45c	102.86±2.37bc	98.68±2.05c
5_FR	109.05±1.06abc	172.21±14.48abcd	181.38±17.32a	142.15±20.63abc	130.09±4.55ab	124.53±17.69abcd	133.34±2.8bc	121.58±13.17c	150.81±2a	107.04±8.56bc
5_ROMP	159.72±52.62a	185.87±26.49abc	133.34±30.89bc	99.55±3.1d	112.64±7.73abcd	152.68±23.29a	141.05±10.82bc	148.88±28.28bc	102.71±1.44bc	104.01±0.98bc
5_URB	119.93±2.76abc	140.64±10.39cde	127.05±6.46bcd	109.46±6.58cd	127.61±3.65abc	111.67±3.95abcd	121.2±7.5c	138.54±6.03bc	101.78±1.69bc	110.73±4.75bc

Note: Means±s.e. followed by different letters present significant differences at $p<0.05$ based on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

Concerning reading 5, it is observed that the maximum value is recorded at 5_ROMP, while the lowest is recorded at 5_CT. Comparing these two values, significant differences are found, although no significant differences are identified between 5_ROMP and the other values (Table 2).

In relation to the substrate α - d - lactose (CH3), the highest value recorded is 107.65, while the lowest value, 95.86, is identified for 3_FR. The difference between the two extremes does not seem to be significant. For reading 4, a maximum value of 176.08 and a minimum of 103.18 is noted. Analysing these data, we can observe significant differences between 4_CT and 4_CR, as well as between 4_CT and the other values. As for reading 5, the maximum value is recorded at 142.15 and the minimum at 97.47, and the difference between them is considerable (Table 2).

For β-methyl-d-glucoside (CH4), the highest value recorded is found to be 166.34. In contrast, for 3_ROMP, we observe the lowest value, which is 100.53. This discrepancy is significant between the two extremes. Regarding the data from reading 4, we observe a maximum value of 154 and a minimum value of 104.43. Therefore, significant differences can be identified between 4_FR and 4_CT. Regarding reading 5, there is a maximum value of 142.15 and a minimum of 97.47 and the difference between them is significant (Table 2).

In substrate d, the maximum value recorded for Xylose or CH5 is 127.15, associated with 3_CT. In contrast, the lowest value, 101.4, is identified in 3_URB, with no significant difference between the two extremes. Concerning reading 4, a maximum value of 133.97 and a minimum of 107.25 is observed. In the light of these findings, no significant differences are shown between 4_CR and 4_URB, or between 4_CR and the other values. For reading 5, the maximum value is 133.04 and the minimum is 97.21, marking a significant difference between them (Table 2).

In substrate i - Erythriol (CH6), the highest value recorded is found to be 111.28 in 3_CR. In contrast, the lowest value is found in 3_FR, being 101.79, with an apparently insignificant difference between the two extremes. For reading 4, a maximum value of 146.33 and a minimum value of 104.31 are shown. Analysing these data, significant differences can be observed between 4_CT and 4_URB. For reading 5, the values recorded are 152.68 and 111.67 respectively, with a significant difference between them (Table 2). In group d - Mannitol (CH7), the highest value is observed in 3_CT, showing a considerable difference compared to the other groups. In contrast, the lowest value occurs in 3_ROMP, indicating significant differences from the maximum value, but not from the other groups. Hence, significant differences are found between 3_URB and 3_CT after 48 hours of incubation. As for reading 4, the maximum value is recorded at 4_CR, while the minimum value is at 4_CT, with significant differences from the other non-overlapping groups. These differences are evident at 72 hours of incubation. For reading 5, the maximum value is recorded at 5_CR and the lowest at 5_CT. Analysis of these values shows no significant differences after 96 hours of incubation (Table 2).

In the context of dataset N, it is observed that group 3_CT stands out with the highest value, indicating a significant discrepancy compared to the other groups. On the other hand, the 3_URB group records the lowest value, showing a significant difference between 3_CT and 3_URB, which may suggest variations in the environmental setting or the impact of pollution. Regarding reading 4, the values for 4_FR and 4_ROMP are significantly different, with the values for 4_ROMP being considerably lower than those for 4_FR. Regarding reading 5, although the maximum value is 148.88 and the minimum is observed at 5_CT, these differences are not significant (Table 2).

In the glucose-1-phosphate group (CH9), it is observed that 3_CR records the highest value of 139.36, while the lowest value of 97.32 is recorded in 3_FR, showing a significant difference between these two. As for reading 4, the values for 4_FR and 4_URB are notably different. In the context of reading 5, the maximum value is 150.81 for 5_FR, while the minimum value is recorded in 5_CR, showing a significant difference between them. Significant differences are also shown between 5_FR and the other groups (Table 2).

Analysing the presence of d,1-α-glycerol phosphate in comparison to the other substrates, it is observed that it shows low levels of uptake. Specifically, glycerol phosphate suggests that the microorganisms in the examined soil are less efficient in their metabolic process. The maximum value is recorded in 3_FR, while the minimum value appears in 3_URB, showing significant differences between them. For reading 4, no significant differences are found between 4_FR, which has the highest uptake, and 4_ROMP, which records the lowest value. This indicates that the substrate present, glycerol phosphate, shows low uptake compared to other carbohydrate types, suggesting that the community of microorganisms capable of metabolising it is relatively smaller than for other elements in the previous groups (Table 2).

In the case of the substrate d-glucosaminic acid (CX1), it is noted that 3_CT shows the highest value. In contrast, 3_URB has the lowest value, showing a significant difference between 3_CT and 3_URB, which may reflect differences in the environment or the impact of pollution. For reading 4, the values for 4_CT and 4_ROMP are significantly different, with the values for 4_ROMP considerably lower than those for 4_FR. For reading 5, the maximum value is 169.34 and the minimum is observed for 5_CT, but these differences are not significant (Table 3).

Regarding the -galactonic acid y-lactone group (CX2), the highest value observed is 167.58 in 3_C, while the lowest value of 100.32 occurs in 3_URB, indicating a significant difference between these two extreme values. For reading 4, the maximum value is 180.87 and the minimum is 103.65, showing significant differences between 4_CT and 4_URB. In reading 5, the maximum value recorded is 109.84 and the minimum is 95.57, but the difference between them is not considered significant (Table 3).

Table 3. Dynamics of carboxylic acids functional groups in long-term contaminated sites

	CX1	CX2	CX3	CX4	CX5	CX6	CX7	CX8	CX9
3_CR	115.27±6.48c	112.14±6.81c	122.99±14.21cd	112.21±1.64b	174.65±40.5ab	184.4±48.12abcd	112.21±3.67c	100.48±9.11cde	110.8±7.88c
3_CT	135.51±1.86bc	167.58±16.25ab	171.95±11.8abc	121.12±8.61b	186.47±19.81a	205.68±28.86abc	165.01±26.8bc	96.21±3.16de	169.42±24.12abc
3_FR	112.99±1.12c	147.04±17.08b	200.7±3.64a	106.94±0.93b	142.48±15.86ab	252.2±40.35a	122.1±2.42c	95.27±6.04e	112.95±7.43c
3_ROMP	104.96±1.97c	105.47±2.33c	109.6±4.07d	109.05±1.26b	124.67±15.5ab	140.52±31.34cde	108.04±1.49c	116.42±4abc	107.51±2.29c
3_URB	104.3±2.31c	100.32±1.09c	103.06±0.44d	107.54±1.11b	105.54±1.11b	102.46±0.3e	102.95±0.39c	100.27±1.69cde	102.89±1.33c
4_CR	218.78±60.23a	112.86±11.33c	176.25±25.41ab	103.14±0.32b	185.1±34.9a	222.4±11.51ab	195.28±87.34b	115.05±7.9abcd	200.36±53.12ab
4_CT	141.1±7.43bc	108.09±0.75c	106.49±6.81d	159.96±18.41a	121.99±4.53ab	123.46±3.5de	162.98±8.75bc	123.37±5.63ab	119.95±5.26bc
4_FR	195.61±12.05ab	180.87±18.96a	175.85±11.04ab	123.87±23.21b	186.39±22.9a	183.49±24.03abcd	338.41±13a	133.13±12.97a	151.1±45.8abc
4_ROMP	106.09±1.08c	103.26±0.92c	148.21±38.9bcd	101.28±0.59b	166.3±60.38ab	158.57±48.63bcde	104.08±1.89c	93.46±1.56e	106.17±4.59c
4_URB	117.53±8.49c	103.65±1.22c	107.92±2.34d	106.3±1.36b	106.06±3.21b	107.35±2.72e	103.97±0.5c	100.81±0.21cde	104±2.83c
5_CR	169.34±47.75abc	107.59±2.26c	172.67±37.5abc	112.46±4.45b	138.86±35.43ab	131.96±21.16de	122.69±4.34c	118.3±5.93abc	135.66±27.22bc
5_CT	112.03±1.39c	95.57±1.7c	102.08±6.51d	109.28±3.84b	103.93±7.46b	106.32±2.92e	105.69±1.38c	109.52±5.69bcde	97.02±2.09c
5_FR	147.5±8.4bc	109.84±2.03c	107.98±3.93d	115.43±13.78b	113.55±6.4ab	113.86±5.84de	122.58±0.86c	101.71±4.16cde	220.34±58.59a
5_ROMP	116.2±13.58c	104.19±2.41c	124.12±21.83cd	101.79±2.51b	123.41±16.03ab	105.46±1.01e	107.2±1.18c	96.87±8.41de	108.41±3.78c
5_URB	154.99±45.63abc	101.97±3.45c	118.94±10.27d	106.72±0.82b	117.89±6.91ab	140.08±10.5cde	101.09±2.69c	104.6±8.39bcde	154.84±47.14abc

Note: Means±s.e. followed by different letters present significant differences at $p<0.05$ based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

Within the d-galacturonic acid or CX3 group, the highest value was recorded at 3_FR, while the lowest value was observed at 3_URB. This shows significant differences between 3_FR and 3_URB after 48 hours of incubation, but not compared to the other groups. In reading 4, the maximum value was recorded at 4_CR and the minimum at 4_CT, indicating significant differences after 72 hours of incubation. For reading 5, the highest value was observed at 5_CR and the lowest at 5_CT, indicating significant differences between these two values, although no significant differences were found between 5_CT and the other values (Table 3).

In the 2-hydroxy benzoic acid substrate (CX4), it is notable that there is no difference between the maximum value, 3_CT, and the minimum value, 3_URB, and regardless of the value, there are no significant differences in the other groups. Regarding reading 4, the highest value is indentified at 4_CT, with 159.96, showing significant differences from the other groups, while the lowest value is at 4_ROMP. Hence, it can be concluded that there are relevant differences between these values after 72 hours of incubation. Regarding reading 5, the highest value is observed at 5_FR and the lowest at 5_CT. Comparing these two values, no significant differences are observed after 96 hours of incubation (Table 3).

For the substrate 4-hydroxy benzoic acid (CX5), the highest value recorded is 186.47 in 3_CT, while the lowest value of 107.54 is identified in 3_URB, showing a remarkable difference between these two extreme values. In reading 4, a maximum value of 186.39 and a minimum value of 106.06 are observed, marking notable differences between 4_FR and 4_URB. In reading 5, the maximum value is 138.86 and the minimum is 103.93, but the difference between them is not considered significant (Table 3).

In the y-hydroxy butyric acid or CX6 group, the highest value recorded is 252.2 and belongs to 3_FR. In contrast, the lowest value of 102.46 is observed in 3_URB, showing a significant difference between the two extremes. For reading 4, the values range from a maximum of 222.4 to a minimum of 107.35, indicating notable differences between 4_CR and 4_URB. For reading 5, the values range from a maximum of 131.96 to a minimum of 105.46, with no significant difference between them (Table 3).

Within the itaconic acid group (CX7), 3_CT has the highest value of 165.01. In contrast, 3_URB has the lowest value of 102.95, indicating that there is no relevant variation between 3_CT and 3_URB. As for reading 4, the values for 4_CR and 4_URB are considerably different. For reading 5, the maximum value of 122.69 is observed at 5_CR and the minimum value at 5_URB, with no significant difference between them. Also, no significant differences are observed between the other groups (Table 3).

Comparing the keto-butyric acid substrate or CX8 with other groups shows that it has lower absorption levels. This indicates that the microorganisms in the soil analysed are less efficient in metabolising this type of substance. At reading 3, the highest value is at 3_ROMP and the lowest is 95.27, with no significant differences. For reading 4, the highest value is 133.13 and the lowest is 93.46, showing significant differences between 4_FR and 4_ROMP. For reading 5, the maximum value is 118.3 and the minimum 96.87, with a significant difference between them (Table 3).

Within the d-malic acid group (CX9), 3_CT has the highest mean value, while 3_URB has the lowest mean value. However, 3_URB is not significantly different from the groups with high, medium or low values, indicating that there are no notable differences in environmental or pollution impact between 3_CT and 3_URB. In contrast, 4_CR and 4_URB show significant differences, with the values for 4_URB being considerably lower than those for 4_CR. The groups 4_FR, 4_ROMP and 4_URB show no significant differences between them or from 4_CR and 4_CT. In group 5, 5_FR has the highest mean value, indicating a significant difference from all other groups. Group 5_CT has the lowest mean value and, although it differs from the groups with high values, it is similar to those with mean values. In comparison, 5_FR and 5_CT are significantly different, with the values for 5_CT being much lower than those for 5_FR. The groups 5_CR, 5_ROMP, 5_CT and 5_URB do not differ significantly from each other and from the other groups (Table 3).

In the Tween 40 group (P1), 3_CR shows the highest value of 138.37. In contrast, the lowest value of 108.18 is recorded for 3_URB, indicating that the differences between 3_CR and 3_URB are not significant. On reading 4, significant differences are observed between 4_FR and 4_URB, suggesting possible environmental changes or the impact of pollution on soil microbiota. For reading 5, the maximum value is 146.97 for 5_FR and the minimum value is 110.35 for 5_CT, with no significant differences between them, suggesting greater homogeneity of environmental conditions or impact on soil microbiota (Table 4).

In the Tween 80 or P2 group, values for 3_URB are observed to be much lower than those for 3_CT, reflecting variations in environmental conditions or the effects of pollution on the soil microbiota at those sites. In term of reading 4, there is a maximum value of 149.37 and a minimum of 113.66. In the light of these findings, no significant differences between 4_FR and 4_URB can be shown. For reading 5, there is a maximum value of 185.44 and a minimum of 125.33 and the discrepancy between them is significant (Table 4).

As for the α-cyclodextrin or P3 group, 3_CT is observed to have the highest value, while 3_ROMP has the lowest value, suggesting that there are no significant differences between these two variables. Regarding reading 4, the values for 4_CT and 4_CR are significantly different. In the context of reading 5, 5_FR records the highest value, while 5_CR records the lowest value, with significant differences (Table 4).

Among the glycogen group (P4), 3_CT is shown to have the highest value, while 3_URB has the lowest value. This significant discrepancy between 3_CT and 3_URB may reflect variations in the environment or the effects of pollution. As for the readings for variant 4, the values for 4_CR and 4_FR are considerably different, with 4_CR having considerably lower values than 4_FR. In reading 5, the highest value recorded is 152.1 and the lowest is observed at 5_CR, confirming the significant differences between these values (Table 4).

It is observed that in the 1-Arginine or AA1 group, the highest value is recorded at 3_CT, while the lowest value is identified at 3_URB, showing significant differences between the two, but not with respect to the other groups. Significant differences between 3_URB and 3_CT after 48 hours of incubation are thus observed. As for reading 4, the highest value is recorded at 4_CR, with significant differences from the other groups whose letters do not coincide, while the lowest value is observed at 4_URB. In this situation, it can be concluded that there are significant differences between these values after 72 hours of incubation. Concerning reading 5, the highest value is recorded at 5_CR, while the lowest is observed at 5_CT. Comparing the two values, it is found that there are no significant differences between them (Table 4).

Within the 1-Asparagine group (AA2), the highest value recorded is 364.34, while in 3_URB the lowest value is found, namely 103.45, indicating a significant difference between the two extremes. As for reading 4, there is a maximum value of 192.03 and a minimum of 102.95. Following these observations, no significant differences can be shown between 4_CR and 4_URB, as well as between 4_CR and the other values. In relation to reading 5, the maximum value recorded is 142.82, while the minimum is 88.46, but the difference between them is not significant (Table 4).

Table 4. Dynamics of Polymers and Amino acids functional groups in long-term contaminated sites

	P1	P2	P3	P4	AA1	AA2	AA3	AA4	AA5	AA6
3_CR	138.37±17.53ab	132.67±18.98abc	109.83±2.6bcd	115.38±3.89de	134.14±13.95cd	221.9±64.88b	108.02±3.84cde	131.08±22.93bc	103.62±2.85d	108.26±3.02cd
3_CT	128.21±2.04ab	136.84±11.03abc	125.29±6.18bc	135.6±4.12bcd	194.14±25.06abc	224.91±31.06b	139.06±6.9abcd	173.8±4.71ab	113.31±8.61bcd	107.71±5.33cd
3_FR	112.23±3.38b	121.74±5.74c	105.05±9.31cd	123.17±5.51de	146±7.69cd	364.34±69.34a	106.39±2.35de	123.87±9.04bc	101.41±2.24d	102.78±0.66d
3_ROMP	118.53±4.46ab	119.82±23.33c	105.82±4.09cd	110.41±5.99de	125.81±18.49cd	158.21±50.54bcd	89±18.11e	113.93±5.49bc	107.41±1.96d	107.66±2.07cd
3_URB	108.18±1.14b	102.91±2.77c	106.2±1.55cd	103.68±1.55e	101.41±1.41d	103.45±1.17cd	105.09±2.62de	107.42±2.55bc	104.89±2.67d	104.46±1.8d
4_CR	146.98±25.52ab	127.88±6.52bc	103.06±1.65d	114.48±11.23de	269.29±77.85a	192.03±15.82bc	128.86±12.17abcde	146.56±22.49bc	101.25±0.31d	105.33±1.72cd
4_CT	140.34±8.4ab	129.53±7.46bc	162±7.28a	128.59±3.09bcde	129.4±4.36cd	121.4±2.21cd	166.08±16.78a	156.96±11.62bc	124.98±2.92bc	117.42±3.11cd
4_FR	170.45±22.09a	149.37±12.11abc	156.86±16.53a	167.89±11.57a	237.91±23.15ab	145.04±14.61bcd	151.4±17.46abc	238.79±62.68a	153.97±2.92a	144.09±3.55a
4_ROMP	115.98±23.36b	136.57±14.03abc	111.26±6.18bcd	115.02±2.09de	164.34±51.36bcd	147.89±44.91bcd	103.64±3.46de	123.72±10.72bc	104.01±1.54d	105.4±4.38cd
4_URB	100.87±11.06b	113.66±4.81c	108.03±2.68cd	124.68±6.42de	106.3±3.75d	102.95±1.82cd	107.86±4.21cde	107.43±2.06bc	103.56±1.72d	102.13±0.75d
5_CR	144.96±35.35ab	181.35±55.92ab	106.14±3.5cd	111.45±7.13de	128.87±21.27cd	142.82±38.37bcd	158.3±11.89ab	130.09±17.08bc	127.86±9.42b	106.25±1.37cd
5_CT	120.98±3.64ab	125.33±3.29c	106.34±2.76cd	119.99±1.94de	86.53±20.15d	88.46±14.37d	115.56±2.51bcde	101.82±6.22c	127.57±13.53b	136.77±19.01ab
5_FR	146.97±10.28ab	142.94±11.44abc	168.97±11.4a	151.2±3.36abc	123.57±5.93cd	97.27±3.33cd	160.87±18.6a	144.23±29.15bc	112.28±1.94cd	121.28±3.59bc
5_ROMP	130.82±36.24ab	185.44±19.66a	112.12±8.86bcd	125.12±22.75cde	116.03±16.06cd	120.76±14.62cd	140.77±36.78abcd	165.49±37.9bc	106.88±2.23d	101.63±2.85d
5_URB	142.87±12.3ab	144.05±13.86abc	129.3±5.29b	152.1±16.05ab	109.39±3.93d	115.02±1.25cd	131.04±21.03abcde	108.62±3.63bc	107.72±3.23d	114.15±5.23cd

Note: Means±s.e. followed by different letters present significant differences at $p<0.05$ based on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 3, 4 and 5 represent the percentage of increase/decrease from 24 to 48 h (3), 48 to 72 h (4), 72 to 96 h (5).

In the 1-phenylalanine (AA3) group, it is observed that the highest value is recorded at 3_CT, while the lowest value is identified at 3_ROMP. These differences are significant compared to the maximum value, but not compared to the other groups. Thus, significant differences are found between 3_CT and 3_ROMP after 48 hours of incubation. For reading 4, the highest value is represented by 4_CT, while the lowest value is identified at 4_ROMP. In this case, it can be concluded that there are significant differences between these values after 72 hours of incubation. As for reading 5, the highest value is observed at 5_FR, while the lowest is recorded at 5_CT. Although there are significant differences between these two values, no significant differences are identified between 5_FR and the other values (Table 4).

For the 1-Serine group (AA4), it is noted that 3_CT records the highest value, i.e. 173.8. In contrast, the lowest value of 107.42 is observed for 3_URB, indicating that there is no significant difference between 3_CT and 3_URB. Concerning reading 4, the values for 4_FR and 4_URB are significantly different. In the context of reading 5, the maximum value is 165.49 for 5_ROMP, while the minimum is recorded at 5_CT, where it is found that there is

no significant difference between them (Table 4).
Group 1-Threonine or AA5 shows no discrepancy between the highest value, 3_CT, and the lowest, 3_FR, while no significant differences are observed between groups at other value levels. Regarding reading 4, the highest value is recorded at 4_FR, totalling 153.97, indicating significant differences from the other groups, while the lowest value is evident at 4_CR. Therefore, it can be concluded that there are significant differences between these values at 72 hours of incubation. Regarding reading 5, the maximum value is observed at 5_CR, while the minimum is recorded at 5_ROMP. Analysing these two values, significant differences can be observed after 96 hours of incubation (Table 4).
In relation to the glycyl-l-glutamic acid group or AA6, the highest value recorded is 108.26, while the lowest value of 102.78 is identified in 3_FR, but the difference between them does not seem to be significant. Regarding reading 4, a maximum value of 144.09 and a minimum of 102.13 is observed. Analysing these data, significant differences can be identified between 4_FR and 4_URB, as well as between 4_FR and the other values. For reading 5, the maximum value recorded is 136.77, while the minimum is 101.63, and the difference between them is significant (Table 4).
Within the methyl ester group of pyruvic acid, or CH1, it is evident that CR has the highest value at 275.79. Conversely, the lowest value, 144.17, is seen in URB, highlighting a significant difference between CR and URB.
Regarding the substrate CH2, or D-cellobiose, it is notable that there is no difference between the highest value, CT, and the lowest value, URB. Additionally, regardless of the value, no significant differences are observed in other groups (Table 5).
In the context of the α-d-lactose substrate (CH3), the highest recorded value is 277.83. In contrast, the lowest value, 135.36, is identified in URB, highlighting a significant difference between these two extremes (Table 5).
In the context of β-methyl-D-glucoside (CH4), the greatest value is recorded for FR. On the other hand, the lowest value is recorded for ROMP, showing significant differences from the maximum value, but not from the other groups. Therefore, significant variations are observed between FR and ROMP (Table 5).
In the context of the substrate D-xylose, or CH5, considering the second reading, with CR having the highest value and ROMP the lowest, we can observe significant differences between these values.
Within the context of the substrate i-erythritol (CH6), it is notable that ROMP shows the highest value. In contrast, URB has the lowest value, highlighting a significant difference between ROMP and URB. This may reflect environmental differences or the impact of pollution (Table 5).
In the d-mannitol group (CH7), the higher value recorded is 494.31 in CR. In contrast, the lower value is 206.43 in URB, indicating a significant difference between these two extremes.
Within the context of the N-acetyl-D-glucosamine group (CH8), it is observed that the greatest value is recorded in FR. Conversely, the lowest value is identified in URB, indicating significant differences compared to the maximum value. Therefore, significant differences are noted between FR and URB after 24 hours of incubation (Table 5).
Among the glucose-1-phosphate (CH9) group, FR has the maximum value, indicating a significant difference from the other groups. URB has the minimum value, being substantially different from the groups with high values, but not from those with medium or low values. Thus, URB and FR are significantly different (Table 5).
For the substrate d,1-α-glycerol phosphate (CH10), it can be observed that the highest recorded value is 154.31 in FR. In contrast, the lowest value is identified in ROMP, reaching 111.34, with a significant difference between these extremes being evident (Table 5).
In the case of the substrate Acid d-glucosamine (CX1), it can be observed that the maximum value recorded is 368.48 in CR. In contrast, the lowest value is found in ROMP, namely 129.19, showing a significant difference between these two extremes (Table 6).

Table 5. Dynamics of carbohydrates functional groups from 24 to 96 hours in long-term contaminated sites

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
CR	275.79±4.64a	412.74±89.3a	107.90±0.56c	168.51±40.7ab	206.75±35.2a	150.67±29.1ab	494.31±33.9b	312.11±55.7ab	161.73±46.3ab	119.14±4.55b
CT	160.63±18.8bc	277.64±44.1ab	277.83±16.0a	169.36±16.9ab	145.25±11.3b	206.84±11.3ab	216.94±25.0b	230.78±21.0ab	168.03±27.3ab	118.71±0.58b
FR	214.09±20.0abc	283.77±49.7ab	204.54±43.3ab	219.58±29.2a	183.18±13.8ab	176.90±25.2ab	409.80±8.88ab	377.82±84.1a	209.85±30.5a	154.31±15.0a
ROMP	237.05±52.1ab	277.23±72.8ab	148.05±35.7bc	104.61±2.67b	127.78±13.2b	238.26±71.4a	246.06±72.0ab	174.44±36.0b	112.83±2.29b	111.34±2.23b
URB	144.17±13.6c	192.19±8.81b	135.36±7.40bc	127.55±11.4b	138.72±5.34b	122.28±5.71b	206.43±78.6ab	166.94±19.7b	111.17±0.90b	115.93±4.51b

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

Within the group of galactonic acid γ-lactone (CX2), it is observed that the highest value is recorded in FR. Conversely, the lowest value is identified in URB, indicating significant differences compared to the maximum value. Therefore, significant differences are noted between FR and URB (Table 6).

In the d-galacturonic acid or CX3 group, according to the second reading, we observe significant differences between the maximum and minimum values, represented by FR and URB, respectively (Table 6).

In the substrate 2-hydroxy benzoic acid (CX4), it is observed that CT has the highest value. In contrast, ROMP has the lowest value, highlighting a notable difference between ROMP and CT. This may indicate environmental differences or pollution effects.

In the case of 4-hydroxy benzoic acid (CX5) substrate, CR presents the highest level, while URB shows the lowest level. This shows substantial distinctions between CR and URB, with no discernible variation between the other groups (Table 6).

Within the y-hydroxy butyric acid or CX6 group, there is a notable contrast in values. FR stands out with a value of 503.11, while URB lags behind with a value of only 153.75, showing a substantial disparity between the two.

In the CX7 itaconic acid category, FR is the one with the highest average, while URB is far behind FR in value. Consequently, FR and URB show notable differences (Table 6).

Examining the substrate keto-butyric acid or CX8, relative to the other groups, shows that it has lower levels of uptake. Specifically, this substrate indicates that the microorganisms in the soil analysed are less efficient in metabolising this substance. Hence, at reading 2, the highest value is observed at CR and the lowest is 104.78, with no significant differences.

In the d-malic acid group (CX9), it is observed that the highest value is recorded at FR. In contrast, the lowest value occurs at ROMP, showing significant differences from the maximum value, but not from the other groups that have overlapping letters. Therefore, significant differences are found between FR and ROMP (Table 6).

Table 6. Dynamics of carboxylic acids functional groups from 24 to 96 hours in long-term contaminated sites

	CX1	CX2	CX3	CX4	CX5	CX6	CX7	CX8	CX9
CR	368.48±41.3a	138.65±25.7bc	346.78±41.8ab	130.28±6.86ab	390.31±33.6a	502.10±72.6a	263.91±112.85bc	135.34±9.84a	273.87±45.8ab
CT	214.44±13.6b	173.47±18.7b	190.05±31.6bc	212.11±31.2a	241.82±44.9ab	273.13±48.5ab	280.20±32.9b	129.77±8.48a	200.55±40.6abc
FR	323.72±3.97a	289.01±31.4a	380.27±22.3a	159.91±50.6ab	309.53±69.3ab	503.11±19.1a	507.17±28.5a	129.22±17.5a	313.77±17.7a
ROMP	129.19±14.4b	113.35±1.35bc	227.10±108.95abc	112.39±2.46b	271.69±126.14ab	265.51±138.19b	120.55±3.50bc	104.78±5.78a	123.92±8.72c
URB	190.80±58.8b	105.93±2.38c	132.85±14.9c	122.00±2.06b	131.64±6.05b	153.75±10.1b	108.19±2.80c	105.98±10.1a	169.73±58.9bc

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

In the second reading of the Tween 40 group (P1), it is observed that FR records the highest value. On the other hand, the lowest value appears at URB, showing notable differences compared to the maximum value. Therefore, after 24 hours of incubation, significant differences are observed between FR and URB.

In the Tween 80 or P2 group, ROMP shows the highest numbers while URB shows the lowest numbers, indicating minimal contrast between ROMP and URB (Table 7).

In group α - Cyclodextrin or P3, FR is found to have the highest figure of 274.79, while CR has the lowest value of 120.16. This highlights a significant discrepancy between FR and CR.

Regarding the glycogen group (P4), it is observed that FR has the highest value while CR has the lowest value, indicating a significant difference between the two. This difference can be attributed to variations in the environment or the impact of pollution.

In the Arginine or AA1 group (Group 1), there's a notable contrast in levels. The highest measurement, 437.79, is observed in the CR condition, whereas the lowest, 117.64, is found in URB. This variation between the highest and lowest values is considerable (Table 7).

In the analysis of group 1-Aspargine (AA2), it is noted that the highest concentration is recorded in CR. In contrast, the lowest level is recorded in URB, indicating significant differences compared to the maximum value, but not compared to the other groups having the same associated letter. Therefore, significant differences are evident between URB and CR.

In the 1-phenylalanine group (AA3) category, the highest value recorded reached 267.04, while the lowest value, 116.10, was observed in ROMP. This notable difference between the highest and lowest value is relevant.

In category 1-Serine (AA4), FR has the highest value of 382.11, while URB has the lowest value of 125.71. This highlights the significant differences between FR and URB (Table 7).

In the first category, threonine or AA5, in reading 2, CT shows the highest value, 182.92, which differs markedly from the values of the other groups. In contrast, the lowest value is observed in URB. Consequently, it suggests notable distinctions between these values.

We can identify significant differences between FR and ROMP within the glycyl-l-glutamic acid or AA6 group, with a maximum value of 179.39 and a minimum of 115.00 (Table 7).

Table 7. Dynamics of polymers and amino acids functional groups from 24 to 96 hours in long-term contaminated sites

	P1	P2	P3	P4	AA1	AA2	AA3	AA4	AA5	AA6
CR	271.88±38.4a	298.05±81.2a	120.16±5.69c	150.62±30.0b	437.79±115.31a	534.45±109.65a	224.75±39.3ab	239.62±41.2bc	134.55±12.8ab	121.13±3.96b
CT	217.21±11.2ab	225.21±33.3a	214.76±3.02b	209.65±12.9b	218.58±64.5ab	240.21±53.9ab	267.04±30.8a	278.36±31.1ab	182.92±31.4a	172.99±25.6a
FR	281.25±43.8a	257.48±18.7a	274.79±28.6a	313.39±31.2a	428.69±48.3a	493.98±38.9a	262.76±54.8a	382.11±30.3a	175.12±1.93a	179.39±3.29a
ROMP	165.47±38.7b	298.63±57.6a	132.62±16.0c	160.14±33.6b	246.49±94.8ab	313.74±171.69ab	116.10±3.74b	241.13±73.5bc	119.62±6.32b	115.00±1.58b
URB	156.46±25.0b	170.30±26.0a	148.34±7.39c	196.07±20.0b	117.64±2.32b	122.52±3.38b	149.89±29.3b	125.71±8.59c	116.97±4.58b	121.53±2.61b

Note: Means±s.e. followed by different letters present significant differences at *p<0.05* based on post-hoc LSD test. Legend: CR - Craica, CT - Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

The results show that substrate water or WAT is highest in CR and lowest in CT. This discrepancy is significant compared to the maximum value recorded, but not so significant compared to the other groups with overlapping letters. Therefore, significant differences are observed between CR and CT (Table 8).

In the amine category, phenyltinamine or AM1 has the highest average value, while URB has the lowest average value. Therefore, there is a significant difference between URB and FR.

As for the putrescine or AM2 group, the maximum value recorded is 236.55, while the lowest value of 124.76 is identified for URB. The difference between the two extremes appears to be negligible (Table 8).

Regarding high carbohydrate levels, such as CH2, CH1 and CH1 in CR(2), CT(3), CR(4) and ROMP(5) soils show intense microbial activity, suggesting more efficient degradation of organic matter. Lower CH1, CH3, CH4, CH5 values in ROMP(2), FR(3), CT(4), CT(5) soils may signal an inhibition of microbial activity due to the presence of heavy metals that are toxic to microorganisms (Figure 1).

The high polymer values, namely P1, P2 AND P4 in FR(2), CR(3), FR(4) and ROMP(5) soils, indicate a significant presence of microorganisms capable of degrading complex materials. At the same time, lower P2, P3 and P4 in CR(2), URB(3), URB(4) and CR(5) soils indicate a decrease in microbial diversity and capacity to break down polymeric substances due to heavy metal contamination (Figure 1).

Table 8. Dynamics of basal and amines/amides functional groups from 24 to 96 hours in long-term contaminated sites

	WAT	AM1	AM2
CR	107.61±1.31a	117.20±7.73b	186.50±59.7a
CT	92.133±3.7925c	219.37±33.5ab	222.74±18.0a
FR	94.884±3.5595bc	343.34±42.0a	236.52±36.4a
ROMP	107.40±2.66a	229.54±115.98ab	201.18±78.7a
URB	102.77±2.61ab	113.57±2.02b	124.76±4.72a

Note: Means±s.e. followed by different letters present significant differences at *p<0.05 based* on post-hoc LSD test. Legend: CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb, URB-Urbis; 2 represent the percentage of increase/decrease from 24 to 96 h.

Increased carboxylic and acetic acid concentrations, namely CX3, CX6, CX7 in FR(2), FR(3), FR(4) and FR(5) soils suggest active communities to stressful environments.

The decreased CX2 and CX8 levels in ROMP(2), FR(3), ROMP(4), CT(5) soils may be a warning sign of inhibition due to heavy metal toxicity (Figure 1).

Elevated values of amino acids, AA1, AA2 and AA4 in FR(2), FR(3), CR(4), ROMP(5) soils indicate active protein synthesis and good metabolic health of microbial communities. Lower values, AA1, AA3 and AA6 in ROMP(2), ROMP(3), URB(4) and CT(5) soils may show metabolic stress caused by metal contaminants, affecting protein synthesis (Figure 1).

The high values of amines and amides, AM1, AM2 in FR(2), CT(3), ROMP(4) and ROMP(5) soils indicate a microbial activity of protein degradation. Smaller concentrations, AM1 and AM2 in URB(2), URB(3), URB(4) and CT(5) soils reveal an inhibition of microbial function caused by the presence of heavy metals (Figure 1).

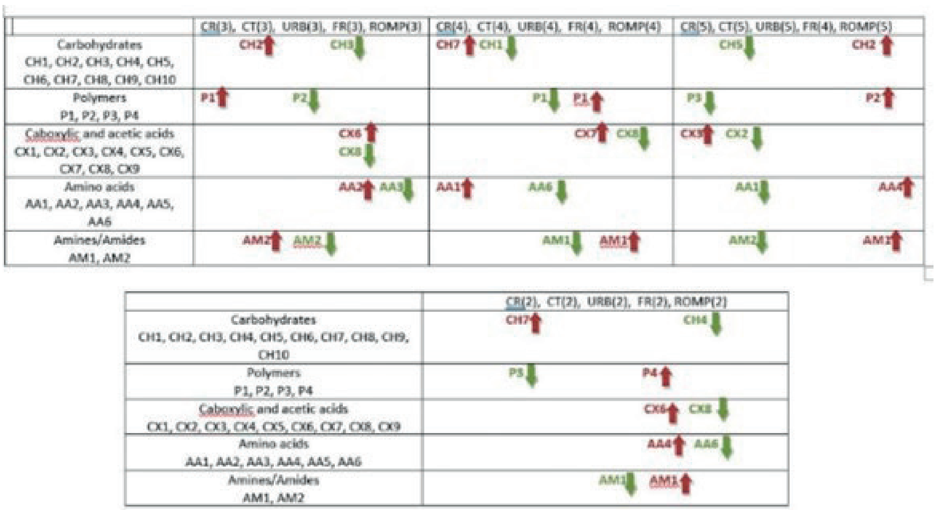

Figure 1. Trends of functional groups asociated with contaminated sites

CONCLUSIONS

The presence of heavy metals significantly alters the functional kinetics of microorganisms in Baia Mare soils. They reduce microbial diversity, moreover they disrupt nutrient cycling and decrease soil fertility. Microorganisms show different responses to contamination. Susceptible ones reduce their diversity and population, while resistant species adapt and proliferate.

This paper highlights significant differences in microbial activity between polluted sites in Baia Mare, such as Craica and Colonia Topitorilor, which show varying microbial functional profiles and growth rates.

The method used effectively measures the functional responses of the microbial community to contamination, while providing detailed insights into how different substrates metabolise in polluted environments.

The research highlights the need to understand soil microbial dynamics in the face of heavy metal pollution, which is essential for developing effective bioremediation strategies and ensuring soil and thus environmental health.

ACKNOWLEDGEMENTS

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STUDY ON WINTER WHEAT PRODUCTION AND QUALITY IN THE PECICA-ARAD AREA

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Abstract

Although at present, both in our country and internationally, in the world grain trade, protein content is an indicator of grain quality, it is positively correlated with other quality indicators such as gluten content, gluten index and sedimentation index. The present study followed the influence of mineral fertilization and pedoclimatic conditions in the Pecica-Arad microzone on the level of production and its quality, expressed by protein content, gluten content, gluten index and sedimentation index. The study was conducted between 2019 and 2021, in the low plain of the Mures Meadow. The variety used in the experimentation was Ciprian, created at SCDA Lovrin. The protein content values achieved at the 5 N doses highlight that it ranges from 11.84% (N0) to 15.34% (N120). Analyzing the production obtained also from the point of view of the values of quality indicators (wet gluten over 25%, gluten index 86.33 and sedimentation index above the threshold of 50) and comparing these values with the regulated limits for each one, we can appreciate the special quality of the harvest.

Key words: *wheat, protein, gluten, quality.*

INTRODUCTION

Wheat, due to its chemical composition and multiple uses in human food, is the cereal with a crucial role in global food security (Erenstein et al., 2022; Gherasimescu et al., 2023). The proportion of land cultivated with this plant, the climate changes in recent years, restrictions on limiting the amounts of fertilizers applied, impose changing the cultivation technology and adapting it at the micro zone level (Smuleac et al., 2020; Gherasimescu, 2023; Sitnicki et al., 2024).

This is currently possible through the use of precision agriculture, which allows the rational use of resources in accordance with ensuring the necessary nutrients, so that we achieve productions both quantitatively and qualitatively while maintaining a clean environment (Smuleac et al., 2022; Dziekanski et al., 2022; Sitnicki et al., 2024). In the production of quality wheat, rational mineral fertilization plays a very important role in terms of the ratio between the three macronutrients N:P:K (Raun et al., 1999; Harrison et al., 2001; Mohammed et al., 2013; Blandino et al., 2015; Kozlovský et al., 2018).

MATERIALS AND METHODS

The purpose of the research was to evaluate the productive potential and the quality of wheat production, through the contribution of mineral fertilization with nitrogen, phosphorus, and potassium under the influence of climatic factors in the Pecica-Arad micro zone. The research was conducted within a bifactorial experiment, using the subdivided plot method with three repetitions and the following gradation of experimental factors: Factor A - the level of fertilization with P and K, with 5 gradations: a1 - P_0K_0; a2 - $P_{40}K_0$; a3 - $P_{80}K_0$; a4 - $P_{40}K_{40}$; a5 - $P_{80}K_{80}$. Factor B - fertilization with N, with 5 gradations: b1 - N_0; b2 - N_{30}; b3 - N_{60}; b4 - N_{90}; b5 - N_{120}. The variety used in the experiment was Ciprian, created at SCDA Lovrin, and the seeding density was: 550 germinable seeds/m^2.

To complement the analysis of wheat quality, particularly protein content as influenced by the fertilization levels, Near-Infrared Spectroscopy (NIR) analysis was employed using a Pfeuffer Granolyser, which analyses whole grains directly. This method involved placing whole wheat grains in the Granolyser, which irradiates the grains with near-infrared light. Multiple

scans of each sample were conducted to minimize random sampling errors and enhance the reliability of the spectral data. This rapid and non-destructive approach allowed for the efficient assessment of protein levels, providing a critical measure of the nutritional quality of wheat influenced by the experimental fertilization regimes.
The processing and interpretation of the experimental results were performed using the variance analysis program – statistics [ANOVA], MSTATC.

RESULTS AND DISCUSSIONS

Regarding the influence of phosphorus and potassium fertilization (Table 1), we note that, in comparison with the unfertilized control variant a1 [P_0K_0], insignificant production increases were obtained, except for $P_{40}K_{40}$ where a distinct significant increase of 8%, respectively 436 kg/ha, was achieved.

Table 1. Influence of phosphorus and potassium fertilization on wheat yield

Factor PK	Yield		Diff. (kg/ha)	Signif.
	kg/ha	%		
V1 - P0K0	5421	100.0	mt	
V2 - P40K0	5533	102.1	112	ns
V3 - P80K0	5698	105.1	277	ns
V4 - P40K40	5857	108.0	436	**
V5 - P80K80	5702	105.2	281	ns

DL 5% = 314 kg; DL 1% = 416; DL 0.1% = 540

Compared to the unfertilized control group b1[N_0], statistically significant yield increases were recorded, with the exception of the N_{30} dose, which resulted in an insignificant increase. The yields that were statistically ensured range from 363 kg/ha to 540 kg/ha, indicating distinct and significantly different increases. The doses N_{60}, N_{90}, and N_{120} surpass the control with an increase ranging from 7% to 10% (Table 2).

Table 2. Influence of nitrogen fertilization on wheat yield

Factor N	Yield		Diff. (kg/ha)	Signif.
	kg/ha	%		
V1 - N_0	5421	100.0	mt	
V2 - N_{30}	5533	102.1	112	ns
V3 - N_{60}	5698	105.1	277	ns
V4 - N_{90}	5857	108.0	436	**
V5 - N_{120}	5702	105.2	281	ns

DL 5% = 314 kg; DL 1% = 416; DL 0.1% = 540

From Figure 1, it can be observed that the yield increases with the PK dose up to $P_{40}K_{40}$, after which it declines. The lowest yield [5421 kg/ha] is obtained at P_0K_0, and the highest [5857 kg/ha] at $P_{40}K_{40}$. There are no significant differences between the yields obtained at the five PK doses. Wheat production increases up to the N_{60} dose, beyond which it decreases. The lowest yield is recorded at the N_0 level [5300 kg/ha], and the highest at N_{60} [5851 kg/ha], as shown in Figure 2.

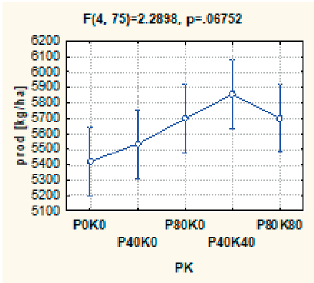

Figure 1. Yield variation under the influence of P and K fertilization

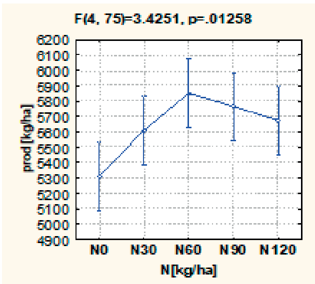

Figure 2. Yield variation under the influence of N fertilization

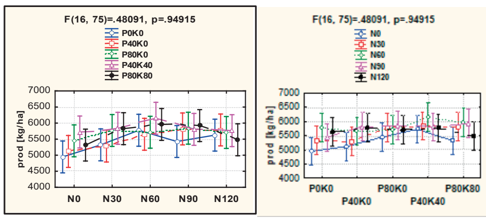

Figure 3. Variation in yield interacting factors by P, K and N fertilization

From Figure 3, it is evident that regardless of the PK doses [a], the lowest wheat production is obtained at N_0, with values ranging from 4948 kg/ha [$P_0K_0N_0$] to 5725 kg/ha [$P_{40}K_{40}N_0$]. The highest wheat production, 6150 kg/ha, is achieved at $P_{40}K_{40}N_{60}$, followed by $P_{80}K_{80}N_{60}$ with 5959 kg/ha, and $P_{80}K_{80}N_{90}$ producing 5925 kg/ha.

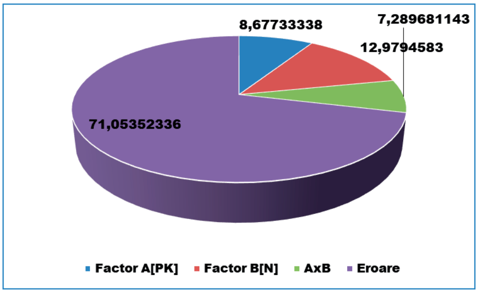

Figure 4. Contribution of factors and interaction of macro-elements with P, K and N in 2019-2021

Figure 4 indicates that phosphorus (P) and potassium (K) fertilizers make up 8.7% of the yield. Nitrogen (N) fertilizers contribute 13%, and the combined effect of using both types of fertilizers (interaction A x B) adds another 7.3%. This information highlights how different nutrients and their combination affect wheat production, stressing the importance of balanced fertilization for improving crop yields.

Table 3. Influence of phosphorus and potassium fertilisers on protein content (%) (2019-2021)

Factor PK	Protein	Diff. (kg/ha)	Signif.
V1 - martor P_0K_0	13.16	mt	
V2 - $P_{40}K_0$	13.41	0.25	ns
V3 - $P_{80}K_0$	13.68	0.52	*
V4 - $P_{40}K_{40}$	13.57	0.41	ns
V5 - $P_{80}K_{80}$	13.73	0.57	*

DL 5% = 0.45 DL 1% = 0.60 DL 0.1% = 0.78

The protein content, based on phosphorus and potassium fertilization, ranged from 13.16% [P_0K_0] to 13.73% [$P_{80}K_{80}$] (Table 3). Compared to the control variant a1 [P_0K_0], the increase in protein content was:

- Insignificant at $P_{40}K_0$ and $P_{40}K_{40}$.
- Significant at $P_{80}K_0$ and $P_{80}K_{80}$. The increases vary between 0.25 and 0.57%.

The analysis of the protein content at the five PK doses [factor A] highlights that it ranges from 13.2% obtained at P_0K_0 to 13.7% obtained at $P_{80}K_0$ and $P_{80}K_{80}$ (Figure 5). This indicates the influence of specific fertilization strategies on the nutritional quality of wheat, demonstrating that higher levels of phosphorus and potassium can enhance protein content within the grains.

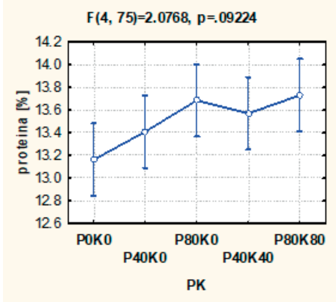

Figure 5. Variation of protein content under the influence of P and K fertilization

The influence of nitrogen fertilizers on the protein content (%), Table 4, recorded values ranging from 10.63% in the N_0 variant to 15.40% in the N_{120} variant.

Table 4. Influence of nitrogen fertilisers on protein content (%) (2019-2021)

Factor PK	Protein	Diff. (kg/ha)	Signif.
V1 - martor N_0	10.63	mt	
V2 - N_{30}	12.77	2.14	***
V3 - N_{60}	13.80	3.17	***
V4 - N_{90}	14.96	4.33	***
V5 - N_{120}	15.40	4.77	***

DL 5% = 0.45 DL 1% = 0.60 DL 0.1% = 0.78

Compared to the control b1[N_0], very significant increases were obtained regardless of the applied nitrogen dose. The increases varied between 2.14-4.77%, being distinctly superior to the control variant. The analysis of the protein content conducted on the 5 nitrogen doses [factor A] from Figure 6, highlights that it ranges between 10.4%-15.4%. The protein content increases with the increase of the applied nitrogen dose. The highest protein percentage, 15.4%, is achieved at N_{120}.

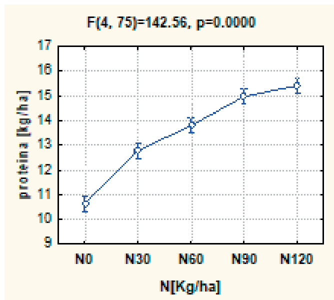

Figure 6. Variation of protein content under the influence of N fertilisation

The analysis of the results regarding the influence of the PK x N interaction [AxB] on the protein content (%), Figure 7, shows that the protein content increases with the nitrogen dose regardless of the PK dose. The highest values of protein content are obtained at N_{90} and N_{120} regardless of PK, and the lowest at N_0.

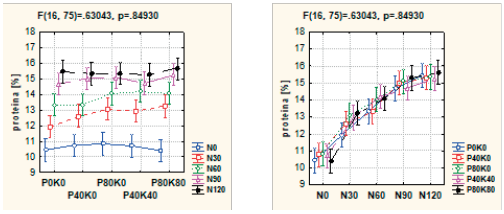

Figure 7. Variation of protein content under the influence of PK x N fertilisation interaction

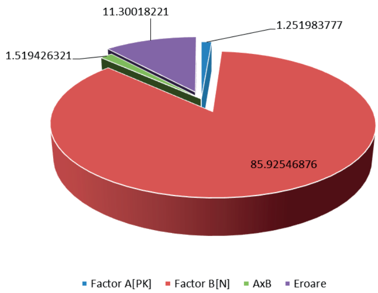

Figure 8. Contribution and interaction of P, K and N fertilization on protein content

Analysing the contribution of experimental factors to the achievement of protein content (figure 8), we observe that nitrogen fertilization contributes 85.93% to the protein yield, while fertilizers with P, K contribute only 1.25%, and the AxB interaction contributes 1.52%.

Although at present, both in our country and internationally, in the global grain trade, the protein content is an indicator of grain quality, it is positively correlated with other quality indicators such as gluten content, gluten index, and sedimentation index.

The quantity and quality of gluten are very important quality indicators in evaluating a variety and for the technological process, contributing to the characterization of the dough, especially its processing capacity and baking potential.

In table 5, the average values of the quality indicators (wet gluten, gluten index, and sedimentation index) are presented, depending on the experimental micro-zone and climatic conditions.

Table 5. Average values of quality indicators, depending on the micro-area of experimentation and climatic conditions

Quality index	Wet gluten	Gluten index	Sedimentation index
Average	26.5	86.63	52.07

Analysing the obtained data and comparing these values with the regulated limits for each one, we can appreciate the exceptional quality of the harvest achieved during the experimental period in the Pecica micro-zone. Thus, besides the values of the protein content ranging between 13%-15.6%, if we also analyse the values of the three quality indicators (wet gluten, gluten index, and sedimentation index), we have certification of the baking quality of the obtained productions.

During the experimental period, the content of wet gluten exceeded on average the percentage of 25%, which demonstrates that in the researched area, very good quality wheat for baking is obtained. The quality assessment of baking wheat based on the gluten index is done according to the following scale:

- "very good" quality > 80;
- "good" quality - 65-80;
- "unsatisfactory" quality < 65.

The values of the sedimentation index on average during the experimental period were 86.33. This value also confirms through this indicator the very good quality of the production obtained. According to the quality assessment scale of baking wheat based on the sedimentation index, varieties with values above 50 are considered category I, those with values between 50-35.01 are category II, category III is 35-20.01, and category IV are those with values below 20. The average value of this quality index recorded during the experimental period was above the threshold of 50, indicating category I productions.

CONCLUSIONS

The results obtained from monitoring the influence of mineral fertilization levels with N, P, and K on the production of the Ciprian wheat variety in the Pecica-Arad micro-zone, which is representative of the wheat culture in the western part of the country, have demonstrated that the most significant influence on production is exerted by the nitrogen dose, followed by the phosphorus dose, and finally, the potassium dose. The highest wheat production of 5851 kg/ha is achieved with a fertilization regime of $P_{40}K_{40}N_{60}$.

In the Pecica-Arad micro-zone, the protein content obtained with any of the N_{30}-N_{120} doses is significantly higher than that of the N_0 dose. The surplus in protein content, compared to the N0 control, ranged between 2.4-4.77%.

By analysing the obtained production and from the perspective of the quality indicator values (wet gluten - 26.5, gluten index - 86.33, and sedimentation index - 52.07) and comparing them with the regulated values for each indicator, we can appreciate the exceptional quality of the harvest produced in the studied area.

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THE INFLUENCE OF ORGANIC AND MINERAL FERTILIZATION ON SUGAR BEET CULTURE IN COVASNA COUNTY

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Abstract

Sugar beet is a species whose importance contradicts the current situation of its cultivation at the national level, namely its increasingly low presence in the crop. The crop technology and the increased attention that the plant needs during the vegetation period, transform the sugar beet crop into a `niche` crop. The work is based on the analysis of the data obtained in the agricultural year 2022-2023 following the establishment of an experimental sugar beet field trial. After fertilizing the variants with the established doses of organic, mineral, or organo-mineral fertilizers, different morphological characters were analyzed, such as the plant length, plant diameter, the length of the parcel, the diameter of the parcel, the weight of the plant, but also the main elements of production, such as the percentage content of sugar, root production per hectare and total sugar content/ha.

Key words: *sugar beet, area, sugar production, correlations, technology.*

INTRODUCTION

Beta vulgaris is a member of the *Chenopodiaceae* and, like many others in the family is a halophyte. It is a highly variable species containing four main groups of agricultural significance: leaf beets (such as Swiss chard), garden beets (such as beetroot), fodder beets (including mangolds), and sugar beet. The storage organ of the sugar-beet plant is usually called the root, although only about 90% is root-derived, the upper 10% (the crown) being derived from the hypocotyl (Elliott et al., 1993).

Sugar beet is a recent crop developed solely to extract the sweetener, sucrose. Breeding and improvement of *Beta vulgaris* for sugar has a rich historical record. Sugar beet originated from fodder beet in the 1800s, and selection increased its sugar content from 4 to 6% to over 18% today. The development of vegetable beets-namely table beet and leaf beet (chard)-predates the creation of sugar beet. Each of these likely shares a common ancestor in the wild relative *B. vulgaris* spp. *maritima*. Beets of all crop types share common disease pressures. Germplasm for breeding and improvement, mostly for disease resistance, is accessible from each of the crop types and wild relatives, as there are no barriers to sexual hybridization. All cultivated types are biennial, with a basic chromosome number of 9, and most new cultivars are diploid. Most sugar beets are hybrids, facilitated by a complex system of cytoplasmic male sterility - CMS (Bohra et al., 2016). Hybrids are typically monogerm, which reduces the labour required for thinning. Genomics and molecular markers are rapidly improving our understanding of the genetic characters controlling sugar beet phenotypes, particularly concerning bolting. Such understanding may allow an expansion of the range of sugar beet cultivation and may help improve yield through earlier planting. Developing beets for new uses, as an energy resource, and for bio-based industrial feedstock, for instance, may further expand the range of beet production for human uses (McGrath et al., 2018).

Mechanical harvest and cleaning of sugar beet, followed by transport, often result in substantial root tip breakage and surface damage through mechanical impact (Gorzelany et al., 2003). The extent of damages from topping or defoliation and root tip breakage highly depends on the harvester settings, and therefore, on the driver (Hoffmann et al., 2018a). In addition to the yield loss through damage, it has been shown that the sugar loss during beet storage is influenced mostly by the amount of damage (Hoffmann & Schnepel, 2016). Furthermore, this and other observations suggest, that the damage susceptibility depends on the sugar beet variety (van Swaaij et al. 2003; Hoffmann et al., 2018b). This can probably be attributed to the stability of the root tissue.

The importance of fertilizing with mineral fertilizers lies in the fact that they intensify physiological processes such as: breathing, transpiration, opening of stomata and photosynthesis. At the same time, mineral fertilizers increase the osmotic pressure and the suction force. They cause a stronger growth of the aerial parts of the sugar beet, especially those based on nitrogen.

It is considered that manure is a complex and inexpensive fertilizer that is available to many sugar beet growers. The application of manure for the sugar beet crop is very beneficial due to the maximum production increases it achieves.

The study's objective is finding the most suitable fertilization method, exclusively mineral, organic or organo-mineral, for the sugar beet crop.

MATERIALS AND METHODS

The research was carried out in Covasna county, Zăbala village, in a demonstration plot of 1.5 ha. Three sugar beet hybrids were taken into study namely: Darvas, Deseda and Tatry and different doses of organic, mineral, or organo-mineral fertilizer were applied, as follows: 20 t/ha manure, 20 t/ha manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, 10 t/ha manure, 10 t/ha manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, only mineral fertilization with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K and the control (unfertilized) (Table 1).

Table 1. The experimental factors

Fertilizer/ Hybrid	a1: DARVAS	a2: DESEDA	a3: TATRY
b1: 20 t manure/ ha	a1b1	a2b1	a3b1
b2: 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K	a1b2	a2b2	a3b2
b3: 10 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K	a1b3	a2b3	a3b3
b4: 10 t manure/ ha	a1b4	a2b4	a3b4
b5: 150 kg/ha N + 150 kg/ha P + 150 kg/ha K	a1b5	a2b5	a3b5
b6: control (unfertilized)	a1b6	a2b6	a3b6

The climatic conditions of the area can be characterised by an average annual temperature of 10.64°C, and 500-600 mm/year precipitations.

The soil on which the experiment was located showed a pH value of 6.13, being classified as acidic soil. Low values were identified for Potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), and Zinc (Zn), normal values for Nitrogen (N), Sodium (Na), Manganese (Mn), and Boron (B) and high values were identified for Iron (Fe) and Copper (Cu).

To assess the efficiency of mineral and organic fertilizers, the variants are compared with the control (unfertilized).

The variants were placed systematically, in four repetitions. The hybrids were sown on May 13, 2023, and harvested on November 13, 2023. The preceding crop was maize. The sowing was carried out mechanized, at a distance of 45 cm between rows and a density of 90,000 germinable seeds/ha.

Darvas hybrid is a new, robust hybrid with a high tolerance to foliar diseases but also to drought.

Deseda hybrid provides high sugar production, high productivity per hectare and excellent tolerance to *Cercospora*.

Tatry hybrid promises a high sugar content, above the usual root yield, with a high juice purity coefficient and a high tolerance to beet Cercosporiosis and abiotic stress.

The morphological characteristics and production elements measurements were carried out in the sugar beet laboratory within the National Research and Development Institute for Potato and Sugar Beet, Braşov.

RESULTS AND DISCUSSIONS

Plant length (cm)

Darvas hybrid fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a1b2) had the highest length, with a value of 29.50 cm in the second repetition. At the variant level, the best average was recorded for Tatry fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, i.e 27.08 cm, 28.26% above the lowest average recorded for the a3b6 (Tatry control variant) (Table 2).

Table 2. Plant length (cm)

	R1	R2	R3	R4	Average
a1b1	24.00	25.70	21.40	23.20	23.58
a1b2	22.00	29.50	23.70	26.40	25.40
a1b3	21.00	29.00	22.30	24.80	24.28
a1b4	21.80	24.50	22.40	23.10	22.95
a1b5	20.00	20.90	26.30	22.10	22.33
a1b6	20.00	20.60	17.20	18.10	18.98
Average a1	21.47	25.03	22.22	22.95	22.92
a2b1	26.20	23.00	24.10	22.70	24.00
a2b2	23.50	26.40	27.10	25.90	25.73
a2b3	19.80	27.50	21.40	22.30	22.75
a2b4	21.00	19.00	21.20	20.40	20.40
a2b5	18.40	16.50	16.20	17.10	17.05
a2b6	17.20	14.40	14.00	15.60	15.30
Average a2	21.02	21.13	20.67	20.67	20.87
a3b1	24.00	28.40	25.90	26.30	26.15
a3b2	28.50	25.80	27.10	26.90	27.08
a3b3	26.50	23.50	22.80	25.10	24.48
a3b4	24.00	20.60	21.30	22.10	22.00
a3b5	26.50	21.50	23.30	23.40	23.68
a3b6	19.50	20.40	18.20	20.00	19.53
Average a3	24.83	23.37	23.10	23.97	23.82
Average	22.44	23.18	21.99	22.53	22.54

Plant diameter (cm)

The highest value for plant diameter was registered for Deseda variant fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, i.e 10.70 cm and in the second repetition. At the average level, the best value/ variant was recorded at Tatry variant fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, i.e 10.18 cm, 25.54% above the lowest average value recorded at a3b6 (Tatry control variant) (Table 3).

Table 3. Plant diameter (cm)

	R1	R2	R3	R4	Average
a1b1	10.40	9.70	10.10	9.80	10.00
a1b2	8.80	8.70	8.60	8.70	8.70
a1b3	8.30	10.00	9.60	8.80	9.18
a1b4	9.10	9.70	9.00	9.20	9.25
a1b5	6.80	7.40	7.60	7.30	7.28
a1b6	6.40	5.70	6.20	6.00	6.08
Average a1	8.30	8.53	8.52	8.30	8.41
a2b1	9.10	8.80	8.80	9.00	8.93
a2b2	8.60	10.70	9.40	10.20	9.73
a2b3	9.00	8.90	9.10	9.20	9.05
a2b4	7.50	7.60	7.80	7.70	7.65
a2b5	8.10	6.60	8.00	6.60	7.33
a2b6	7.80	7.90	6.80	7.80	7.58
Average a2	8.35	8.42	8.32	8.42	8.38
a3b1	9.80	8.20	9.20	9.10	9.08
a3b2	10.50	9.80	10.30	10.10	10.18
a3b3	9.00	8.60	8.90	8.50	8.75
a3b4	9.80	8.50	9.20	9.40	9.23
a3b5	8.80	8.20	8.00	8.00	8.25
a3b6	8.30	7.00	7.60	7.40	7.58
Average a3	9.37	8.38	8.87	8.75	8.84
Average	8.67	8.44	8.57	8.49	8.55

Neck length (cm)

The recorded average values for the neck length were between 2.48 cm at a1b6 (Darvas control variant) and 4.58 cm at Darvas variant fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K. The difference between the maximum and the minimum average values was45.84% (Table 4).

Table 4. Neck length (cm)

	R1	R2	R3	R4	Average
a1b1	4.00	4.50	4.80	4.60	4.48
a1b2	3.90	4.80	4.90	4.70	4.58
a1b3	3.00	5.30	4.20	4.10	4.15
a1b4	4.00	3.20	4.20	3.80	3.80
a1b5	3.20	2.40	3.10	2.70	2.85
a1b6	2.10	2.60	2.40	2.80	2.48
Average a1	3.37	3.80	3.93	3.78	3.72
a2b1	2.90	2.80	2.30	2.70	2.68
a2b2	2.90	3.50	3.10	3.00	3.13
a2b3	3.50	3.00	2.80	2.90	3.05
a2b4	2.00	2.30	2.90	2.60	2.45
a2b5	2.70	2.80	2.50	2.50	2.63
a2b6	2.20	3.60	2.10	2.30	2.55
Average a2	2.70	3.00	2.62	2.67	2.75
a3b1	3.60	4.20	3.80	3.90	3.88
a3b2	4.50	3.90	4.10	4.10	4.15
a3b3	3.80	3.50	3.00	3.30	3.40
a3b4	3.00	3.10	3.00	3.20	3.08
a3b5	4.00	3.70	3.10	3.50	3.58
a3b6	3.00	2.40	2.70	2.90	2.75
Average a3	3.65	3.47	3.28	3.48	3.47
Average	3.24	3.42	3.28	3.31	3.31

Neck diameter (cm)

Regarding the neck diameter, in the Tatry variant fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K was registered the highest value of 5.90 cm, in the second repetition. The best average value at the variant level was recorded for Tatry variant fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, i.e 5.53 cm, 47.55% above the lowest average value recorded for a1b6 (Darvas control variant) (Table 5).

Table 5. Neck diameter (cm)

	R1	R2	R3	R4	Average
a1b1	5.20	5.30	5.60	5.10	5.30
a1b2	5.60	5.20	5.80	5.40	5.50
a1b3	5.20	4.80	5.30	5.10	5.10
a1b4	4.10	5.00	4.50	4.60	4.55
a1b5	4.10	3.80	3.90	4.00	3.95
a1b6	3.10	2.90	2.70	2.90	2.90
Average a1	4.55	4.50	4.63	4.52	4.55
a2b1	4.40	4.60	4.90	4.70	4.65
a2b2	4.60	4.70	5.10	5.00	4.85
a2b3	4.30	4.80	4.20	4.50	4.45
a2b4	3.40	3.60	3.70	3.60	3.58

a2b5	3.70	3.70	3.90	3.90	3.80
a2b6	3.00	3.10	3.10	3.20	3.10
Average a2	3.90	4.08	4.15	4.15	4.07
a3b1	4.80	4.70	4.40	4.70	4.65
a3b2	5.30	5.90	5.40	5.50	5.53
a3b3	4.50	5.10	4.80	4.30	4.68
a3b4	4.70	4.10	4.10	4.30	4.30
a3b5	4.10	4.30	3.90	4.00	4.08
a3b6	3.80	3.40	3.90	3.60	3.68
Average a3	4.53	4.58	4.42	4.40	4.48
Average	4.33	4.39	4.40	4.36	4.37

Plant weight (g)

The plant weight recorded the highest value for Darvas variant fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K variant, i.e. 1341.21 g, in the fourth repetition. At the average level, the best value/variant was recorded at Darvas variant fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, namely 1,318.20 g, 77.08% above the lowest average value recorded at a1b6 (Darvas control variant) (Table 6).

Table 6. Plant weight (g)

	R1	R2	R3	R4	Average
a1b1	1021.60	1292.20	1274.30	1185.30	1193.35
a1b2	1361.40	1239.50	1330.70	1341.21	1318.20
a1b3	908.30	626.10	821.60	863.74	804.94
a1b4	731.50	775.10	698.20	726.31	732.78
a1b5	533.10	486.70	465.50	520.64	501.49
a1b6	317.80	226.00	302.50	362.12	302.11
Average a1	812.28	774.27	815.47	833.22	808.81
a2b1	835.50	820.00	877.30	852.66	846.37
a2b2	942.80	1078.80	1110.40	1123.52	1063.88
a2b3	796.50	691.30	772.20	718.49	744.62
a2b4	561.80	541.50	469.30	522.37	523.74
a2b5	494.40	450.40	478.80	487.87	477.87
a2b6	499.30	481.90	420.10	440.27	460.39
Average a2	688.38	677.32	688.02	690.86	686.15
a3b1	1040.00	1116.80	865.30	1002.02	1006.03
a3b2	994.10	1284.70	1129.10	1098.45	1126.59
a3b3	905.50	884.60	901.20	896.35	896.91
a3b4	828.60	784.30	809.90	799.42	805.56
a3b5	622.80	699.30	774.20	710.87	701.79
a3b6	521.70	584.60	503.20	543.91	538.35
Average a3	818.78	892.38	830.48	841.84	845.87
Average	773.15	781.32	777.99	788.64	780.28

Sugar content (%)

Regarding the sugar content, the variant with the best value registered was Tatry fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, i.e 18.408%, in the first repetition. The best average at the variant level was recorded for Tatry fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K variant, namely 18.270%, 23.21% above the lowest average value recorded for a1b6 (Darvas control variant) (Table 7).

Table 7. Sugar content (%)

	R1	R2	R3	R4	Average
a1b1	17.196	16.988	17.022	16.952	17.04
a1b2	17.386	17.402	17.320	17.296	17.351
a1b3	16.690	16.503	16.466	16.557	16.554
a1b4	16.404	16.200	16.112	16.331	16.262
a1b5	15.346	15.236	15.047	15.029	15.165
a1b6	14.086	14.021	13.866	14.140	14.028
Average a1	16.185	16.058	15.972	16.051	16.067
a2b1	18.114	18.033	18.102	18.023	18.068
a2b2	17.450	17.384	17.416	17.295	17.386
a2b3	16.630	16.694	16.273	16.334	16.483
a2b4	15.566	15.403	15.742	15.648	15.590
a2b5	15.136	15.016	14.981	15.084	15.054
a2b6	14.144	13.921	14.069	14.032	14.042
Average a2	16.173	16.075	16.097	16.069	16.104
a3b1	17.512	17.436	17.220	17.361	17.382
a3b2	18.408	18.347	18.112	18.214	18.270
a3b3	17.200	17.416	17.118	17.374	17.277
a3b4	16.224	16.339	16.347	16.263	16.293
a3b5	15.316	15.441	15.130	15.120	15.252
a3b6	14.784	14.226	14.297	14.343	14.413
Average a3	16.574	16.534	16.371	16.446	16.493
Average	16.311	16.223	16.147	16.189	16.217

Root production (t/ha)

The production of roots (t/ha) recorded average values between 49.25 t/ha at Tatry control variant and 60.05 t/ha at Tatry fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K. The difference between the maximum and minimum average was of 17.98% (Table 8).

Table 8. Root production (t/ha)

	R1	R2	R3	R4	Average
a1b1	58.40	59.30	54.70	59.10	57.88
a1b2	60.70	60.10	59.30	57.60	59.43
a1b3	54.30	52.60	55.10	58.90	55.23
a1b4	52.80	53.10	52.40	52.50	52.70
a1b5	50.20	51.30	53.30	52.70	51.88
a1b6	49.60	48.90	50.10	48.70	49.33
Average a1	54.33	54.22	54.15	54.92	54.40
a2b1	59.70	59.30	58.00	50.20	56.80
a2b2	59.90	60.30	58.70	59.80	59.68
a2b3	56.80	58.10	57.80	54.90	56.90
a2b4	54.60	53.70	53.10	52.90	53.58
a2b5	52.30	52.80	51.60	52.00	52.18
a2b6	50.30	51.40	50.80	50.70	50.80
Average a2	55.60	55.93	55.00	53.42	54.99
a3b1	59.10	58.70	58.40	58.90	58.78
a3b2	60.60	59.50	60.20	59.90	60.05
a3b3	58.40	57.30	58.60	58.40	58.18
a3b4	54.60	52.90	53.80	54.20	53.88
a3b5	53.80	52.40	51.30	53.20	52.68
a3b6	49.30	47.20	51.30	49.20	49.25
Average a3	55.97	54.67	55.60	55.63	55.47
Average	55.30	54.94	54.92	54.66	54.95

Sugar production (t/ha)

Sugar production is strongly influenced by all previously analyzed characteristics, and results from the sugar content percentage and the root production of the plot. The highest average sugar production was recorded at Tatry fertilized with 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K variant, with a value of 10.971 t/ha of sugar, and the lowest average value was recorded at Darvas control variant (a1b6), i.e. 6.919 t/ha of sugar. The difference between the maximum and minimum average value was of 36.93% (Table 9)

Table 9. Sugar production (t/ha)

	R1	R2	R3	R4	Average
a1b1	10.04	10.07	9.31	10.02	9.86
a1b2	10.55	10.46	10.27	9.96	10.31
a1b3	9.06	8.68	9.07	9.75	9.14
a1b4	8.66	8.60	8.44	8.57	8.57
a1b5	7.70	7.82	8.02	7.92	7.87
a1b6	6.99	6.86	6.95	6.87	6.92
Average a1	8.84	8.75	8.68	8.85	8.78
a2b1	10.81	10.69	10.50	9.05	10.26
a2b2	10.45	10.48	10.22	10.34	10.38
a2b3	9.45	9.70	9.41	8.97	9.38
a2b4	8.50	8.27	8.36	8.28	8.35
a2b5	7.92	7.93	7.73	7.84	7.86
a2b6	7.11	7.16	7.15	7.11	7.13
Average a2	9.04	9.04	8.89	8.60	8.89
a3b1	10.35	10.24	10.06	10.23	10.22
a3b2	11.16	10.92	10.90	10.91	10.97
a3b3	10.05	9.98	10.03	10.15	10.05
a3b4	8.86	8.64	8.79	8.82	8.78
a3b5	8.240	8.091	7.762	8.044	8.034
a3b6	7.29	6.72	7.33	7.06	7.10
Average a3	9.32	9.09	9.15	9.20	9.19
Average	9.06	8.96	8.91	8.88	8.95

CONCLUSIONS

Following the measurements, Darvas (a1b2), Deseda (a2b2) and Tatry (a3b2) variants registered the highest values, being fertilized with 20 t manure/ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K.

The importance of mineral fertilization is related to the intensification of the physiological processes such as: breathing, transpiration, stomata opening and photosynthesis.

The importance of organic fertilization results from the fact that manure is a complex and inexpensive fertilizer that is available to many sugar beet growers. The application of manure for the sugar beet crop is very beneficial through the maximum production increases, as long as it is administered in optimal quantities and periods.

The combination of the two types of fertilizers leads to the harmonious development of the plant and to superior production and sugar content values than individual fertilizations, only with mineral fertilizers or only with organic fertilizers.

In conclusion, sugar beet is an intensive, very profitable crop, that efficiently capitalizes on fertilization, soil, or irrigation water, and is also a good preceding plant for most crops.

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THE INFLUENCE OF MICROBIAL BIOFERTILIZERS ON THE BALANCE OF NUTRITIONAL ELEMENTS ON SOILS WITH DIFFERENT DEGREES OF EROSION

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Abstract

Currently, the problem of degradation of the effective soil fertility of the Republic of Moldova is reaching a critical scale, being unsatisfactory on 90% of the agricultural land. The annual uncompensated losses of organic matter as a result of mineralization exceed the level of 700 kg/ha, and the total deficit resulting from erosional losses is 1100 kg/year. The main factors that conditioned the establishment of a negative balance of organic matter are the lack of crop rotations, the intensification of erosion processes, the increased costs of chemical fertilizers, as well as the unfavorable climatic conditions manifested by excessive droughts with annual frequency. All together lead to the intensification of chemical, physical and biological degradation processes. In this article, the results of the research are presented, regarding the influence of microbial biofertilizers from the PGPB group (plant growth promoting bacteria) on the balance of nutrients on soils with different degrees of erosion. It was demonstrated that the application of bacteria suspensions, Ps. aureofascens, Az. chroococcum and Ps. fluorescens, contributes to the more intensive accumulation of some nutritional elements, which leads to balancing plant nutrition, as well as increasing the fertility of degraded soils.

Key words: *degradation, erosional perder, organic matter, biofertilizers, fertility.*

INTRODUCTION

Soils are the main natural wealth of every country. The food security of the country, the well-being of the population in both rural and urban areas, as well as the quality of the environment, largely depend on their quality and production capacity. However, the long exploitation of soils without observing the laws of agricultural science and the technologies of cultivation of crops leads to their degradation. In the last 20-25 years, all forms and types of soil degradation have intensified and expanded, especially through erosion, dehumification, drying up of nutrients, salinization and alkalization, destructuring, secondary compaction, reduction of biodiversity, etc. Erosion is the most serious and widespread form of soil degradation. Surface and deep erosion is conditioned by natural and anthropogenic factors (Dobrovolyskij et al., 2008). The natural factors are: the geomorphological conditions (the bumpy relief, especially in the central and southern areas of Moldova), the torrential character of the atmospheric deposits, the low degree of soil coverage with vegetation in the critical erosion season, the low resistance of the soils to erosion. The main anthropogenic actions that led to the acceleration of erosion processes refer to: the excessive capitalization of the land fund (about 74% of the total) with the inclusion in the agricultural circuit of lands with an increased degree of inclination; abandonment of zonal anti-erosion embankments; quota increased grazing crops on sloping land; the lack of drainage regularization strips on the slopes; the lack of the simplest agrotechnical and phytotechnical anti-erosion measures on the land in Soil erosion is a natural process and has an irreversible character (Dimitrov et al., 2004). The fertile soil washed from the slope by erosion is lost practically forever. The speed and result of the manifestation of erosion depend on the natural conditions and human activities. The surface of eroded soils expanded over 44 years (1965-2009) by 284.0 thousand ha, increasing annually by 6.6 thousand ha. According to the Land Cadastre of the Republic of Moldova from 01.01.2009, agricultural lands with different degrees of erosion constitute 878 thousand ha or 35% of the total.

Depending on the degree of erosion, soil fertility decreases from 20% for slightly eroded soils to 60-80% for strongly eroded ones. The damages caused to the national economy by erosion are colossal. Annual soil losses amount to about 26 million tons. This amount of fertile soil contains 700 thousand tons of humus and 84 thousand tons of nitrogen and phosphorus. The cost of agricultural production, lost due to soil erosion, is estimated at 873 million lei. In total, the annual direct and indirect damages as a result of erosion processes amount to 2 billion 723 million lei. Erosional processes also have a negative impact on soil biota. Microorganisms, which facilitate the absorption of nutrients or increase their availability, stimulate plant growth, are commonly called biofertilizers. Biofertilizers are considered as an alternative or complement to chemical fertilization to increase the production of agricultural crops at low cost and to improve the quality of eroded soils. The products (derivatives) of plant growth-stimulating bacteria, so-called PGPB, are most often used. There are some PGPB, the best studied, that can fix nitrogen, solubilize mineral nutrients and mineralize organic compounds. Data from the literature indicate an increased tendency to increase production yield due to the incorporation of nitrogen-fixing PGPB bacteria into the rhizosphere (Martínez-Viveros et al., 2010). Based on microorganisms and metabolites, a series of new biological substances are created, which allow a significant reduction in the amount of chemical substances used in agriculture. Various aspects remain unstudied, especially in the direction of the selection of bacterial strains, taking into account the specifics of growth conditions and plant species, as well as the complex use of microelements with microorganisms in order to increase their efficiency and reduce the level of erosion and environmental pollution.

MATERIALS AND METHODS

The experiments were mounted on moderately eroded clay-loam carbonate chernozem. The total thickness of the humifier profile is 80 cm. The glomerular-bulky structure is characteristic only for the arable layer (0-31 cm). The chernozem of the experimental batch is characterized by a low content of carbonates in the arable layer (2.3%), with depth their amount increases, reaching the maximum in the BC horizon in the 81-95 cm layer (12.8%). The pH_{H_2O} - value is 8.4-8.6 and is stable throughout the profile. The soil of the experimental lot is weak humus (2.67%) in the arable start, but has a deep humus profile, in the state 81-95cm it contains 1.39% humus (Table 1). The sum of exchangeable cations (Ca^{++}, Mg^{++}) is 31.4 me/100 g sol.

Table 1. Physico-chemical characteristics of clay-clay carbonate chernozem

Genetic horizon	The depth, cm	Humus, %	$CaCO_3$, %	pH_{H2O}	Exchangeable cations, me/100 g sol		
					Ca^{++}	Mg^{++}	Sum
A_p	0-31	2.67	2.3	8.4	27.5	3.9	31.4
A	31-46	2.42	3.5	8.5	27.4	3.1	30.5
B^1	46-65	1.62	4.9	8.5	25.7	3.9	29.6
B^2	65-81	1.39	6.8	8.6	21.3	3.5	24.8
BC	81-95	1.39	10.8	8.6	18.2	5.9	24.1
C	95-150						

Vines, Sauvignon variety, young plantation (5-6 years old) served as the object of study. The plants were foliarly treated with bacterial metabolites during the vegetation period five times with an interval of 12-15 days (5 true leaves, 3-4 days before flowering, after flowering, in the phase of intensive plant development, before ripening of the baccalaureate). Based on the preliminary research, the strains of *Pseudomonas aureofascens* and *Ps. fluorescens* were selected. Plants treated with water served as a control. The bacteria were grown on liquid nutrient medium for 24 hours at a temperature of 27˚C with a titer of 10^{10} UFC/ml and applied in the form of metabolites. To obtain the bacterial metabolites, the concentrated suspensions were centrifuged at 8 thousand rpm for 20 minutes in order to precipitate the bacterial cells and obtain the metabolic products. Soil samples for analysis were taken from the experimental lot at two depths - 0-30 and 30-60 cm. The following indices were determined in the soil and plant organs: the content of NPK, total N, NH_4, trace elements Fe, Cu, Mn, Zn, humus, pH, carbonates ($CaCO_3$), exchangeable cations (Ca^{++}, Mg^{++}).

The results were analyzed statistically, using the software - the Statistica programming package on the computer. The data are presented in tables and figures showing the general arithmetic mean of the results from three experiments.

Table 2. Content of total nitrogen, phosphorus and potassium accessible in the rhizosphere under plants of vines, the Codrinski variety. The experience from the vegetable complex

	N, %	P_2O_5, mg/100 g	K_2O, mg/100 g
Control	0.13	4.4	15.0
Az. chroococcum, in soil	0.12	4.0	15.2
Ps. fluorescens, in soil	0.13	4.4	15.2
Az. chroococcum + Ps. fluorescens, in soil	0.12	5.2	15.1
Az. chroococcum + Ps. fluorescens in soil + micoelemente foliar;	0.12	5.2	15.2
Az. chroococcum +Ps. fluorescens, in soil+ microelemente foliar	0.12	6.7	15.1
Az. chroococcum, foliar	0.11	9.6	15.2
Ps. aureofaciens, foliar	0.11	6.7	15.0
Az. *chroococcum + Ps. aureofaciens,* foliar	0.11	1.4	20.2
Az. chroococcum + Ps. aureofaciens + microelements foliar	0.12	8.76	18.1
$DL_{0.5}$	0.1	1.7	0.8

As a rule, the number of phosphorus compounds in the soil is quite high, but most of it is not available for plant nutrition. In addition, a significant part of inorganic phosphorus compounds used as fertilizer is chemically immobilized in a relatively short period and becomes inaccessible to plants, which limits production (Naoko & Wasaki, 2010). Thus, the solubilization and mineralization of phosphorus by phosphate-mobilizing bacteria is an important feature of PGPB. The content of exchangeable potassium increased only in the variants with foliar fertilization with *Az. chroococcum* + *Ps. aureofascens* applied without and with microelements. Analogical effect was mentioned by some researchers (Avis et al., 2008). The soil analysis in the experiment demonstrated insignificant changes in the content of nutritional elements. A statistically significant increase in ammoniacal nitrogen was found in the variant with the application of *Ps. aureofaciens + Az. chroococcum,* foliar + micoelements foliar. A mathematically assured increase in nitric nitrogen concentration has also been demonstrated. Especially for the variants with the application of the suspension of *Ps. fluorescens + Az. chroococcu*m, in soil + microelements, foliar, as well as in the variant with application of metabolites of *Ps. aureofaciens + Az. chroococcum*, foliar. A trend of increasing mobile phosphorus in the plant rhizosphere was observed in the experimental variants. In the specialized literature, it is mentioned that the rhizospheric bacteria, especially the *Azotobacter* and *Pseudomonas* strains, contribute to increasing the amount of nutrients available in the soil. On the other hand, it is known that the increased accumulation of nitric nitrogen (NO_3) in the soil leads to a decrease in the consumption of nitrates by In our variants with the application of rhizobacteria and microelements, the growth of plants was much more intensive compared to the control, correspondingly - and the consumption of nitrates from the soil by the plants, which can explain the decrease in the content of nitric nitrogen in the soil (Martínez-Viveros et al., 2010). The content of exchangeable potassium increased only in the variants with foliar fertilization with *Az. metabolites, chroococcum* + *Ps. aureofaciens* applied without and with micoelements. The data obtained in general coincide with those obtained by some researchers in experiments with other plant species, who established that rhizospheric bacteria in particular contribute to increasing the amount of nutrients in the soil accessible to plants, especially mobile phosphorus.

Table 3. The content of nutrients in the rhizosphere of vine plants, the Presentable variety, the experience from the vegetation complex, mg/100 g soil

Experimental variant	N-NH_4	N-NO_3	P_2O_5	K_2O
Control	0.8	2.58	2.8	24.4
Ps. fluoreiscens + *Az. chrocoocum,* in soil	0.9	3.43	3.0	22.0
Ps. fluorescens + *Az. chroococcum,* in soil + trace elements, foliar	0.9	5.37	3.1	19.2
Ps. aureofaciens + *Az. chrocoocum*, foliar	1.0	6.16	2.9	20.4
Ps. aureofaciens + *Az. chroocoum,* foliar + trace elements, foliar	1.4	3.43	2.9	20.4
$DL_{0.5}$	0.2	1.5	1.2	1.6

The role of microelements in plant metabolism is difficult to overestimate (Gartel, 1974).

They are involved in plant nutrition directly as nutrients and as activators of many metabolic processes. The ionic forms of Fe, Zn, Cu, Mn, B and Ni are part of or act as cofactors in numerous enzymes. The determination of the content of the mobile forms of microelements in the soil of the experimental lot with the developed scheme demonstrated the following tendency: the incorporation of bacterial suspensions into the soil contributed to the decrease of the Fe content, but the foliar application of the metabolites maintained the Fe content at the level of the control (Table 4). Iron is the fourth most abundant element on earth. In aerobic soils, it is poorly absorbed by bacteria or plants, because iron ions, which prevail in nature, are poorly soluble, so their amount, which is available for assimilation by living organisms, is extremely low. And microorganisms and plants need a high level of iron. All bacterial products contributed to the increase of Mn content in the rhizospheric soil; bacterial suspensions and metabolites, applied together with microelements, significantly increased the Zn content in the rhizospheric soil; the content of Cu in the given case is almost in all variants at the level of the control and practically does not depend on the application of fertilizers (Marleny, 2006).

Table 4. The content of microelements in the rhizosphere, the experience from the plant complex, the Codrinski variety, (mg/kg)

Experimental variant	Cu	% control	Fe	% control	Zn	% control	Mn	% control
Control	4.0	100	504.0	100	2.3	100	15.2	100
Az. chroococcum, in soil	4.0	100	448.0	88.9	2.3	100	17.1	112.5
Ps. fluorescens, in soil	4.0	100	392.0	77.8	3.9	169.6	18.7	123.0
Az. chroococcum + *Ps. fluorescens,* in soil	4.6	115	448.0	88.9	2.3	100	22.2	146.1
Az. chroococcum + *Ps. fluorescens* in soil + microelements, foliar	4.0	100	448.0	88.9	7.0	304.3	22.2	146.1
Az. chroococcum + *Ps. fluorescens* + microelements, foliar	4.0	100	448.0	88.9	7.3	317.4	16.4	107.8
Az. Chroococcum, foliar	4.4	112	448.0	88.9	7.0	304.3	19.9	130.9
Ps. aureofaciens, foliar	4.0	100	504.0	100	4.6	200.0	16.4	107.9
Az. chroococcum + *Ps. aureofaciens,* foliar	4.0	100	504.0	100	7.9	343.5	18.7	123.0
Az. chroococcum + *Ps. aureofaciens* microelements foliar	4.0	100	616.0	122.2	2.3	100	17.6	115.8
$DL_{0.5}$	0.1		1.1		2.5		1.5	

It is known that pseudomonads, including *Ps. fluorescens*, in order to survive in conditions with limited reserves of available iron, synthesize soluble yellow-green fluorescent pigments - siderophores, with low molecular weight, molecules with a particularly high affinity for Fe $^{3+}$, as well as membrane receptors capable of binding to siderophore complexes of iron, thus facilitating the absorption of iron by microorganisms (Asada, 1999). Binding of iron to pseudomonad siderophores results in restriction of phyto-pathogen growth and enhancement of plant growth. According to the data presented, the lowest iron content in the soil was in the variant with the incorporation of the *Ps. fluorescens* (77.8% compared to the control), and the foliar fertilization of plants with a solution of metabolites of *Az. chroococcum* + *Ps. aureofaciens* + microelements, foliar contributed to increasing the iron level in the rhizosphere (by 20% compared to the control). The supply of iron to plants is particularly important under conditions of exposure of plants to stress factors, especially caused by heavy metals. The determination of the content of mobile forms of microelements in the rhizosphere of the cuttings at the end of the experiment highlighted the fact that, when planting the cuttings, the application of the bacterial suspension of *Ps. fluorescence* + *Az. chroococcum* in soil separately and by combining extraradicular fertilization with microelements, foliar caused an increase in the level of Zn in the soil. This fact indicates the increase in the content of accessible forms of these elements for plants (Table 5). Grapevine being a perennial crop, it is very sensitive to the imbalance of microelements, and the rhizospheric bacteria acted as shock absorbers that balanced the trophic stresses in the soil and plants (Fuentes-Ramires & Caballero-Mellado, 2006).

Table 5. The content of accessible forms of microelements in the rhizosphere under the action biofertilizers, mg/kg soil

Experimental variant	Zn	Cu	Fe	Mn
Control	1.6	4.2	175.7	6.4
Ps. fluorescens+ Az. chroococcum, in soil	5.9	4.4	186.6	7.4
Ps. fluorescens+ Az. chroococcum, in soil + microelements foliar	1.,0	4.2	175.2	6.9
Ps. aureofaciens+ Az. chrocoocum, foliar	2.0	5.5	177.7	5.5
Ps. aureofaciens+ Az. Chroococcum + microelements, foliar	1.4	4.3	167.8	6.6
$DL_{0.5}$	2.3	0.9	1.4	0.8

The content of microelements in the soil under the plants per fruit in the experiment on the experimental plot was determined in the second half of the vegetation period - over a month after the triple foliar fertilization with bacterial metabolites and microelements. The content of Cu in the soil under the fruit plants was significantly increased, 14.4 and 4.0 mg/kg, due to multiple treatment with preparations containing copper against *Plasmopara viticol*a (Table 6). There is the opinion that it accumulates in the superficial layers of the soil and does not migrate on the soil profile in depth. The data presented show the increased content of Cu on 0-30 cm. Previously, it was demonstrated that the increased share of Cu in the soil is accompanied by the decrease in the content of the accessible forms of microelements Fe and Zn, compared to the soil occupied by annual plants.

Table 6. Content of accessible forms of microelements in soil under grapevines after foliar fertilization, experimental lot, Codrinski variety, mg/kg

Experimental variant	The depth, cm	Cu	% control	Fe	% control	Zn	% control	Mn	% control
Control	0-30	14.40	100	475.0	100	3.46	100	34.55	100
	30-60	8.80	100	409.0	100	3.45	100	34.52	100
Ps. aureofaciens, foliar	0-30	15.20	105.6	382.0	80.4	4.32	124.8	43.20	125.0
	30-60	12.80	145.5	426.0	104.2	4.47	129.6	44.70	129.6
Az. chroococcum, foliar	0-30	12.80	88.9	455.0	95.8	4.47	129.2	44.70	129.4
	30-60	14.00	97.2	455.0	111.2	5.70	165.2	56.95	165.1
$DL_{0.5}$		2.5		3.6		1.2		1.5	

The tendency to decrease the content of Cu in the soil in the variants with Microcom is clearly pronounced, which confirms our assumption about the effect of microelements, prepared with microelements, in reducing the excessive content of this element in the soil under perennial plants.

CONCLUSIONS

Application of suspensions and metabolites of bacteria with plant growth stimulating function, *Az. chroococcum, Ps. aureofaciens* and *Ps. fluorescens* (PGPB) contributes to the more intense accumulation of nutrients by plants, which contributes to the reduction of the erosion process.

The application of the complex of micro-elements and together with the suspensions and metabolites of bacteria with plant growth stimulating function, *Az. chroococcum, Ps. aureofaciens* and *Ps. fluorescens* (PGPB) contributes to increasing the accessibility of nutrients to grapevine plants, due to the bacteria's ability to efficiently dissolve inaccessible forms of nutrients.

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WESTERN CORN ROOTWORM (*Diabrotica virgifera virgifera* Le Conte) - APPEARANCE AND DISTRIBUTION IN CENTRAL-SOUTH BULGARIA

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Abstract

From a plant-protection point of view, corn is attacked by many enemies (multivorous and specialized), which significantly reduce the yield, in individual years they multiply massively and are able to completely compromise the harvest. In recent years, a new corn enemy has appeared, the western corn rootworm (Diabrotica virgifera virgifera Le Conte), which is causing significant problems. For this purpose, the appearance and population dynamics of the species were followed. The studies were conducted during the period 2022-2023 in corn fields in the region of Chirpan and Plovdiv on an area of 1000 ha. Carrying out monitoring in the region of Central- South Bulgaria is important for establishing the spread of the species and organizing a whole complex of phytosanitary measures to limit the spread of the western corn rootworm.

Key words: *Diabrotica virgifera virgifera, monitoring, Central-South Bulgaria, maize.*

INTRODUCTION

Maize is attacked by a number of enemies, of which the western corn rootworm *(Diabrotica virgifera virgifera* Le Conte) is an economically important species. Costs in the US for control of this enemy and production losses are over US$1–2 billion annually and are among the largest for insect control (Metcalf, 1986; Steffey et al., 1994; Sappington et al., 2006). Losses of €300 billion are currently looming, with forecasts of as high as €500 billion.

338 species belong to the genus *Diabrotica* (Wilcox, 1972). Of these, 10 species are of economic importance (Krysan & Mileer, 1986), and three species damage maize (McDonald, 1989): *Diabrotica barberi* Smith & Laurence, *Diabrotica undecimpunctata howardi* Barber and *Diabrotica virgifera virgifera* Le Conte (Smith & Lawrence, 1967). The species: *Diabrotica barberi* and *Diabrotica virgifera virgifera* are of greatest economic importance. In the USA, out of 32000000 ha of corn areas, 12000000 ha are affected by *Diabrotica virgifera virgifera* (Frolov, 2012).

In 1909, damage by *Diabrotica virgifera virgifera* was first recorded on corn in the United States. In Europe, the species entered in 1992 from America (Krysan & Smith, 1987) and was first discovered in the area of Surčin, near Belgrade Airport in 1992 on an area of nearly 60 ha by the Serbian entomologist Franja Baća (Baća, 1994; Miller et al., 2005). In the following years, the expansion grew and the pests entered a number of European countries, such as: Hungary (1995), Croatia (1995), Romania (1996), Bosnia and Herzegovina (1997), Montenegro (1998), Bulgaria (1998), Italy (1998)) and others. (Edwards et al., 1998; Edwards et al., 2010).

In Bulgaria, the western corn rootworm (*D. virgifera virgifera*) was detected for the first time in 1998 in the western part of the country in Vidin region (Orsoya village), and later in Montana region (Bregovo city, Archar village). Today the species can be found in many regions of our country.

Both adults and larvae cause damage to plants. At first the adults make damage that resembles that of the wheat leech (*Lema melanopa* L.), later they feed on the silk by gnawing at the tip and it stands as if cut with scissors. This is the reason for the poor pollination of the cobs. Initially, the larvae feed on the young roots of the plants, later they enter the root system and the root collar, and as a result of feeding, the plants lag behind in their development, the damage site resembles a goitre because growth occurs in the ground part, the stems become

unstable and in rain or wind the plants lie down.
According to Chaing (1973), at 29 larvae on the roots, the plant died.
A special place in the fight against the western corn rootworm (*D. virgifera virgifera*) is monitoring for adults and larvae. In adults, it is carried out by visual observations, using pheromone and pherocone traps.
The density of the lavas is established through soil excavations and observations during the growing season for the appearance of "goose neck" damage.
To limit the spread and multiplication of this enemy, a number of measures are carried out such as: crop rotations, soil treatments, optimal fertilization and use of resistant varieties.
According to Lance & Sutter (1990); Metcalf et al. (1987) plants of the family *Cucurbitaceae* attract adults. For this reason, they are one of the main ingredients in granular food baits for adult forms. When organizing a timely fight against adults, their density and multiplication is significantly reduced, as a result of which losses are reduced and the harvest is preserved.

MATERIAL AND METHODS

The studies were conducted during the period 2022-2023 in corn fields with hybrid DEKALB® in the region of the city of Plovdiv and hybrid DKC 4709 in the city of Chirpan on a total area of 1000 ha.
For adult monitoring the standard entomological methods were used: visual observations and placement of pheromone traps (Figure 1).

Figure.1 Pheromone traps

Visual observations were carried out on all areas sown with maize during the emergence period. Through them, the phase and state of the surveyed culture were established, as well as the moments of appearance of the individual stages of the enemy. They also give an idea of the density of the species
Pheromone traps were placed during July and counted once every 10 days. They were used to track the appearance of adult individuals in corn fields, as well as their density during the growing season. One pheromone trap is placed every 0.6 ha.
The degree of larval attack was reported on the Iowa scale (1 to 6).

RESULTS AND DISCUSSIONS

The first adult forms of the enemy in the pheromone traps were detected on July 12 - three in the Plovdiv area and one in Chirpan.

As the weather warmed, the species increased its numbers and in the second half of August, the species reached its highest density, 14 in the fields in Plovdiv and 10 in Chirpan. At the end of August, the density of beetles decreased (Figure 2).
In 2023, the first adults were detected three days earlier than the previous year - the first ten days of July, due to the higher average day-night temperatures for the period in both regions. They reached 27.4ºC for Plovdiv and 26.4ºC for Chirpan and are a consequence of the higher maximum temperatures for the month (Figure 3, Table 1).
The density of the species began to increase in both areas, with a peak in the multiplication of the species being registered during the first ten days of August. 19 adults were found for the Plovdiv region, respectively 11 for Chirpan. Extremely high temperatures for the period combined with prolonged droughts led to the early harvesting of maize, which explains the lower density of the species at the end of August.
For the period of the study in 2022, 93 adults were caught in Plovdiv and 60 in the corn fields in Chirpan (Figure 4).

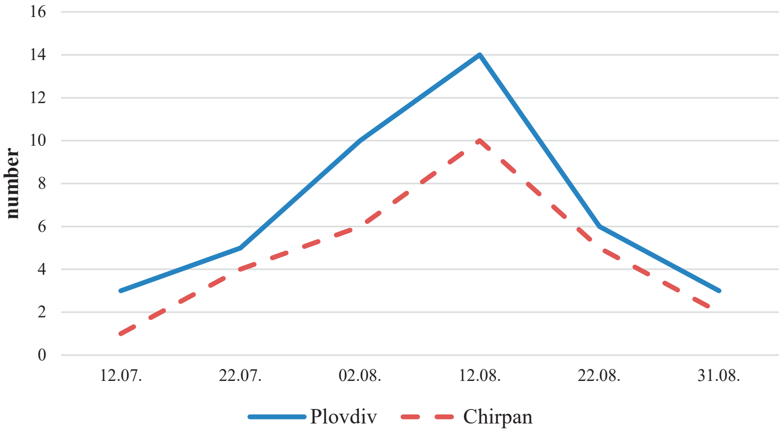

Figure 2. Population dynamics of western corn rootworm in 2022
in corn fields in the regions of Plovdiv and Chirpan

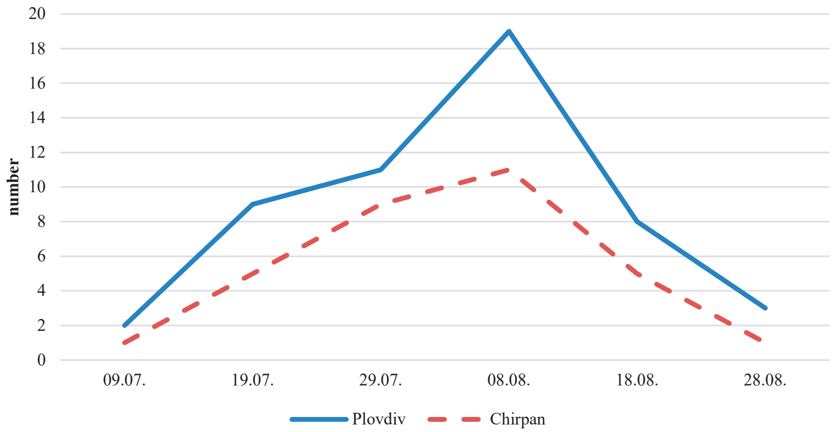

Figure 3. Population dynamics of western corn rootworm in 2023
in corn fields in the regions of Plovdiv and Chirpan

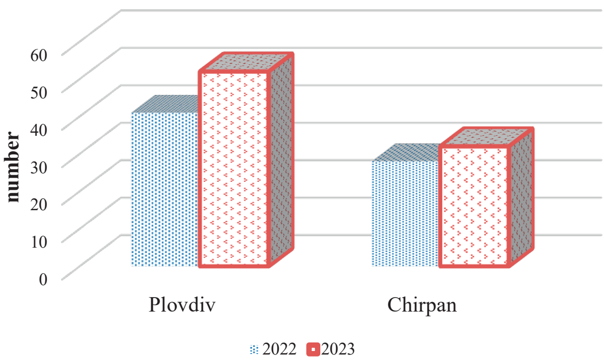

Figure 4. Captured adults of western corn rootworm established in 2022-2023
in corn fields in Plovdiv and Chirpan regions

Damage from the larvae and adults of the western corn rootworm was also reported during the surveys.

In 2022, 5±0.01% damage by larvae was recorded, and in 2023, respectively, 10±0.01% (Figure 5). It's a 2 on the assault. Iowa scale. In 2022, *Diabrotica virgifera virgifera* was lower in abundance, resulting in 25±0.01% damaged plants by adults.

The conditions of the environment in 2023 favored the development of the species, as a result of which the established damages from the adult forms reached 35±0.01% (Figure 6).

Table 1. Weather conditions in 2022-2023

	Chirpan			Plovdiv		
Month / Year	VII	VIII	IX	VII	VIII	IX
Rainfall, mm						
2022	7.7	68.8	34.9	37.3	27.4	16.8
2023	25.4	26.4	30.1	20.4	25.3	35.8
Average T^0C						
2022	25.1	25.2	18.9	25.3	25.2	19.7
2023	26.4	26.2	21.9	27.2	26.4	21.9
Min T^0C						
2022	15.4	17.8	12.1	14.7	13.8	5.2
2023	18.0	17.8	14.8	13.9	13.1	10.9
Max T^0C						
2022	33.1	33.2	27.2	38.6	36.8	35.8
2023	34.8	33.6	29.5	40.8	41.7	35.0

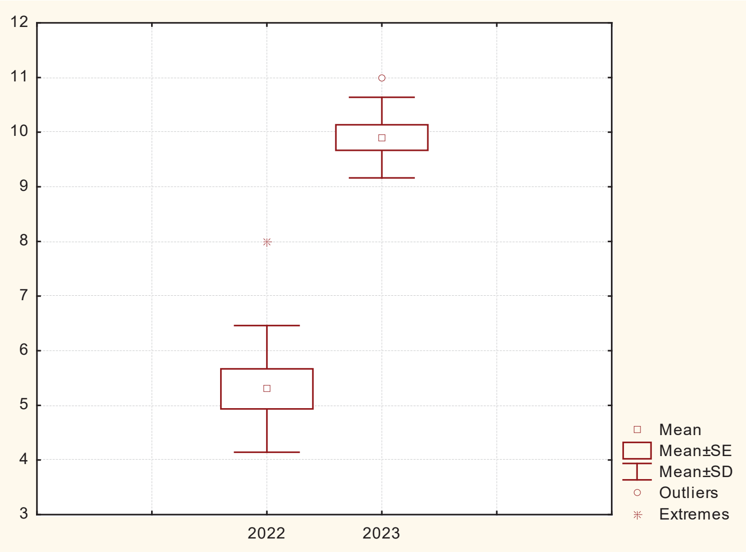

Figure 5. Larval damage (%) in 2022-2023

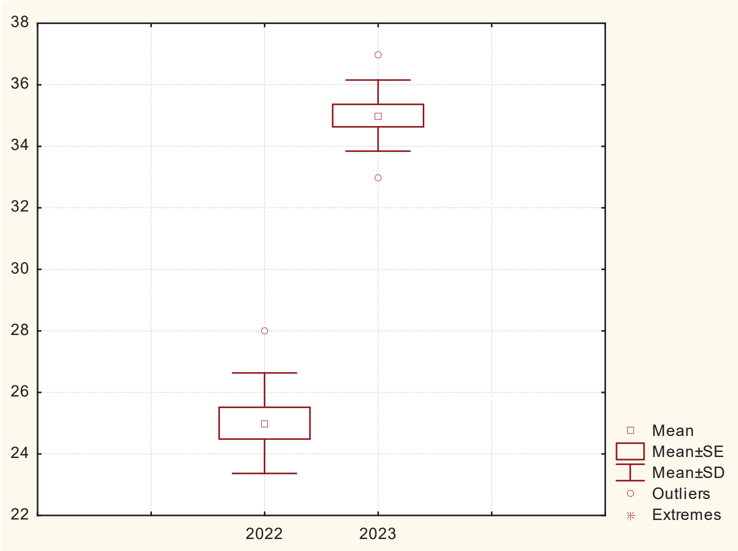

Figure 6. Adult damage (%) in 2022-2023

CONCLUSIONS

As a result of the observations, the following conclusions and recommendations can be made:

- In recent years, a significant increase in the density of the western corn rootworm (*Diabrotica virgifera virgifera* Le Conte) has been observed in the region of Central- South Bulgaria.
- The first adult individuals in the corn fields were found on July 12, 2022 and on July 9, 2023. The peak in the multiplication of the species was found in the middle of August in both years of the study.
- In 2022, the registered damage from the larvae of *Diabrotica virgifera virgifera* was 5±0.01%, and in 2023, respectively, 10±0.01%.
- In 2022, *Diabrotica virgifera virgifera* was found in a lower density, as a result of which 25±0.01% damaged plants were reported from the adults. The environmental conditions in 2023 favored the development of the species, with 35±0.01% of the damage recorded by the adult forms.

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THE MANAGEMENT OF WEEDS USING NEW GENERATION HERBICIDES IN MAIZE

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Abstract

Maize (Zea mays L.) is one of the most versatile multi-purpose crop used as feed and food crop beside other no-food uses. During 2023 year a field experiment in a randomized complete block design with three replications and fourteen treatments was carried out in the Agricultural and Research Station Caracal with the aim of evaluation of weed control in maize, using new generation herbicides. The treatments were composed of isolated and associated herbicides and all were considered selective in maize via pre-emergence (PRE) and post-emergence (POST) applications. The efficacy evaluation was done at 7, 14, 21 and 28 days since each treatment targeting CHEAL, HIBTR, DATST, POLSS, CONAR, ECHCG, SETVI, SORHA, CYNDA, DIGSA, MATSS, AMBEL, GALPA, POROL, SOLNI. Results revealed that, among the herbicidal treatments, the best efficacy was recorded by SAE 053 H/01 + Baracuda doze p.c. 1.2 + 0.5; SAE 053 H/01 + Nico 40 OD doze p.c. 1.2 + 0.5; SAE 053 H/01 + Baracuda +Nico 40 OD doze p.c. 1.2 + 0.5 + 0.5.

Key words: *efficacy, weeds management, new herbicides, Zea mays L*

INTRODUCTION

Since its domestication some 9,000 years ago, maize (*Zea mays* L., also commonly known as corn) has played an increasing and diverse role in global agri-food systems (Awika, 2011; Kennett et al. 2020).

Nowadays, multiple roles and uses of maize (*Zea mays* L.) is still explored, due to its genetic diversity and economical importance, primarily as a feed globally and also as a food crop, besides other non-food uses.

In terms of production volume, maize is currently the most popular cereal and is expected to overtake all other crops as the most frequently grown and traded commodity in the next ten years, being so versatile in a wide range of climates and soils (Borleanu et al., 2012; Dragomir et al., 2022; Guzzon et al., 2021; Lamichhane et al., 2023; Partal et al., 2012a; Partal et al., 2012b; Partal and Paraschivu, 2020).

According to previous studies, over the next 30 years, the earth's average surface temperature will rise at a rate of about 0.2 degrees Celsius every decade having a significant impact on the growth and health of natural plant species and crops and their interactions with abiotic (changes in temperatures, warmer than long-term means or unseasonal frosts and precipitation including snow, hail or extreme intensity, variable humidity, drought, salinity, heat, etc.) and biotic (invasive species, weeds, pests, pathogens) constrainers, leading even at new reports about them in different world areas (Elad and Pertot, 2014; Solomon, 2007; Bernstein et al., 2008; Paraschivu et al., 2019; Răduțoiu and Băloniu, 2021; Răduțoiu et al., 2023; Soare et al., 2010a; Soare et al., 2010b; Velea et al., 2021; Zală, 2021; Zală et al. 2023a). Moreover, by 2080, global temperature is anticipated to increase by 4,5-degree Celsius declining by 6% in productivity per each degree Celsius (Asseng et al., 2015). All these constrainers impact directly natural vegetation features and crops production with economic consequences (Eschen et al., 2021; Feng et al., 2020; Hedlund et al., 2020; Păunescu et al., 2022; Răduţoiu, 2022; Răduţoiu and Stan, 2022; Răduțoiu, 2023; Sawicka and Egbuna, 2020; Tripathi et al., 2016).

There are several possible strategies including breeding, technical progress and improving fertilizer and pesticides efficiency to increase

crops production (Lipianu et al., 2023; Paraschivu et al., 2022; Sălceanu et al., 2022; Zală et al., 2023b).
Current problems related to the consequences caused by using pesticides along with those caused by agricultural pollution (Bonciu et al, 2020; Bonciu, 2023b, 2023c; Torrens & Castellano, 2014) require essential changes in plant breeding technologies (Bonciu, 2023a). One of the most modern such technologies is agricultural biotechnology and genomics (De Souza and Bonciu, 2022a, 2022b), which is able to ensure the creation of varieties and forms of plants with targeted performances: increased productivity and quality, resistance to biotic constrainers and tolerance to unfavourable climatic factors.
Among the biotic factors, weeds are one of the critical factors. The negative effects of annual and perennial weed species on maize yield have been documented in many studies previously (Absy, 2019; Idziak et al., 2022; Mhlanga et al., 2016; Samant et al., 2015; Tesfay et al., 2014; Zhang et al., 2013).
Reports have estimated around a 37% global loss in total maize production due to weeds (Oerke & Dehne, 2004; Sharma and Rayamajhi, 2022). It's possible that the maize plants won't be able to grow enough roots in weedy fields, but the main obstacle to increased maize yields is related to managing and controlling weed growth (Güncan & Karaca, 2014).
The amount of the loss depends on the weed flora's composition, when the weeds arise in relation to the crop, their density and intensity, and the crop's developmental stage in relation to the competition period (Singh et al., 2016). Since this is the phase when the components relevant to grain yield are established, competition with maize at the stage of five fully grown leaves has the greatest detrimental effect on the crop (Duarte et al, 2002).
Other studies showed than when weeds interference in maize from 36 weeks after sowing (WAS) significantly depressed the growth parameters and grain yield of maize, leading to 28-100% yield losses (Imoloame & Omolaiye, 2016; Jagadish et al., 2016). Moreover, hand weeding and hoeing methods were effective in coping with the annual weeds, but they were not effective in controlling perennial weeds (Idziak et al., 2022).
Therefore, the use of pre-emergence and post-emergence herbicides can be an effective way to manage weeds in maize, due to their fast results, easy application and low cost (Idziak et al., 2022). Also, compared to other methods, the chemical control method is quicker, more efficient, and requires less labour (Kakade et al., 2020; Qu et al., 2021; Sharma & Rayamajhi, 2022).
In practical, farmers use both pre-emergence and post-emergence herbicides intensively in maize fields. The effect of pre-emergence herbicides applied to the soil lasts about 40–50 days, but the secondary weed infestation, requires post-emergence foliar application (Delchev, 2021). However, when the combined use of pre-emergence and post-emergence herbicides targets both annual and perennial weeds, it will have more effects on weeds. In the future, herbicides will still be a useful tool in agriculture for controlling weeds as part of an integrated weed management strategy.
The aim of current study was to evaluate the selectivity and efficacy of combined effects of new generation herbicides for weeds management in maize with the different bioactive ingredients in natural conditions from ARDS Caracal, Romania.

MATERIALS AND METHODS

A field investigation was carried out in the field of the Agricultural Research Station Caracal (ARDS) of the University of Craiova, Romania (44°11’N and 24°37’E) during 2023 year to study the relative efficacy of new generation of herbicides on weed control in maize combined with different bioactive ingredients.
The trial was conducted in a split–split-plot design with the main plots arranged in a randomized complete block (RCBD – Fisher model) with three replicates. The size of each plot was 25 m^2.
All recommended cultural practices (i.e. fertilization with 250 kg ha-1 NPK 15:15:15 and spring dressing with 200 kg/ha NH_4NO_3 was performed, etc.) and other management (two times disc harrowing and two times cultivation before sowing) were applied.

The treatments were composed of isolated and associated herbicides and all were considered selective in maize via post-emergence (POST) applications.
The experiment included the following treatments:
V1. Untreated – control;
V2. SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – 1.2 l/ha;
V3. SAE 053 H/01 + Kaishi (80 g/l mesotrione + 30 g/l nicosulfuron + aminoacides) – 1.2 l/ha + 2 l/ha;
V4. SAE 053 H/01 + Improve 5 in 1 (80 g/l mesotrione + 40 g/l nicosulfuron + citric acid + aminoethanol) – 1.2 l/ha + 100 ml/100 l solution;
V5.Elumis OD (75 g/l mesotrione + 30 g/l nicosulfuron) – 1,2 l/ha;
V6. Elumis OD + Kaishi (75 g/l mesotrione + 30 g/l nicosulfuron + aminoacides) – 1.2 l/ha + 2 l/ha;
V7.Crew Ace OD + Baracuda (40 g/l nicosulfuron + 100 g/l mesotrione) – 1 l/ha + 1l/ha.
The herbicide products were applied post-emergent in BBCH 14-16, when maize had 4-6 leaves. The volume of the spraying solution was 400 l/ha. In the study, a back sprayer with a 25 L tank capacity, gasoline engine, and fan nozzles was used for herbicide application. Prior to the establishment of the trials, weed species and their densities were noted. In this regard, a 1 m^2 frame was used in the trial area, randomly replaced, and the weed species, growth stages, and the number of each weed species in the covered area or m^2 were recorded. Thus, the first evaluation of weeds spectrum was done before spraying targeting the following species: *Hibiscus trionum* (HIBTR), *Convolvulus arvensis* (CONAR), *Digitaria sanguinalis* (DIGSA), *Portulaca oleracea* (POROL), *Solanum nigrum* (SOLNI), *Xantium strumarium* (XANTIST), *Atriplex patula* (ATRPL), *Cirsium arvense* (CIRAR), and *Amaranthus retroflexus* (AMARE). The density and periodicity of weed population emergence determine the critical period of crop – weed competition. Thus, the densities of each species were calculated according the following equation:

$$\text{Density (plants/m}^2\text{)} = B/m,$$

where, "B" indicates the total number of individual plants in the samples and "m" represents the total number of meters (Odum & Barrett, 1971).
In addition, the scale suggested by Üstüner and Güncan (2002) was used to determine the density of the species (Table 1).

Table 1. Density scale of the weeds

Scale	Density level	Density (plants/m^2)
A	High dense	10+
B	Dense	1-10
C	Middle dense	0.1-1
D	Low dense	0.01-1
E	Rare	Less than 0.01

The efficacy of the studied herbicides on weed population and weed species, changes in weed population and species were observed four times at regular intervals after herbicide treatments on the 7th, 14th, 21th and on the 28th day after application.
The percentage of reduction in weed population was determined by comparing the treated plots with the weedy control plots. Each assessment specifies the phenology of the weeds and the effects on the weeds. The Abbott formula was used for determination of the effect on weeds at the species level and the effects on all weeds (Snedecor et al., 1967):

$$HPE = (CWN – TWN) X 100/CWN$$

where, "HPE" indicates Herbicide Percentage Effect, "CWN" indicates Number of Weeds in Control, "TWN" indicates Number of Weeds in Treatments.

The selectivity of the herbicides was evaluated by the 9 score scale of EWRS as described by Zhelyazkov et al. (2017) (at score 0 there are not damages on the crop, and at score 9 the crop is completely destroyed).
Statistical analysis of collected data was performed by using ANOVA and mathematical functions of MS Office Excel 2013 facilities. For relevant statistical differences ($p<0.05$) was used complementary test for multiple comparations Newman-Keuls.

RESULTS AND DISCUSSIONS

Worldwide, the production of maize is severely affected by weeds, which lowers crop yields and reduces farmer earnings, due to their competition with maize plants for space, light, water, and nutrients (Gianessi, 2013; Acharya et al., 2022; Chauhan, 2020; Maqsood et al., 2020). In this context, minimizing the detrimental effects of weeds on maize yield requires efficient weed management.

Sutton et al. (2002) explained that, in contrast to costly labor for weed eradication, the chemical approach of weed management is stress-free, adaptable, and affordable.

During the experiment, the effect of the applied herbicides varied according to the active ingredients of the herbicide and the weed species. In addition, the effectiveness of herbicides varied according to the assessment times. Also, many previous studies have been done on the efficacy and selectivity of herbicides in maize (Delchev, 2021; Grzanka et al., 2022; Iqbal et al., 2020; Jagła et al., 2020).

However, factors such as locations, maize cultivar, bioactive compound of herbicides, mode of actions of the herbicides, as well as weed species and their densities, are also critical predictors in weed management.

In maize crop the most representative weed species are: monocotyledons (*Setaria* sp., *Echinochloa crusgalli*, *Sorghum halepense* (seed and rhizomes), *Elymus repens*, *Eriochloa villosa*) and dicotyledons: *Amaranthus retroflexus*, *Chenopodium album*, *Solanum nigrum*, *Sinapis arvensis*, *Raphanus raphanistrum*, *Stellaria media*, *Thlaspi arvensis*, *Hibiscus trionum*, *Datura stramonium*, *Abutilon theophrasti*, *Cirsium arvense*, *Convolvulus arvensis*, *Sonchus arvensis*) (Popescu et al, 2009).

In the maize field trail in ARDS Caracal the structure of weeds was diverse, leading to an 85% of infestation degree, with mono- and dicotyledonous weeds ratio of 6:94). Most of the weeds were annual and perennial dicotyledonous plants, depending on previous crop and pedo-climatic conditions, as follows:

-Annual monocotyledonous: *Digitaria sanguinalis* (DIGSA) (6%); no perennial monocotyledonous was present.

- Annual dicotyledonous: *Portulaca oleracea* (POROL) – 16%, *Solanum nigrum* (SOLNI) – 36%, *Xantium strumarium* (XANTIST) – 1%, *Atriplex patula* (ATRPL) – 30%, *Amaranthus retroflexus* (AMARE) – 1%, *Hibiscus trionum* (HIBTR) – 4%;

Perennial dicotyledonous: *Convolvulus arvensis* (CONAR) – 5%; Cirsium arvense (CIRAR) – 1% (Figure 1).

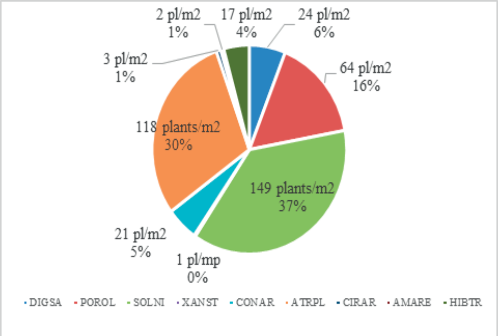

Figure 1. Weeds structure from maize crop on the experimental field

Amid the weeds observed, the highest density of weed species were found as *Atriplex patula* (ATRPL) (30%), *Solanum nigrum* (SOLNI) (37%), and *Portulaca oleracea* (POROL) (16%).

The assessment according with the scale suggested by Üstüner and Güncan (2002) showed that weeds density (weeds/m^2) ranged between Dense (B) and High-Dense (A). The results indicated that weeds density was high (A) for all weeds targeted species and all variants after 7 days from treatments application. At 14 days after treatments only in variants 4 (SAE 053 H/01 + Improve 5 in 1 (80 g/l mesotrione + 40 g/l nicosulfuron + citric acid + aminoethanol) – 1.2 l/ha + 100 ml/100 l solution), 5 (Elumis OD (75 g/l mesotrione + 30 g/l nicosulfuron) – 1.2 l/ha) and 6 (Elumis OD + Kaishi (75 g/l mesotrione + 30 g/l nicosulfuron + aminoacides) – 1.2 l/ha + 2 l/ha) was noticed a lower weeds density (B).

At 21days after treatments application weeds density was diminished but still high for variants 2 (SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – 1.2 l/ha) and 3 (SAE 053 H/01 + Kaishi (80 g/l mesotrione + 30 g/l

nicosulfuron + aminoacides) – 1.2 l/ha + 2 l/ha).
At 28 days after treatments only in variant 2 (SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – 1.2 l/ha) the weeds density was still high (A), while for all other treatments led to dense (B) (Figure 2).

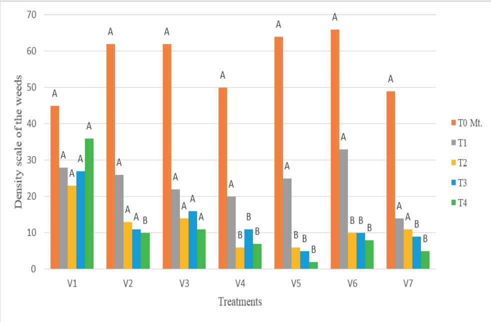

Figure 2. Weeds density according with the treatments applied and the assessment moments

According to the products utilized, the use of herbicide treatments had a substantial impact on the control of annual and perennial weed species in the treated version as compared to the untreated plot.
The herbicides efficacy (HPE - Herbicide Percentage Effect) ranged between 0 to 100% accordingly with selectivity, moment of application and assessment, the stage of weeds, the infestation degree and climatic conditions (Şerban et al., 2021).
Figure 3 shows the average efficacy results (%) obtained in the early post-emergence application of SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – 1.2 l/ha (V2).

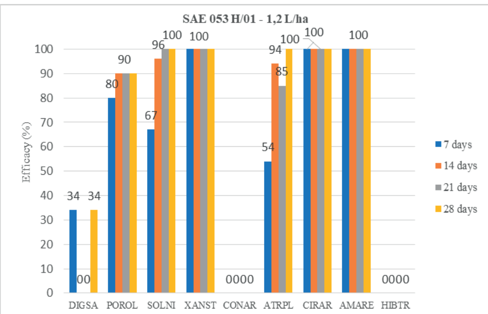

Figure 3. Efficacy (%) of SAE 053 H/01 postemergently applied in annual and perennial weeds controlling of maize crop, in 2023 (7, 14, 21, 28 days after treatment)

The results showed a control effect of 85-100% at 14, 21 and 21 days after treatments for annual dicotyledons (*Portulaca oleracea* (POROL), *Solanum nigrum* (SOLNI), *Xantium strumarium* (XANTIST), *Atriplex patula* (ATRPL), *Amaranthus retroflexus* (AMARE) and perennial dicotyledons *Cirsium arvense* (CIRAR). The annual monocotyledons *Digitaria sanguinalis* (DIGSA) was uncontrolled (34%).
Kaishi is a bio-stimulant of vegetal origin and enzymatic hydrolysis with role of increasing metabolism and boosting general growth of plants, but also improving absorption in plant tissues of fertilizers and plant protection products. Also, it decreases adverse effects generated by abiotic constrainers, such as vigour reductions caused by herbicide applications and a faster recovery of vegetative growth. The average efficacy results of the combination of herbicide SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – (1,2 L/ha) and Kaishi (aminoacides - 2 L/ha) showed a good degree of control to weed species, especially at 14, 21 and 28 days after treatments. Thus, this combined treatment efficacy ranged between 85-100% after 14, 21 and 28 days after treatment. Excepting the annual dicotyledons *Hibiscus trionum* (HIBTR), all assessed weeds showed a high degree of control. At 7 days after treatment only the annual monocotyledonous *Digitaria sanguinalis* (DIGSA) and annual dicotyledons *Solanum nigrum* (SOLNI), *Atriplex patula* (ATRPL) was not complete controlled, the efficacy of the herbicide SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – (1.2 l/ha) and Kaishi (aminoacides - 2 l/ha) ranging between 50-70% (Figure 4).

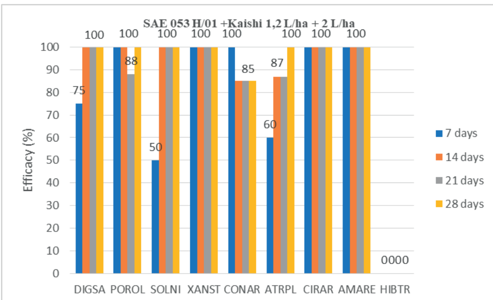

Figure 4. Efficacy (%) of SAE 053 H/01 + Kaishi postemergently applied in annual and perennial weeds controlling of maize crop, in 2023 (7, 14, 21, 28 days after treatment)

When the herbicide SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – (1.2 l/ha) was mixed with Improve 5 in 1 (80 g/l mesotrione + 40 g/l nicosulfuron + citric acid + aminoethanol) – (1.2 l/ha + 100 ml/100 l solution) (V4) The results show a control effect greater than 90% for the annual monocotyledons (*Digitaria sanguinalis* (DIGSA) and annual dicotyledons *Amaranthus retroflexus* (AMARE), *Solanum nigrum* SOLNI), *Xanthium strumarium* (XANST), *Portulaca oleracea* (POROL), *Atriplex patula* (ATRPL) and perennial dicotyledons (*Cirsium arvense* (CIRAR) at 14, 21 and 28 days. This combination proved low efficacy at 7 days for *Portulaca oleracea* (POROL), *Solanum nigrum* SOLNI) and *Atriplex patula* (ATRPL). The lowest efficacy was noticed in perennial dicotyledons Convolvulus arvensis (CONAR) that ranged between 65 to 82% (Figure 5).

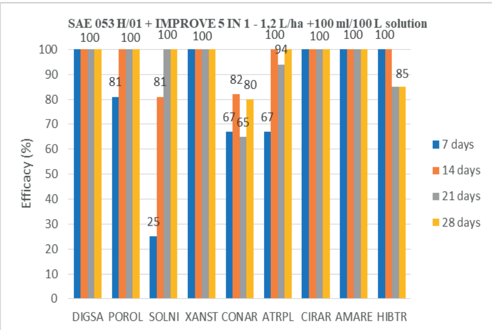

Figure 5. Efficacy (%) of SAE 053 H/01 + Improve 5 in 1 postemergently applied in annual and perennial weeds controlling of maize crop, in 2023 (7, 14, 21, 28 days after treatment)

Ones of the best results in controlling maize weeds were noticed when the herbicide Elumis OD (1.2 l/ha) was applied even after 7 days with efficacy between 93-100%.

In case of the annual monocotyledonous weed *Digitaria sanguinalis* (DIGSA) the herbicide Elumis OD showed lower efficacy (75%) after 7, 14 and 21 days after treatment, but proved 100% control at 28 days after treatment.

The best control with 100% efficacy was observed at all assessment moments after treatment for annual dicotyledons *Amaranthus retroflexus* (AMARE), *Xanthium strumarium* (XANST), *Portulaca oleracea* (POROL), *Hibiscus trionum* (HIBTR) and perennial dicotyledonous *Convolvulus arvensis* (CONAR), *Cirsium arvense* (CIRAR) (Figure 6). For the weeds *Digitaria sanguinalis* (DIGSA), *Solanum nigrum* (SOLNI), *Atriplex patula* (ATRPL) the herbicide efficacy was lower at 7 days after treatment (75%, 30%, respectively 81%).

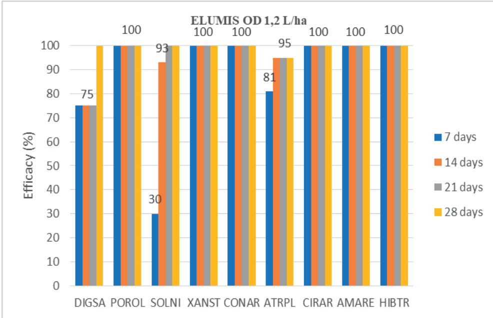

Figure 6. Efficacy (%) of ELUMIS OD post-emergently applied in annual and perennial weeds controlling of maize crop, in 2023 (7, 14, 21, 28 days after treatment)

When the herbicide Elumis OD was combined Kaishi the efficacy at 7 days ranged between 49% to 100%.

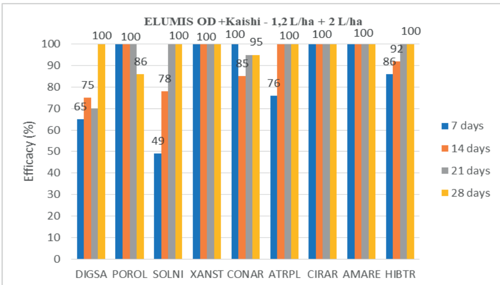

Figure 7. Efficacy (%) of ELUMIS OD + Kaishi post-emergently applied in annual and perennial weeds controlling of maize crop, in 2023 (7, 14, 21, 28 days after treatment)

The results showed a better control of weeds and a increased efficacy of Elumis OD at14 and 21 days after treatment when it was applied alone than in the variant when it was applied combined with Kaishi. A possible explanation might be the stimulant effect of Kaishi also on weeds not only maize plants, as slightly side effect. For the annual monocotyledons *Digitaria sanguinalis* (DIGSA), the annual dicotyledons *Xanthium strumarium* (XANST), *Amaranthus retroflexus* (AMARE), the perennial dicotyledonous *Convolvulus arvensis* (CONAR), *Cirsium arvense* (CIRAR) the efficacy of ELUMIS OD + Kaishi was 100% after 7, 14, 21 and 28 days post-treatment.

When the combination Crew Ace OD + Baracuda (40 g/l nicosulfuron + 100 g/l mesotrione) – 1 l/ha + 1 l/ha was applied all assessed weeds were controlled 100% at 7, 14, 21 and 28 days after treatment, excepting *Portulaca oleracea* (POROL), *Solanum nigrum* (SOLNI), *Atriplex patula* (ATRPL) that showed good efficacy at 14, 21 and 28 days after treatment. The annual dicotyledons *Hibiscus trionum* (HIBTR) was uncontrolled (20-35%) (Figure 8).

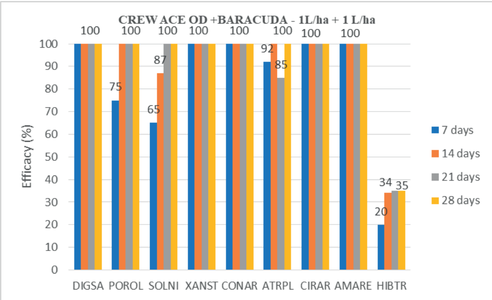

Figure 8. Efficacy (%) of CREW ACE OD +BARACUDA post-emergently applied in annual and perennial weeds controlling of maize crop, in 2023 (7, 14, 21, 28 days after treatment)

In the experimental field, the selectivity assessment for all herbicides variants had no phytotoxic effects (EWRS scale = 0) (Table 2).

Table 2. The selectivity (%) of herbicide treatments post-emergently applied at the maize crop 2023 (7- 14 - 21- 28 days after treatment)

Var.	Treatments	Dose	Time*	Selectivity %			
				7	14	21	28
1	Untreated – control	-	-	No phytotoxic effects**			
2	SAE 053 H/01	1,2 l/ha	P-EM				
3	SAE 053 H/01 + Kaishi	1,2 l/ha + 2 l/ha	P-EM				
4	SAE 053 H/01 + Improve 5 in 1	1,2 l/ha + 100 ml/100 l solution	P-EM				
5	Elumis OD	1,2 l/ha	P-EM				
6	Elumis OD + Kaishi	1,2 l/ha + 2 l/ha	P-EM				
7	Crew Ace OD + Baracuda	1 l/ha + 1l/ha	P-EM				

*P-EM = Post-Emergent in BBCH 14-16, when maize had 4-6 leaves
** (EWRS scale = 0, where 0 means not damages on the crop, and score 9 means the crop is completely destroyed).

The results of the experiment show that chemical control of the weed species existing in the maize crop is an important and necessary technological measure.

CONCLUSIONS

Managing weeds through pre-emergence, post-emergence and sequential use of herbicides will be an ideal means for controlling the weeds in the view of economics and effectiveness in maize.

All herbicide treatments used in the experiment had a good selectivity for maize plant without exhibiting phytotoxic effects. The results of the experiment revealed that weed density at 21 and 28 days after sowing (DAS) was significantly affected by all weed control treatments, excepting variants 2 (SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – 1.2 l/ha) and 3 (SAE 053 H/01 + Kaishi (80 g/l mesotrione + 30 g/l nicosulfuron + aminoacides) – 1.2 l/ha + 2 l/ha).

The herbicide SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – 1.2 l/ha) showed a control effect of 85-100% at 14, 21 and 21 days after treatments for annual dicotyledons *Portulaca oleracea* (POROL), *Solanum nigrum* (SOLNI), *Xantium strumarium* (XANTIST), *Atriplex patula* (ATRPL), *Amaranthus retroflexus* (AMARE) and perennial dicotyledons *Cirsium arvense* (CIRAR). The average efficacy results of the combination of herbicide SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – (1.2 l/ha) and Kaishi (aminoacides - 2 L/ha) showed a good degree of control to weed species, especially at 14, 21 and 28 days after treatments. Thus, this combined treatment efficacy ranged between 85-100% after 14, 21 and 28 days after treatment. When the herbicide SAE 053 H/01 (80 g/l mesotrione + 30 g/l nicosulfuron) – (1.2 L/ha) was mixed with Improve 5 in 1 (80 g/l mesotrione + 40 g/l nicosulfuron + citric acid + aminoethanol) – (1.2 l/ha + 100 ml/100 l solution) (V4) The results show a control effect greater than 90% for the annual monocotyledons (*Digitaria sanguinalis* (DIGSA) and annual dicotyledons *Amaranthus retroflexus* (AMARE), *Solanum nigrum* SOLNI), *Xanthium strumarium* (XANST), *Portulaca oleracea* (POROL), *Atriplex patula* (ATRPL) and perennial dicotyledons *Cirsium arvense* (CIRAR) at 14, 21 and 28 days.

Ones of the best results in controlling maize weeds were noticed when the herbicide Elumis OD (1,2 L/ha) was applied even after 7 days with efficacy between 93-100%. The results showed a better control of weeds and a increased efficacy of Elumis OD at 14 and 21 days after treatment when it was applied alone than in the variant when it was applied combined with Kaishi.

When the combination Crew Ace OD + Baracuda (40 g/l nicosulfuron + 100 g/l mesotrione) – 1 l/ha + 1 l/ha was applied all assessed weeds were controlled 100% at 7, 14, 21 and 28 days after treatment, excepting *Portulaca oleracea* (POROL), *Solanum nigrum* (SOLNI), *Atriplex patula* (ATRPL) that showed good efficacy at 14, 21 and 28 days after treatment.

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PRODUCTIVITY AND STABILITY OF BULGARIAN TRITICALE CULTIVARS UNDER DIFFERENT LEVELS OF NITROGEN FERTILIZATION AND CONTRASTING ENVIRONMENTS

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Abstract

In order to determine suitable combinations between genotypes and agronomy practices based on productivity, stability and adaptability, four triticale genotypes (Kolorit, Bumerang, Respekt and Atila) were studied in three contrasting growing periods and under four levels of nitrogen fertilization. The productivity, stability and adaptability were determined by using the method of Eberhart and Russell and by AMMI analysis. Cultivar Bumerang was with the highest productivity, in all levels of nitrogen fertilization, while cultivar Respekt was with the lowest yields. A tendency was observed toward higher effect of the genotype, while the effect of the year conditions decreased with the higher nitrogen nutrition; the effect of the genotype x environment remained almost constant. The highest stability, averaged for this experiment, was that of cultivar Bumerang, and the lowest - of cultivar Kolorit. With the exception of the variant without nutrition, both cultivars had a comparatively stable response to the other nitrogen norms, which makes them suitable for growing under the soil and climatic specificity of Bulgaria at varied nutrition regimes.

Key words: *adaptability, nitrogen nutrition, fertilizer norm, triticale, stability.*

INTRODUCTION

Increasing the yields from the crop plants under field conditions is a key task of agricultural production. It arises from the constantly increasing demands for quality food and feed sources worldwide. In this relation, there are two main approaches to increase the productivity of a crop – to choose a more productive genotype or to apply agronomy practices suitable for growing of the respective genotype. With the use of both approaches under conditions of a changing climate, and in a certain geographic region – under conditions of dynamic meteorological parameters, it becomes possible both the genotype and the applied agronomy practice to ensure predictable, i.e. stable yields.

Triticale, being a part of the forage or grain production in an agricultural farm, is characterized by a high production potential with regard to both grain yield and biomass productivity. This is related to its biological an economic properties allowing to grow the crop in a wide area of environmental conditions and under high levels of biotic and abiotic stress. According to data provided by the Ministry of Agriculture and Foods, in 2019 a mean yield from triticale of 2657 kg/ha was registered in Bulgaria. During 2010-2022, the mean annual yields varied from 2.453 t/ha in 2012 to 3.193 t/ha in harvest year 2014, the trend for grain yield being positive. In 2022, seventeen Bulgarian triticale cultivars were included in the official varietal list of Bulgaria (Executive Agency of Variety Testing, Field Inspection and Seed Control, 2022).

One of the ways to increase triticale grain production is to use more efficiently the potential of the crop. Similar to any biological entity, triticale is characterized by a certain susceptibility to some biotic and abiotic factors at genotype level. This determines the different production potential of the developed varieties and lines and is the reason for varied responses of a given genotype to variable conditions of the environment. When conducting Multi Environment Trials (METs), regardless of the crop, the genotypes are ranked differently under different environments. This relates to the effect of the genotype x environment interaction and to the different stability resulting from this interaction. There are various methods for assessment of the stability of a set of genotypes subjected to investigation. According to Becker and Leon (1988), stability can be static and dynamic depending on the point of view: biological (the changes of the environmental conditions are not considered) or agronomic (the changeable environmental conditions are taken into account, as well as the tendency towards change of the average level of productivity of the set of cultivars).

Currently, a large number of parameters have been identified, which can be adequately used for assessment of stability and adaptability (Crossa et al., 1990). According to Tsenov et al. (2022), the use of one method only is not a correct approach since the different approaches reflect to different degrees the yield-stability combination. These authors point out that the adequate solution in this case would be the use of multiple methods, which, however, need to be adequately integrated. Some of the most widely used methods for stability assessment are those of Eberhart and Russell (1966) and the AMMI Stability Model (according to Gauch, 1992). Silveira et al. (2016) emphasized that the use of conventional methods such as the parameters according to Eberhart and Russell (1966) should be complemented with such methods as AMMI. Regardless of this, the methods and approaches for assessment of stability and adaptability are based on the genotype x environment interaction.

In practice, however, it is often necessary to evaluate other interactions, as well: genotype x fertilization, genotype x growing system, genotype x sowing date, etc. Against the background of several periods of conducting this experiment, a tri-factor interaction needed to be assessed. This presented us with a challenge in the interpretation and evaluation of the stability of the genotypes. There are a significant number of researches on triticale, which give assessment on productivity and stability against the background of different growing periods. Scarce are the investigations on stability under different levels and forms of fertilization not only in triticale, but in cereals in general.

The aim of this study was to compare the grain yields from triticale cultivars and to determine their ontogenetic adaptability and stability in the formation of productivity under the agro-ecological conditions of Central South Bulgaria.

MATERIALS AND METHODS

Plant material

During 2014-2017, a three-factor field experiment was carried out at the Institute of Field Crops – Chirpan, Bulgaria. Winter triticale cultivars developed at Dobrudzha Agricultural Institute – General Toshevo were subjected to comparative study. The experiment was designed according to the block method in four replications, the size of the harvest plot being 18 m^2. Sowing was done with 550 germinating seeds after previous crop sunflower.

The following factors and levels were investigated: factor A cultivar – a_1) Kolorit, a_2) Atila, a_3) Bumerang and a_4) Respekt; factor B nitrogen fertilization – b_1) N_0 (without nitrogen fertilization), b_2) N_6 (nutrition with 6 kg/da a.m. N_20), b_3) N_{12} (nutrition with 12 kg/da a.m. N_20) and b_4) N_{18} (nutrition with 18 kg/da a.m. N_20). Cultivar Kolorit has been a standard for Bulgaria since 2015. Nutrition was done manually with ammonium nitrate (NH_4NO_3) at tillering stage in spring. Background phosphorus fertilization with triple superphosphate was used at norm P_2O_5/da, incorporated in autumn in all variants of nitrogen fertilization with a follow-up cultivation. Grain yield was determined as per harvest plot after harvesting and was re-calculated to kg/da. The agronomy practices were according to the standard methodology for growing of cereal crops.

Soil and climate conditions

The soil type was Vertisol with 80-115 cm humus horizon. It was characterized by low to moderate mineral nitrogen reserves, low content of mobile phosphorus and good reserves of available potassium.

The climatic data were obtained from the agro-meteorological station on the territory of the Institute of Field Crops in Chirpan. Agro-climatic assessment was done on the available moisture reserves in soil. The moisturizing coefficients of Ivanov were determined over months for the vegetative growth period. The formula К = Sr/E, where: К – moisturizing coefficient; Sr – monthly sum of precipitation (mm); E – monthly evaporation (mm) was applied. To calculate evaporation (E), the formula used was E = 0.0018 x (t + 25)2 x (100-a), where: t – mean monthly air temperature, (°C); a – mean relative air humidity (%). It was accepted that values of К<0.3 indicated drought, while values of К >2.0 signaled excessive moisture.

The Institute of Field Crops is situated in the transitional continental sub-region (Sabev and Stanev, 1963). Typical for this region are soft winters, hot summers and high variation of temperatures during the vegetative growth period both over years and within the year.

The period 2014/2015 was characterized as warmer and more humid with regard to temperature sums and precipitation, marking it as different from the long-term tendency. Such conditions were a prerequisite for good development of the crop. During the winter period, the mean monthly temperature was higher in December, January and February (Table 1). January was rather warm, with accumulated 79.2ºC more than the long-term mean value (Table 2).

The amount of autumn and winter rainfalls was higher and therefore, according to the hydro-thermal coefficient (HTK), excessive moisture was registered (Table 3). The temperature sum in February was with 184.2ºC more than the climatic mean value, and as a result the mean temperature was also higher. In combination with the snowfall in January, which caused excessive moisture, it led to tillering and early resumed vegetative growth.

The temperatures and precipitation during 2016-2017 were close to the mean data. An exception was the sum in January –160.7ºC below zero, or with 159.3ºC less. Retarded development of the crop was observed and late occurrence of stage 3rd leaf in January. The highest HTK of moisturizing was registered (Table 4).

Statistical analysis

The results obtained on yield were summarized and averaged over genotype, fertilizer norm, year, and in total. A two-way ANOVA over years was carried out according to the model genotype x fertilization and in general according to the model genotype x environment, considering each fertilization norm in each vegetative growth period as representing separate growing conditions. A three-way ANOVA was done according to the model genotype x fertilization norm x year.

To determine the stability and adaptability of the investigated genotypes both under different fertilization norms and for each one individually, the method of Eberhart and Russell (1966) was applied, calculating the regression coefficient (b_i) and the mean square deviation of the regression (s^2_{di}). AMMI Stability Analysis was applied according to Gauch (1992) for each level of fertilization, and for the total of all levels and years of the experiment.

To summarize and average the data, Microsoft Office Excel 2003 was used, IBM SPSS Statistics v.19 was applied for the ANOVA, to calculate the stability parameters – the internet-based platform StabilitySoft (Pour-Aboughadareh et al., 2019), and for AMMI analysis – AMMISoft (Gauch and Moran, 2019).

Table 1. Air temperature (mean diurnal) during the vegetative growth (tº C)

Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
2014/15	12.6	7.6	4.4	2.4	3.4	6.2	11.3	18.9	20.3
2015/16	12.7	10.0	3.7	-0.3	8.1	8.8	14.7	16.1	22.7
2016/17	12.4	6.7	0.8	-5.2	1.7	9.3	11.9	16.6	22.1
1928/13	***12.9***	***7.2***	***2.0***	***-0.2***	***2.1***	***6.1***	***11.8***	***16.8***	***20.8***

Table 2. Temperature sums Σ (tº C)

Period	Ocr	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Σ
2014/15	391.9	227.2	138.0	74.8	96.0	192.7	340.4	586.0	608.9	2656.9
2015/16	392.8	299.3	115.1	-8.7	233.6	273.5	439.8	498.0	679.8	2923.2
2016/17	385.0	201.7	26.2	-160.7	45.6	289.2	355.7	513.7	664.2	2321.5
1928/13	***396.7***	***215.9***	***61.1***	***-4.4***	***49.4***	***188.9***	***357.9***	***511.5***	***630.7***	***2407.7***

Table 3. Sum of precipitation (mm)

Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Σ
2014/15	135.4	36.9	142.3	50.3	61.7	134.9	15.1	58.8	78.1	713.5
2015/16	76.6	50.2	1.3	73.9	28.3	53.1	26.6	75.0	15.0	400.0
2016/17	12.0	47.7	5.9	69.8	23.8	51.3	22.6	59.5	84.3	376.9
1928/13	***37.5***	***43.3***	***54.0***	***44.3***	***37.7***	***37.0***	***45.2***	***64.1***	***65.4***	***428.5***

Table 4. Hydrothermal coefficient (HTK) of Ivanov

Период	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
2014/15	**4.4**	**2.4**	**10.9**	**1.5**	**2.8**	**5.9**	0.2	0.7	0.8
2015/16	1.7	1.0	*0.1*	**5.1**	0.9	1.3	0.3	1.1	*0.1*
2016/17	*0.2*	1.7	0.3	**9.6**	1.5	1.2	0.3	0.7	0.8

К>2.0 *excessive moisture*

К<0.3 drought

RESULTS AND DISCUSSIONS

Results

The results on the grain yield from the investigated genotypes (Table 5) revealed a tendency towards increase with the higher levels of fertilization during the three harvest years and for all cultivars. At the same time, regardless of the applied fertilization, the most favorable conditions were observed during harvest year 2017, and the highest yield for this period was obtained from cultivar Bumerang, followed by Kolorit. The highest productivity in this growing period was realized by cultivar Respekt. In 2016, the highest values were registered in cultivar Atila – 282.22 kg/da, 417.22 kg/da, 484.72 kg/da and 514. 17 kg/da at levels N_0, N_6, N_{12} and N_{18}, respectively. The lowest productivity was that of cultivar Respekt, regardless of the fertilization used.

In harvest year 2015, an identical tendency in the different cultivars depending on the nitrogen fertilization was not observed. In the variant without using nitrogen fertilizer, cultivars Bumerang (300.69 kg/da) and Respekt (308.19 kg/da) demonstrated high and similar yields; at level N_6 the highest values were registered in cultivar Bumerang (434.25 kg/da), at N_{12} - in cultivars Bumerang (476.36 kg/da) and Atila (460.50 kg/da), and at N_{18} – in Kolorit (554.89 kg/da) and Bumerang (545.36 kg/da). In this economic year, cultivar Atila gave the lowest yield at level N_0, and under all other levels the lowest productivity was observed in cultivar Respekt. These data show that the productivity of the cultivars depended both on the applied mineral fertilization and on the conditions of the environment.

To determine the effect of the year conditions and the cultivar against the background of different nitrogen norms, a two-way ANOVA was carried out (Table 6). Based on the obtained data, it can be assumed that when using certain nitrogen fertilizer doses, the effect of the genotype and the conditions of the environment varied in strength, as well as the effect of their interaction. Under N_0, 79.7% of the total yield variation was due to the conditions of the year. The factor genotype had a statistically significant effect, but of small size – only 3.6%. The effect of the genotype x environment interaction accounted for 10.1%, which was within the normal range for such a crop as triticale. Under N_6, the yield was determined to almost equal degree by the effects of the genotype and of the year conditions, and the effect of their interaction was 7.9%.

Such distribution was not typical for the crop, although the effect of the year conditions was dominant. With the next higher norms of nitrogen fertilization, N_{12} and N_{18}, the effect of the cultivar was dominant for the variation of grain yield, 43.4% and 58.3% from the total variation, respectively. The effect of the environmental conditions determined under N_{12} accounted for 19.3% of the differences in the mean arithmetic value of all cultivars, and under N_{18} – for only 8.4%. A tendency was observed with the higher nitrogen norms towards higher effect of the genotype at the expense of the effect of the conditions of the year. At the same time, the effect of the genotype x environment interaction remained relatively the same for all four variants of fertilization, 10.1%, 7.9%, 8.3%, 13.1%, respectively.

The results from the analysis of the variance for the total of the four variants of fertilization revealed that the factors cultivar and year, as well as the interaction cultivar x year, had significant effects on the yield from the cultivars. The highest influence on the changeability of yield was that of the factor year – 68.1%. The cultivar had a relatively low impact on this parameter – 15.0%, while the genotype x environment interaction was also low – 7.9%. These values were normal for triticale, such results having been reported in previous studies for similar sets of cultivars.

Table 5. Yield over cultivars, levels of fertilization and years (kg/da)

Genotype	2015			
	N_0	N_6	N_{12}	N_{18}
Kolorit	283.56	361.53	431.64	554.89
Bumerang	300.69	434.25	460.50	545.36
Respekt	308.19	336.72	349.61	377.50
Atila	277.83	402.75	476.36	504.08
Genotype	2016			
	N_0	N_6	N_{12}	N_{18}
Kolorit	193.06	382.50	415.83	438.33
Bomerang	216.11	409.44	464.72	511.39
Respekt	197.78	328.06	397.78	419.72
Atila	282.22	417.22	484.72	514.17
Genotype	2017			
	N_0	N_6	N_{12}	N_{18}
Kolorit	486.39	511.39	532.36	555.00
Bomerang	496.11	517.22	546.11	601.81
Respekt	356.67	377.08	399.72	407.22
Atila	442.08	476.39	508.89	531.94

The results given in Table 5 and Table 6 corresponded to those obtained from the three-way ANOVA. The analysis of the factors cultivar, year and fertilization (Table 7) revealed a low but significant effect of the interactions cultivar × year (3.8%), cultivar × fertilization (1.9%) and year × fertilization (8.2%). The values of η on the total variation of the factors (G x Yr x F) explain the low dependence of the differences between the levels of the factors with regard to yield. The highest effect on the value of yield was determined for the factor fertilization – 40.6%.
The significant effect of the studied factors and their interaction on the yield from the triticale cultivars allowed the assessment of the parameters related to the stability and adaptability of the genotypes investigated in this research.

Table 6. Two-way ANOVA of the genotype x environment interaction by level of fertilization and in total

Source	SS	df	Mean Square	η(%)	F	Sig.
	N_0					
G	18751.900	3	6250.633***	3.6	6.481	0.001
E	416041.062	2	208020.531***	79.7	215.691	0.000
G * E	52569.664	6	8761.611***	10.1	9.085	0.000
Error	34719.744	36	964.437			
Total	522082.370	47				
	N_6					
G	76382.263	3	25460.754***	37.4	28.479	0.000
E	79742.146	2	39871.073***	39.0	44.598	0.000
G * E	16080.476	6	2680.079*	7.9	2.998	0.018
Error	32184.642	36	894.018			
Total	204389.527	47				
	N_{12}					
G	93339.367	3	31113.122***	43.4	17.892	0.000
E	41518.168	2	20759.084***	19.3	11.938	0.000
G * E	17804.058	6	2967.343ns	8.3	1.706	0.148
Error	62602.275	36	1738.952			
Total	215263.868	47				
	N_{18}					
G	155954.196	3	51984.732***	58.3	35.089	0.000
E	22590.844	2	11295.422**	8.4	7.624	0.002
G * E	35713.053	6	5952.176**	13.1	4.018	0.004
Error	53333.670	36	1481.491			
Total	267591.763	47				
	Total					
G	305848.364	3	101949.455***	15.0	80.293	0.000
E	1385554.807	11	125959.528***	68.1	99.202	0.000
G * E	160746.613	33	4871.109***	7.9	3.836	0.000
Error	182840.330	144	1269.725			
Total	2034990.115	191				

The analysis on the mean yield showed that the maximum value was obtained for cultivar Bumerang (458.64 kg/da), and the lowest – for cultivar Respekt (354.67 kg/da), the average yield of the experiment being 421.35 kg/da. Based on the regression coefficient (b_i) according to Eberhart and Russell (1966), it is possible to estimate the response of the cultivars to the changeable growing conditions. Such a condition in this study was the nitrogen fertilization. Values of b_i close to 1.00 implied wide adaptability of the genotype, while values below or above it indicated narrow adaptability to the favorable growing conditions or narrow adaptability to unfavorable growing conditions. In this research, the adaptability of the cultivars was determined through b_i for each level of the factor fertilization and for each cultivar.

Table 7. Three-way ANOVA according to the investigated factors (cultivar, year and level of fertilization)

Source	SS	df	Mean Square	η (%)	F	Sig.
G	305848.364	3	101949.455***	15.0	80.293	.000
Yr	392383.566	2	196191.783***	19.3	154.515	.000
F	825662.587	3	275220.862***	40.6	216.756	.000
G * Yr	78274.869	6	13045.812***	3.8	10.275	.000
G * F	38579.362	9	4286.596***	1.9	3.376	.001
Yr * F	167508.654	6	27918.109***	8.2	21.988	.000
G * Yr * F	43892.382	18	2438.466*	2.2	1.920	.018
Error	182840.330	144	1269.725			
Total	2034990.115	191				

Table 8 demonstrates several facts. Firstly, with the increase of the levels of fertilization from N_0 to N_{18}, the significance of b_i for cultivar Kolorit also increased. Secondly, the cultivar possessed the narrowest adaptability to favorable environments, but its yield was not the highest, in comparison to the other cultivars. This means that this cultivar was with the highest requirements to the favorable climatic factors and agronomy practices used for its growing. Cultivar Bumerang demonstrated a similar response to the conditions of the environment, with b_i values 1.26, 1.10, 1.33 and 1.71 under N_0, N_6, N_{12} and N_{18}, respectively, in combination with the highest mean yield. The regression coefficient of cultivars Respekt and Atila varied within 0.52-0.65 and 0.35-0.77, respectively. These values determine narrow adaptability to unfavorable conditions of growing and they have low response to change in the growing conditions and the environment as a whole. Under nitrogen deficiency, as was the variant without using synthetic fertilizer (N_0), grain yield from cultivars Respekt and Atila will not demonstrate a significant decrease in comparison to the rest of the cultivars.

Such characteristics of the cultivars regarding their adaptability can also be accepted based on the values of b_i for all levels of fertilization. The coefficient b_i determined for cultivar Respekt under fertilization variant N_{18} was negative (-0.20) and it can be assumed that this cultivar did not interact with the conditions of the environment, i.e. it was not responsive to the high nitrogen norms, regardless of the year conditions. Therefore, the suggestion could be made that under low-productive environment the grain yield from Respekt would be higher than the yields of the other cultivars. Alternatively, under intensive agriculture with the use of higher nitrogen norms, cultivar Bumerang will exceed the yields of the investigated cultivars.

Table 8. Parameters of stability and adaptability over fertilization levels and total for the investigated set of cultivars

Cultivar	Yield (kg/da)	b_i (Plasticity)	s^2_{di} (Stability)
N_0			
Kolorit	321,00	1,32	0,34
Bumerang	337,64	1,26	1,21
Respekt	287,55	0,65	331,73
Atila	334,05	0,77	273,18
N_6			
Kolorit	418,47	1,61	29,08
Bumerang	453,64	***1,10***	45,90
Respekt	347,29	0,52	5,69
Atila	432,12	0,77	14,19
N_{12}			
Kolorit	459,94	1,69	88,38
Bumerang	490,44	1,33	8,44
Respekt	382,37	0,52	131,39
Atila	489,99	0,47	0,71
N_{18}			
Kolorit	516,07	2,14	372,45
Bumerang	552,85	1,71	5,85
Respekt	401,48	-0,20	126,23
Atila	516,73	0,35	31,86
Total			
Kolorit	428,87	1,22	1051,84
Bumerang	458,64	1,22	190,87
Respekt	354,67	0,63	772,14
Atila	443,22	0,93	774,61
Average	421,35		

The stability coefficient s^2_{di} gives an idea of the grain yield expression under different environments. Its mathematical origin is from the deviations of the actual from the theoretical yield values, and statistically speaking, it is the dispersion of variability of the yield values. It is accepted that the higher s^2_{di} value implies lower stability. In this research, stable were considered different cultivars depending on the amount of mineral fertilizer applied. Under N_0, these were cultivars Respekt and Atila, under N_6 – Bumerang, under N_{12} – Respekt, and under N_{18} – Kolorit. At level N_0, cultivar Kolorit demonstrated the highest stability, under N_6 – Respekt, under N_{12} – Atila, and under N_{18} – Bumerang.

Having in mind Table 8, it can be assumed that the productivity of cultivars Kolorit and Bumerang is above the average of the studied triticale genotypes under favorable condition. Based on the regression coefficient b_i = 1.22, cultivar Bumerang was characterized by a narrow adaptability to favorable environments, demonstrating high stability – s^2_{di} = 190.87. At the same time cultivar Kolorit exhibited low stability at significance of s^2_{di} = 1051.84 and identical adaptability – b_i = 1.22. This means that under unfavorable conditions Kolorit will produce comparatively lower and highly variable yields in comparison to the other cultivars, while under good agronomy practices and favorable climatic conditions grain yield will be high but differing and with low predictability relative to the rest of the cultivars. On the other hand, the yields of Bumerang will be high under variable conditions of the environment, and the more favorable they are, the higher the yield will be.

In cultivar Atila, the significance of b_i was close to 1 - 0.93. This implied comparatively wide plasticity under the investigated conditions of the environment and fertilization norms. Its stability, however, was comparatively low suggesting lower yield predictability under changeable meteorological conditions or agronomy practices. In cultivar Respekt, summarized for the study, narrow adaptability to unfavorable conditions of the environment was observed. This showed that the cultivar would respond with higher productivity to unfavorable conditions or to lower levels of nitrogen fertilization. At the same time, however, this cultivar was also characterized by low stability.

The applied AMMI analysis allowed identifying the genotypes, which combined productivity and at the same time were in low interaction with the growing conditions. To visualize the results from the analysis, biplots were constructed for each of the used fertilization norms and in total for the experiment. The genotypes positioned close to the x axis and to the right of the y axis were characterized by lower interaction with the conditions of the environment and were more productive, while those, positioned at a distance from axis x and to the left of axis y, were characterized by higher interaction with the conditions of the environment and were with lower productivity. At level of fertilization N_0 (Figure 1), the lowest interaction with the environmental conditions was that of cultivars Atila, and the interaction of cultivar Respekt was the highest. Opposite but identical reaction was observed in cultivars Kolorit and Bumerang. At the same time, the best combination of productivity with stability was found in cultivars Bumerang and Atila. In Kolorit, the high yield was combined with lower stability, while in Respekt the low yield was in combination with low stability.

Similarly, after using N_6, the highest yield according to the mean (413.0 kg/da) was registered in Bumerang, which was in low interaction with the environment since it was positioned close to the abscissa (Figure 2). Second ranked cultivar Atila, which was characterized by identical but opposite interaction with the conditions of the environments as compared to Bumerang. Under N_0 and N_6, the position of Kolorit (G1) relative to the ordinate remained identical with regard to the mean yield under both levels of fertilization. Additionally, it was positioned at the greatest distance from the abscissa, which determined its lower yield stability. The lowest productivity in combination with the lowest stability was again observed in cultivar Respekt.

Under N_{12}, the tendency of cultivar Kolorit being positioned close to the line determining the mean yield of the cultivars and at distance from the abscissa was also observed (Figure 3). Cultivars Bumerang and Atila were with similar and higher yields than the average under fertilization with N_{12} and had a comparatively lower interaction with the conditions of the environment, the tendency towards simultaneous combination of low productivity and low stability remaining the same. It should be underlined that since the interaction genotype x environment was not significant at level above 95%, the conclusions drawn were valid at level of significance 85%.

Under N_{18}, cultivar Bumerang combined high stability with the highest yield (Figure 4). Its position was closest to the abscissa, and it determined low interaction with the environment. Cultivar Kolorit was in strong interaction with the conditions of the environment and demonstrated the lowest stability. This tendency was valid also for cultivars Atila and Respekt as compared to the other levels of fertilization.

It can be summarized that cultivar Bumerang, under all levels of nitrogen fertilization, demonstrated the highest productivity and low interaction with the conditions of the environment implying very good stability (Figure 5). In cultivar Respekt, the lowest grain yield was accompanied by comparatively low stability as compared to the rest of the cultivars. Concerning cultivar Atila, the observed productivity was lower than the productivity of Bumerang, but close, and in combination with interaction with the environment with opposite values. Cultivar Kolorit, on its part, was characterized by yields close to the mean, averaged for the period of study but at the same time its interaction with the environment was exceptionally high.

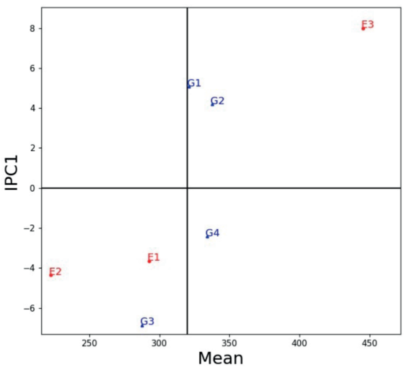

Figure 1. AMMI1 Biplot for fertilization level N_0
(G1-Kolorit; G2-Bumerang; G3-Respekt; G4-Atila; E1-2014/2015; E2-2015/2016; E3-2016/2017)

The results from the AMMI analysis for all four levels of nitrogen nutrition (Figure 5) allow characterizing not only the individual genotypes by their ability to interact with the environment, i.e. to assess in a sense their stability, but also to estimate the degree, to which each combination of year conditions with fertilization relates to the occurrence of genotype interaction.

The highest negative interaction with the genotypes was that of the year conditions during 2014/2015 and 2015/2016 under level N0, and the highest positive – of the year conditions in 2014/2015 and 2016/2017. Respectively, these were the conditions of the environment, under which the lowest and the highest yields were obtained, averaged for the four studied genotypes.

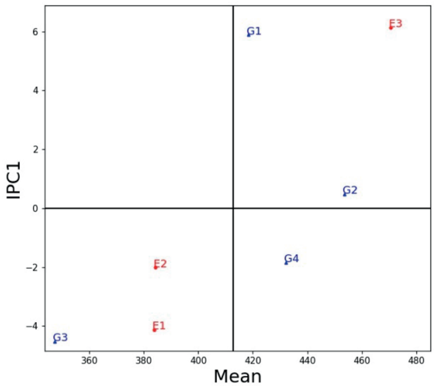

Figure 2. AMMI1 Biplot for fertilization level N_6
(G1-Kolorit; G2-Bumerang; G3-Respekt; G4-Atila; E1-2014/2015; E2-2015/2016; E3-2016/2017)

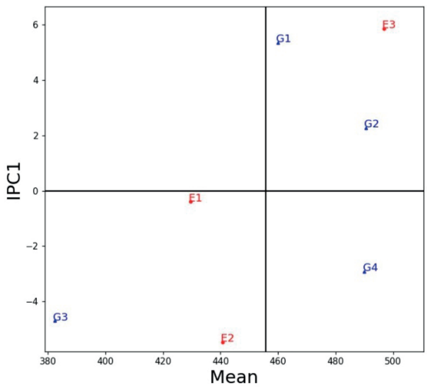

Figure 3. AMMI1 biplot foe reftilization level N_{12}
(G1-Kolorit; G2-Bumerang; G3-Respekt; G4-Atila; E1-2014/2015; E2-2015/2016; E3-2016/2017)

The conditions of 2014/2015 had the lowest interaction with the genotypes under level N12, and to some degree – the conditions of 2014/2015 and 2015/2016 under level N6.
The other combinations of environmental conditions with norms of nitrogen fertilization had comparatively moderate interactions with the genotypes. A tendency was observed of negative to positive change of the interaction with the higher nitrogen norms. A tendency was also observed of cultivar Bumerang having the lowest interaction with the conditions under all levels of nitrogen fertilization in 2016/2017 growing period.

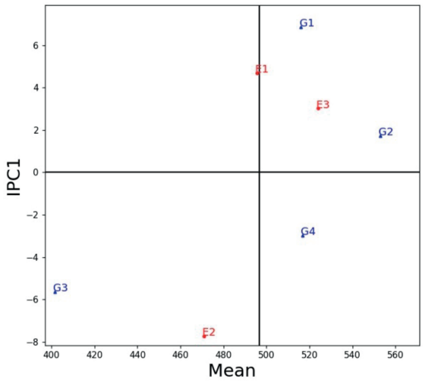

Figure 4. AMMI1 biplot for fertilization level N_{18}
(G1-Kolorit; G2-Bumerang; G3-Respekt; G4-Atila; E1-2014/2015; E2-2015/2016; E3-2016/2017)

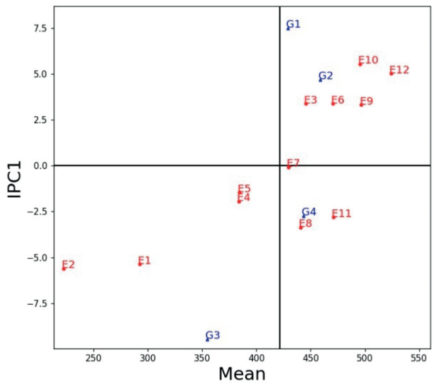

Figure 5. AMMI1 biplot for all levels of fertilization and years
(G1-Kolorit; G2-Bumerang; G3-Респект; G4-Atila; E1-2014/2015, N_0; E2-2015/2016, N_0; E3-2016/2017, N_0; E4-2014/2015, N_6; E5-2015/2016, N_6; E6-2016/2017, N_6; E7-2014/2015, N_{12}; E8-2015/2016, N_{12}; E9-2016/2017, N_{12}; E10-2014/2015, N_{18}; E11-2015/2016, N_{18}; E12-2016/2017, N_{18})

The lowest was the interaction of cultivar Atila with the conditions of 2015/2016 under levels N12 and N18. Kolorit and Respekt were at a significant distance from any combination of year conditions with fertilization norm. This was an indication of their rather low stability and narrow adaptability to the more favorable and more unfavorable environments, respectively. The low response of Bumerang's interaction to multiple specific periods revealed its wider adaptability to the conditions of the year, respectively, and also to the different norms of nitrogen fertilization. Similar was the response of cultivar Atila, but its adaptability was comparatively narrower and tended towards the more unfavorable conditions.

Discussions

The analysis of the cultivars in a series of statistical analyses allowed evaluating their yield and characterizing their adaptability and stability in response to the conditions of the environment. A peculiarity of the parameters for evaluation of the stability and adaptability under differing controlled factors (as a part of the growing conditions) was the possibility to identify tendencies and to determine the genotypes valuable for practice with a view of the needed agronomy practices. The results we obtained undoubtedly showed that the higher norms of nitrogen nutrition increased productivity regardless of the investigated cultivar. At the same time, under all norms and year conditions, the values of the yield from the studied triticale cultivars were within the range normal for this crop. This was also confirmed by the results obtained from our previous researches on the same or similar varieties (Baychev, 2006; Baychev, 2009; Baychev and Petrova, 2011; Baychev, 2012; Stoyanov and Baychev, 2016; Stoyanov et al., 2017; Stoyanov and Baychev, 2018; Stoyanov, 2018; Stoyanov, 2020a; Stoyanov, 2020b, Stoyanov, 2022; Stoyanov and Baychev, 2021; Muhova and Kirchev, 2020; Dobreva et al., 2018), and from foreign studies on other genotypes under different environments (Bespalova et al., 2012; Ponomarev, 2012; Borovik, 2016; Abdelaal et al., 2019; Lalević et al., 2019; Babaitseva et al., 2021). A peculiarity of the four investigated genotypes was their tendency towards maintaining their ranking by mean productivity regardless of the applied norm of nitrogen nutrition. This related to the lower effect of the genotype x environment interaction, although under its definite presence. Stoyanov (2018) and Stoyanov (2022) demonstrated that cultivars Bumerang and Atila were with higher productivity than cultivar Kolorit, while cultivar Respekt was characterized by rather low productivity under variable environments, the ranking of these genotypes being different under contrasting conditions.

The results of Dobreva et al. (2018) confirmed our data on the four cultivars, observing an identical tendency when investigating them under the same fertilizer norms, but in variants with and without leaf fertilization - towards productivity increasing with the higher nitrogen norms. Addy et al. (2020) also confirmed that the yield values increased with the increase of N input. Bielski et al. (2020), too, observed higher grain yields with the higher nitrogen norms. These authors reported that the yield at norm 16 kg N was higher than the yield at fertilization with 12 kg N da, but, similar to the results from our research, the difference was not significant. Usevičiūtė et al. (2022) observed increase of the yield from spring triticale after applying combined NPK fertilizer in comparison to the untreated check variant; they also found that the increased amount of the applied organic charcoal from pines increased the productivity of the genotype they investigated, too.

The results from the above studies undoubtedly support the results we obtained. On the other hand, however, the tendencies of yield change were not identical for the individual cultivars under different levels of fertilization (Figure 6) related to their differing stability.

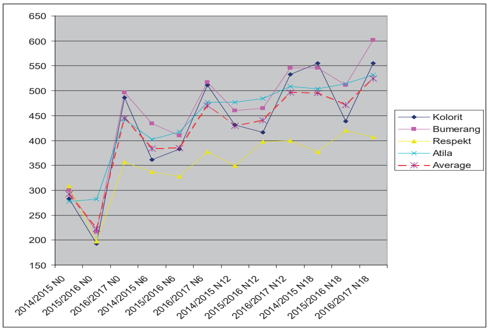

Figure 6. Tendency in the yield of the studied triticale cultivars under different year conditions and norms of nitrogen nutrition

Obtaining high yields from the crop plants is, on the one hand, related to the growing of cultivars, which are adapted to certain soil and climatic conditions (Stoyanov, 2020a). On the other hand, however, certain changes in the technology of growing can destabilize the yields form a given genotype relative to the conditions of the environment.

The results from the dispersion analysis over fertilization levels and in total revealed a definite tendency of lower effect of the year conditions with the higher fertilizer norms. It was also evident that the effect of the cultivar increased with the increase of the fertilization norm. A possible explanation of this tendency is that the genotypes differed in the absorption and utilization of N depending on the environment (Belete et al., 2018). Such a phenomenon is understandable since soil and moisture are important for the nitrogen fertilizer uptake in direct relation to the meteorological conditions. On the other hand, the genotype exercises its influence through its ability to develop under certain conditions, the nutrients uptake being in direct relation to the well-formed roots typical for each variety. Generalized for this study on the variance of yield, the effect of the year conditions was dominant, which was normal for such a crop as triticale. In world literature, a small number of researches on triticale investigated the stability and adaptability of yield and its components under different levels of nitrogen nutrition. While investigating Bulgarian triticale cultivars by the method of Eberhart and Russell, Kirchev et al. (2016) found out that the regression coefficient in the variant without fertilization varied among the cultivars; in two of them (AD-7291 and Zaryad) it tended towards narrow adaptability to the more unfavorable conditions of the environment, in another two (Sadovets and Rozhen), the values showed close to wide adaptability, and in cultivar Rakita narrow adaptability to the favorable conditions was observed.

This tendency was not present under the other levels of fertilization, with the exception of cultivar Rakita. Quite impressive is the fact that with the increase of nitrogen nutrition, the observed regression coefficients also increased, and at the highest level (18 kg/dca), they were close to 1.00 in four of the five studied cultivars. Not all cultivars demonstrated this tendency; the regression coefficients of some of the genotypes became even lower with the higher nitrogen norms. In Kolorit and Bumerang, the regression coefficient increased with the increase of nitrogen nutrition, while in Respekt and Atila it decreased. Such a phenomenon can be explained by the fact that under unfavorable environments the productivity of Atila and Respekt changed to a much lesser extent in comparison to the favorable environments, while in Kolorit and Bumerang the favorable conditions of the environment caused much higher values of the yield (Figure 6).

Concerning stability, the same study (Kirchev et al., 2016) showed that the mean square deviation of the regression increased with the higher nitrogen nutrition.

The results obtained from the AMMI analysis confirmed to some extent the tendencies of values b_i and s^2_{di}. Kolorit was in a strong interaction with the conditions of the environment, with rather variable yields and narrow adaptability to favorable conditions of the environment. On the other hand, Respekt showed similar results, although opposite in direction, related to this cultivar's narrow adaptability under unfavorable environments. This thesis was also confirmed by the fact that in the absence of nitrogen nutrition, cultivar Respekt was characterized by higher productivity, but only under the conditions of harvest year 2015. The results reported by Oral (2018) from an AMMI-analysis on an experiment including two triticale genotypes, two vegetative growth seasons and five norms of fertilization, show that the two genotypes reacted similarly to Respekt and Kolorit, in opposition to each other.

At the same time, a similar tendency was also observed – the IPCA value increased with the higher nitrogen nutrition, when it affected positively the yield. Paderewski et al. (2016), when expanding the AMMI model with the aim to study more complex relationships, demonstrated that the investigation on different agronomy practices led to the division of certain mega-environments, and to the identification of the narrow adaptability of the genotypes to specific regions, way of growing or climatic conditions.

The use of a similar analysis in this study allowed the definite characterization of cultivar Bumerang as comparatively more widely adaptable because of its comparatively low interaction with some fertilizer norms and with certain year conditions. A narrower adaptability was observed in Atila under some of the lower norms. The obtained results clearly demonstrated the excellence of cultivar Bumerang as widely adaptable and comparatively stable, but also high-yielding, relative to the other three studied genotypes. At the same time, regardless of the applied nitrogen nutrition, this genotype was with the best response under more favorable conditions of the environment.

On the other hand, the high productivity of such a genotype as Atila, in combination with similar stability but narrower adaptability to unfavorable growing conditions allowed the cultivar to realize high productivity under meteorological conditions differing form the long-term tendency, but under higher nitrogen nutrition levels. This peculiarity gives good reasons to reintroduce cultivars Atila and Bumerang in mass production and distribute them in the various soil and climatic regions of Bulgaria.

CONCLUSIONS

Based on the above results, the following conclusions can be made:

1. During the investigated economic years, the triticale cultivars differed by their mean productivity, harvest year 2016/2017 being the most favorable for growing of the crop. Cultivar Bumerang was characterized by the highest productivity, averaged for the period of study, while the lowest yields were that of cultivar Respekt.
2. The highest productivity of the cultivars was observed at level of fertilization 18 kg/da nitrogen; this was valid for all cultivars, regardless of the growing conditions, indicating the high potential of the crop for intensive growing.
3. A tendency was observed towards higher effect of the genotype and lower effect of the year conditions with the higher levels of nitrogen fertilization, while the effect of the genotype x environment interaction remained almost constant.
4. The widest adaptability (tending towards narrow under favorable conditions of the environment), regardless of the fertilizer norm, was that of cultivar Bumerang; cultivar Kolorit was with very narrow adaptability to favorable conditions, while Atila and Respekt demonstrated a narrower adaptability to unfavorable environments. A tendency was observed in Kolorit and Bumerang towards higher values of b_i with the higher fertilizer norms, while in Atila and Respekt this value decreased.
5. Cultivar Bumerang was with the highest stability, averaged for the experiment, Kolorit was with the lowest, while Atila and Respekt were with similar results with regard to s^2_{di}. Based on the AMMI carried out, Bumerang and Atila had the lowest interaction with the environment.
6. A tendency was observed in cultivar Bumerang towards lower interaction with the environmental conditions under all levels of nitrogen nutrition in economic year 2016/2017, and in cultivar Atila towards the lowest interaction with the conditions of economic year 2015/2016 at levels N12 and N18. Except for the variant without fertilization, both cultivars had comparatively stable responses to the other fertilizer norms, which makes them suitable for growing under the varied soil and climatic conditions of Bulgaria and under different regimes of nitrogen nutrition.

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IRRIGATION WATER PRODUCTIVITY UNDER DRIP IRRIGATION OF TWO CORN HYBRIDS

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Abstract

A field experiment with two maize hybrids was conducted to analyze the influence of irrigation and feeding, with nutrients, on productivity, irrigation efficiency and irrigation water productivity. The experiment was conducted under non-irrigated and irrigated conditions. The object of the research are two maize hybrids: Premeo and Knezha-461. Optimizing the irrigation regime provides an increase in corn yields for grain by 237.1% in Premeo and by 233.1% in Knezha-461 compared to the non-irrigated option. Optimizing the irrigation and nutrition regime leads to an increase in productivity by 340.8% in Premeo, and in Knezha-461 by 313.5%. The corn hybrid Premeo 11.4 kg/mm stands out with the highest irrigation water productivity, followed Knezha-461 with 10.3 kg/mm, average for the study period. A trend towards an increase in the productivity of irrigation water with an increase in the productivity of the maize hybrids was found. It was established that the Premeo corn hybrid has a higher effect per 100 m^3 *of irrigation water, on average for all variants - 722.2 kg/ha, while for Knezha-461 the effect is 656.1 kg/ha.*

Key words: *corn, irrigation, fertilization, yield, productivity.*

INTRODUCTION

Maize is an important grain forage and food crop. The market in recent decades has offered a wide variety of hybrid varieties of corn. The correct selection of suitable hybrids depends on ecological plasticity, resistance to pest attacks, etc. It is necessary to take into account the agroclimatic resources of the area, such as temperatures (sum of the effective temperatures), precipitation (amount and distribution of the fallen precipitation), stocking of the soil with macro and micro elements, etc. in the cultivation of hybrids of corn.

This requires producers to know their qualities well and the agro-meteorological conditions in the area where they will be grown. Factors that affect productivity, such as climate change, diminishing natural resources, and others, lead to a reduction in the productivity potential of crops (Lobell et al., 2008; Batisti et al., 2009; Tsenov et al., 2009; Easterling, 2011; Sevov, 2013; Nelson et al., 2014). The uneven moistening and the increase in air temperatures bring to the fore the question of the nature of the relationship between corn productivity and climate elements (Zhivkov et al., 2006; Popova, 2006; Simić et al., 2023).

Research and updating of technologies and policies can have a significant impact on more efficient use of water resources (Ziad et al., 2010; Moteva et al., 2016; Kireva et al., 2018; Żarski et al., 2023). Against the backdrop of global warming and drying, Rank et al. (2023) believe that it is necessary to search for and implement water-saving technologies in agriculture, combined with fertilization, mulching and other approaches to reduce the water footprint of crops.

The assimilation of nutrients throughout the growing season is linked in a number of agronomic practices that contribute to optimizing the process and guaranteeing high yields. These practices include structuring the soil, having enough readily available moisture for the plants, combating water deficit and nutrient deficiency. The microelements available in the soil are not sufficient for the development of plants. It is necessary to carry out foliar feeding during the growing season of the crop (Oldham, 2019; Luță et al., 2022).

A number of studies have established the positive influence of mineral fertilization with macroelements on the quantity and quality of the harvest (Samodova, 2008; Ivanov et al., 2021). The one-sided application of mineral fertilizers leads to a disruption of the ecological balance and a decrease in the quality of production (Brzozowska, 2008). The need to supplement nutritional micronutrients is a problem that has been recognized for a long period of time (Blaziak et al., 2003; Suwara et al., 2007; Xue et al., 2023). According to Racz et al. (2021) foliar fertilization cannot replace main fertilization, but it can help plants overcome stressors. Jakab-Gábor et al. (2017), are of the same opinion who consider that foliar fertilization only acts as a corrective to the main fertilization.

Another researcher considers foliar feeding to be the best approach for micronutrient supplementation (Kashyap et al., 2022). Foliar fertilizers act as biostimulators and enhance plant defense mechanisms (Haraga et al., 2023). The team of scientists reported an increase in plant height, higher biomass values, and higher yields. The productivity of corn under different feeding regimes is the subject of research by a number of researchers in different agro-climatic regions of the country. Fields studies prove the positive influence of fertilization on biometric parameters, such as number of rows in one cob, number of grains in one row, number of grains in one cob and weight of grain in one cob (Petrovska et al., 2010; Kuneva et al., 2014). Ma et al. (2015) found that localized application of phosphorus (P) plus nitrogen (N) improved maize grain yield, number of kernels per ear and agronomic nitrogen use efficiency.

The combined application of soil and foliar fertilizers increases the productivity of corn for grain found Ivanova et al. (2023). The influence on the biometric parameters grain yield, weight of 1000 grains, cob length, number of rows in the cob, number of grains in the cob, number of grains in the row, weight of the grain in the cob and weight from the cob has also been proved. The highest values were obtained in the joint action of ammonium nitrate (norm 12 kg/day) + 250 ml/day NPK 2.5 SO_3 with a composition of 3.0 N: 1.5 P: 10 K: 2.5 SO_3 + 250 ml/day SO_3 10% Zn, 3% N (zinc and nitrogen sulphides). Kovalenko et al. (2023) also confirmed the positive effect of applying foliar nutrition to crops, analyzing the formation of biometric parameters of maize hybrids of different maturity groups. Araújo et al. (2021) after conducting a comparative study of several maize hybrids in the conditions of Brazil found variations in the agronomic results of maize hybrids, such as significant differences in the percentages of dry matter, mineral matter, crude protein, etc.

The aim of the present study is to analyze the influence of irrigation and feeding with nutrients during the growing season on the productivity of two maize hybrids, as well as to determine the effectiveness of irrigation and the productivity of irrigation water in two maize hybrids.

MATERIALS AND METHODS

To fulfill the set goal, an experiment was conducted with corn hybrids. A field experiment was carried out under controlled conditions, in the experimental field of Trakia University, Stara Zagora. The study of ecological plasticity in two hybrids of corn was carried out in the period 2021-2022. The experiment was conducted under non-irrigated and irrigated conditions. The object of the research are two maize hybrids: Premeo (FAO 400) representative of a new generation of hybrids from the Artesian technology and Knezha-461: a hybrid from the selection of Bulgarian hybrids of the Institute of Maize, Knezha. The hybrids were grown on a soil type, typically meadow-cinnamon soil.

The productivity of the two maize hybrids was studied under different feeding regimes. The experiment was carried out using the method of fractional plots in 4 repetitions, with the size of a harvest plot 15 m^2. Sowing was carried out in an optimal period for the culture. Irrigation was done with a drip irrigation system. Pre-irrigation humidity was maintained at 80% FC (field capacity), and the irrigation rate was calculated for an active soil layer of 0-80 cm.

Variants of the field experience include var. 1. Control - without fertilization and irrigation; var. 2. Without fertilization + irrigation; var. 3. Feeding with foliar fertilizer (aminosol, nutriplant) + irrigation; var. 4. Feeding with foliar fertilizer (Kinsidro Grow, N-lock) + with irrigation. In options 2, 3 and 4, post-sowing, pre-emergence fertilization with N_{14} was performed. At var. 3 feeding with aminosol, zinc and boron is applied in the 4-6 leaf phase, and with nutriplant it is treated in the 8-10 leaf phenophase. At var. 4 the Kinsidro Grow biostimulator is applied in the phenophase 2-8 leaf, and the nitrogen stimulator in the 3-6 leaf phenophase.

The productivity of the irrigation water in the studied hybrids was established. Irrigation water productivity was defined as the ratio between yield and irrigation rate.

Statistical analysis was performed with Anova.

RESULTS AND DISCUSSIONS

Climatic conditions

In climatic terms, the area in which the experimental field is located refers to the European-continental climatic area. The years of the field climate study are characterized by significant differences in the measured decennial mean temperatures compared to the multi-year period (1930-2020). The average temperature during the corn growing season for the two-year period is 20.3ºC, which is 0.9ºC higher than the average multi-year temperature values for the region (19.4ºC). Regardless of the narrow range of fluctuations of the temperature sums in the two years of the field experience, the data show that differences were recorded in July and August. In August 2021, the average monthly temperature was 2.0°C above normal, and in 2022 it was 2.5°C. In the months of July and August, deviations from the temperature norm were recorded, and in 2021 they were 10.68% and 8.55%. In 2022, an increase in temperature was again measured compared to the norm. The increases in July and August were 9.81% and 10.68%. The recorded temperature values confirm the trends towards climate warming.

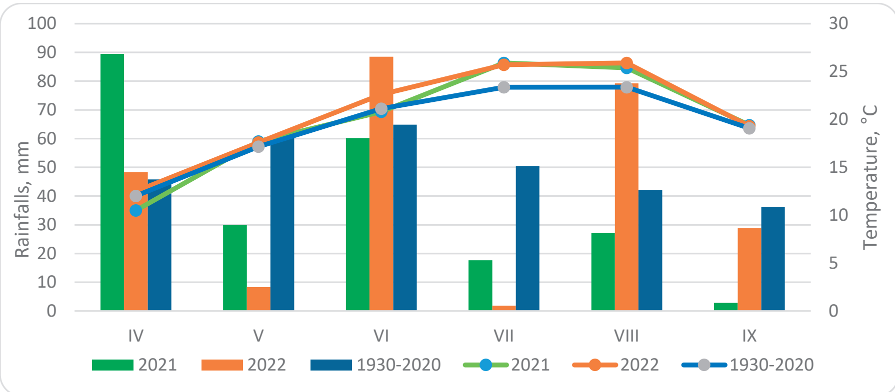

Figure 1. Climatogram for the growing season of corn for the period 2021-2022, the area of the city of St. Zagora

In terms of precipitation, with a precipitation rate of 300.1 mm, 24.3 % less precipitation was recorded in the first year and 15.1% in the second year. The period of the field study is characterized by uneven distribution of precipitation. In 2021, the measured rainfall is relatively evenly distributed, but the 17.7 mm recorded in July is insufficient to provide an optimal amount of moisture for maize development.

Precipitation is more unevenly distributed in the second year. Only 8.3 mm is the amount of precipitation that fell in May, while in June 88.5 mm was recorded. In July, only 1.8 mm was measured. The analysis shows the uneven distribution of the rainfall during the maize growing season on the one hand, and on the other hand, the tendency towards drought is confirmed again.

Given the change of climatic elements and their influence on the growth, development and fruiting of crops, the humidity coefficient and the hydrothermal coefficient (HC) have been determined. The active temperature sums were used to determine the climatic indicators and coefficients. The active temperature sums are determined by the equation:

$$\Sigma\ T\ ºC > 10º = (t_1 + t_2 + t_3 + \ldots + t_n),$$

where: $\Sigma\ T\ ºC > 10º$ - sum of the effective temperatures (for the period with an average daily temperature >10º) in ºC; $t_1, t_2, t_3, \ldots t_n$ - consecutive observations of average day and night air temperatures in ºC; 1, 2, … n - index for the consecutive number of the day during the established period.

Ivanov's (1941) humidification coefficient is determined by the following relationship:

$$E = 0.0018*(t + 25)^2*(100-a),$$

where: t - average monthly (decade) air temperature, °C; a - average relative humidity of the air, %.

Values of the hydrothermal coefficient (according to Selyaninov) were calculated by the formula:

$$K = P*10/\sum T^o,$$

where P is the sum of precipitation (mm) for a period of time, ∑To is the sum of the average daily air temperatures (°C) for the same period.

Table 1. Coefficients characterizing the humidity

Year	Ivanov's humidification coefficient																	
	IV			V			VI			VII			VIII			IX		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
2021	0.74	0.45	0.14	0	0.04	0.17	0.12	0.28	0.17	0.27	0.06	0	0.01	0.03	0.1	0	0	0,01
2022	0.02	0.47	0.12	0.03	0.02	0.02	0.1	0.05	0.02	0	0.01	0	0.05	0.14	0.2	0.05	0.14	0.01
Hydrothermal coefficient																		
2021	2.93	2.06	0.84	0	0.24	0.99	0.68	1.18	1	2.06	0.48	0	0.08	0.29	0.72	0	0.04	0.1
2022	0.15	2.66	0.82	0.21	0.11	0.17	0.66	0.33	0.12	0	0.07	0.01	0.46	1.16	1.47	0.34	1.03	0.07

The humidification coefficient and the moisture balance coefficient reflect the warmth and moisture availability of the area. Ivanov's empirical formula for calculating evaporation shows the degree of humidification per ten days for the period during which plants need a sufficient amount of soil moisture. In the last ten days of July, the humidity in the first year is 70% lower than the second. Water deficit during these periods requires irrigation to increase the water supply in the soil.

According to Ivanov's classification, at less than 0.3 there is drought, and at greater than 2.0 there is overwetting. When calculating Selyaninov's hydrothermal coefficient, it is taken into account that if it is less than 0.5, drought is observed, and if it is greater than 2.0, it is correspondingly overwet.

Humidification is distinguished by a coefficient of variation, VC = 23.64%. The values of the hydrothermal coefficient range from 0.00 to 4.93 for 2021, which indicates the nature of the year. High variability is also characteristic of HC during the VI-VII period. Coefficient of variation of the moisture balance for the entire period at HC is very high VC = 31.56 %.

Productivity of the corn hybrids

The results of the irrigation options show that when the moisture in the soil horizon is optimized, the grain yields increase. By ensuring a sufficient amount of readily available moisture, in combination with the nutritional regime, an increase in yield values was reported for all hybrids. According Żarski et al. (2023) the complex influence of irrigation and fertilization contribute to yield increases, on average by approximately 25% and over 80% during dry seasons.

Knezha-461 is a hybrid that is responsive to irrigation and that is why the results show times higher yields under irrigated conditions. At var. 1 yields range from 2833.0 kg/ha to 4850.0 kg/ha. Var. 2 is characterized by higher productivity – 15150.0 kg/ha and 10440.0 kg/ha, in the first and second year. The increase, on average for the period, is 233.1% and is the result of the irrigations. The statistical processing of the results shows a high level of reliability ($p \leq 0.1\%$). Optimizing soil moisture contributes to the increase in hybrid productivity. The yields are higher with the top dressing variants. During the first year of the study, var. 4 has the highest yields – 20950.0 kg/ha.

While in the second year, var. 3 stood out with 11153.3 kg/ha, which was 333.3 kg/ha more than var. 4 on average for the period, the analysis shows that at var. 4 the highest yield was reported – 15885.0 kg/ha. Next var. 3 with 13810.2 kg/ha. At var. 2, irrigation was carried out without feeding during the vegetation period, but the yields were also relatively high. On average for the period, 12795.0 kg/ha were registered. The increase in the var. 4, compared to the non-irrigated option, is 313.5%, on average for the study period.

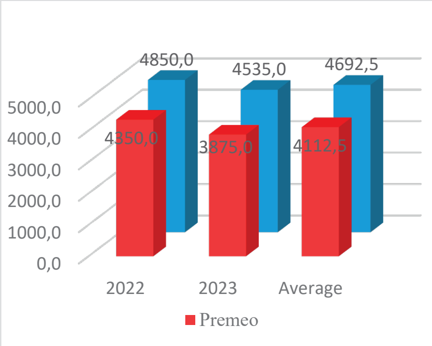

Figure 2. Grain yield for the 2021-2022 period, kg/ha

Premeo is a hybrid of Artesian technology, i.e. these hybrids are distinguished by greater yield resistance in years with insufficient rainfall or when the soil horizon is not sufficiently provided with available moisture. These characteristics of the hybrid enable it to produce a yield of 3500.0 kg/ha in the first year, under non-irrigated conditions and without feeding. The second experimental year is also characterized by high productivity in var. 1 of 4350.0 kg/ha. The responsiveness of maize to irrigation water is again expressed in an increase in the hybrid's productivity. When optimizing the moisture in the soil horizon (var. 2), an increase in the productivity of corn is observed. On average for the period of the study, yields were 13233,0 kg/ha, and by year it was as follows 16733,0 kg/ha and 9733,0 kg/ha. After feeding during the growing season with foliar fertilizers, a jump in the parameters of the yields is reported at Premeo. The increase in the var. 3 and 4 is higher and is due to the efficiency of the assimilation of the applied foliar fertilizers rich in nutritional elements. Irrigation contributes to a 237,1% increase in yield. On average for the period, the highest results were registered for var. 3 (240,8 %). When comparing the irrigation variants with the highest yield, stands var. 3.

Productivity of the irrigation water

During the field study period, a different number of irrigations were conducted in different years.In 2021, 6 irrigations were carried out to maintain the moisture in the soil horizon. In 2022, 4 waterings were implemented. Maize responds to water stress by leaf curling during the hot hours of the day, reducing water transpiration from the crop. In the highly stressed plants, curling of the leaves has been reported already early in the morning. Water stress can lead to lower leaf area index (LAI) and biomass due to reduced intercepted photosynthetically active radiation (IPAR) and radiation use efficiency (RUE) studies have shown by Song et al. (2019). In the non-irrigated variants, under the influence of water deficit, the corn remains in this condition at night. Permanent water deficit reduces the productivity of the culture. During the growing season of corn for grain, the time for supplying irrigation water was established based on the amount and distribution of precipitation and as a result of tracking moisture in the soil horizon in the 0-80 cm layer.

Table 2. Irrigation parameters, productivity of maize hybrids for grain, irrigation norms and irrigation water productivity, for the period 2021-2022

Hybrids of corn	Variant	Yield (Y), kg/ha		Irrigation norm (M), Mm		Irrigation water productivity (IWP), kg/mm	
		2021	2022	2021	2022	2021	2022
Premeo	1	3500.0	4350.0	-	-		-
	2	16733.0	9733.0	1800	1200	9.30	8.11
	3	21467.0	13133.0	1800	1200	11.93	10.94
	4	19633.0	10627.0	1800	1200	10.91	8.86
Knezha -461	1	2833.0	4850.0	-	-	-	-
	2	15150.0	10440.0	1800	1200	8.42	8.70
	3	16467.0	11153.3	1800	1200	9.15	9.29
	4	20950.0	10820.0	1800	1200	11.64	9.02

The productivity of the irrigation water is the ratio between the yield and the irrigation rate. The results of the present experience show that this indicator shows a dynamics of values that varies by year and variant. According to variants in both hybrids in var. 2 lower values of irrigation water productivity are calculated. In the case of the Premeo hybrid, the highest productivity was found in var. 3, ranging from 10.94 kg/mm to 11.93 kg/mm over the years (Table 2). Knezha-461 stands out with 11.64 kg/mm at var. 4 (2021). In the second year, the productivity of the irrigation water is the highest with lime. 3. As per Wang et al. (2022) determination of water productivity is important in the construction of hydromelioration systems and in combating global climate change.

Table 3. Grain yield, additional yield, irrigation standards and effect of 100 m^3 of irrigation water for the period 2021-2022

Hybrids of corn	Variant	Yield (Y), kg/ha		Additional yield (AY), kg/ha		Irrigation norm (M), mm		Effect of 100 m^3 of irrigation water, kg/ha	
		2021	2022	2021	2022	2021	2022	2021	2022
Premeo	1	4210.0	4095.0	-	-	-	-		
	2	9200.0	11997.4	13233.0	5383.0	1800	1200	735.17	448.58
	3	11733.0	13268.8	17967.0	8783.0	1800	1200	998.17	731.92
	4	10113.0	13576.9	16133.0	6277.0	1800	1200	896.28	523.08
Knezha -461	1	4850.0	4535.0	-	-	-	-		
	2	10440.0	12320.7	12317.0	5590.0	1800	1200	684.28	465.83
	3	11153.3	13754.8	13634.0	6303.3	1800	1200	757.44	525.28
	4	10820.0	13138.9	18117.0	5970.0	1800	1200	1006.50	497.50

The effect of 100 m^3 of irrigation water is determined by calculating the ratio between the additional yield and the irrigation rate. Table 3 shows that the values of this indicator are highest in the first experimental year with var. 4 (1006.5 kg/ha). With the same variant, in the second year, 497.5 kg/ha are reported, which is 50.5% less, regardless of the smaller number of irrigations realized. The phenophase of corn for grain is important when implementing irrigation. A number of authors report dependencies between the phenological development of crops and the irrigation regime. (Brar et al., 2016; Katiyar et al., 2018). Studies have established a positive linear relationship between the duration of some phenophases and irrigation, which predetermines and increase grain yield. Lower parameters characterize the effect of 100 m^3 in the second year.

On average for the period at Knezha-461, the highest effect per 100 m^3 of irrigation water is recorded at var. 4 (752.0 kg/ha). Average for the period, with the highest effect per 100 m^3 of irrigation water, var. 3 (Premeo) with 865.0 kg/ha followed by var. 4 (Knezha-461) with 752.0 kg/ha. The analysis of the results of the two corn hybrids studied shows that Premeo is characterized by a higher effect per 100 m^3 of irrigation water, an average of 722.2 kg/ha for all variants. With Knezha-461, the average effect of the variants was calculated to be 656.1 kg/ha.

CONCLUSIONS

Optimizing the irrigation regime provides an increase in corn yields for grain by 237.1% in Premeo and by 233.1% in Knezha-461 compared to the non-irrigated option. Optimizing the irrigation and nutrition regime leads to an increase in productivity by 340,8 % (var. 3) in Premeo, and in Knezha-461 by 313.5% in var. 4.

The corn hybrid Premeo (var. 3) 11.4 kg/mm stands out with the highest irrigation water productivity, followed by var. 4 (Knezha-461) with 10.3 kg/mm, average for the study period. A trend towards an increase in the productivity of irrigation water with an increase in the productivity of the maize hybrids was found.

It was established that the Premeo corn hybrid has a higher effect per 100 m^3 of irrigation water, on average for all variants – 722.2 kg/ha, while for Knezha-461 the effect is 656.1 kg/ha.

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THE INFLUENCE OF MINERAL FERTILIZATION TYPE ON THE PROTEIN CONTENT OF RAPESEED SEEDS AND MEAL

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Abstract

Rapeseed (Brassica napus) stands as one of the preeminent oil-bearing crops globally, attributable to its elevated seed oil concentration, robust hybrid production capabilities within the extant crop, and its versatile applications. The advent of erucic acid and glucosinolate-free hybrids has significantly broadened the utility of rapeseed-derived cakes and meal, characterized by a substantial protein content ranging from 38% to 41.9%. This study aimed to elucidate the impact of mineral fertilizer type on the protein content of both rapeseed and its resultant meal. Three distinct mineral fertilizers were tested in the experiment: E34 (10:24:0 + 0.1Zn + 0.1Br + 20 SO_3), DAP (18:46:0), and the 20:20:0 fertilizer type. The findings unequivocally underscored the discernible impact of fertilizer type on the protein content of rapeseed seeds. Notably, the protein content ranged from 19.80% in the 20:20:0 fertilization variant to 22.04% in the E34 fertilization variant. Similarly, the protein content of the resultant meal exhibited variance, oscillating between 38.07% in the 20:20:0 fertilization variant and 39.81% in the E34 fertilization variant. These outcomes accentuate the pivotal role of mineral fertilization in modulating the nutritional composition of rapeseed and its derivatives.

Key words: *fertilization, protein, rapeseed.*

INTRODUCTION

According to the US Department of Agriculture/Foreign Agricultural Service (USDA/FAS, 2018/19), rapeseed meal is the second largest global production of protein meals in the world, following soybean meal (Gherasimescu et al., 2023). The emergence of erucic acid-free and glucosinolate - free rapeseed hybrids has expanded the use of rapeseed for fodder in cattle feed, being an important source of proteins and amino acids that stimulate lactation, and more recently in human nutrition as a source of plant protein due to its special fatty acid composition (Bătrîna et al., 2021; Şuveţ et al., 2021; Dziekanski et al., 2022; Sitnicki et al., 2024).

Rapeseed-based feeding is the most important source of non-GMO proteins in Europe and is very important for dairy and meat producers in meeting the market demand for non-GMO products (Gofferje et al., 2015; Reichert et al., 2020; Schafer et al., 2018; Bătrîna et al., 2020).

Another use of the cakes resulting from oil extraction is to produce natural fertilizers (Suvet et al., 2023).

Many studies (Sanchez-Vioque et al., 2001; Chang and Nickerson, 2014, 2015; Jang et al., 2011; Shin et al., 2011; Bandara et. al., 2017; Zhang et al., 2018; Fetzer et. al. 2019; He et al., 2019;) mention the use of rapeseed proteins for use in the production of adhesives, polymers, adhesives, and lubricants.

This study aimed to elucidate the impact of mineral fertilizer type on the protein content of both rapeseed and its resultant meal.

MATERIALS AND METHODS

The study examining the impact of various mineral fertilizers on the protein content of rapeseed seeds and meal was carried out on cambic chernozem soil. This research also explored how different fertilization levels interact with the specific climatic conditions experienced during 2021-2022 at the Educational Station of the University of Life Sciences 'Regele Mihai I' from Timişoara. The experiment was focused on the influence of three types of mineral fertilizers, namely: E34 (10:24:0 + 0.1Zn + 0.1Br +20 SO_3), DAP (18:46:0) and fertilizer type 20:20:0. The fertilizers were applied fractionally: 200 kg for preparing the germination bed and 200 kg in spring. The used rapeseed hybrid was Astronom. The preceding plant was autumn wheat.

In order to assess the protein content, rapeseed meal and seeds were first defatted, then proteins were extracted using a strong alkali solution, followed by hydrolysis to break down proteins into amino acids. These amino acids are derivatized using phenyl isothiocyanate to form volatile derivatives suitable for GC-MS analysis. The total protein content was estimated by quantifying these derivatives using calibration curves based on standard proteins.

RESULTS AND DISCUSSIONS

The study of seed protein content [8.5% moisture]

The data regarding the protein content in seeds, presented in Table 1 and Figure 1, indicate that it ranged from 19.80% in the fertilization variant a3 (20:20:0) to 22.04% in the fertilization variant a1 (E34). The average protein content recorded for the experiment was 20.63%. Compared to the experiment's average, the a1 fertilization variant registered an increase of 1.42%, a difference statistically marked as highly significant. The protein content in the case of a3 fertilization level was 0.83% below the experiment's average, recording a negative increase, statistically significant in a negative sense.

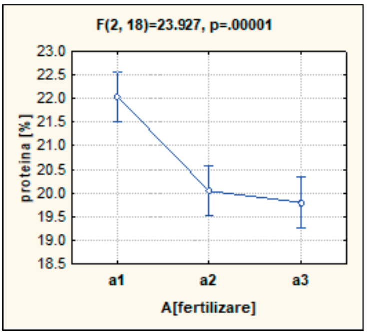

Figure 1. Variation of seed protein content in the 3 types of fertilization (factor A)

In conclusion, factor A (type of fertilization) has a significant action on protein synthesis; among the three levels of fertilization followed in the experiment, there are significant differences regarding the protein content.

Table 1. Protein content in the seed, depending on the type of fertilization

Factor A	Protein (%)	Diff.	Signif.
a1 - E34	22.04	1.42	***
a2 - DAP	20.04	-0.59	ns
a3 - 20:20:0	19.80	-0.83	0
Average	20.63	Mt	

DL 5% = 0.75 %; DL 1% = 1.02; DL 0.1% = 1.40

The protein fluctuations from one level of fertilization to another are significant.

The protein content decreases with the level of fertilization, showing a descending trend. Protein content varies between 22% and 19.8%. The differences between fertilization levels are highly significant (p<0.00).

The protein content in seeds, based on the experimental year, is presented in Table 2 and Figure 2.

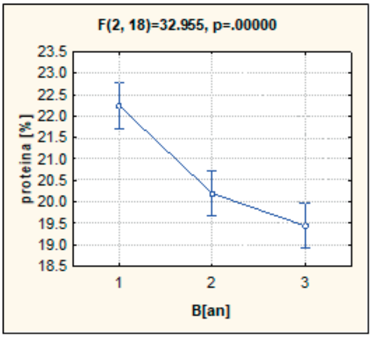

Figure 2. Yearly variation of protein content in seeds [factor B]

Table 2. Protein content in seed by experimental year

Factor B	Protein (%)	Diff.	Signif.
b1 - 2019	22.24	1.61	***
b2 - 2020	20.20	-0.43	ns
b3 - 2021	19.44	-1.18	**
Average	20.63	mt	

DL 5% = 0.75 %; DL 1% = 1.02; DL 0.1% = 1.40

Beyond the level of mineral fertilization, the protein content in seeds is also influenced by climatic conditions, namely temperature and humidity during the fruiting period. As a result, the protein content in the experimental cycle from 2019 to 2021 ranged from 19.44% in 2021 to 22.24% in 2019, a year characterized by very high temperatures and a lack of moisture. Therefore, compared to the experiment's average of 20.63%, in 2019 the protein content exceeded the control value by 1.61%, a difference statistically assured as highly significant. The protein content in 2020

registered a value roughly equal to the experiment's average.
In years with a precipitation deficit during the fruiting period, the protein content was higher, but seed production was lower. In conclusion, we can state that factor B (year) has a highly significant impact, the difference between production years being highly significant. The annual fluctuations in protein content are highly significant (meaning they are statistically assured at the α=0.001% level).
The protein content in seeds resulting from the interaction of the factors fertilization level x experimental year (A x B) and the significance of the content differences compared to the control are presented in Table 3 and Figure 3.

Table 3. Protein content in seed obtained from the interaction between A x B
and the significance of the production differences compared to the control

Factor B (year)	Factor A (fertilization)								
	a1 - E34			a2 - DAP			a3 - 20:20:0		
	protein %	Diff. [%]	Signif .	protein %	Diff. [%]	Signif.	protein %	Diff. [%]	Signif.
b1 - 2019	23.74	3.11	***	21.71	1.08	ns	21.27	0.64	ns
b2 - 2020	21.78	1.15	ns	19.72	-0.91	ns	19.10	-1.53	0
b3 - 2021	20.62	-0.01	ns	18.68	-1.95	ns	19.03	-1.60	0
Average	20.63								

DL 5% = 1.295%; DL 1% = 1.774% DL 0.1% = 2.418%

Regardless of the experimental year, the protein content in seeds shows a descending trend relative to the level of fertilization (from E34 to 20:20:0), except for the year 2021, which has a descending trend from a1 to a2, and from a2 to a3, the trend is ascending.

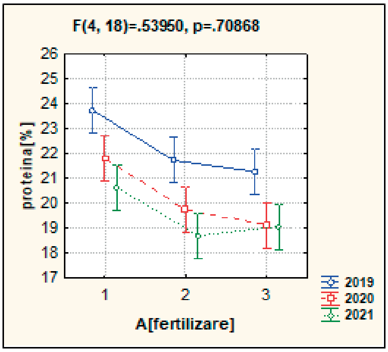

Figure 3. Variation in protein content
(A x B interaction)

The contribution of factors A (fertilization), B (year), and the interaction AxB to the achievement of protein content in rapeseed is presented in Figure 4.
Factor A (fertilization) contributes to the achievement of production by 35.73%, factor B (year) contributes 49.22%, and the interaction A x B contributes 1.61%. Therefore, the greatest contribution comes from factor B(year), followed by factor A (fertilization) and the interaction A x B.

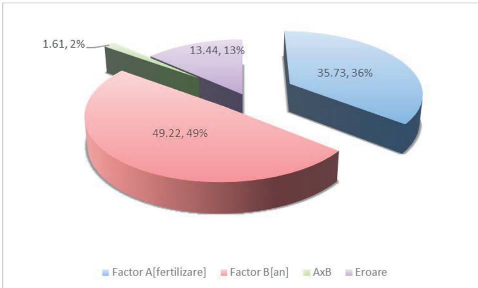

Figure 4. Contribution of factors A (fertilization),
B (year) and A x B interaction

The protein content in meal [12% moisture]

The protein content in meal is presented in Table 4 and Figure 5. The obtained data indicate that it ranged from 38.07% in the fertilization variant a3 (20:20:0) to 39.81% in the fertilization variant a1 (E34).

Table 4. Protein content in meal, depending
on the type of fertilization

Factor A	Protein (%)	Diff.	Signif.
a1 - E34	39.81	1.00	ns
a2 - DAP	38.55	-0.26	ns
a3 - 20:20:0	38.07	-0.74	ns
Average	38.81	Mt	

DL 5% = 1.20 %; DL 1% = 1.65; DL 0.1% = 2.25

These results confirm the exceptional value of the meal obtained from oil extraction as fodder, considering that the Astronom hybrid is free from erucic acid and glucosinolates, and the resulting fodder contains approximately 38%

protein. Depending on the experimental year, the protein content in the meal (Table 5 and Figure 6) varied according to climatic conditions from 37.68% (in 2021) to 40.27% (in 2019).

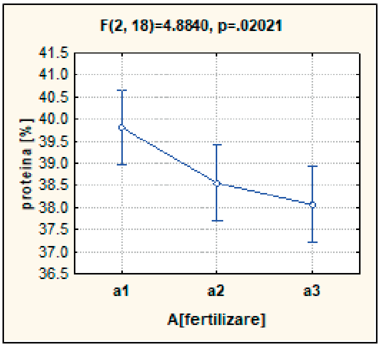

Figure 5. Protein content variation in meal across the three types of fertilization (factor A)

The protein content decreases with the level of fertilization; the two variables are inversely proportional. The evolution of protein content is descending. The protein content varies between 39.8% (a1), 38.5% (a2), and 38% (a3). The differences between the levels of fertilization are significant (p = 0.02, i.e., p<0.05).
The protein content in meal depending on the experimental year is presented in Table 5 and Figure 6. From the data analysis, it is observed that it is directly correlated with the protein content in the seed.

Table 5. Protein content in meal by experimental year [Factor B]

Factor B	Protein (%)	Diff.	Signif.
b1 - 2019	40.27	1.46	***
b2 - 2020	38.48	-0.33	ns
b3 - 2021	37.68	-1.13	**
Average	38.81	mt	

DL 5% = 1.20 %; DL 1% = 1.65; DL 0.1% = 2.25

The highest protein content, around 40.3%, was obtained in b1 (year 2019) – class B, a content that significantly differs from the protein content obtained in b2 (year 2020) and b3 (year 2021).

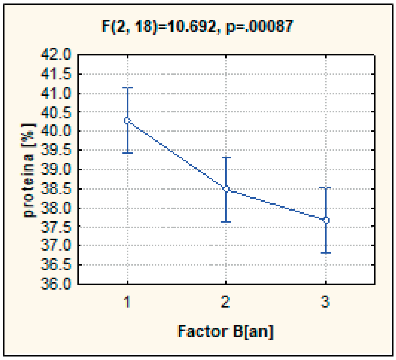

Figure 6. Yearly variation of protein content in seeds (factor B)

The protein content in the meal under the interaction of experimental factors, fertilization and year, is presented in Table 6 and Figure 7.

Table 6. Protein content in meal obtained from the interaction between A x B and the significance of the production differences compared to the control

Factor B (year)	Factor A (fertilization)								
	a1 - E34			a2 - DAP			a3 - 20:20:0		
	protein %	Diff. [%]	Signif.	protein %	Diff. [%]	Signif.	protein %	Diff. [%]	Signif.
b1 - 2019	41.69	2.88	**	40.62	1.81	ns	38.51	-0.30	ns
b2 - 2020	39.19	0.38	ns	37.87	-0.94	ns	38.37	-0.44	ns
b3 - 2021	38.54	-0.27	ns	37.17	-1.64	ns	37.34	-1.47	ns
Average	38.81 %								

DL 5% = 2.09%; DL 1% = 2.86% DL 0.1% = 3.90%

Compared to the control – the average of the experiment, only at a1 variant in the year 2019, was there a significantly distinct difference in the protein content in the meal, while for the other variants, the differences were not significant. Regardless of the production year, the highest protein content was obtained at the fertilization level a1, and the lowest protein content was achieved at the fertilization level a3. The protein contents obtained at a1, over the three production years, varied between 41.7% and 38.5%, and those from a3 varied between 38.5% (b1), 38.4% (b2), and 37.3% (b3).
The contribution of factors A (fertilization), B (year), and the interaction A x B to achieving the protein percentage in the meal is presented in Figure 8. Factor A (fertilization) contributes to the production by 18.06%, factor B (year) contributes 39.54%, and the interaction AxB contributes 9.2%. Therefore, the greatest

contribution comes from factor B (year), followed by factor A (fertilization) and the interaction A x B.

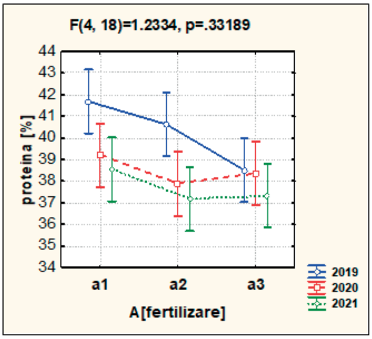

Figure 7. Meal protein content variation (A x B interaction)

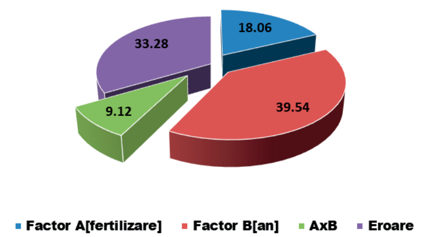

Figure 8. The contribution of factors A (fertilization), B (year), and the interaction A x B to the protein content in the meal

CONCLUSIONS

The protein content in seeds fluctuated between 19.80% for the fertilization variant with the 20:20:0 type of fertilizer and 22.04% for the E34 fertilization variant. The average protein content recorded for the experiment was 20.63%, a value close to the protein content in soybeans. These results underline the significance of the type of fertilizer on protein production, with significant differences in protein content among the three types of fertilization used in the experiment. The fluctuations in protein content from one fertilization level to another are significant.

Beyond the type of mineral fertilization, the protein content in seeds is also influenced by climatic conditions, namely temperature and humidity during the fruiting period. Accordingly, the protein content in the experimental cycle from 2019 to 2021 varied between 19.44% in 2021 and 22.24% in 2019, a year characterized by very high temperatures and a lack of moisture. Thus, compared to the experiment's average of 20.63% in 2019, the protein content exceeded the control value by 1.61%, a difference statistically significant as highly significant. The protein content in 2020 recorded a value approximately equal to the experiment's average.

In the production of seed protein content, the type of fertilization contributed 35.73%, climatic conditions contributed 49.22%, and the interaction of fertilization type x climatic conditions contributed 1.61%.

The protein content in meal varied between 38.07% for the 20:20:0 fertilization variant and 39.81% for the E34 fertilization variant. Depending on the experimental year, the protein content in the meal fluctuated due to climatic conditions from 37.68% (in 2021) to 40.27% (in 2019).

The contribution of the fertilization type to the protein content in the meal was 18.06%, climatic conditions contributed 39.54%, and the interaction of fertilization type x climatic conditions contributed 9.2%.

These findings confirm the exceptional value of the meal resulting from oil extraction as fodder, considering the Astronom hybrid is free from erucic acid and glucosinolates, and the fodder obtained contains approximately 38% protein.

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FEATURES OF GROWTH AND DEVELOPMENT OF *Hyssopus officinalis* L. IN THE CONDITIONS OF THE SOUTHERN STEPPE OF UKRAINE

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Abstract

Each year, the pharmaceutical industry`s demand for high-quality medicinal raw materials increases, leading to the need for the cultivation of the most commonly used medicinal plants. According to statistics, over 40% of all drugs, including 75% of medicines, are produced from medicinal plants. In the southern regions of Ukraine, the cultivation of stress-resistant crops with high productivity and enhanced quality characteristics of raw materials is essential, especially those that can thrive under high temperatures and low relative humidity conditions. Medicinal and essential oil crops could be suitable for this region. Hyssop (Hyssopus officinalis L.) is a non-traditional plant from the Lamiaceae family, which has been cultivated in Ukraine over the past decade. The article presents the results of phenological observations and biological growth and development characteristics of Hyssopus officinalis L. under introduction conditions. The growth dynamics of the plants are studied, and three developmental stages are considered: the latent stage, the vegetative stage, and the generative stage. The obtained results can be used in breeding work and in providing practical recommendations for the cultivation and propagation of hyssop.

Key words*: introduction, Hyssopus officinalis L., medicinal hyssop, ontogenesis periods, phenological observations.*

INTRODUCTION

In the face of global climate change, a critical task worldwide is the enrichment and preservation of plant biodiversity. Enriching biological diversity of plants and expanding the assortment of valuable plants can be achieved through the introduction and cultivation of new, non-traditionally grown species.

Many plants of the *Lamiaceae* Martynov family are valuable for their aromatic, essential oil, medicinal, vitamin, and decorative properties. One of the most common species in the *Hyssopus* L. genus of the *Lamiaceae* Martynov family is *Hyssopus officinalis* L. Its native region is the Mediterranean and Western Europe (Druțu et al., 2014). It is cultivated for medicinal, decorative, and aromatic purposes (Kovalenko & Andriychenko, 2018; 2019; Kovalenko, 2022; Dobrovolskyi, 2021; Dumitru et al., 2020). It is used in medicine in various countries.

The herb of *Hyssopus* is included as an official raw material in the pharmacopeias of France, Portugal, Romania, Sweden, and Germany. In Indian medicine, the herb is used for asthma, coughs, as a digestive and digestive aid. Bulgarian medicine recommends an infusion of the herb for chronic bronchitis, intestinal catarrh, and as an antiseptic. Locally, hyssop decoctions are used for eye rinses, gargles for stomatitis, for soothing hoarseness, as compresses for bruises and as a wound-healing agent (Rabotyagov et al., 2003). Hyssop essential oil has antimicrobial properties and is used for purulent skin infections of staphylococcal origin (Svidenko and Derevjanko, 2005; Svidenko, 2005). Additionally, the plant is used as a culinary raw material for the production of fish products, pickling cucumbers and tomatoes, as well as in the preparation of meat and vegetable soups, sauces, stews, roasts, salads (Nair, 2022). Hyssop essential oil and extracts are used in winemaking, canning, and the perfume-cosmetic industry (Wesolowska et al., 2010; Judžentienė, 2016; Kazazi, et al., 2007). *Hyssop officinalis* L. is a valuable honey plant, and the honey produced from it is of high quality.

The widespread use of *Hyssopus officinalis* L. in medicine, essential oil, and the food industry necessitates the expansion of its cultivation areas (Kizil et al., 2016; Salachna, 2023; Stan et al., 2019). The success of its introduction largely depends on a comprehensive study of the process, which thoroughly characterizes the individual development of the plant during specific calendar periods, known as the ontogenesis of the plant. In this study, we investigated the age periods and stages that *H. officinalis* undergoes under the conditions of introduction.

MATERIALS AND METHODS

The study utilized seed populations of *Hyssopus officinalis* L., specifically the 'Nikit'sky Bily' variety. Research on the growth and development characteristics of hyssop was conducted in the conditions of the South Steppe. Ecological and phenological observations were carried out on the plants, along with biometric measurements using commonly accepted methodologies. The study examined three developmental stages: the latent stage, the vegetative stage, and the generative stage. Based on these stages, the annual growth cycle was divided into four phenophases with corresponding subphases: the vegetative phase, the budding phase, the flowering phase (initiation of flowering, full flowering, wilting), and the fruiting phase (initiation of fruit formation, initiation of seed setting, seed setting).

RESULTS AND DISCUSSIONS

In the cultivation of *Hyssopus officinalis* in the South Steppe conditions, the plants typically reach a height of 70-80 cm and a bush diameter of 100-130 cm. The stems are four-angled and become woody at the base. Each shrub may have up to 100 flowering shoots. The leaves are sessile, linear-lanceolate, opposite, and entire. The leaf blade is pubescent on both sides, measuring 3.0-3.5 cm in length and 0.7-0.9 cm in width. Essential oil glands on the upper and lower leaf plate are clearly visible (Figure 1, a) The small flowers are clustered in false whorls in the leaf axils and form spike-type inflorescences at the upper part of the stem (Figure 1, b and c). The flower corolla (7.5-10.5 mm in length) is usually blue-violet, but there are also white and pink-colored forms. The calyx is tubular-bell-shaped with five pointed teeth.

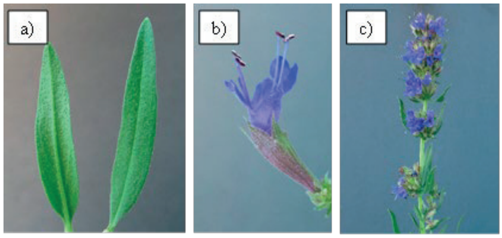

Figure 1. Morphological features of organs of *Hyssopus officinalis* L.: a - leaf plate (lower and upper part); b - flower; c - inflorescence

Hyssopus officinalis plants can be propagated through seeds, woody and green cuttings, or by dividing the shrub. Seed propagation is commonly preferred.

In the context of our study on the introduction of *H. officinalis*, we identified three age periods of ontogenesis: the latent period, vegetative, and generative. Within these, we distinguished six age states of the species: seeds, seedlings, juvenile, immature, vegetative, and generative.

Latent Period. This period spans from the moment the seeds fully mature until they germinate. It typically lasts for a minimum of 8-9 months. The seeds have an elongated-ovate shape, ranging from dark brown to black, with three angles, and are approximately 2.5-3.0 mm long and 1.0-1.2 mm wide (Figure 2, a-c). Along the seed, the seed scar is well visible. On average, the weight of 1000 seeds is 0.96 g. At a temperature of 20°C, the seeds begin to germinate on the 3rd to 4th day. Laboratory germination rates range from 90-95%. Seed germination decreases after three years.

Vegetative Period. This period starts with the emergence of seedlings and ends with the formation and development of generative shoots. During this time, the seedlings undergo several morphological changes and eventually resemble adult plants. This period is divided into multiple age states.

Seedlings. During the germination process, the cotyledons swell, and the embryo increases in size. Initially, the root appears, followed by round cotyledonal leaves (Figure 2, b and c). On experimental plots where seeds are sown in the spring (first to second decade of April) at a depth of 1.0-1.5 cm, seedlings emerge after 14 days (in dry spring years, it might take up to 20-25 days). Field seedling similarity is 75%.

Juvenile state. The appearance of the first true leaf corresponds to the beginning of the juvenile age state. At this stage, juvenile plants still retain their cotyledonal leaves. The first pair of true leaves appears in seedlings after 5-7 days, approximately 18-20 days from sowing. They are elongated-ovate and dark green in color, measuring 1.3-1.5 cm in height, with a root length of 1.7-1.9 cm. The stem is rounded in cross-section and has an anthocyanin coloration. During the third decade of growth, the second pair of true leaves emerges (Figure 2, h). At this point, the plants reach a height of 4.0-4.3 cm, and the root length increases to 2.3-2.8 cm. The first pair of true leaves measures 1.7-2.0 cm in length and 0.7-0.8 cm in width. The leaves are pubescent, especially on the lower side, and the essential oil glands are visible to the naked eye on the leaf blade. Towards the end of the third decade in May, the third pair of true leaves appears, followed by the fourth pair within 5-6 days. After the emergence of the third pair of true leaves, the plant's growth accelerates.
In the leaf axils of the central (main) shoot, the formation of first-order lateral shoots begins (Figure 2, e). At this stage, the seedlings reach a height of 8.0-8.5 cm. In the bushing phase (first decade of July), the plants reach heights of 15-20 cm with a bush diameter of 10-15 cm. In the leaf axils of the central shoot, starting from the 3rd-4th pair and above, first-order lateral shoots develop, ranging from 2 to 5 cm in length, which, in turn, give rise to second-order shoots.
Immature state. The beginning of the immature age state is characterized by the withering of cotyledonal leaves, the development of leaves resembling those of adult individuals, and intensive root system growth. One significant change in leaf shape becomes more prominent in immature individuals. Unlike juvenile elongated-ovate leaves, immature individuals have lanceolate leaf blades.
Vegetative state. This stage is characterized by significant height growth and the accumulation of reserve plastic substances necessary for plants to transition into flowering and fruiting. There is an intensive development of lateral shoots, and the base of the shoots becomes four-angled. Within a decade, the plants reach a height of 35 cm and a bush diameter of 27 cm. Vegetative plants have significantly larger organ sizes. In the first year of development, a shrub is formed with a main shoot, 8-13 pairs of first-order shoots, and 26-32 pairs of second-order shoots.er.

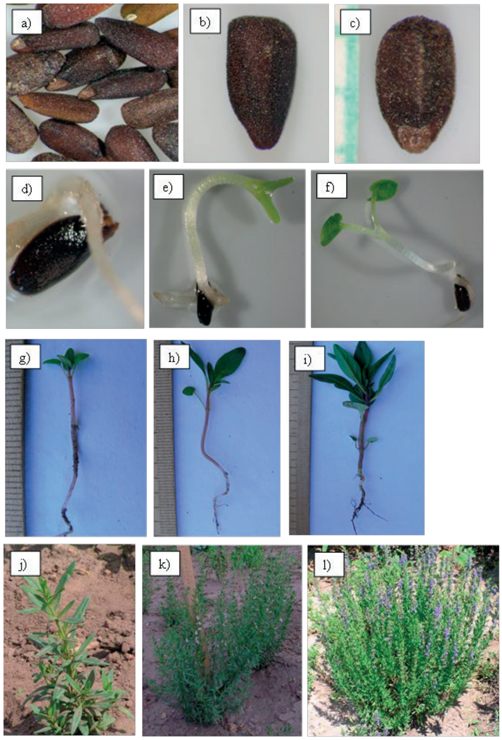

Figure 2. Seedling development:

a…c - latent period (seeds); d…j - virginal period: d - root germination, e…f - appearance of cotyledon leaves, g…i - juvenile plants, j - immature plants; k…l - generative period: j…k - budding, the beginning of flowering in a seedling of the first year of life, *F. rosea*, l - mass flowering in a seedling of the second year of life, *F. coeruleus*

Generative period. In the first year of the plant's life, it forms inflorescences. Both lateral and central shoots become generative with a closed type of development. In young generative individuals, the phase of budding begins at the end of the second decade of July. The beginning of flowering is observed at the end of the first decade of August. Mass flowering of the experimental plants in their first year is noted at the end of the second and the beginning of the third decade of August.
During the mass flowering phase, first-year plants reach a height of 55 cm and a shrub diameter of 51 cm. Seedlings grown from seeds have a well-developed root system, and the

bushes branch well. During the spring and summer, they require good care, including soil loosening and watering in hot months.

The vegetative recovery of plants in the second year begins in the third decade of March. The budding phase begins in the second to third decade of June. The phase of initial flowering occurs in the first decade, and mass flowering begins in the second decade of July. The phase of initial flowering in forms with white flowers usually occurs 3-4 days earlier. Flowering begins with the central (main) inflorescence and then the lateral ones bloom.

The end of the flowering phase occurs in the third decade of August, with a total flowering duration of two months. During this time, the stems become woody in the middle and lower parts. The seeds ripen in September, and they are easily shed. The plants dry up in November. The average duration of the vegetative period is 195 days.

When studying the growth dynamics of hyssop, it was established that the maximum growth of plants is observed during the budding phase and the beginning of flowering. After that, plant growth almost stops (Figure 3).

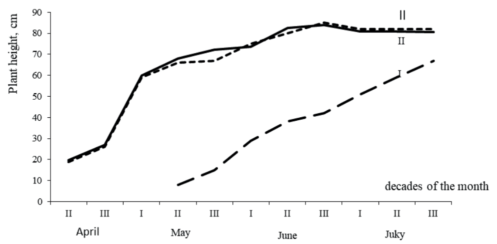

Figure 3. Dynamics of growth and development of *H. officinalis* in the conditions of the Kherson region:

I - plants of the first year of life; II - plants of the second year of life; III - plants of the third year of life

CONCLUSIONS

We have examined three age periods of *H. officinalis* (latent, vegetative, generative), each of which consists of distinct age stages. At the initial stages, seedlings undergo a series of morphological changes associated with plant height and biomass accumulation. The maximum plant growth occurs at the beginning of the generative period during the budding and early flowering phase. Additionally, we have found that in the conditions of introduction, the plants go through all the phenological development phases and produce viable seeds. During the first year of their life, *H. officinalis* seedlings have a single branching shoot that flower and bears fruit. Starting from the second year of life, the number of vegetative and generative shoots in the bush increases. The obtained results can be utilized in breeding work and in providing practical recommendations for cultivation and propagation.

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STUDY OF THE EARLY - PRODUCTION RELATIONSHIP IN MAIZE HYBRIDS GROWN AT CARACAL, ROMANIA

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Abstract

Choosing maize hybrids is one of the most important decisions a farmer makes each year, the yield potential, disease resistance and herbicide/pest resistance being the main factors that need to be addressed and balanced. Also, a key factor in the choice of the maize hybrid is its earliness. In stress environments, stable genotypes are valuable for their predictable and consistent yields. In the period 2020-2023, on the chernozem from Caracal located in South Romania, an assortment of maize hybrids was tested, the precocity-production relationship being studied. The obtained results showed that in years favorable to maize cultivation, hybrids from the FAO groups 260-340, 340-390 and 400-460 can be grown on the black soil from Caracal in South Romania with similar results. If there are also unfavorable years, considering that we cannot know from the beginning what the weather will be like, it is preferable to cultivate hybrids from the FAO groups 260-340 and 350-390.

Key words: *maize, hybrid, precocity, FAO group.*

INTRODUCTION

Maize crops are of particular importance, as maize kernels are used for human consumption, as animal feed and also as raw material in industry. According to FAO data, approximately 21% of world production is used in human food, 72% in animal feed and 7% as raw material in the food industry (Stoica, 2020).

Maize earliness refers to the period between the date of sowing and the date of harvest, this being a key factor in the choice of the maize hybrid. It is important to consider the early ripening period and soil conditions in the region. The later the maize hybrid, the longer its growing period. The degree of maturity of maize (*Zea mays* L.) hybrids, usually grown in central and northwestern Romania, varied from FAO 100 to 400 (Haș et al., 2012). Late stock normally shows too much moisture at harvest and then presents storage problems. This severely limits the scope of germplasm that can be used in a breeding program located in the area. Selection for early flowering and low moisture at harvest adapts the material to areas requiring earlier maize (Ortiz et al., 2008).

Obtaining maize hybrids which, among other characteristics, must also be early is an important concern of maize breeders. Early maize must grow faster and mature earlier in cooler conditions than late maize to produce mature kernels in a shorter time. Early maize grows faster, especially in the spring when the weather is cool. The maturity zones are based on the useful degrees (GDU) accumulated during the frost-free period: GDU = [Max Temp (≤ 30ºC) + Min Temp (≥ 10ºC)]/2 – 10ºC.

In northern and premontane areas, due to limited thermal resources, precocity is necessary to reach maturity (Cristea, 2004; Căbulea et al., 1999). In southern areas, earliness is used to alleviate the moisture stress of late drought by sowing earlier and earlier.

Maize can be grown under different environmental conditions, but nevertheless, its production is strongly influenced by genotypes, technologies (rotation, earliness and sowing date) and their interaction (Ali et al., 2017). Maize is sensitive to drought at almost all growth stages, but is most sensitive during the flowering period (Spitkó et al., 2014). Thus, when a water deficit occurs at flowering, there

is a delay in spike growth and then silk emergence (Edmeades, 2013).
In stress environments, stable genotypes are valuable for their predictable and consistent yields. However, such stability would be less valuable in a less stressed environment, where genotypes through their adapted responses could produce higher yields, taking advantage of favorable environmental conditions (Kusmec et al., 2018). Genotype is the hereditary makeup of an individual and its performance is based on the ecosystem in which it is placed (Andorf et al., 2019).
Choosing maize hybrids is one of the most important decisions a farmer makes each year. Yield potential, disease resistance and herbicide/pest resistance are the main factors that need to be addressed and balanced. Another important factor is relative maturity. A hybrid can take more advantage of the available heat units and produce more, contrary to what its earliness group foreshadowed (Stefan et al., 2018).
Growers may choose hybrids of different relative maturity for various reasons. Michigan State University recommends choosing a hybrid with varying relative maturity to use flowering and pollination as a drought shield and to vary harvest dates. Usually, not all hybrids from the same FAO group are chosen because sometimes the spring or summer can be particularly cool or a frost can set in earlier.
Silva et al. (2022) evaluated seven maize hybrids from different seed companies/ marke-ters to study the effect of water and nitrogen deficit on grain yield and yield components. The hybrids tested were DK619, Lerma, NK703, Rio Cisnes, Rio Maipo, Rio Negro and Rio Trancura. Hybrids were arranged in randomized blocks with 4 replicates. Plot size was 18 m^2 (four rows spaced 0.75 m x 6 m long). The main differences in production showed that the influence of the environment (SE=1,054 kg/ ha) was greater than that caused by the hybrid (SE=240 kg/ha). Production varied between 12,802 and 16,572 kg/ ha. Differences in grain yield were associated with biomass ($r = 0.89$; $p = 0.008$) and number of grains/m^2 ($r = 0.70$; $p = 0.080$). Water stress and nitrogen stress caused a 72% and 57% decrease in production compared to the production from the non-stress environment (Silva et al., 2022).
Based on the results recorded in the United States (Kansas), Araya et al. (2017) concluded that maize production will decrease in the coming years, on average by 18-33%. The shortening of the maize vegetation period (9-18% reduction in days to maturity), due to high temperatures, could cause a decrease in production.
Studies carried out in Turda recommended earlier sowing as a technological measure to reduce the influence of high temperatures, considering that in this way the plants would more effectively use the existing water reserve in the soil, from the first months of the year (Haş et al., 2021).

MATERIALS AND METHODS

In the period 2020-2023, on the chernozem from Caracal in South Romania, numerous maize hybrids from globally recognized seed producing companies were tested - BASF, Biocrop, Corteva, Donau Saat, KWS, Lidea, MAS Seeds, Saaten Union, Syngenta and with vegetation periods placed in almost all FAO earliness groups.
From a climatic point of view, the years 2020, 2021 and 2023 were favorable for maize cultivation, but the year 2022 was extremely dry and the hybrids placed in the FAO 400-490 group and above FAO 500 had extremely low productions.
The precocity-production relationship was studied through the lens of correlations between production and humidity at harvest, production and classification in the FAO group, production and its coefficient of variability within the FAO groups, but also by calculating the differences in production using the Newman-Keuls test.

RESULTS AND DISCUSSIONS

For the period in which the climatic conditions were favorable for maize cultivation, the Newman-Keuls test shows distinctly significant differences between the productions of hybrids belonging to the FAO 150-250 group and all other groups, between the FAO group greater than 460 and the hybrids from the FAO 260-340 groups, 350-390 and 400-450, but does not show any difference between the productions

of the hybrids from the FAO 260-340 group and the 350-390 and 400-450 groups, nor between the 350-390 and 400-450 groups (Table 1).
For the period in which the values from the year 2022 were also entered in the production average, a year in which the climatic conditions were extremely unfavorable, the Newman-Keuls test allows a different interpretation. Distinctly significant differences were recorded between the yields of hybrids belonging to the FAO group greater than 460 and all other groups, as well as between the FAO 150-250 group and hybrids from the FAO 260-340 and 400-450 groups. The production differences in hybrids belonging to the 150-250 group and those from the 350-390 group, on the one hand, and those between the FAO 350-390 and FAO 400-450 groups, on the other hand, are significant. There was no difference between the yields of the hybrids in the FAO 350-390 group and the FAO 260-340 group, but also between the FAO 260-340 and 400-450 groups (Table 2).

Table 1. Presentation of the results of the Newman-Keuls test for maize tested at Caracal – average of the years 2020, 2021 and 2023 (unfavorable year excluded)

Experienced FAO Group	Experienced FAO Group				
	FAO 150-250 (48.64 q/ha)	FAO > 460 (58.00 q/ha)	FAO 260-340 (70.37 q/ha)	FAO 350-390 (70.41 q/ha)	FAO 400-450 (73.63 q/ha)
FAO 150-250 (48.64 q/ha)	0	9.36**	21.73**	21.77**	24.99**
FAO > 460 (58.00 q/ha)		0	12.37**	12.41**	15.63**
FAO 260-340 (70.37 q/ha)			0	0.04*ns*	3.26*ns*
FAO 350-390 (70.41 q/ha)				0	3.22*ns*
FAO 400-450 (73.63 q/ha)					0

*p < .05, * * p < .01

Table 2. Presentation of the results of the Newman-Keuls test for maize tested at Caracal - average of the years 2020-2023 (unfavorable year included)

Experienced FAO Group	Experienced FAO Group				
	FAO > 460 (25.36 q/ha)	FAO 150-250 (32.30 q/ha)	FAO 350-390 (37.67 q/ha)	FAO 260-340 (41.08 q/ha)	FAO 400-450 (42.43 q/ha)
FAO > 460 (25.36 q/ha)	0	6.94**	12.01**	15.72**	17.07**
FAO 150-250 (32.30 q/ha)		0	5.37*	8.78**	10.13**
FAO 350-390 (37.67 q/ha)			0	3.41*ns*	4.76*
FAO 260-340 (41.08 q/ha)				0	1.35*ns*
FAO 400-450 (42.43 q/ha)					0

*p < .05, * * p < .01

We can conclude that in the years favorable for maize cultivation, hybrids from FAO groups 260-340, 350-390 and 400-460 can be grown on the Caracal chernozem with similar results. If there are also unfavorable years, considering that we cannot know from the beginning what the weather will be like, it is preferable to cultivate hybrids from the FAO groups 260-340 and 350-390.
Research on new maize hybrids that differ significantly in characteristics related to earliness such as anthesis, silking, anthesis-silking intervals as well as those related to physiological maturity (grain filling period and rate of their filling) were also studied by other researchers (David, 2008; Ibraheem and Abdel-Moneam, 2015; Borozan et al., 2021).

The most productive maize hybrids were those placed in the FAO 400-450 group, whose average in 2020 was 8097 kg/ha. At the opposite pole were the hybrids from the FAO > 460 group whose average production was 338 kg/ha (Figure 1).

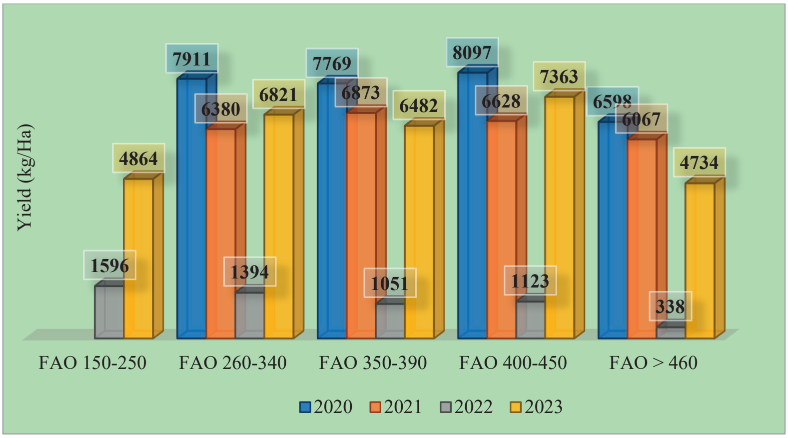

Figure 1. Average yields of maize hybrids tested at Caracal according to FAO group and year of testing

The largest weight is the productions over 6000 kg/ha registered in all FAO groups except 150-250. In three of the groups, namely FAO 260-340, FAO 350-390 and FAO 400-450, in 3 years out of the 4 tests, the aforementioned productions were recorded.
The extremes of the coefficients of variability were: 3.3% for productions in the FAO group 150-250 and 76.8% for productions in the FAO group over 460, both in the year 2022.
The coefficient of determination shows us that 33% of the variability of the coefficient of variability is the consequence of the changes in the yields of the maize hybrids (Figure 2).

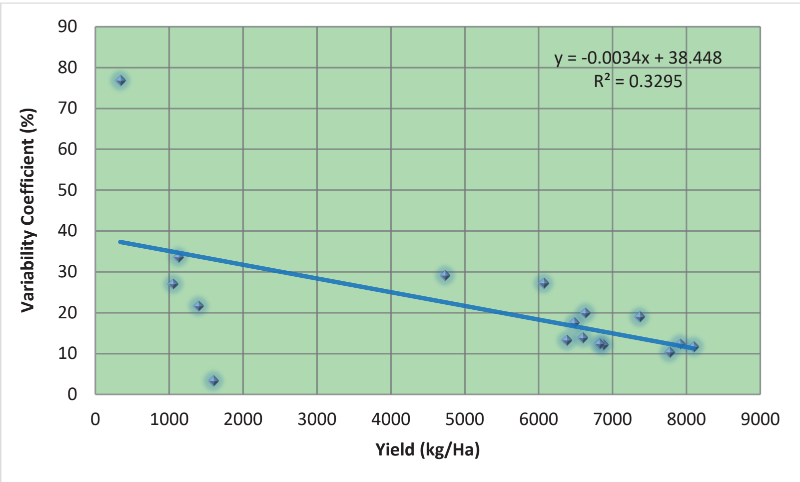

Figure 2. The relationship between the production of maize hybrids - production variability coefficient

In the case of the relationship between the production of maize hybrids and the humidity at harvest in 2021, the coefficient of determination shows us that 56% of the variability of humidity is given by the variability of the productions of maize hybrids (Figure 3). In the year 2023, this coefficient of determination is lower, so that 43% of the variability of moisture at harvest is closely related to the variability of the productions obtained on the chernozem from Caracal (Figure 4).

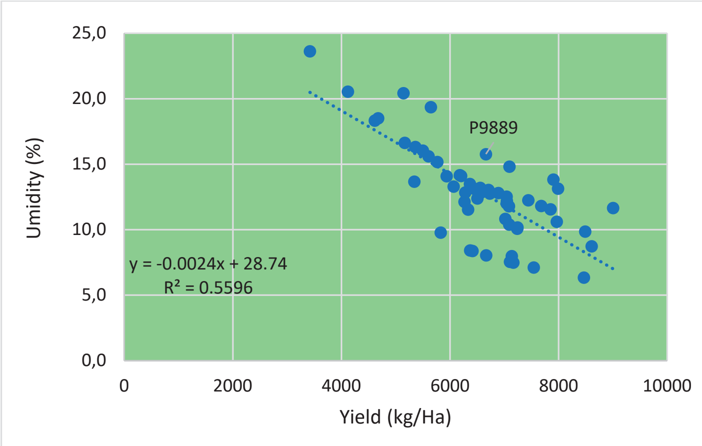

Figure 3. The relationship between the production of maize hybrids and the humidity at harvest in 2021

The correlations clearly show that in 2 of the 4 years tested, the relationship of yield with moisture at harvest was strongly negative. The later the hybrid was (high moisture at harvest) the less productive it was and vice versa.

In the year 2021, the points representing the values of the obtained productions are almost uniformly distributed along the line representing the linear equation. The hybrid Corteva P9889 stood out, which at a high humidity (15.8%) obtained a relatively increased production (6661 kg/ha).

In the year 2023, the hybrid Corteva P9911 presented a positive deviation from the line showing the correlation, which, although it recorded a high moisture at harvest (17.8%), also had a high production (8040 kg/ha) (Figure 4). With such a production, it was expected that the hybrid would belong to an FAO group above 400, more precisely FAO 410.

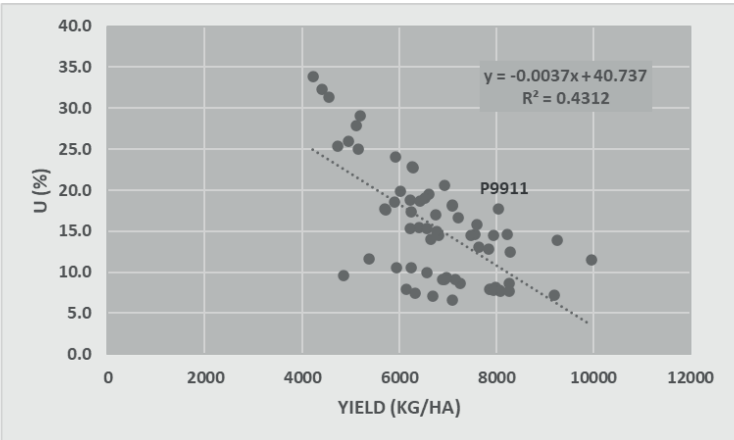

Figure 4. The relationship between the production of maize hybrids and the humidity at harvest in 2023

CONCLUSIONS

In years favorable to maize cultivation, hybrids from the FAO groups 260-340, 350-390 and 400-460 can be grown on the Caracal black soil with similar results. If there are also unfavorable years, considering that we cannot know from the beginning what the weather will be like, it is preferable to cultivate hybrids from the FAO groups 260-340 and 350-390.

The extremes of the coefficients of variability were: 3.3% for productions in the FAO group 150-250 and 76.8% for productions in the FAO group over 460, both in the year 2022. The variability was so diversified, from very stable to unstable, which suggests that it is extremely difficult to choose a performing hybrid with productive stability for the Caracal chernoziom.

Following the correlations between yields and humidity at harvest, two maize hybrids from Corteva (P9889 and P9911) were highlighted which, although they showed high humidity, also recorded high yields. They thus broke the particularly strong but negative relationship in the sense that a hybrid with high moisture at harvest has a low yield.

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THE EVOLUTION OF PESTICIDES USE IN THE CONTEXT OF SUSTAINABILITY OF AGRI-FOOD SYSTEMS

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Abstract

Future agriculture strategies and policies need to be developed to reduce the use and risks of pesticides, support agricultural production and food security, and encourage the development and adoption of sustainable practices. In the world, in 2021, more than 3.5 million tons of pesticides were used, i.e. 1.4 million tons more than in 2000. The amount of herbicides used in 2021 was higher by about 8.5 million tons compared to 2000, and fungicides and insecticides by 2-3 million t. For the European Union and Romania, there was a decrease in the quantities of all categories of plant protection products. This reflects the permanent concern of today`s society, decision-makers, and citizens to improve the quality of life and the environment, biodiversity, and safe agri-food products through the sustainable use of pesticides and to promote the wider use of alternative ways of crops protecting against pests, weeds, and diseases.

***Key words**: agri-food system, health, pesticides, sustainability.*

INTRODUCTION

Agriculture in the last period of time has been constantly progressing. Particularly productive biological forms were created, rich in useful substances (proteins, lipids, essential amino acids etc.), with high adaptability, resistant to pests and diseases, and cultivation methods were established for all agricultural plants, on the most different soils and ecological zones, with specific fertilization methods and techniques, methods of weed, disease and pest control, and agricultural machinery systems were greatly improved (Alengebawy et al., 2021). As a result, agricultural production has increased significantly. Despite all these advances made, contemporary agriculture is threatened by great dangers. Pollution, which leads not only to the modification of ecosystems, but also to the denaturation and degradation of agricultural products, to the loss of biodiversity, the emergence of resistance of harmful organisms, to the application of various plant protection products, but also to the reduction of the quality of life in general (European Environment Agency, 2022).

Current agricultural technologies must contribute to the permanent improvement of the environment, landscapes, human health, plants and animals (Mușat et al., 2021). The sources of pollution are numerous and among them an important role is played by the pollution generated by the irrational use of pesticides and fertilizers with negative effects on the water used to irrigate crops, as well as the effects of greenhouse gases diffused into the atmosphere. The problems facing agriculture are generated precisely by the need to continuously increase the production of food and raw materials for industry for a population that is becoming more and more numerous, over 9 billion people by 2030 (UN, 2022). The nature of these problems is very diverse and we can mention, among others, the climate changes manifested in recent years through the increase in multiannual average temperatures, the occurrence of extreme weather phenomena, socio-economic problems, migration, the various conflicts in some parts of the world. Taking all these aspects into account, the crops damage caused by diseases and pests and weeds is substantial. It is difficult to advance a synthetic figure regarding the value of these damages, because the situation is very different in various regions of the globe, but sometimes losses of 100% can be reached. The problems regarding the use of pesticides for crop protection are becoming very topical. Different organizations, international institutions have defined the notion of pesticides. For example, FAO defines it as pesticides "any substance or mixture of substances of chemical or biological ingredients intended for repelling, destroying or controlling any harmful organism or for regulating plant growth" (FAO, 2021). The EU's Common Agricultural Policies define pesticides as "something that prevents, destroys, or controls harmful organisms, or protects plants or plant products during production, storage and transport" (EU, Green Deal, 2019). Also, The World Health Organization (WHO) defines pesticides as "substances used in public health to kill vectors of disease, such as mosquitoes, and in agriculture to kill harmful organisms that damage crops" (WHO, 2020).

Over 1000 different pesticides are used around the world (WHO, 2020). Due to the danger that these substances have on the environment, the legislation of recent years in all world, including EU countries, has imposed some actions to achieve a sustainable use of pesticides (EU, Green Deal, 2019). Thus, the Directive 2009/128/EC of the European Parliament and of The Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. In 2019, the European Commission has adopted a proposal for a new Regulation on the Sustainable Use of Plant Protection Products, including EU wide targets to reduce by 50% the use and risk of chemical pesticides by 2030, in line with the EU's Farm to Fork and Biodiversity strategies (Commission Directive (EU) 782, 2019). Integrated Pest Management (IPM) focuses on pest prevention and prioritises alternative pest control methods, with chemical pesticides only used as a last resort.

Currently, in the world there is a great demand for new, innovative and safe crop protection products that increase the ability to control weeds, diseases and pests in crops (Barbas et al., 2023), in response to current legislative requirements.

Under these conditions, the current paper presents the evolution of crops pesticide use in Romania by comparison with the European Union and world in context of sustainability of agri-food system.

MATERIALS AND METHODS

The results presented in this paper are based on the study of specialized literature, as well as the statistical databases of FAO and Eurostat, which present centralized data at the global, European and Romanian level regarding the total quantities of pesticides used, the categories of pesticides used, as well as use per area of cropland and use per value AP (Agricultural Production).

FAO methodology highlights pesticides (total) as the sum of active ingredients in the following categories of pesticides: Fungicides & Bactericides, Herbicides, Insecticides, Plant Growth Regulators, Seed Treatment Fungicides, Seed Treatment Insecticides, Mineral Oils, Rodenticides, and Disinfectants and Other pesticides, nes (not elsewhere specified) (FAO, 2024).

The reporting period is 1990-2021. The year 1990 was chosen as the reference year when the Romanian Revolution took place in December 1989 and the transition to the market economy and with the turning point in 2007, as the year in which Romania became a member of the European Union and had to respect and align the legislation and the rules regarding the use of pesticides in agriculture.

RESULTS AND DISCUSSIONS

The worldwide quantity of pesticides applied to agricultural land has experienced a permanent increase since 1990, reaching in 2021 a value of more than 3.5 million tons, respectively with more than 1.70 million tons more than in 1990 (Figure 1). Also, on the continents, the increasing quantities were recorded for America with over 1.16 million tons in 2021 (1,772,194.51 tons), by comparison with 1990 (608,728,21 tons) and at the opposite end is the European Union, which in recent years recorded decreases from 373,053.18 tons to 354,082.32 in 2021.

For Romania, the total amount of pesticides used for agricultural land decreased continuously, reaching 5,590 tons in 2021 (Figure 2).

From the point of view of the quantities used per unit of cultivated area, it can be seen that the greatest increase was observed on the American continent, where the amount used in 1990 was 1.62 kg/ha, and in 2021 the amount was 4.7 kg/ha, a trend that was also maintained for the other continents (Figure 3).

For Romania, the amount used per ha has decreased constantly in the last 30 years, reaching 0.62 kg/ha in 2021 (Figure 4).

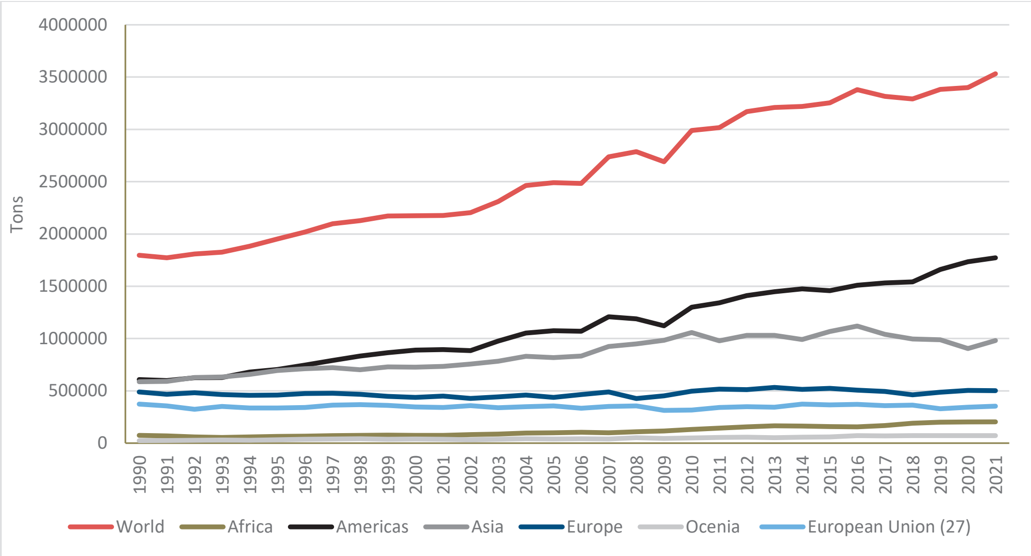

Figure 1. Total pesticides for agricultural use in the world, continents and European Union (FAO, 2024)

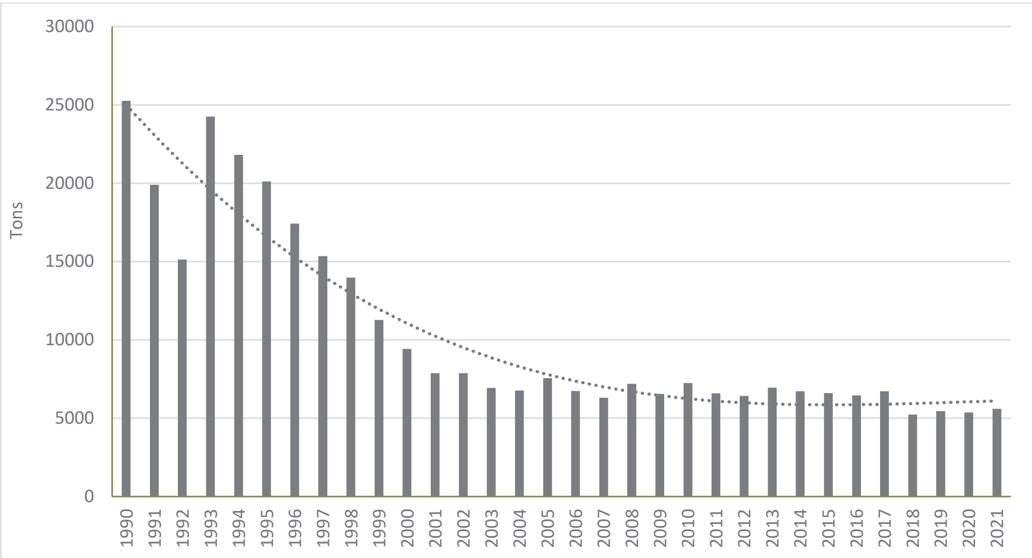

Figure 2. Total pesticides for agricultural use in Romania (FAO, 2024)

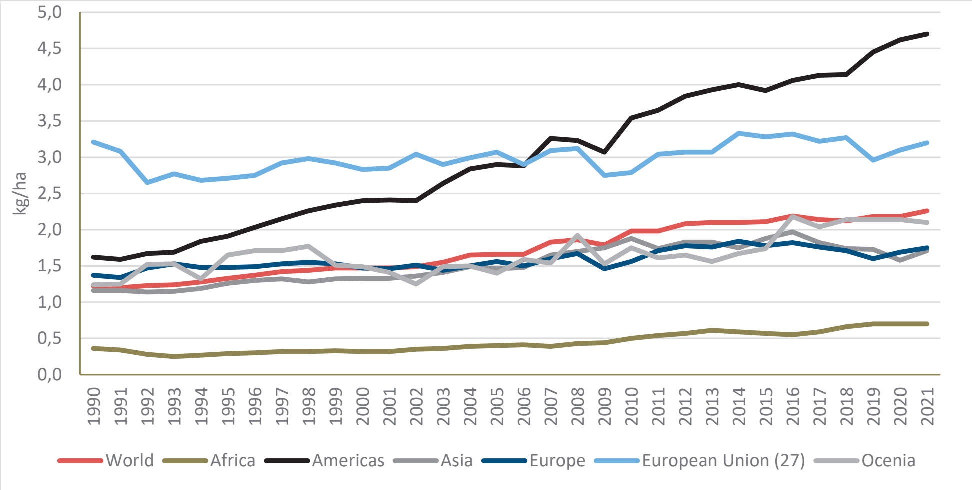

Figure 3. Pesticides used per area of cropland in the world, continents and European Union (FAO, 2024)

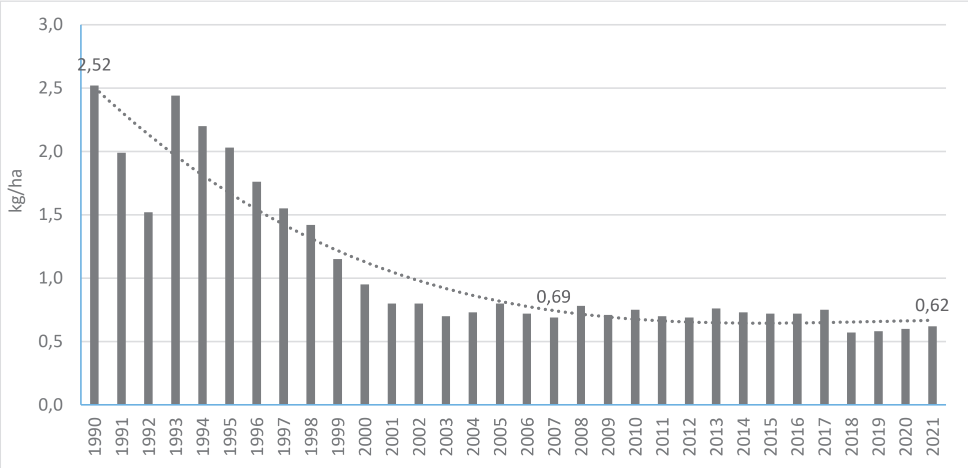

Figure 4. Pesticides used per area of cropland in Romania (FAO, 2024)

From the point of view of the categories of pesticides used, according to the data presented in next figures, it can be observed that the largest quantities used were herbicides. At the global level, the positive evolution of the herbicides use is exemplified by 699602.28 tons in 1990, to 1730303.03 tons in 2021. Also, on the continents, increases of 10-15% were observed throughout the analysed period. In the European Union, the values remained relatively constant, respectively, 10-11 million tons on agricultural land (Figure 5).

There is also a significant increase in the use of insecticides worldwide, from 699,602.28 tons in 1990 to 1,730,303.03 in 2021, or about 2.5 times more (Figure 6).

For fungicides and bactericides, the situation is similar, the quantities increasing in 2021 by about 1.8 times compared to 1990. But, the European Union recorded a decrease in the quantities used, by about 10-12% (Figure 7).

In Romania, the quantity of herbicides has been continuously reduced, reaching 2,969 tons in 2021, compared to 7,567 tons in 1990. Also, for insecticides, there is a decrease in quantities from 5,797 in 1990 to 822 in 2021. This decrease is also valid for fungicides (Figure 8).

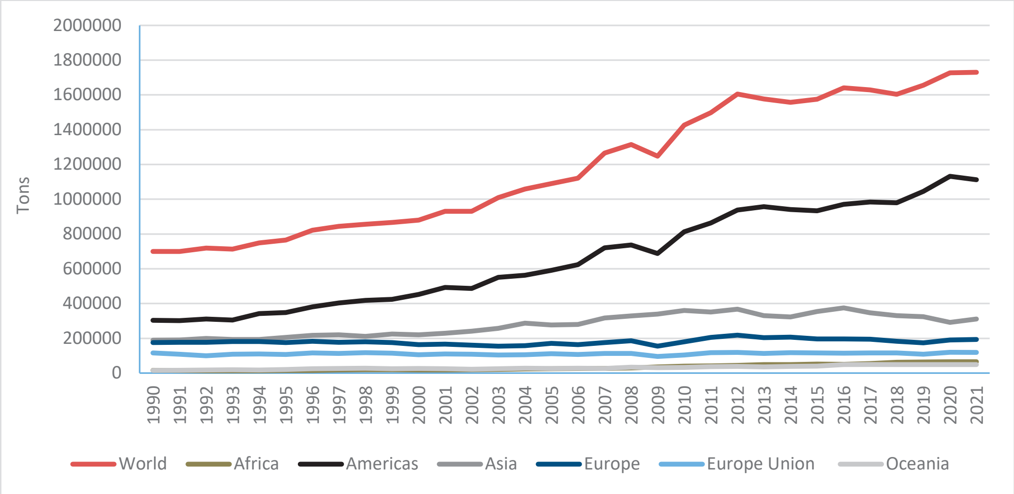

Figure 5. Herbicides for agriculture use (FAO, 2024)

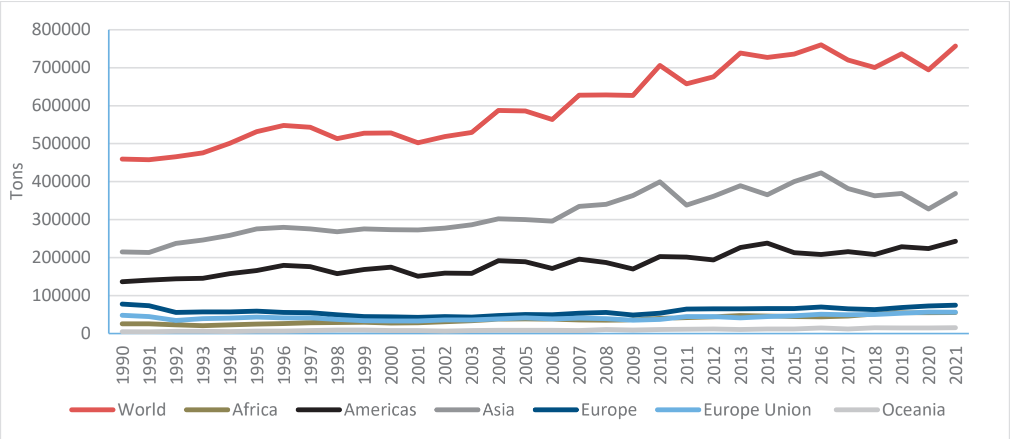

Figure 6. Insecticides for agriculture use (FAO, 2024)

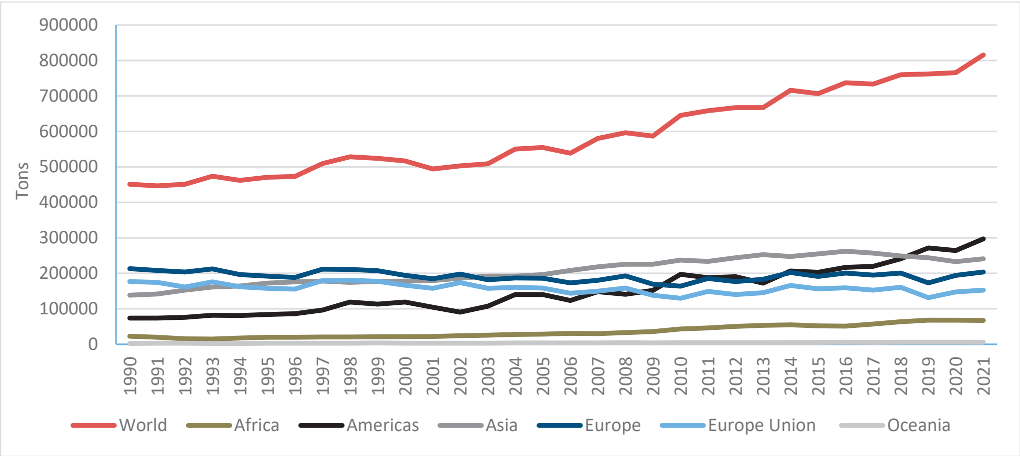

Figure 7. Fungicides and bactericides for agriculture use (FAO, 2024)

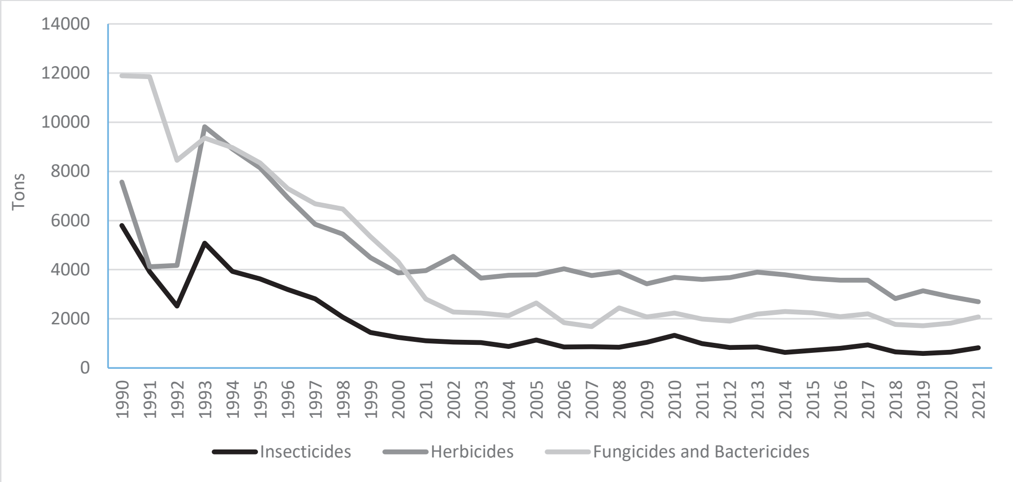

Figure 8. Pesticides categories for agriculture use in Romania (FAO, 2024)

The first countries with the highest consumption of pesticides per agriculture use is present in Figure 9. The largest pesticide user country is Brazil, which started with 51,120 tons in 1990 and reached 719,507 tons in 2021. Also, Argentina started with 26156 in 1990 and reached 241,519,98 in 2021. For the other countries, the values remained relatively constant, with increases of up to 60-70% (Figure 9). Regarding the consumption per area of cropland, it can be observed that for all countries the consumption has increased. Brazil stands out again, where consumption has increased 10 times in the 30 years, from 0.91 to 10.90 kg/ha. For Argentina, the values increased steadily, reaching 5.58 kg/ha in 2021. For Europe Union, the presence of Spain is noteworthy, both for the consumption used on agricultural land and for crop land (Figure 10).

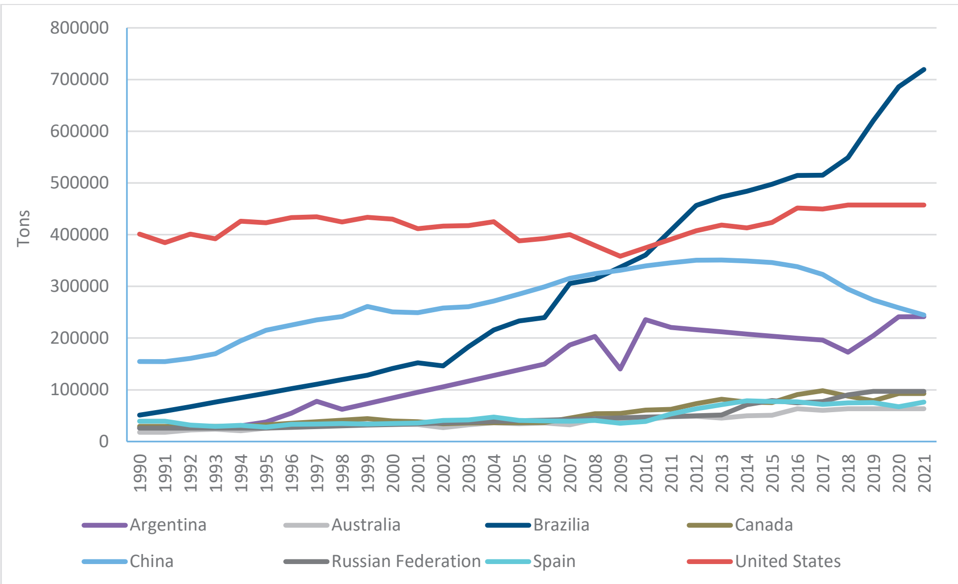

Figure 9. The first countries with the highest consumption of pesticides for agriculture use (FAO, 2024)

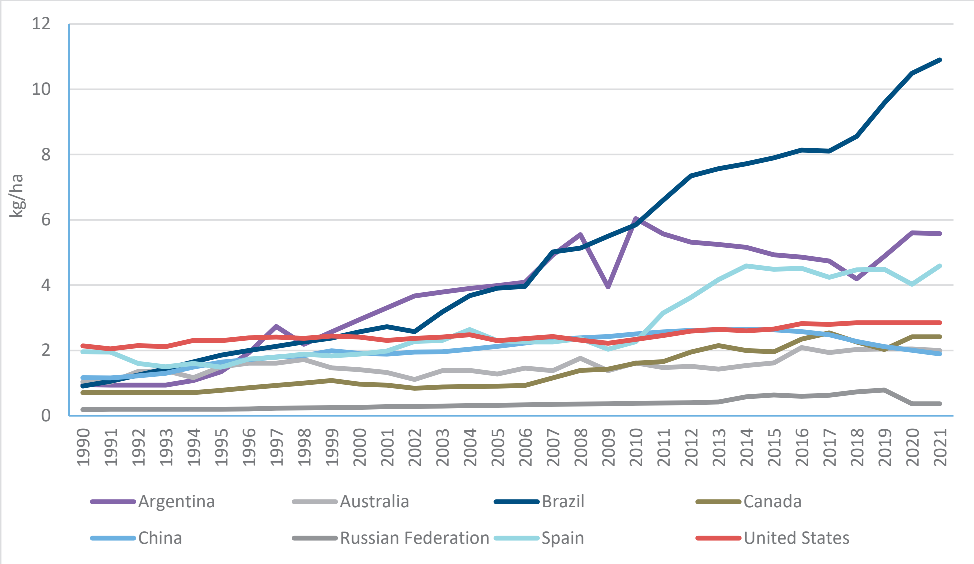

Figure 10. The first countries with the highest consumption of pesticides per area of cropland (FAO, 2024)

The evolution of the amount of pesticides traded in the last 30 years is presented in Figure 11. From the data analysis, it is noted that starting with the year 2020, the quantities traded, both for imports and exports, have decreased by about 0.5 million tons (Figure 11). Regarding the market value, it has continuously increased, but in the last two years the value of the market has decreased by about 3 million dollars (Figure 12) This was primarily due to the lower consumption of pesticides worldwide imposed by the legislation worldwide, but also in the European Union. For Romania, the quantities traded for both import and export increased continuously until 2020 (Figure 13). In 2021, a decrease in quantities was recorded by 8,179 tons, but the market value of these products increased by more than 41 million dollars (Figure 14). Among the causes reported by the experts in the field are mentioned: interrupted supply chains, very high transport costs, significant logistics problems, skyrocketing energy prices, lack of labour, and missing stocks. Taken together, these issues make sourcing more difficult, drive up prices, and make the supply chain particularly susceptible to the smallest disruptions.

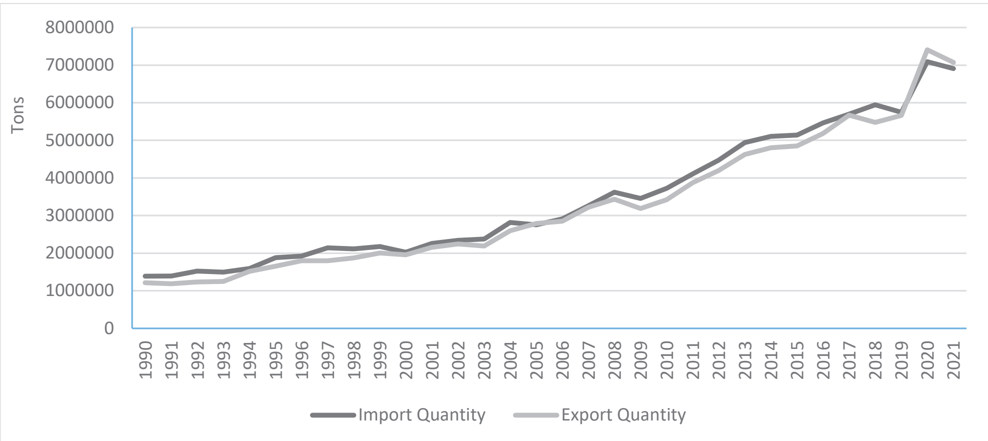

Figure 11. Import and Export quantity in the world (FAO, 2024)

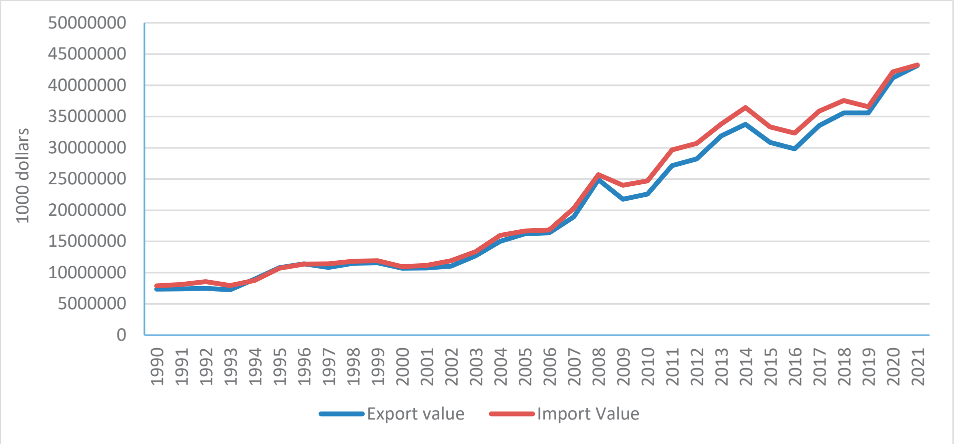

Figure 12. Import and Export values in the world (FAO, 2024)

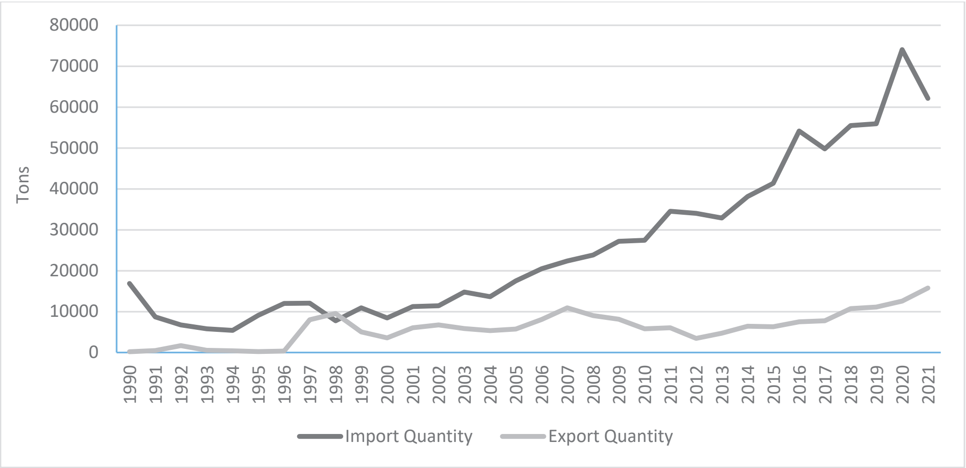

Figure 13. Import and Export quantity in Romania (FAO, 2024)

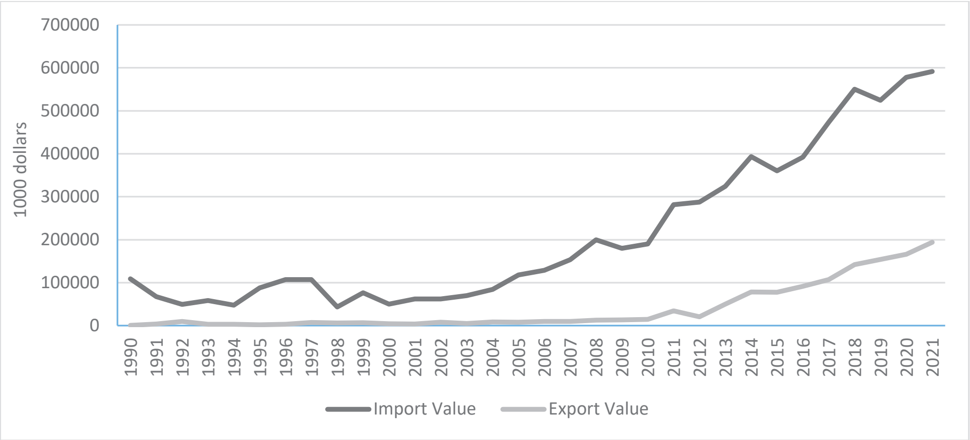

Figure 14. Import and Export values in Romania (FAO, 2024)

As it emerged from the statistical analysis carried out in this study, pesticides are widely used in agricultural production to prevent or combat pests, diseases, weeds and other plant pathogens, in order to reduce or eliminating production losses and maintaining a high quality of products (Zhang et al., 2023). Although pesticides are developed through very strict regulatory procedures to work with a reasonable certainty and with minimal impact on human health and the environment, exists serious concerns about health risks arising from occupational exposure and from food and drinking water residues (Damalas & Eleftherohorinos, 2011).
Also, the integrated pest management (IPM) of the harmful organisms emphasizes the growth of healthy crops and a cleaner environment through methods that affect agrosystems as little as possible and encourage natural pest control mechanisms (EU – Toolbox, 2023).
Pesticide treatments often prove counterproductive because they kill beneficial species, such as natural enemies of pests, and increase the chances of developing resistance pests to pesticides (Damalas & Eleftherohorinos, 2011). Also, the impacts of chemical pesticides on the environment, including biodiversity, water, air and soil, and on human health, have become a major concern for civil society and consumers (Ali, 2023). They are also a major issue for the sustainability of agricultural systems. Recently, the Farm to Fork and Biodiversity European strategies set an ambitious target of reducing the use and risks of chemical pesticides by 50% by 2030 (Mora et al., 2023). For reducing the use of pesticides and indirectly the risks to the health of people, the landscape, animals and plants, prevention and/or eradication of harmful organisms should be achieved or supported by several methods and in particular by: crop rotation; use of appropriate cultivation techniques (eg sowing technique, sowing dates and densities, sub-sowing, conservation tillage, clearing and direct seeding); the use, as appropriate, of resistant/tolerant varieties, as well as seeds and standard/certified planting material; use of balanced fertilization, liming and irrigation/drainage practices; preventing the spread of harmful organisms through hygiene measures (for example by constantly cleaning machinery and equipment); protecting and spreading important beneficial organisms, for example through appropriate plant protection measures or the use of ecological infrastructures inside and outside production sites; organic agriculture, etc.
Organic agriculture prohibits use synthetic pesticides, it protects the environment, preserves and increases soil fertility, helps to obtain foods of high nutritional value without residues (Nastase & Toader, 2016).
The necessity of having an alternative agriculture method that can be functioned in a friendly Ecosystem while sustaining and increasing productivity. Organic farming is recognized as the best-known alternative (Gamage at al., 2023).

CONCLUSIONS

Pesticides are widely used to protect food production and meet global food demand. Without their use, in some situations, harvest losses can be 100%
However, despite their usefulness, pesticides cause adverse effects on water quality, biodiversity and human health. The use of pesticides is one of the main factors in the negative impact of modern industrial agriculture on the environment. Due to their toxicity, pesticides can harm plants, animals and humans. To limit the impact on the environment, current policies recommend a series of measures to reduce the amount of pesticides. Including the promotion of agricultural systems that use non-polluting technologies for plants, animals and the environment, maintain and improve soil fertility and lead to the sustainability of agri-food systems.
As a result, the prospects of reducing the use of pesticides both in terms of agricultural land and crops or finding alternative solutions for control of harmful organisms can lead to the creation of sustainable practices beneficial both for farmers and for consumers in general.

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RESEARCH ON THE INFLUENCE OF THE CULTIVATED GENOTYPE AND THE SOWING SCHEME ON THE GRAINS QUALITATIVE PARAMETERS AT TWO-ROW BARLEY

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Abstract

The quality of two-row barley grains, the main raw material in the beer manufacturing sector, is directly influenced by the management of some variables related to the cultivation technology, so that the selection of the most valuable genotypes and the establishment of an optimal density of plants per unit area represent the guarantee the success of this culture. The present research aimed to testing the behavior at new two-row barley genotypes and to identify the most optimal sowing scheme so that, at harvest, the grains meet the requirements imposed by the standards in force regarding the physical and chemical parameters which define the quality of the grains. The results of the research highlighted the superiority of the Salamandre variety which, by ensuring a sowing density of 350-450 germinating grains/m^2, was clearly superior to the Tepee and Bosut varieties in terms of grain quality in the soil-climatic conditions specific to the N-W area of the Romanian Plain.

Key words: *two rows barley, sowing scheme, genotype, physical quality parameters, chemical quality parameters.*

INTRODUCTION

Research in recent years has focused on improving the characteristics of current varieties of barley and barley for beer, as well as on using genetic improvement programs to create new genotypes that ensure high productivity and at the same time increase the technological value of the harvest for the brewing sector (Matthies et al., 2014).

Sing et al. (1974) mentioned that barley varieties with 2 rows are preferred for malting over those with 4 or 6 rows. Genotypes with a high grain uniformity value, containing more than 60% starch in grains and as low as possible in protein, are preferred for the purpose of obtaining a quality malt, there being a negative correlation between barley and barley varieties in terms of extracted malt yield and diastatic strength (Therrein et al., 1994).

Verma et al. (2004) reported as the most suitable for malting barley varieties with a mass of 1000 grains of at least 42 g, with 9-11% protein content of the grains, with a malt extract of at least 80% and a diastatic strength between 80ºL-120ºL. The superiority of barley genotypes on 2 rows was also mentioned by Sing (2005) who reported after research that higher weight grains with wrinkle uniformity were obtained which reported from its research that higher weight grains were obtained, with high uniformity, higher starch content and lower protein levels in grains, compared to 6-row barley genotypes.

The choice of valuable varieties that achieve superior yields in terms of quantity and quality is the key to success both on the grain market and in the processing sector for malt and, implicitly, for beer manufacturing (Križanova et al., 2010).

In this context, the research underlying the elaboration of this paper was also incriminated, research aimed at the management of variables of barley culture technology for beer in order to identify the most valuable barley genotypes whose genetic dowry to potentiate, under optimal conditions of crop technology, the productive capacity and technological value of the final grain production as a raw material in the brewing industry.

MATERIALS AND METHODS

The research underlying the preparation of this paper was carried out on medium samples of grains harvested from the experience with barley for beer mounted in the agricultural year 2021-2022 Experimental Field from Moara Domnească belonging to the University of Agronomic Sciences and Veterinary Medicine form Bucharest. The experience was a bifactoral one and aimed to evaluate the behavior of new barley varieties sown at different densities, with following experimental factors:

- Genotype of cultivated barley - Factor A, with graduations:

a1 - Tepee genotype
a2 - Bosut genotype
a3 - Salamandre genotype

- Sowing norm - Factor B, with graduations:

B1 - 250 germinating grains (gg)/m^2
B2 - 350 germinating grains (gg)/m^2
B3 - 450 germinating grains (gg)/m^2

The total area of the experiment was 3000 m^2, with 9 experimental variants arranged according to the method of plots subdivided into 3 repetitions, the calculation and interpretation of the research results being made by the method of variance analysis.

When collecting the experiment, 3 average samples of grains from each variant and experimental repetition were retained in order to determine the main physical and chemical parameters defining the qualitative value of barley grains intended for malting as the main raw material in the brewing process. The determinations which have been carried out after the grain has undergone its seminal rest period and covered the following indicators:

- mass of 1000 grains-MMB (g), by the method of two repetitions of 500 grains (SR 6124/1999);
- volumetric weight (MH) (kg), using Granomat Analyzer (SR 6123/1999);
- uniformity of grains (%), with the help of the Sortimat sieving machine;
- grain moisture (%), with the Granolyser Analyzer (SR ISO 712:99);
- germination energy of grains (%), by germination envelope method (SR 1634:1999);
- protein content of grains (%), using the Granolyser Analyzer;
- starch content of grains (%), using the Granolyser Analyser.

RESULTS AND DISCUSSIONS

The physical purity of barley grains harvested from the three genotypes taken in the study exceeded in all experimental variants the minimum limit of 93% imposed by the standards in force for the purpose of brewing, there being no significant differences in the experimental variants in terms of this physical indicator of grain quality (Table 1).

Table 1. The influence of experimental factors on varietal purity of the grains

Experimental Variant	Varietal purity (%)	Difference (%)	Significance degrees
V1-Tepee-250 gg	94	-0.9	-
V2-Tepee-350 gg	95	0.1	-
V3-Tepee-450 gg	96	1.1	-
V4-Bosut-250 gg	93	-1.9	-
V5-Bosut-350 gg	94	-0.9	-
V6-Bosut-450 gg	96	1.1	-
V7-Salamandre-250 gg	95	0.1	-
V8-Salamandre-350 gg	96	1.1	-
V9-Salamandre-450 gg	96	1.1	-
Experimental average (Control)	**94.9**	Control	Control
	$DL_{5\%}$ = 2.049; $DL_{1\%}$ = 3.022; $DL_{0,1\%}$ = 4.711		

Following the determination of the humidity of the beans, values varied between 11.1% and 11.8%, values that fall within the limits allowed by the brewing chain, the STAS wich specifying a maximum value of this physical quality parameter of maximum 14% (Table 2).

Table 2. The influence of experimental factors on humidity content of the grains

Experimental Variant	Humidity (%)	Difference (%)	Significance degrees
V1-Tepee-250 gg	11.7	0.3	xx
V2-Tepee-350 gg	11.6	0.2	-
V3-Tepee-450 gg	11.7	0.3	xx
V4-Bosut-250 gg	11.6	0.2	-
V5-Bosut-350 gg	11.4	0.0	-
V6-Bosut-450 gg	11.2	-0.2	-
V7-Salamandre-250 gg	11.1	-0.3	xx
V8-Salamandre-350 gg	11.3	-0.1	-
V9-Salamandre-450 gg	11.8	0.4	xx
Experimental average (Control)	**11.4**	Control	Control
	$DL_{5\%}$ = 0.201; $DL_{1\%}$ = 0.295; $DL_{0,1\%}$ = 0.458		

The differences between the experimental variants tested during the research were insignificant (-) in most experimental variants, but the Tepee and Salamandre varieties were highlighted which, at a sowing norm of 250 and 450 germinable grains/m^2, showed a better water retention capacity in grains, differences from the average experience taken as experimental control having distinctly significant positive statistical assurance (xx).

The weight of the grains was directly influenced by the two experimental factors tested during the research (genotype cultivated and sowing density). It is thus observed that there was a great variability between the experimental variants in terms of mass of 1000 grains, the values of this physical parameter of grain quality being between 45.6 g, minimum value recorded for the Bosut variety against the background of a sowing density of 350 germinable grains/m^2 and 50.8 g for the Salamander variety, against the background of the sowing density of 250 germinable grains/m^2 (Table 3).

Table 3. The influence of experimental factors on grains weight

Experimental Variant	Weight of 1000 grains (g)	Difference (g)	Significance degrees	Volumetric weight (kg/hl)	Difference (kg/hl)	Significance degrees
V1-Tepee-250 gg	49.4	0.95	xxx	66,6	0.28	xx
V2-Tepee-350 gg	49.2	0.75	xxx	66.8	0.48	xxx
V3-Tepee-450 gg	48.8	0.35	xxx	66,5	0.18	x
V4-Bosut-250 gg	45.9	-2.55	ooo	66.7	0.38	xxx
V5-Bosut-350 gg	45.6	-2.85	ooo	64.7	-1.62	ooo
V6-Bosut-450 gg	47.4	-1.05	ooo	65.3	-1.02	ooo
V7-Salamandre-250 gg	50.8	2.35	xxx	68.5	2.18	xxx
V8-Salamandre-350 gg	49.6	1.15	xxx	67.9	1.58	xxx
V9-Salamandre-450 gg	49.4	0.95	xxx	68.2	1.88	xxx
Experimental average (Control)	**48.45**	Control	Control	**66.32**	Control	Control
	$DL_{5\%}$ = 0.942; $DL_{1\%}$ = 0.140; $DL_{0,1\%}$ = 0.222			$DL_{5\%}$ = 0.142; $DL_{1\%}$ = 0.220; $DL_{0,1\%}$ = 0.376		

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Compared to the average experience taken as a control, very positive differences were recorded (xxx) in Tepee and Salamandre varieties, while in Bosut varieties the differences were very significantly negative (ooo), regardless of the sowing scheme practiced at the establishment of the crop. However, it should be noted that the mass values of 1000 grains exceeded the minimum allowable value of 42 g laid down for barley and barley grains as raw material for brewing.

The volumetric weight of the beans is not a parameter that influences the quality of beer, but it is of interest to processors in terms of estimating the storage space they need. Analyzing the behavior of the three barley genotypes taken in the study (Table 3), it is found that, regardless of the sowing norm used when setting up the crop, grains with a volumetric weight higher than the minimum value required by the brewing industry, namely 65 kg/hl, except for the Bosut variety where, due to the use of a sowing norm of 350 germinable grains/m^2, the hectoliter mass of the grains was 64.7 kg/hl. In the Salamander variety, the highest values of the hectoliter mass of grains were recorded, values that varied between 67.9 and 68.5 kg/hl with very significantly positive differences (xxx) from a statistical point of view from the average experience (control).

In order to determine the uniformity of barley grains, as the main raw material in the brewing industry, the sifting of grains was performed with the help of the Sortimat sieving machine, a machine that was equipped with a set of 3 sieves with holes of different diameter, respectively 2.8 mm, 2.5 mm and 2.2 mm. After sifting, the grains remaining on the surface of each sieve were classified in quality classes according to the requirements imposed by the national and international standards used as a benchmark in assessing the uniformity of barley and barley grains destined for the brewing chain.

Table 4. The influence of experimental factors on the grains uniformity

Experimental Variant	Class I 2.8 mm (%)	Diff. (%)	Signif. degrees	Class II 2.5 mm (%)	Diff. (%)	Signif. degrees	Class III 2.2 mm (%)	Diff. (%)	Signif. degrees
V1-Tepee-250 gg	85	2.89	-	9	-1.88	94	6	-1.22	-
V2-Tepee-350 gg	83	0.89	-	10	-0.88	93	7	-0.77	-
V3-Tepee-450 gg	80	-2.11	-	12	1.12	92	8	0.78	-
V4-Bosut-250 gg	81	-1.11	-	10	-0.88	92	9	1.78	-
V5-Bosut-350 gg	80	-2.11	-	11	0.12	91	9	1.78	-
V6-Bosut-450 gg	78	-4.11	-	14	3.12	92	8	0.78	-
V7-Salamandre-250 gg	86	3.89	-	8	-2.88	94	6	-1.22	-
V8-Salamandre-350 gg	84	1.89	-	10	-0.88	94	6	-1.22	-
V9-Salamandre-450 gg	82	0.11	-	14	3.12	96	6	-1.22	-
Experimental average (Control)	**82.11**	Control	Control	**10.88**	Control	Control	**7.22**	Control	Control
	$DL_{5\%}$ = 5.079; $DL_{1\%}$ = 7.666; $DL_{0,1\%}$ = 12.453			$DL_{5\%}$ = 3.441; $DL_{1\%}$ = 4.908; $DL_{0,1\%}$ = 7.165			$DL_{5\%}$ = 3.408; $DL_{1\%}$ = 5.161; $DL_{0,1\%}$ = 8.431		

Analyzing the results obtained from the assessment of the uniformity of barley grains in the 2022 harvest, it is found that all three barley genotypes exceeded the minimum allowable values of grains with a diameter greater than 2.5 mm (80%) - according to international standard), against the background of the three sowing norms taken into account when setting up the crop, with insignificant differences (-) from the average of the experimental variants (Control).

Thus, the percentage of grains with a thickness greater than 2.8 mm, corresponding to Class I of quality, varied between 78% and 86%, the percentage level of grains with a diameter greater than 2.5 mm, classified in the Class II of quality, was between 8% and 14%, and the percentage of grains retained on the sieve with holes of 2.2 mm oscillated between 6% and 9%, there were no significant differences between the test variants analysed (Table 4).

From the point of view of germination energy of barley grains for beer, it is observed that all barley genotypes tested in the research performed very well in terms of this physiological parameter, exceeding the minimum allowable value of 92% provided by the standard in force, regardless of the sowing scheme practiced (Table 5).

Table 5. The influence of experimental factors on the grains germination energy

Experimental Variant	Germination energy (%)	Difference (%)	Significance degrees
V1-Tepee-250 gg	97	0.89	-
V2-Tepee-350 gg	97	0.89	-
V3-Tepee-450 gg	98	1.89	-
V4-Bosut-250 gg	95	-1.11	-
V5-Bosut-350 gg	94	-2.11	-
V6-Bosut-450 gg	95	-1.11	-
V7-Salamandre-250 gg	96	-0.11	-
V8-Salamandre-350 gg	96	-0.11	-
V9-Salamandre-450 gg	97	0.89	-
Experimental average (Control)	**96.11**	Control	Control
	$DL_{5\%}$ = 2.181; $DL_{1\%}$ = 3.310; $DL_{0,1\%}$ = 5.425		

The Tepee and Salamandre varieties were also highlighted in terms of germination energy, whose grains exceeded after 72 hours a germination rate of 95% according to EBC recommendations (2010), except for the Bosut variety where a 94% germination percentage was recorded due to the use of a sowing norm of 350 germinable grains/m^2. In all experimental variants differences from the mean experience had insignificant statistical certainty (-).

The amplitude of protein content of grains belonging to the three analyzed barley varieties varied between 9.6% and 12.4% (Table 6), most of the experimental variants falling within the limits provided by both the European Standard and the National Standard regarding the use of barley and barley grains as essential raw material in brewing, respectively 9.5-11.5%.

Table 6. The influence of experimental factors on the grains protein and starch content

Experimental Variant	Protein content (%)	Difference (%)	Significance degrees	Starch content (%)	Difference (%)	Significance degrees
V1-Tepee-250 gg	10.2	-0.2	oo	59,6	-1.0	ooo
V2-Tepee-350 gg	9.8	-0.6	ooo	60,2	-0.4	ooo
V3-Tepee-450 gg	10.4	0.0	-	61,4	0.8	xxx
V4-Bosut-250 gg	11.3	0.9	xxx	59,5	-1.1	ooo
V5-Bosut-350 gg	10.9	0.5	xxx	59,8	-0.8	ooo
V6-Bosut-450 gg	11.4	1.0	xxx	59,7	-0.9	ooo
V7-Salamandre-250 gg	9.6	-0.8	ooo	61,6	1.0	xxx
V8-Salamandre-350 gg	9.8	-0.6	ooo	61,9	1.3	xxx
V9-Salamandre-450 gg	10.3	-0.1	-	62,1	1.5	xxx
Experimental average (Control)	**10.4**	Control	Control	**60.6**	Control	Control
	$DL_{5\%}$ = 0.131; $DL_{1\%}$ = 0.194; $DL_{0,1\%}$ = 0.301			$DL_{5\%}$ = 0.090; $DL_{1\%}$ = 0.130; $DL_{0,1\%}$ = 0.195		

The results obtained after determining the protein content of the grains revealed that by practicing the three sowing schemes, the barley genotypes tested during the research efficiently used the nitrogen administered in a balanced dose when setting up the crop. Knowing that barley and barley varieties with a low protein content of grains are preferred for brewing, we can say that the most valuable in terms of grain quality were Tepee and Salamandre varieties with the lowest protein content in grains (9.6-9.8%), given the use of sowing norms of 250 and 350 germinable grains/m^2, with distinctly significant negative (oo) and very significantly negative (ooo) towards the experience average (Control).

Following the determination of the starch content of grains, a great variability was observed between experimental variants, the values of this chemical indicator of grain quality oscillating between 59.5% and 62.1% (Table 6). The Tepee and Salamandre varieties were noted, for which, against the background of ensuring a sowing density of 450 germinable grains/m^2, the content of grains in starch exceeded the minimum allowable value of 60% stipulated in the standards with very significantly positive differences (xxx) from the mean of control experience. In the Bosut variety, regardless of the sowing scheme practiced, the starch content was below the permissible mimin level provided by the standards, but very close to this value (59.5-59.8%) with very significantly negative statistical assurance (ooo) compared to the experience control.

CONCLUSIONS

Based on the results obtained from determining the physical and chemical parameters of the barley grain samples taken from the 2022 harvest, we can draw the following conclusions:

There were no significant differences in terms of physical purity of the beans, in all experimental variants taken in the studio the values of this quality indicator exceeding the minimum value imposed by the standard for the destination of brewing.

With grain moisture values between 11.1% and 11.8%, all experimental variants were below the maximum scale of 14% specified by the grain quality standard, as raw material for the chain - Brewing.

All barley varieties have exceeded the minimum limit of 42 g imposed by the standard for the mass of 1000 grains, but the Tepee and Salamandre varieties are significantly superior to the Bosut variety, in terms of the values of this physical indicator of grain quality, exceeding the limit of 48 g, irrespective of the sowing scheme practiced,

Irrespective of the sowing standard used when setting up the crop, for all three barley varieties analysed the volumetric weight of the grains exceeded the minimum limit of 65 kg/hl required by the brewing industry, except for the Bosut variety where the value of this physical parameter it was under the conditions of using a sowing norm of 350 germinable grains/m^2, of 64.7 kg/hl.

The results of the research revealed that the experimental factors tested had a direct and significant influence on the uniformity of the grains, the grains classified in the assortment Class I + Class II quality exceeding the percentage value of 91%, regardless of the genotype of barley cultivated or the density provided per unit area at the establishment of the crop.

The grains belonging to the three varieties of barley recorded, after 72 hours from the moment of laying on the germination layer, a germination rate of over 92% (the minimum limit imposed by the standard), noting the Tepee and Salamandre varieties with a germination energy exceeding 94%.

The most valuable in terms of protein content of grains were Tepee and Salamandre varieties, with values of this chemical quality parameter varying between 9.6% and 10.3%, regardless of the sowing scheme practiced.

The barley variety Salamander was clearly superior to the other varieties also in terms of grain content in starch, the values of this grain quality indicator exceeding 61% in all three sowing schemes practiced at the establishment of the crop, thus exceeding the minimum scale required by the brewing industry.

Based on the results obtained, we can say that, against the background of optimizing the sowing scheme, the three barley genotypes taken in the study performed very well in the specific conditions of the research location, achieving grain productions with high technological value in accordance with the norms imposed by the final destination, beer manufacturing.

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CHLOROPHYLL CONTENT, PHENOLOGY AND MORPHOLOGICAL TRAITS OF WHEAT UNDER SALINITY STRESS

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Abstract

Natural and anthropogenic salinization through intense and progressive periods of drought, as well as due to the use of fertilizers or the soil parental material, leads to degradation of soil quality. In this context, wheat, one of the most important crops, is threatened. Specific objectives include measuring chlorophyll content and morphological parameters such as grain number, fresh and dry biomass for stems and spikes. The experiment was set up in field conditions, in mesocosms, under six saline doses of 15-30-45-60-75 mM NaCl and a control without salt, in five replicates. The results highlighted different effects depending on the tested variety and the applied salinity doses. The most concentrated dose of 75 mM NaCl drastically reduced the values of almost all morpho-physiological parameters in all varieties. Transilvania, Arieșan, Faur, Ciprian, Pădureni, and Bezostaia had higher morpho-physiological parameter values. Otilia is the wheat variety most sensitive to salinity stress. Different tolerance patterns and trends were observed based on the interaction between the variety and salinity dose.

***Key words**: abiotic stress, growth, SPAD units, wheat varieties.*

INTRODUCTION

Soil salinization results from water-soluble salt accumulation in the substrate due to environmental factors and anthropogenic activities (Zinck and Metternicht, 2009). This human-induced salinity, also called secondary salinity, involves intensive irrigation practices, malfunctioning drainage systems, or poor farm management practices (Okur and Örçen, 2020). Environmental factors, such as the soil profile (Bui, 2017) and severe drought episodes caused by global climatic changes (Qafoku, 2015), combined with the anthropogenic activities mentioned above, change the soil-water balance (Zinck and Metternicht, 2009), leading to soil salinization. This poses a substantial threat to soil quality and, finally, to plants, having effects on vegetal organisms similar to those caused by drought (Uddin et al., 2016).

The imminent danger of soil salinity is increasingly acute in the context of climate change, posing a significant challenge to global food security because vast regions of arable land are either saline or susceptible to salinity (Butcher et al., 2016).

Salt stress adversely affects plants by inducing osmotic stress and disrupting physiological and metabolic processes, ultimately leading to inhibited growth and alterations in morphological characteristics (Arif et al., 2020). The ultimate consequence is a slow development on the Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) scale or even skipping some growth stages, resulting in plants with premature leaf senescence (Lutts et al., 1996). From an economic perspective, the repercussions of agricultural production have profound implications for global food security (Kopittke et al., 2019). In the contemporary context of climate change and an ever-increasing population, achieving the "zero hunger" sustainable development goal (SDG) of the UN Agenda also means achieving global food security (United Nations, 2024). *Triticum* *aestivum* L. is the second most produced cereal, with 783 million tons of global production (Statista, 2023), and stands out as the world's most vital agricultural crop. Wheat, through its by-products, constitutes a large part of the daily diet. Wheat grains and flour are rich sources of nutrients, minerals, vitamins, fiber, and proteins (Yigider et al., 2023). In addition, wheat is a major crop present in almost all crop rotations in agricultural ecosystems globally (Dixon et al., 2009; Giraldo et al., 2019). Therefore, in the current context of massive population growth, the focus on crop cultivation must be increased. Salt stress has varying effects on wheat plant phenological stages. Both BBCH growth and development stage, biomass, and relative chlorophyll content, through which photosynthetic activity can be revealed, are parameters through which the effects of salt stress on wheat plants can be highlighted (EL Sabagh et al., 2021). Wheat root and stem biomass are affected by salinity stress at high concentrations (Ahmadi et al., 2018; Pour-Aboughadareh et al., 2021). The relative water content of wheat leaves is low under salinity stress conditions, even under osmotic regulation (Boyer et al., 2008). Plant water content decreases with increasing salinity (El-Bassiouny and Bekheta, 2005). Abiotic stress effects can vary depending on climatic and soil conditions, but especially on the genetic information of each wheat variety (Hossain et al., 2021). The identification and maintenance of wheat varieties are essential to ensure food security under a changing climate and massive population growth. To provide resilience under salinity conditions it is essential to establish the tolerance of a large germplasm collection. This information is valuable to farmers facing this issue. In addition, improved farming practices and the implementation of advanced technologies can contribute to optimized yields and reduced salinity stress.

In light of the above, the aim of this study was to test and establish ten wheat varieties tolerant to salinity stress in order to provide information necessary for agronomists to address this issue and mitigate its impact. The objective of this study was to assess the morpho-physiological changes induced by salinity. Assessments of leaf relative chlorophyll in the beginning and at the end of the experiment were done. The number of emerging stems, spikes, and grains, fresh and dry biomass for stems and spikes, were also measured and the water content for stems and spikes was determined.

MATERIALS AND METHODS

The experiment was set in the middle of October 2022, in the Agro-Botanical Garden of UASVM Cluj-Napoca field conditions. Ten *Triticum aestivum* L. varieties from the ARDS (Agricultural Research and Development Station) Turda were sown in mesocosms with a diameter of 24 cm, filled with a clay-loam soil type, and subjected to salinity stress with no irrigation regime.

The soil properties are presented in Table 1.

The 10 wheat varieties were Andrada (V1), Arieșan (V2), Bezostaia (V3), Ciprian (V4), Faur (V5), Fundulea (V6), Miranda (V7), Otilia (V8), Pădureni (V9), and Transilvania (V10) from ARDS Turda. A total of 30 *Triticum aestivum* L. seeds, in five replications, were tested under six saline treatments T1-0 mM NaCl, T2-15 mM NaCl, T3-30 mM NaCl, T4-45 mM NaCl, T5-60 mM NaCl, and T6-75 mM NaCl. To avoid saline treatments infiltration into the experimental field, the mesocosms were placed above a polypropylene film hydro insulation system.

Table 1. Soil properties at the beginning of the experiment (Si) and at the end of the experiment in all treatments (T1-T6) with another control (Sf) at the end without treatment or plants

	T1	T2	T3	T4	T5	T6	Si	Sf
pH	6.43 ±0.05	6.45 ±0.03	6.53 ±0.02	6.48 ±0.05	6.67 ±0,10	6.55 ±0.03	6.00 ±0.06	6.35 ±0.08
EC (mS)	70.90 ±1.77	73.38 ±2.99	75.68 ±3.23	79.60 ±2.48	82.95 ±2.08	88.45 ±0.71	63.67 ±0.32	65.37 ±0.45
Humus (%)	3.17 ±0.48	3.62 ±0.49	2.57 ±0.23	2.66 ±0.32	2.89 ±0.25	3.10 ±0.22	3.59 ±0.07	2.74 ±0.09
P-AL (ppm)	29.91 ±6.20	22.59 ±3.00	21.86 ±5.10	24.22 ±2.52	17.90 ±4.59	21.36 ±0.86	10.12 ±0.07	9.50 ±0.10
K-AL (ppm)	121.59 ±13.82	93.38 ±5.97	87.69 ±6.91	93.85 ±7.59	92.75 ±9.92	83.13 ±9.70	81.30 ±0.07	103.43 ±0.10
Total N %	0.13 ±0.02	0.12 ±0.01	0.11 ±0.01	0.12 ±0.01	0.10 ±0.01	0.12 ±0.01	0.14 ±0.00	0.10 ±0.00
Soluble Ca (ppm)	446.27 ±14.33	444.24 ±9.16	466.40 ±13.93	449.24 ±3.35	441.26 ±12.49	456.00 ±4.89	442.03 ±0.26	473.47 ±0.37
Soluble Mg (ppm)	82.00 ±1.41	82.07 ±0.76	81.95 ±0.17	82.74 ±0.21	81.73 ±1.25	81.50 ±0.90	86.57 ±0.11	84.39 ±0.15
Chloride (mg/100 g soil)	16.54 ±0.51	22.16 ±0.89	24.22 ±1.35	26.59 ±1.26	36.15 ±3.03	44.84 ±5.59	15.02 ±0.12	17.72 ±0.12
Exchangeable Na (m.e./100 g soil)	0.31 ±0.10	0.25 ±0.04	0.46 ±0.12	0.40 ±0.06	0.52 ±0.07	0.60 ±0.08	0.36 ±0.03	0.13 ±0.04

Relative chlorophyll content was assessed in two evaluation dates by reading the SPAD units value with MC-100 S/N Apogee Instruments Chlorophyll meter. The parameter was two times assessed on 4 November 2022, at the beginning of the experiment, when plants where in BBCH 11 developmental stage with the first true leaf unfolded, and on 23 May 2023, at the end of the experiment when plants were in the BBCH 51 developmental stage, at the beginning of inflorescence (Meier, 2003).

At the end of the experiment, the total number of plants grown and the spike number of a variety in a treatment were determined for the whole set of replicates. The total stem production biomass per variety per treatment and water content was determined for each set of repetitions. The same way it was proceeded with spikes biomass. As for the wheat grains, they were collected from each entire set of replicates for each variety and treatment to determine their total number. The total biomass productions for stems and spikes were measured using gravimetric methods with an analytical balance before and after the vegetal material samples were subjected to 105°C (ISO, 2015) for 48 hours in a drying oven. The lost water content (WC) was measured, according to the ISO 18134-3 standard method (ISO, 2023), for one gram of fresh stem and spike biomass using the formula:

$$Water\ Content = \frac{Fresh\ biomass - dry\ biomass}{Fresh\ biomass} \times 100$$

Data analysis was done in RStudio console, version 4.0.5. Basic statistics was done with psych package, average and standard errors (SE) were automatically calculated. The Analysis of Variance (ANOVA) table and LSD test were performed with agricolae package (de Mendiburu, 2021; Stoian et al., 2024). All the figures were made with the help of online free tools, produced by Plotly.js (v2.24.1) (Statskingdom, 2024). Therefore, for box plots, the inclusive quartile was set and the median value appears in the figures with statistics LSD test, different letters highlight differences between treatments at $p<0.05$ threshold.

RESULTS AND DISCUSSIONS

Chlorophyll content in relation to salinity

Relative chlorophyll content is an important parameter in determining how *Triticum aestivum* plants of the 10 varieties are affected by salt treatment. On the first measurement date (Figure 1), the highest value was observed in plants of the Transilvania variety, plants subjected to the most concentrated dose of T6 treatment.

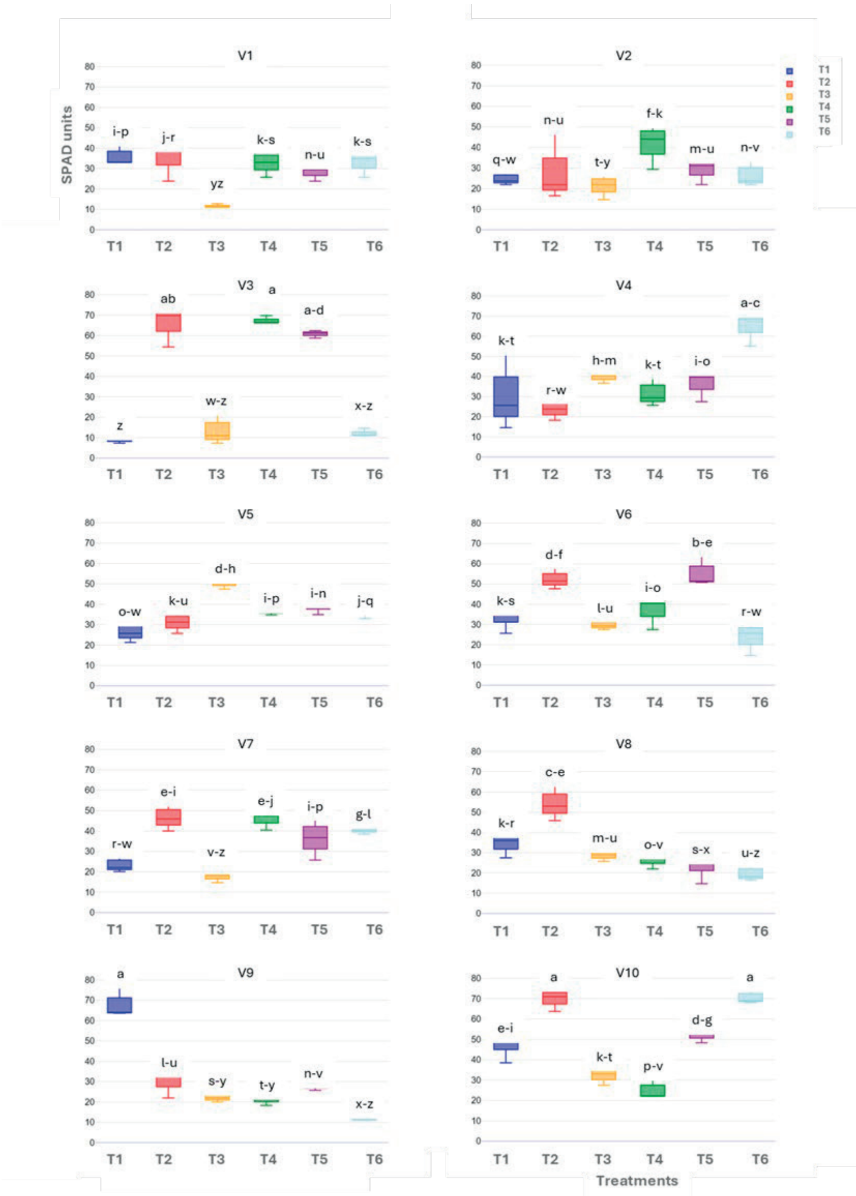

Figure 1. Chlorophyll content in the first assessment

Legend: V1=Andrada, V2=Arieșan, V3=Bezostaia, V4=Ciprian, V5=Faur, V6=Fundulea, V7=Miranda, V8=Otilia, V9=Pădureni, V10=Transilvania wheat varieties; T1=0 mM NaCl, T2=15 mM NaCl, T3=30 mM NaCl, T4=45 mM NaCl, T5=60 mM NaCl, T6=75 mM NaCl saline treatment; ANOVA: F (variety)=14.51, p (variety)<0.001; F (treatment)=20.26, p (treatment) <0.001; F (variety × treatment)=14.75, p (variety × treatment)<0.001

The same variety recorded on average the highest chlorophyll values compared to the other varieties (Figure 1.). Other high values were recorded in the varieties Pădureni treated with T1, Bezostaia with T4, T2, T6 and Ciprian with insignificant decreases of 3%, 5%, 7%, 14% and 8% compared to the maximum. The fact that Pădureni variety reached the maximum value of relative chlorophyll content only under T1 treatment, indicates the negative influence of salt stress on this parameter. The lowest value recorded at this measurement date was observed in the Bezostaia variety treated with T1, with a decrease of about 87% of the maximum recorded values. Other low values were recorded in plants of varieties Andrada at T3, Bezostaia and Pădureni subjected to T6 treatment. It can be observed that the chlorophyll level is strongly influenced by salinity in varieties Arieșan, Andrada and Pădureni.

Salinity represents an increasingly accentuated and threatening abiotic stress, which is why the need to evaluate the potential of the current germplasm collections as tolerant to it has been highlighted. Testing this germplasm collection allowed the development of reaction patterns in terms of chlorophyll content. The morpho-physiological parameters of all ten wheat varieties assessment highlight the salinity different effects. Monitoring leaf chlorophyll content is the physiological parameter of interest in the evaluation of the salinity tolerance degree of different wheat varieties (Cuin et al., 2010). Changes in leaves relative chlorophyll content reveal the trend whereby long-time exposure to saline treatment affects this parameter evolution in time.

Regarding the relative chlorophyll content of the leaves in the first evaluation date, when plants were in BBCH11 developmental stage, the Transilvania variety stood out (Figure 1). The high values of chlorophyll content recorded in this wheat variety under the second and the most concentrated salt treatment suggest a significantly higher degree of tolerance to salt stress compared to the other varieties. Chlorophyll content represents a parameter of interest, being an indicator of the proper functioning of physiological processes and plant productivity, being related to plant nitrogen content (Bannari et al., 2007). The optimal and vigorous plants growth that ensures production is a consequence of an efficient photosynthesis process (Brestic et al., 2018), which is the reason why the Transilvania wheat variety can be classified, from the point of view of this parameter, in the salinity-tolerant category, with the expectation of recording high values of quantitative parameters. All the other varieties can be classified with a lower degree of tolerance, pointing out that Otilia and Pădureni wheat registered a trend of decreasing chlorophyll content values with increasing salinity treatment.

The values of chlorophyll content decrease with increasing salt treatment and also due to prolonged exposure to salt treatment (Azizpour et al., 2010). The varieties Andrada, Ciprian, Pădureni, and Fundulea were placed in the moderately tolerant category. Varieties Faur, Transilvania, Miranda, and Otilia were more sensitive than the others. For the variety Transylvania, long exposure to salt treatment doses had negative effects, falling from the tolerant group to the opposite extreme.

It can be stated that long-time exposure to salinity stress affects the evolution of chlorophyll content (Figure 2). The values recorded are also a consequence of a combined accumulation of factors including climatic and soil conditions, but also the genetic level of resistance to abiotic stressors of each wheat variety. Similarly, drought stress, which is one of the precursors of salinity, has a negative impact on the chlorophyll content of wheat leaves, an impact that is more pronounced the lower the genetic tolerance of each variety to abiotic stress (Naeem et al., 2015). Once chlorophyll levels are reduced, the photosynthesis process is affected. This is a concerning fact because photosynthesis is a whole physiological system that depends on environmental factors to have the best yields in production (Calzadilla et al., 2022).

On the last date of evaluations, the highest value of relative chlorophyll content was recorded in plants of the variety Andrada V1 treated with 0 mM NaCl, T1 (Figure 2).

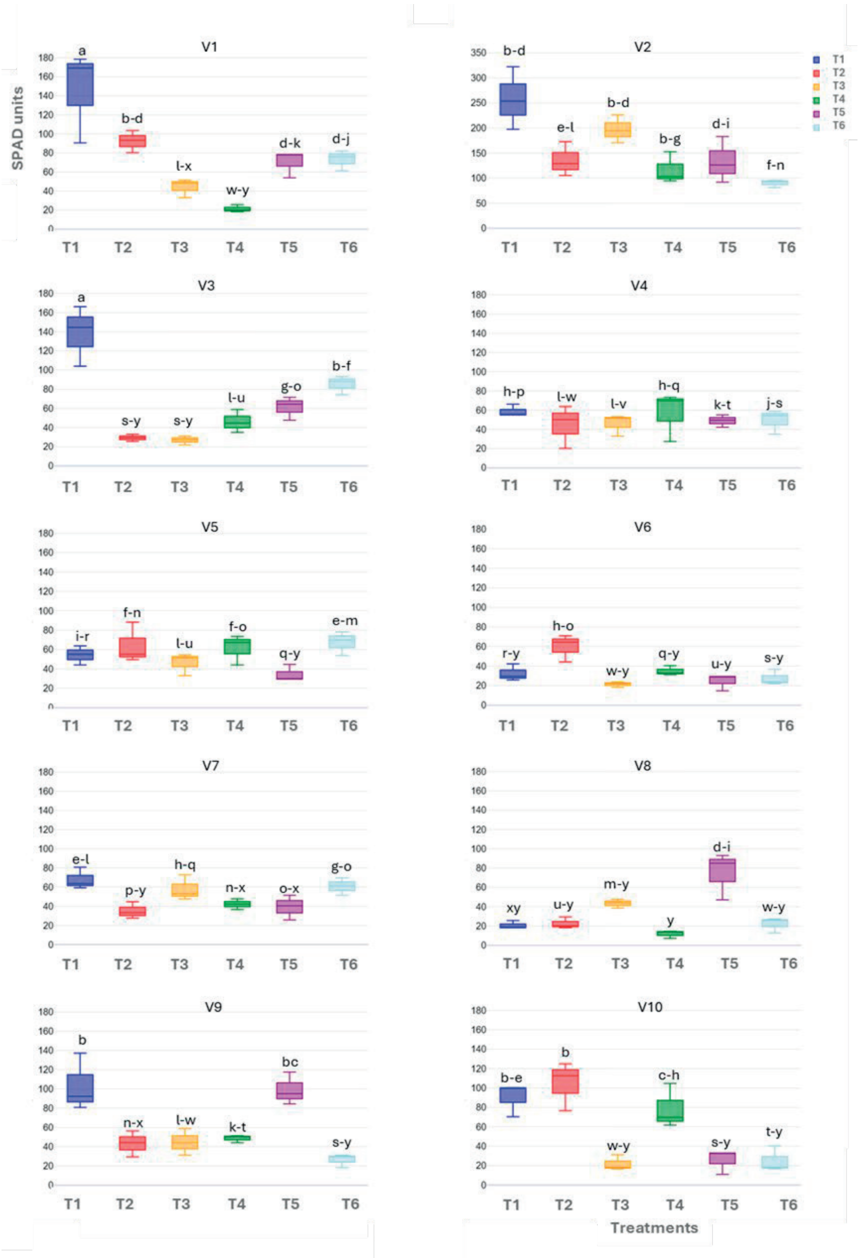

Figure 2. Chlorophyll content in the fifth assessment:
Legend: V1=Andrada, V2=Arieșan, V3=Bezostaia, V4=Ciprian, V5=Faur, V6=Fundulea, V7=Miranda, V8=Otilia, V9=Pădureni, V10=Transilvania wheat varieties; T1=0 mM NaCl, T2=15 mM NaCl, T3=30 mM NaCl, T4=45 mM NaCl, T5=60 mM NaCl, T6=75 mM NaCl saline treatment; ANOVA: F (variety)=19.14, p (variety)<0.001; F (treatment)=22.52, p (treatment)<0.001; F (variety × treatment)=8.65, p (variety × treatment)<0.001.

The top of the highest chlorophyll levels is completed by the values of plants of the variety Bezostaia V3 from T2 with an insignificant decrease of 5 percent from the maximum. High values, but with significant decreases of 28% and 29% compared to the highest value were observed for the varieties Transilvania in T2 and Pădureni in T1. The lowest value was observed in Otilia from T4, with a drastic decrease of 92% compared to the maximum recorded. The same variety treated with T1 and T6, as well as the variety Fundulea from T3, completed the top of the list of minimum values of the measured parameter. At the last measurement date, the best-performing varieties in terms of relative chlorophyll level averages were Arieșan, Andrada, and Bezostaia. On the contrary, the worst performers were Otilia, Fundulea, and Miranda.

Plant traits in different salinity conditions

In terms of the **total number of stems** developed from the thirty seeds from all five replicates initially germinated, Faur was the best-performing and the low-performing variety was Otilia (Table 2). The values of the Faur variety plants ranged between 29 and 30 plants, registering the maximum average values. These were recorded in plants treated with T2, T5, and T6 doses. This demonstrates the tolerance of the variety even at the last two highest salt treatment concentrations. These performances of Faur are statistically higher than those of Transilvania, Fundulea, Ciprian, Miranda, and Andrada. The worst results were recorded for Otilia, with values in the range 19-24. The minimum values of this variety were observed at T5 and the maximum at T3. The lowest value recorded for this parameter was observed in the variety Fundulea, which represents a decrease of 40% from the maximum recorded in the variety Faur (Figure 3). The stems number could be influenced by the germination capacity, but also by the plants growth and development. Altogether could provide inside for the optimal usage purpose primarily for food or bioenergy (Șandor et al., 2015). In terms of the parameter analyzed St, the varieties Faur, Arieșan, Bezostaia and Pădureni were classified as having a high degree of tolerance to salt stress, recording the highest yields. Transilvania, Fundulea, Ciprian and Miranda were ranked in the moderately tolerant category, while Transilvania and Otilia were moderately sensitive. While the Otilia variety maintains the same trend of reduced tolerance in the presence of this stressor, the Transilvania wheat follows a different trend. This indicates that the germination together with the growth and development processes of Otilia variety are salt stress affected (Adjel et al., 2013).

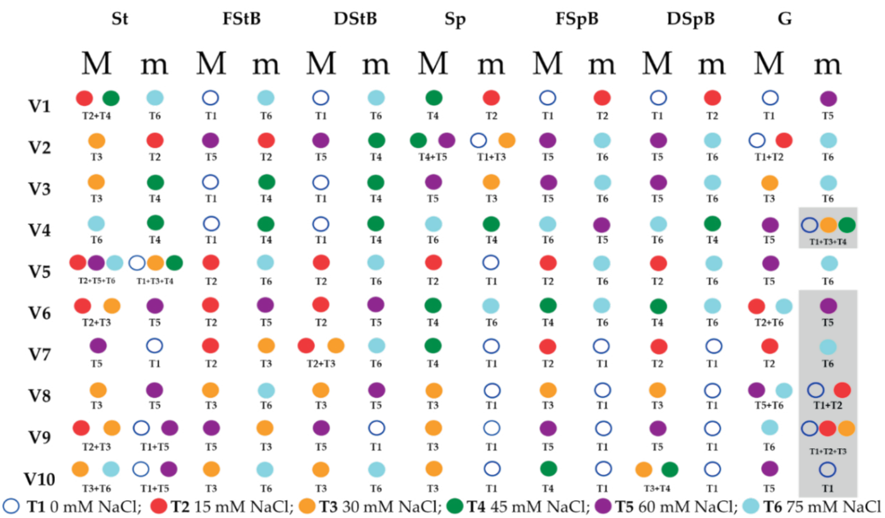

Figure 3. Minimal (m) and maximal (M) performance of wheat varieties morphological parameters in relation with salinity gradient.
Legend: Wheat varieties – V1=Andrada, V2=Arieșan, V3=Bezostaia, V4=Ciprian, V5=Faur, V6=Fundulea, V7=Miranda, V8=Otilia, V9=Pădureni, V10=Transilvania. Parameters: number of stems (St), fresh stems biomass (FStB), dry stems biomass (DStB), number of spikes (Sp), fresh spikes biomass (FSpB), dry spikes biomass (DSpB) and number of grains (G)

Table 2. Number of stems (St), fresh stems biomass (FStB), dry stems biomass (DStB), number of spikes (Sp), fresh spikes biomass (FSpB), dry spikes biomass (DSpB) and number of grains (G) for ten wheat varieties assessed at the end of the experiment

	St	FStB	DStB	Sp	FSpB	DSpB	G
V1	24.00±1.00**cd**	5.44±0.64**bc**	4.84±0.54**bc**	16.17±2.09**cd**	1.81±0.65**bc**	1.56±0.56**b-d**	20.50±10.50**bc**
V2	27.67±0.56**ab**	6.05±0.28**b**	5.25±0.18**b**	24.67±1.54**a**	3.98±0.36**a**	3.57±0.33**a**	71.33±8.11**a**
V3	27.17±1.74**a-c**	5.83±0.52**b**	5.27±0.47**b**	23.33±1.23**ab**	2.20±0.12**b**	1.99±0.10**bc**	27.67±4.25**b**
V4	25.00±1.21**bc**	4.51±0.52**cd**	4.05±0.46**cd**	18.17±2.63**b-d**	0.85±0.11**de**	0.75±0.10**de**	2.33±1.23**c**
V5	29.50±0.22**a**	4.05±0.25**d**	3.59±0.17**d**	14.50±1.45**d**	0.69±0.09**e**	0.60±0.08**e**	3.17±0.70**c**
V6	25.50±1.57**bc**	4.32±0.33**cd**	3.60±0.19**d**	16.17±1.94**cd**	0.80±0.13**de**	0.74±0.12**de**	2.17±0.65**c**
V7	24.50±1.34**bc**	5.21±0.07**b-d**	4.38±0.02**b-d**	21.17±1.45**a-c**	1.24±0.05**c-e**	1.14±0.04**c-e**	4.67±1.69**c**
V8	21.00±0.73**d**	2.73±0.17**e**	2.46±0.10**e**	17.17±1.40**cd**	0.77±0.11**de**	0.72±0.10**de**	5.00±2.57**c**
V9	26.67±0.56**a-c**	7.65±0.67**a**	7.10±0.68**a**	20.00±2.19**a-d**	1.66±0.48**b-d**	1.55±0.46**b-d**	37.50±17.06**b**
V10	25.50±1.77**bc**	5.40±0.36**bc**	4.96±0.31**bc**	19.33±3.69**a-d**	2.23±0.52**b**	2.09±0.49**b**	20.00±7.22**bc**
F	3.82	9.75	11.35	2.48	9.18	9.03	8.84
p	0.001	p<0.001	p<0.001	p<0.050	p<0.001	p<0.001	p<0.001

Note: Means±SE followed by different letters indicate significant differences at p<0.05. Legend: V1=Andrada, V2=Arieșan, V3=Bezostaia, V4=Ciprian, V5=Faur, V6=Fundulea, V7=Miranda, V8=Otilia, V9=Pădureni, V10=Transilvania

Higher values of **spikes** were recorded for Arieșan, Bezostaia, and Miranda varieties and no statistical significant between them only compared to Faur variety (Table 2). The maximum value was recorded for the Transilvania variety in T3. The minimum value was recorded for the same variety, with a decrease of about 90% from the highest value. The maximum values of the spikes number for all varieties were obtained in the proportion of about 36% in T4, 27% in T3, 18% in T5, and 9% in T6 and T2 (Figure 3).

The number of spikes is an important quantitative parameter in agriculture, and its monitoring has been useful in selecting wheat varieties capable of flowering and producing grain (Dreccer et al., 2019). Varieties Arieșan and Bezostaia show similar trends of Sp to those observed for the number of spikes, being classified again in the category of those with a high degree of salinity tolerance. Miranda, Pădureni, and Transilvania were also classified in the same category. The fact that Transilvania had a higher yield of spike number, registering a trend opposite to the one observed for the number of stems, suggests that this variety can be grown in saline conditions to produce yield crops, but cannot be recommended for obtaining biomass for biofuel production. Plants of the varieties Ciprian, Otilia, Andrada, and Fundulea were included in the tolerant category. The lowest degree of tolerance to salt stress was observed in the Faur wheat, which recorded a trend opposite to the one observed in the number of stems, which is why the cultivation of this variety under salinity conditions is recommended strictly for biomass production.

The **number of grains** is an important parameter both in agriculture and for improving wheat plants to be as rich and productive as possible. The highest average value of the parameter was obtained in the variety Arieșan, a value significantly higher than all the other values obtained in the other varieties. On the other hand, the lowest mean value recorded was observed in the variety Fundulea with a significant decrease of about 97% from the highest mean value (Table 2).

Wheat stems and spikes biomass in different salinity conditions in different salinity conditions

Regarding the **wheat stem fresh biomass** (FStB), the best performance of the mean values was recorded in the variety Pădureni, with values in the range 6.19-10.54, statistically higher than those recorded in all other varieties (Tabel 2). At the opposite pole, low performances were observed for Otilia with values in the range of 2.44-3.54 (Table 2). The maximum values recorded were reached for Pădureni wheat variety in T5. On the other hand, the lowest value of dry biomass, with a decrease of about 77% compared to the maximum, was reached for the variety Otilia in the treatment with the highest salinity dose. The maximum values of FStB obtained for all varieties were reached as follows: 30% in plants treated with T1 and T2 doses and 20% in those treated with T3 and T5 (Figure 3).

Pădureni wheat maintains a similar trend to those recorded for spike number, fresh stem biomass had maximum values probably due to an increased degree of tolerance to salt stress (Chețan et al., 2024). Ariesan, Bezostaia, Transilvania, and Miranda were included in the moderately tolerant category. Wheat of Ciprian, Fundulea, and Faur varieties are the least tolerant to salt stress, and the most sensitive was Otilia, a variety with approximately the same trend as those observed previously, with the lowest yields.

The **dry biomass** (DStB) of wheat stems is a parameter that maintains, in terms of performance, the same trend visible in the fresh biomass of plant material. Thus, the highest yielding variety was Pădureni, and the lowest-performing was Otilia. The values for dry biomass recorded for the variety Pădureni were in the range of 5.80-9.98 (Table 2), and the maximum could be observed in T5 (Figure 3). The mean dry biomass values of all other varieties and treatments were significantly lower than the highest value recorded (Table 2). The lowest value recorded was reached in Otilia wheat at the fifth dose of salt treatment, with a decrease of about 78% (Figure 3).

The dry biomass of the stems shows approximately the same trends as those recorded for their fresh biomass.

Wheat production depends mainly on the qualitative and quantitative performance of the wheat spikes. The best performance of the average values of the **wheat spikes fresh biomass** was recorded in plants of the variety Arieșan, and the lowest in wheat of the variety Faur with a significant decrease in average values of about 83% (Table 2). The maximum values recorded for FSpB were reached by wheat variety Arieșan in T5 (Figure 3). With a decrease of about 98% of the maximum, the minimum values were observed for Transilvania variety in T1. Plants of all varieties and all treatments reached the maximum in proportion of 30% in T5 treatment, 20% in T4, 20% in T2, 10% in T3, 10% in T6, and another 10% in T1.

Fresh spike biomass registered higher values for Arieșan wheat variety and it can be considered to have the highest degree of tolerance to salinity, and it can be recommended for growth when salts are present in the soil. The varieties Transilvania, Bezostaia, Andrada, and Pădureni can be classified according to their FSpB values as moderate resistant to salt stress. Miranda, Ciprian, Fundulea, and Otilia showed moderate sensitivity to salinity, while the lowest values of FSpB was recorded in the most sensitive variety, Faur. The salt stress sensitivity trend of Faur wheat, both in stem and spike biomass, suggests that this variety is not recommended to be grown under salinity conditions. The spike dry biomass shows similar trends in the varieties compared to the fresh spike biomass.

The highest average value of **dry biomass** of wheat (DSpB) was recorded in Arieșan, which is significantly higher than all the others. The lowest average value was observed in Faur, with a decrease of about 83% from the highest value recorded (Table 2). The Arieșan variety maintains the same trend as for the determined fresh biomass, recording the maximum dry biomass value at T5. At the opposite pole, the minimum of the recorded values was observed also in the Transilvania variety in T1.

Wheat stem and spike water content in different salinity conditions

In terms of the average **stems water content**, the highest average value was recorded for Miranda and is higher than Pădureni, Transilvania, and Otilia (Figure 4.). The lowest average water content, with a reduction of about 53% from the highest value, was observed in Pădureni. The maximum value of water content was recorded in Fundulea variety of wheat, treated with the second dose of salt treatment. At the opposite pole, the lowest value, with a decrease of about 84% of the maximum, was recorded for the variety Pădureni in T4.

The highest value for **spike water content** parameter was recorded at Andrada variety. Lower values compared to the highest average value recorded were observed for Fundulea, Miranda, Otilia, Pădureni, and Transilvania.

The spike water content does not maintain the same trends as in stems. Thus, the most tolerant wheat varieties to the six doses of salt treatment were Andrada, Faur and Ciprian. A similar trend to the previously analyzed parameter can be observed in the variety Ariesan which remains moderately tolerant to salinity. Bezostaia was also in the same category. Pădureni, Miranda, and Fundulea are moderately sensitive, while the most sensitive to salinity was Otilia, showing a similar pattern compared to the other parameter.

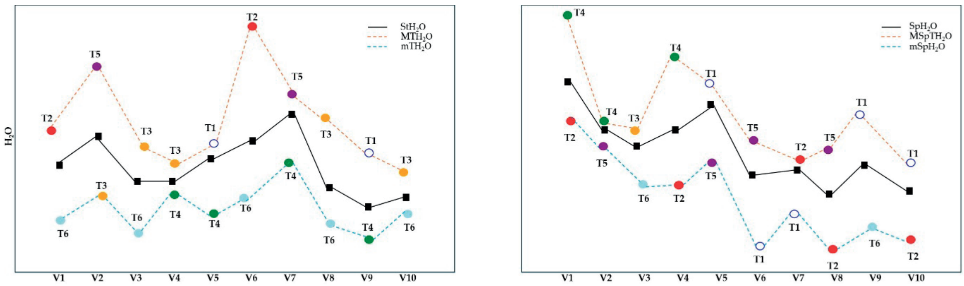

Figure 4. Stems water content (StWC) and spike water content (SpWC) for ten wheat varieties
Legend: V1=Andrada, V2=Arieșan, V3=Bezostaia, V4=Ciprian, V5=Faur, V6=Fundulea, V7=Miranda, V8=Otilia, V9=Pădureni, V10=Transilvania. Upper line – Maximum recorded for each parameter; Middle black line – average recorded for each parameter; Lower line – minimum recorded for each parameter

CONCLUSIONS

The parameters assessment values outline the plant responses variability to salinity stress.
Transilvania and Arieșan wheat variety can be classified, based on leaf relative chlorophyll content, in the salinity-tolerant category.
The varieties Faur, Arieșan, Bezostaia and Pădureni had high number of stems.
Varieties Arieșan and Bezostaia registered the higher number of spikes.
Arieșan variety had the higher fresh spike biomass.
Andrada, Faur and Ciprian registered the most higher spike water content.
The results highlighted the importance of nutrition and environmental conditions, including salinity stress, in chlorophyll synthesis and physiological mechanisms as integrated parts of plant growth and development.
Salinity stress threat is increasing, especially in the context of rapidly expanding populations and drastic climate change.

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INHERITANCE IN F1 AND TRANSGRESSIVE VARIABILITY IN F2 POPULATIONS OF MAIN SPIKE LENGTH SOFT WINTER WHEAT

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Abstract

The character of inheritance of the main spike length in F1 and transgressive variability in F2 populations was studied by hybridization of the maternal form of early maturing winter wheat varieties with medium early, medium early and medium late varieties. It was found that in most combinations of crosses, the inheritance of the length of the main spike was positive dominant. In 2018-2019, with the exception of the Kolchuga/Chornyava combination (2018), positive hypothetical heterosis for the length of the main spike was determined and 35 out of 40 hybrids had positive true heterosis. The significant influence of hybridization components and year conditions on the main spike length, degree of phenotypic dominance and heterosis was established. The majority of F_2 populations significantly exceeded the parental forms in terms of the extreme maximum length of the main spike, indicating a significant formation process and the possibility of conducting selections for the studied trait. In 2019-2020, 26 out of 40 F_2 populations showed a positive degree and frequency of transgressions in spike length.

Key words: *heterosis, hybrid, main spike length, F2 populations, varieties.*

INTRODUCTION

In Ukraine, winter wheat (*Triticum aestivum* L.) plays a leading role in grain production (Burdeniuk-Tarasevych & Lozinskyi, 2015; Sydiakina & Dvoretskyi, 2020) and is cultivated on an area of 6.2-6.7 million hectares (Verner, 2021), which is evidence of its important economic importance (Kiriiak & Kovalenko, 2015; Zhemela et al., 2020). The high ecological plasticity of wheat and its ability to form crops in different geographical zones and agroclimatic conditions (Burdeniuk-Tarasevych & Lozinskyi, 2015; Ray et al., 2013), as well as its excellent nutritional value, have contributed to its widespread use as a staple food (Zhemela et al., 2020; Havryliuk & Kalenych, 2018). The level of wheat yield achieved under production conditions does not meet modern requirements and indicates a low realisation of the crop's genetic potential (Bazalii et al., 2010). At the same time, climate change can have a significant impact on grain production (Tavares et al., 2015; Machold & Honeremeier, 2016). Another important task is to increase wheat production with high quality indicators.

An important factor in the stabilization and growth of winter soft wheat production is the development and introduction of new high-yielding varieties adapted to specific growing conditions (Burdeniuk-Tarasevych & Lozinskyi, 2014; Morhun et al., 2014; Egamov et al., 2021, Lozinskiy et al, 2023). The improvement of new varieties should be based on environmental sustainability, taking into account the specific interactions between environment and genotype, which will provide new approaches to the formation of highly productive agrophytocenoses (Adhikari et al., 2020; Bondareva & Vashchenko, 2021). Success in breeding and genetic research is due to many factors, the most important of which is the search for and creation of new source materials with high productivity, quality and adaptability to biotic and abiotic environmental factors (Morhun et al., 2015). At the same time, in the genetic improvement of wheat, genetic resources play a crucial role in practical breeding work.

Spike length is often used in practical breeding for the selection of valuable genotypes as it has a clear phenotypic manifestation and is a convenient morphological marker for identifying valuable genotypes (Lozinskyi & Ustynova, 2020).

Today, transgressive breeding based on the selection of the best individuals in a hybrid population is one of the main methods of improving self-pollinated crops (Orliuk & Bazalii, 2013).

The aim of the research was to study the mode of inheritance of the length of the main spike in F1 and to establish transgressive variability in F2 populations obtained by hybridization of the maternal form of early maturing winter wheat varieties.

MATERIALS AND METHODS

The research was conducted in the experimental field of the research and production center of the Bila Tserkva National Agrarian University in 2017-2020. 20 F_1 hybrids and their F_2 populations were studied. Varieties of different maturity groups were used as parental forms: Mironovskaya early ripening (Mir. early), Kolchuga, Belotserkovskaya semi-dwarf (B.Ts. s/d.) – early ripening; Zolotokolosa (Zolotokol.), Chernyava – medium early; Stolichna, Vidrada, Antonovka, Yednist – medium ripening; Dobirna and Vdala – medium late ripening.

Seeds $F_{1\text{-}2}$ were sown with a manual sowing machine in accordance with the scheme: mother form (♀), hybrid (population), male form (♂). The hybrid generation was worked with the pedigree method. Phenological observations were made during the wheat growing season and, after the onset of full grain maturity, biometric analysis of the study material was carried out on an average sample of 75 plants (Volkodav, 2003). The predecessor of soft winter wheat was grain mustard. The soil of the trial field is a typical deep, low-humus, coarse-dusted medium and light loamy black soil. According to the 2016 agrochemical survey, the humus content is 3.4-3.8%, alkaline hydrolyzed nitrogen is 118-134 mg/kg soil, mobile phosphorus is 180-208 and exchangeable potassium is 73-91 mg/kg soil. The reaction of the soil solution is slightly acidic and close to neutral. The main fertilizer was a phosphorus-potassium fertilizer at 60 kg/ha N in the form of superphosphate and potassium salt. Ammonium nitrate was applied at 60 kg/ha N to restore the spring vegetation. Herbicides and irrigation were not used in the nursery.

Hypothetical (Ht) and true (Hbt) heterosis by the length of the main spike in F1 was determined by Matzinger D.F. (1962) and S. Fonseca, F. Patterson (1968):

$$Ht\ (\%) = (F_1 - MP)/MP \times 100,$$
$$Hbt\ (\%) = (F_1 - BP)/BP \times 100,$$

where: F_1 – average arithmetic value of the hybrid;

BP – the highest manifestation of a trait of one of the parents;

MP – the average arithmetic value of the indicator of both parental forms.

The degree of phenotypic dominance (hp) was determined by the method B. Griffing (1950):

$$h_p = (F_1 - MP)/(BP - MP),$$

where: h_p – degree of domination;

F_1 – average arithmetic value of the hybrid;

BP – arithmetic mean of the parental component with the stronger manifestation of the trait;

MP – the average arithmetic value of the indicator of both parental forms.

The data obtained were grouped by classification G. M. Beil, R. E. Atkins (1965): positive dominance (heterosis) $h_p > +1$; partial positive dominance $+0.5 < h_p \leq +1$; intermediate inheritance $-0.5 \leq h_p \leq +0.5$; partial negative inheritance $-1 \leq h_p < -0.5$; negative dominance (depression) $h_p < -1$.

The degree (Tc) of positive transgression in F_2 populations was determined by the following method (Vasylkivskyi & Kochmarskyi, 2016):

$$Tc\ (\%) = (M_F - Mp) / Mp \times 100,$$

where: M_F – is the maximum value of a particular quantitative feature in F_2;

Mp – maximum value at the best parental form.

The frequency (Ff) of transgression was determined by the number (%) of individuals of F_2 populations exceeding (+T) the extreme manifestation of the trait in parental forms.

The results of the experimental data were statistically processed using the "Statistica" 12.0 programme.

At the time of sowing (last ten days of September), meteorological conditions in 2017-2019 were favorable for simultaneous germination and growth and development of soft winter wheat in autumn. The amount of precipitation during this period exceeded (2017) or was at the level of long-term average indicators. Soft winter wheat stopped vegetation in the autumn period on 20 November (2017), 12 November (2018) and 21 November (2019), which contributed to the successful hardening of the plant. Winter precipitation was well above the long-term average (112 mm) in the 2017/2018 and 2018/2019 growing years and was slightly lower than in 2019/2020. The winter temperature regime contributed to the successful overwintering of the plants (Table 1).

Table 1. Meteorological conditions in 2018-2020 (according to the Bila Tserkva meteorological station)

Month	Decade	Precipitation, mm					Air temperature, ºC				
		2017	2018	2019	2020	long-term data	2017	2018	2019	2020	long-term data
September		53.2	47.9	19.2		35	16.1	16.2	15.3		13.8
October		50.4	22.0	66.1		33	8.0	9.9	10.6		7.9
November		36.4	23.1	23.4		41	3.2	-0.1	5.0		2.0
December		92.3	71.1	35.1		44	1.6	-2.0	2.5		-2.4
January			30,5	56.8	22.6	35		-2.7	-4.8	0.4	-5.9
February			34,6	21.4	38.4	33		-4.2	0.4	2.2	-4.4
March			74.0	23.4	17.2	30		-2.1	4.7	5.9	0.3
April	I		1.5	-	-	14		10.3	9.6	7.9	7.0
	II		1.3	14.2	5.5	17		13.8	7.3	8.0	7.8
	III		5.3	31.3	7.7	16		15.7	13.2	11.7	10.4
May	I		3.7	26.7	30.8	16		20.4	12.1	12.8	13.3
	II		19.1	15.3	17.6	12		15.9	18.3	13.2	15.3
	III		-	12.0	53.9	18		18.8	19.3	11.5	15.8
June	I		2.2	35.3	7.1	23		19.4	21.1	18.5	17.3
	II		23.3	-.	50.4	27		21.9	23.6	23.2	17.4
	III		33.2	43.9	3.2	23		19.1	21.4	22.0	18.7

The temperature regime after the resumption of vegetation in 2018 (4 April) was characterized by elevated temperatures, which accelerated the growth and development of soft winter wheat. The average monthly temperature in April (15.5°C) was significantly higher than the long-term average (8.4°C), and the average air temperature in May and June was 3.5 and 2.3°C higher, respectively. At the same time, the amount of precipitation in April and May was less than the long-term average by 17.1 and 21.6 mm, respectively.

The vegetation of soft winter wheat from the time of recovery (02.03. - 2019, 28.02. - 2020) occurred during the month with low average monthly temperatures with their gradual increase. The amount of precipitation in March (23.4 mm) and the first two decades of April (14.2 mm) in 2019 was significantly lower than the long-term average. Precipitation in March and April 2020 was 46.6 mm below the long-term average.

Thus, the meteorological conditions during the years of research were characterized by contrasting indicators in terms of temperature and rainfall distribution.

RESULTS AND DISCUSSIONS

Experimental data show that in 2018, the length of the main spike in parental forms was 6.1-9.5 cm. According to the international classifier, short spike (6.1-7.5 cm) were formed by the varieties Yednist, Zolotokolosa, Vdala, Vidrada, Myronivska early ripening, Stolichna and Dobirna. The average spike length was observed in Antonivka, Bilotserkivska semi-dwarf, Kolchuga (7.6-8.8 cm) and Chorniava (9.5 cm). The hybrids we obtained had an average spike length (Table 2).

Table 2. Heterosis and the degree of phenotypic dominance of the main spike length in F_1 (2018 p.)

Crossing combinations	Spike length, cm			Heterosis, %		h_p
	♀	♂	F_1	Ht	Hbt	
♀ early ripening / ♂ early ripening						
Mir. early/B.Ts. s/d.	7.3	7.7	9.1	21.3	18.2	8.0
Mir. early/Kolchuga	7.3	8.8	9.9	22.2	12.5	2.5
B.Ts. s/d./ Kolchuga	7.7	8.8	8.6	3.6	-2.3	0.6
♀ early ripening / ♂ medium early						
Mir. early/Zolotokol.	7.3	6.7	8.6	22.9	17.8	5.3
Mir. early/ Chernyava	7.3	9.5	10.5	25.0	10.5	1.9
B.Ts. s/d./ Zolotokol.	7.7	6.7	8.9	23.6	15.6	3.4
B.Ts. s/d./ Chernyava	7.7	9.5	9.4	9.3	-1.1	0.9
Kolchuga/ Chernyava	8.8	9.5	8.9	-3.3	-6.3	-1.4
♀ early ripening / ♂ medium-ripening						
Mir. early/Antonovka	7.3	7.6	8.8	17.3	15.8	9.0
Mir. early /Yednist	7.3	6.1	9.7	44.8	32.9	4.6
B.Ts. s/d./ Antonovka	7.7	7.6	8.8	14.3	14.3	23.0
B.Ts. s/d./ Yednist	7.7	6.1	8.8	27.5	14.3	2.4
B.Ts. s/d./ Vidrada	7.7	7.2	8.6	14.7	11.7	5.5
Kolchuga /Antonovka	8.8	7.6	8.8	7.3	-	1.0
Kolchuga / Yednist	8.8	6.1	8.7	16.0	-1.1	0.9
Kolchuga / Vidrada	8.8	7.2	8.9	11.3	1.1	1.1
Kolchuga / Stolichna	8.8	7.5	9.7	18.3	10.2	2.4
♀ early ripening / ♂ medium late						
Mir. early / Vdala	7.3	7.0	9.6	33.3	31.5	16.3
Mir. early / Dobirna	7.3	7.5	9.0	21.6	20.0	16.0
B.Ts. s/d./ Dobirna	7.7	7.5	8.4	10.5	9.1	8.0

In most F1 hybrids the spike length exceeded that of the parent forms. In the Kolchuga/Antonovka hybridization, the studied index was at the level of the mother form with a greater manifestation of the trait. In the combinations of crossing Bilotserkivska semi-dwarf/Chernyava, Kolchuga/Yednist and Bilotserkivska semi-dwarf/Kolchuga the length of the spike approached the parental component with a greater manifestation and only in the hybrid Kolchuga/Chernyava there was an intermediate formation of the trait between the original forms.

Indicators of hypothetical heterosis for main spike length in F1 were positive, with the exception of Kolchuga/Chernyava (Ht = -3.3%), and ranged from 3.6-44.8%. Positive true heterosis (1.1-32.9%) was observed in 15 out of 20 hybrids. High values of heterosis were observed in the hybrids Myronivska early ripening/Yednist (Ht = 44.8%, Hbt = 32.9%) and Myronivska early ripening/Vdala (Ht = 33.3%, Hbt = 31.5%).

Positive superdominance for the length of the main spike (hp = 1.1-24.0) was determined in 15 out of 20 hybrids. Inheritance of partial positive dominance was observed in the crossing combinations - Bilotserkivska semi-dwarf/Kolchuga, Bilotserkivska semi-dwarf/Chernyava, Kolchuga/Yednist, Kolchuga/Antonovka, and Kolchuga/Chernyava was characterized by negative dominance.

In 2019, the length of the main spike in the parental forms ranged from 6.1 cm (Yednist) to 9.7 cm (Chernyava). Short ears (6.1-7.3 cm) were formed by varieties Yednist, Zolotokolosa, Vdala, Myronivska early ripening. The average length of the spike was observed in all other studied varieties (7.6-9.7 cm). The hybrids obtained in 2019 had some differences in the length of the main spike compared to 2018. The average spike (9.3-10.4 cm) was formed by Bilotserkivska semi-dwarf/ Yednist, Bilotserkivska semi-dwarf/Zolotokolosa, Bilotserkivska semi-dwarf/Chernyava, Myronivska early ripening/Yednist, Kolchuga/Yednist, Myronivska early ripening/Bilotserkivska semi-dwarf, Myronivska early ripening/Antonovka, Kolchuga/Vidrada, Bilotserkivska semi-dwarf/Vidrada, Myronivska early ripening/Dobirna. Other hybrids had a large spike (10.9-11.6 cm) (Table 3).

Table 3. Heterosis and the degree of phenotypic dominance of the main spike length in F_1 (2019 p.)

Crossing combinations	Spike length, cm			Heterosis, %		h_p
	♀	♂	F_1	Ht	Hbt	
♀ early ripening / ♂ early ripening						
Mir. early/B.Ts. s/d.	7.3	7.8	10.2	35.1	30.8	10.6
Mir. early/Kolchuga	7.3	9.0	10.7	31.3	18.9	3.0
B.Ts. s/d./ Kolchuga	7.8	9.0	10.9	29.8	21.1	4.2
♀ early ripening / ♂ medium early						
Mir. early/Zolotokol.	7.3	6.3	10.8	58.8	47.9	8.0
Mir. early/Chernyava	7.3	9.7	11.6	36.5	19.6	2.6
B.Ts. s/d./ Zolotokol.	7.8	6.3	9.6	36.2	23.1	3.4
B.Ts. s/d./ Chernyava	7.8	9.7	9.7	11.0	0.1	1.01
Kolchuga / Chernyava	9.0	9.7	11.4	21.9	17.5	5.9
♀ early ripening / ♂ medium-ripening						
Мир. ран./ Antonovka	7.3	7.9	10.2	34.2	29.1	8.7
Mir. early / Yednist	7.3	6.1	9.8	46.3	34.2	5.2
B.Ts. s/d./ Antonovka	7.8	7.9	11.0	40.1	39.2	63.0
B.Ts. s/d./ Yednist	7.8	6.1	9.3	33.8	19.2	2.8
B.Ts. s/d./ Vidrada	7.8	7.6	10.4	35.1	33.3	27.0
Kolchuga / Antonovka	9.0	7.9	11.4	34.9	26.7	5.4
Kolchuga / Yednist	9.0	6.1	10.0	32.5	11.1	1.7
Kolchuga / Vidrada	9.0	7.6	10.3	24.1	14.4	2.9
Kolchuga / Stolichna	9.0	7.7	11.4	36.5	26.7	4.7
♀ early ripening / ♂ medium late						
Mir. early / Vdala	7.3	6.7	10.8	54.3	47.9	12.7
Mir. early / Dobirna	7.3	8.0	10.4	35.9	30.0	7.9
B.Ts. s/d./ Dobirna	7.8	8.0	10.9	38.0	36.3	30.0

In 2019, the vast majority of F_1 hybrids obtained by using the maternal form of early maturing varieties exceeded the original forms in terms of ear length, and only in the combination Bilotserkivska semi-dwarf/Chernyava its length was at the level of the parental form with a greater manifestation of the trait.

In all F_1 hybrids, positive values of both hypothetical and true heterosis were established, the indicators of which were significantly influenced by the parental components of hybridization and the conditions of the year. The highest values of hypothetical and true heterosis were observed in hybrids: Myronivska early ripening/Zolotokolosa (Ht = 58.8%, Hbt = 47.9%); Myronivska early ripening/Vdala (Ht = 54.3%, Hbt = 47.9%); Myronivska early ripening/Yednist (Ht = 46.3%, Hbt = 34.2%); Bilotserkivska semi-dwarf/Antonovka (Ht = 40.1%, Hbt = 39.2%); Bilotserkivska semi-dwarf/Dobirna (Ht = 38.0%, Hbt = 36.3%); Myronivska early ripening/Dobirna (Ht = 35.9%, Hbt = 30.0%); Bilotserkivska semi-dwarf/Vidrada (Ht = 35.1%, Hbt = 33.3%); Myronivska early ripening/Bilotserkivska semi-dwarf (Ht = 35.1%, Hbt = 30.8%).

Positive superdominance by the length of the main spike was determined in all studied hybrids with modification of the degree of phenotypic dominance (hp = 1.01-30.0) depending on the selected parental forms.

In 2019, out of 20 crossing combinations in F_2, the average population index of the length of the main spike only in Myronivska early ripening/Kolchuga and Kolchuga/Yednist was 10.6 cm and 12.1 cm, respectively, indicating a long spike, while the rest of the spike was average (8.8-10.5 cm) (Table 4).

In 16 out of 20 F2 populations created by hybridization with the maternal form of early ripe varieties, the extreme maximum length of the main spike (10.5-15.0 cm) significantly exceeded the parental forms (9.0-10.5 cm), indicating a significant formation process and the possibility of conducting selections for the studied trait. In the populations Bilotserkivska semi-dwarf/Kolchuga and Myronivska early ripening/Chernyava the maximum values were at the level of parental forms with a greater manifestation of the trait. It should be noted that the length of the main spike (10.3-12.1 cm) was observed in populations where Kolchuga was used as the mother form. The extreme maximum values reached 13.0-15.0 cm.

Table 4. The degree and frequency of positive transgressions in the length of the main spike in populations F_2 (2019 p.)

Population F_2	Length of the main spike, cm					Transgression,%	
	average			maximum expression			
	♀	♂	F_2	P	F_2	Tc	Th
♀ early ripening / ♂ early ripening							
Mir. early B.Ts. s/d	7.3	7.8	10.3	10.0	12.5	25.0	50.0
Mir. early/Kolchuga	7.3	9.0	10.6	10.5	12.0	14.3	40.0
♀ early ripening / ♂ medium early							
Mir. early/ Zolotokol.	7.3	6.3	10.3	9.0	11.5	27.3	86.7
B.Ts. s/d./ Zolotokol	7.8	6.3	9.3	10.0	12.0	20.0	13.3
♀ early ripening / ♂ medium-ripening							
Mir. early/Antonovka	7.3	7.9	10.1	9.0	12.5	38.9	86.7
Mir. early / Yednist	7.3	6.1	9.6	9.0	11.5	27.8	63.3
B.Ts. s/d./ Antonovka	7.8	7.9	10.1	10.0	12.0	20.0	46.6
B.Ts. s/d./ Yednist	7.8	6.1	9.9	10.0	12.0	20.0	27.6
B.Ts. s/d./ Vidrada	7.8	7.6	9.5	10.0	10.5	5.0	3.3
Kolchuga/Antonovka	9.0	7.9	10.3	10.5	13.0	23.8	40.0
Kolchuga / Yednist	9.0	6.1	12.1	10.5	15.0	42.9	83.3
Kolchuga / Vidrada	9.0	7.6	10.5	10.5	13.0	23.8	48.1
Kolchuga / Stolichna	9.0	7.7	10.5	10.5	13.0	23.8	33.3
♀ early ripening / ♂ medium late							
Мир. ран./ Vdala	7.3	6.7	10.2	9.0	12.0	33.3	86.7
Мир. ран./ Dobirna	7.3	8.0	9.8	9.5	11.0	15.8	60.7
B.Ts. s/d./ Dobirna	7.8	8.0	9.3	10.0	11.0	10.0	10.0

The conducted studies show that in 16 out of 20 F_2 populations a positive degree and frequency of transgressions in the length of the main spike was established. High rates were noted in the populations of: Kolchuga/Yednist (Tc = 42.9%; Th = 83.3%); Myronivska early ripening/Antonovka (Tc = 38.9%; Th = 86.7%); Myronivska early ripening/Vdala (Tc = 33.3%; Th = 86.7); Myronivska early ripening/Yednist (Tc = 27.8%; Th = 63.3%); Myronivska early ripening/Zolotokolosa (Tc = 27.3%; Tc = 86.7%); Myronivska early ripening/Bilotserkivska semi-dwarf (Tc = 25.0%; Th = 50.0%).

In 2020, the average length of the main spikelet in F_2 populations (8.9 cm) was significantly lower than in 2019 – 10.1 cm, with the average population index of 8.6-10.1 cm. At the same time, in the Bilotserkivska semi-dwarf/Kolchuga population, the average length of the main spike in 2020 (9.2 cm) was 0.4 cm longer than last year (Table 5).

Table 5. The degree and frequency of positive transgressions in the length of the main spike in populations F_2 (2020 p.)

Population F_2	Length of the main spike, cm					Transgression, %	
	average			maximum expression			
	♀	♂	F_2	P	F_2	Tc	Th
♀ early ripening / ♂ early ripening							
B.Ts. s/d./ Kolchuga	8.4	9.4	9.2	10.5	11.0	4.8	3.3
♀ early ripening / ♂ medium early							
Mir. early/Zolotokol.	8.7	8.5	9.2	10.0	11.0	10.0	3.3
Mir. early/Chernyava	8.7	10.5	10.1	12.5	14.0	12.0	6.7
B.Ts. s/d./ Zolotokol.	8.4	8.5	8.9	10.0	11.5	15.0	16.7
♀ early ripening / ♂ medium-ripening							
B.Ts. s/d./ Vidrada	8.4	7.1	8.6	9.5	10.0	5.3	3.3
Kolchuga/Antonovka	9.4	8.7	8.9	10.5	11.0	4.8	3.3
Kolchuga/Stolichna	9.4	8.3	9.3	10.5	11.0	4.8	10.0
♀ early ripening / ♂ medium late							
Mir. Early/Vdala	8.7	8.5	9.4	10.0	11.0	10.0	10.0
Mir. early / Dobirna	8.7	9.1	8.9	10.0	11.0	10.0	10.0
B.Ts. s/d./ Dobirna	8.4	9.1	8.9	10.0	10.5	5.0	3.3

Exceeding the extreme maximum manifestation in the length of the main spike of parental forms and a positive degree of transgression in 2020 was determined in 10 out of 20 populations with indicators ranging from 4.8% to 15.0% and a recombinant frequency of 3.3-26.7%, which is significantly lower compared to 2019 and indicates the influence of the year's conditions. The following combinations stood out among the populations with a positive degree of transgression: Bilotserkivska semi-dwarf/Zolotokolosa (Tc = 15.0%; Th = 16.7%), Myronivska early ripening / Vdala (Tc =

10.0%; Th = 10.0%) i Myronivska early ripening/Dobirna (Tc = 10.0%; Th = 10.0%).

Within two years, a positive degree of transgression was found in all populations obtained by crossing early ripening varieties with medium late varieties and in combinations Myronivska early ripening/Zolotokolosa, Bilotserkivska semi-dwarf/Zolotokolosa, Bilotserkivska semi-dwarf/Vidrada, Kolchuga/Antonovka, Kolchuga/Stolichna, which indicates a stable increase of long spike recombinants in them regardless of meteorological factors.

Inclusion of early maturing winter wheat varieties in maternal hybridization with medium early, medium early and medium late varieties promotes the formation of F_2 populations by main spike length, with the possibility of selecting F_2 genotypes that combine high main spike length with other economically valuable traits and characteristics.

CONCLUSIONS

The formation of the length of the main spike in F_1 in 2018-2019 and indicators of heterosis and the degree of phenotypic dominance are due to the components selected for hybridization and the conditions of the year.

Stably high positive indices of hypothetical and true heterosis in 2018-2019 by the length of the main spike were determined in the combinations of crosses Myronivska early ripening/Yednist (Ht = 44.8-46.3%, Hbt = 32.9-34.2%) and Myronivska early ripening/Vdala (Ht = 33.3-54.3%, Hbt = 31.5-47.9%).

Using the maternal form in hybridization of early maturing winter wheat varieties with medium early, medium early and medium late varieties with different combinations of parental pairs, it was found that positive dominance was the most common type of inheritance, established in 39 out of 40 hybrids studied.

Indicators of the degree and frequency of positive recombinants for the length of the main spike in F_2 populations are determined by both hybridization components and year conditions. Thus, in 2019 a significantly higher number of second generation hybrid populations (80.0%) with a positive degree of transgression was identified compared to 2020 – 50.0%. In most of these populations, the frequency of positive recombinants was also higher in 2019.

In 2019-2020, 26 out of 40 F_2 populations showed a positive degree of transgression (4.8-42.9%) by the length of the main spike. The frequency of transgressive recombinants was 3.3-86.7%, depending on the parental forms involved in the hybridization.

The involvement of early-ripening winter soft wheat varieties in hybridization with medium-early, medium-ripening and medium-late varieties by the mother form significantly extends the formation process in terms of the length of the main ear.

The prospect of further research is to evaluate the conducted selections of soft winter wheat for a number of economically valuable traits to create a new source material with high productivity and adaptability to the unfavorable conditions of the Forest-Steppe Ukraine.

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SOYBEAN CROP DEVELOPMENT, YIELD AND HARVEST QUALITY UNDER TWO SOWING DATE

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Abstract

The influence of the sowing date, genotype and climatic conditions on the soybean crop development, yield and seed quality, was studied in a field experiment placed at Research and Development Station for Agriculture (RDSA) Turda, in three consecutive years. The ten soybean genotypes were sown in: 2021, 2022 and respectively 2023, in the experimental field of the Soybean Breeding Laboratory. The biological material consisted of very early (Perla, T12-295), early (Onix, Felix, Cristina TD, Caro TD, T14-4203) or semi-early (Raluca TD, T26-6126, Avatar) genotypes. Based on the experimental results, a decrease in yield was obtained when late genotypes were sown in the second date. For early soybean biological material, the results for production were similar in both sowing dates. In terms of quality, delayed sowing date contributed to an increase in protein content from 35.70% to 36.26%, while the oil content decreased by 0.40% by practicing late sowing.

Key words*: soybean, quality, sowing date, yield.*

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) is one of the most important food crops (Negrea et al., 2023) but also the most widespread leguminous crop worldwide (Ren et al., 2023), due to the wide range of use of its seeds in many branches of the food and technical industry (Guzeler & Yildirim, 2016; Suciu et al., 2022).

With a seed content of about 40% protein and about 20% fat (Staniak et al., 2021), soybeans are used in many countries predominantly in human food and the other parts are used in animal feed. This crop is also a source of many important compounds such as fibre, lecithin, vitamins and mineral salts (Bellaloui et al., 2015).

As one of the cheapest sources of protein, soybean plays an important role in the diet of people in developing countries, where human beings face protein deficiencies (Chaudhry, 1985).

Climate change can have negative effects on temperate zone agriculture due to scarcity of water available to plants in summer, as well as increasing decline in water resources available in soil throughout the growing season (Falloon & Betts, 2010; Kasperska-Wołowicz et al., 2021).

These climatic stress conditions in the soybean reproductive stage can reduce seed yield even by 74% (Jumrani & Bhatia, 2018) if measures are not taken to reduce adverse conditions.

The quality and quantity of soybean harvest may be affected by the genetic potential of the variety, environmental conditions during the growing season and applied cultivation technology (Ionescu et al., 2016; Yilmaz, 2003). Stressors can disrupt plant metabolic processes, cause damage to cell structure, inhibit plant growth and development, and cause seed production and quality to decline and deteriorate (Hou et al., 2006; Michałek & Borowski, 2006).

The growth and development of soybean plants are strongly influenced, besides climatic conditions and sowing time (Albrecht et al., 2008) but also by applied technology.

Research carried out so far has shown that sowing soybeans too early could lead to slow emergence and growth in the early stages of development, but drought stress could be avoided during critical periods, when rainfalls are very low and high temperatures persist for several days, and that delayed sowing leads to significant decreases in production, these are most often associated with reductions in both vegetative and reproductive periods (Bateman et al., 2020).

In the context in which the climate is constantly warming and plants need to adapt to climate change, identifying the factors that influence the formation of crop production, especially climatic ones, is of great importance.

The purpose of this study was to determine the influence of climatic conditions and sowing date on soybean yield and quality, but also on the number of days from sowing to the most important vegetation stages, for soybeans grown in pedo-climatic conditions in the Transylvanian Plateau.

MATERIALS AND METHODS

The purpose of this study consisted in evaluation of ten soybean genotypes in terms of crop development harvest and quality as influenced by:

- technological factors sowing date;
- genotype;
- climatic conditions.

To achieve these goals, an experiment was accomplished at the field of Research and Development Station for Agriculture (RDSA) Turda, in three different under climatic factors, but consecutive years.

The experiment had three replications and was based on the method of subdivided plots. Each plot had an area of 10 m^2. The soybean genetics studied consisted of very early, early or semi-early genotypes that were sown in the experimental field of the Soybean Breeding Laboratory in: 2021, 2022 and respectively 2023. In each of the three experimental years, the delaying sowing date influence on soybean crop yield and quality was studied, sowing being carried out, depending on the climatic conditions of each year, on:

- Year 2021: April 12 and May 5, respectively;
- Year 2022: April 6 and May 3, respectively;
- Year 2023: April 21 and May 2, respectively.

During the growing season, observation and notations of soybean crop development in terms of the main phases were made according to American Code (FEHR and CAVINESS, 1977). Thus, during the paper are presented data on the difference in the number of days from sowing to: beginning of flowering (R1), beginning of maturity (R7), respectively end of maturity (R8) for each of the 10 soybean studied cultivars and three years, between the two sowing dates. Also, at maturity, the experimental variants were harvested and weighed, the yield obtained on each plot being reported per ha.

The seeds of each experimental variant were analysed in the laboratory using near-infrared spectrophotometry to determine the main soy quality indices (protein, fat, stearic acid, oleic acid, linoleic acid and linolenic acid).

The data obtained were statistically processed using ANOVA, and the graphical exemplification of the results was performed with Microsoft Excel.

RESULTS AND DISCUSSIONS

RDSA Turda is located in the north-western part of Turda, the climate of the area, according to the Köppen classification, is rendered by the D.f.b.x. formula, which defines the boreal climate with continental influences, with four distinct seasons. The meteorological data were recorded at Turda Meteorological Station, at an altitude of 427 m and having the following geographical coordinates: longitude 23°47’ and latitude 46°35’. The climatic conditions in the three experimental years varied in terms of temperatures and rainfall, which was reflected in the growth and development of soybean plants and in the level of grain yields obtained from one year to another (Figure 1).

The three experimental years were very different in terms of thermal and pluviometric regime. In general, the first two months of spring were characterized by lower temperatures and rainfall compared to the multiannual average, which led to a late emergence of soybean plants when the first sowing date was experienced (the date of emergence being noted about three weeks after sowing). Also, plant emergence was uneven in the third year of study. In the three experimental years, the rainfall and high temperatures registered in May, led to a uniform emergence of soybean plants sown in the second date (2 weeks, one week, respectively 3 weeks after sowing). The data presented by Dima (2006) are similar to those recorded in our experiment regarding the number of days from sowing to emergence, namely that for the first time of sowing the period between sowing and emergence is longer than for the second date of sowing.

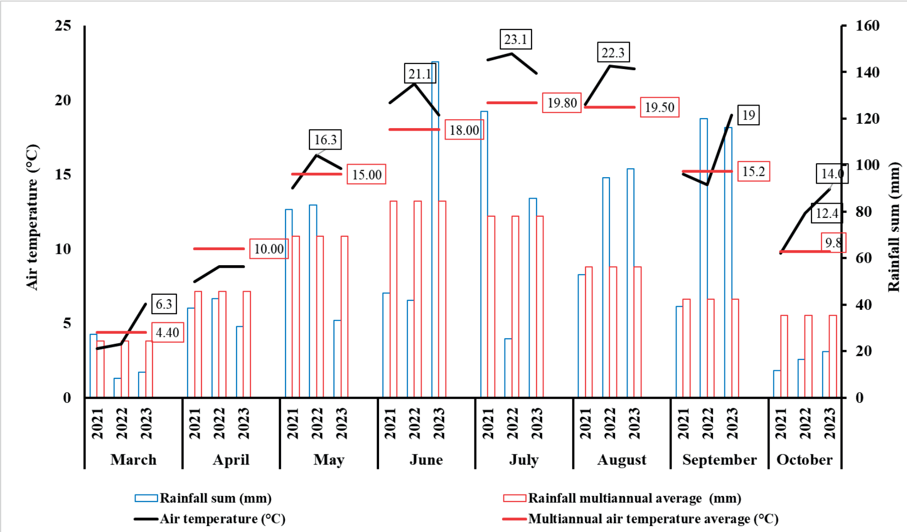

Figure 1. Mean air temperature and rainfall of each month during soybean growing season (Turda, 2021-2023)

On average, the date of flowering (R1) was 6 days shorter when late sowing was practiced and was noted approximatively at the same calendar date, regardless of the sowing time or experimental year (Figure 2).

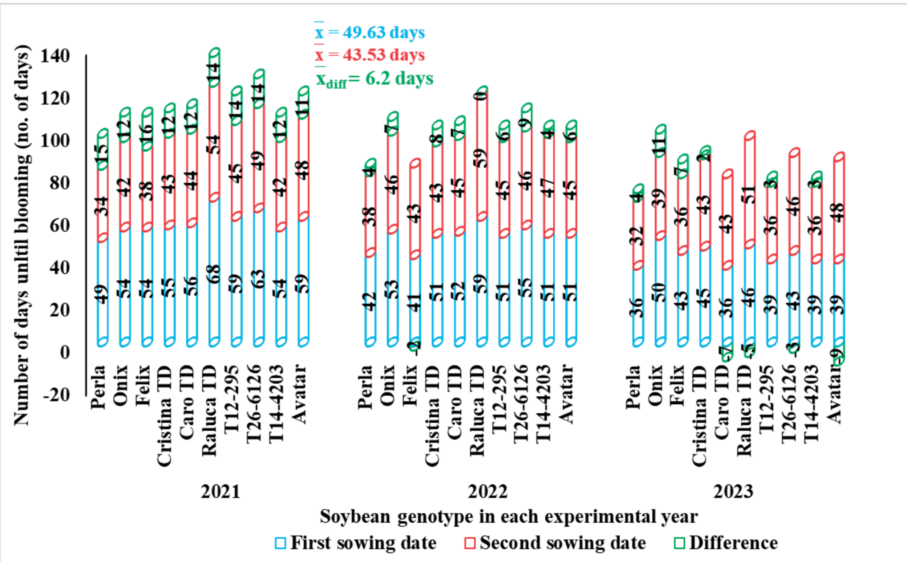

Figure 2. Number of days until blooming (R1) for each soybean genotype and sowing date experimented (Turda, 2021-2023)

The obtained data reveals a variable length of vegetative period of each genotype, the generative stage starting at different time, in each experimental year and sowing date.
In 2023, Perla, the very early variety of soybean created by RDSA Turda, reached flowering 32 days after emergence when it was sown on the second date.
As expected, the beginning of flowering for Raluca TD semi-early soybean variety was noted on July 5, 68 days after emergence, when it was sown on April 12.
On average, when delayed sowing was practiced, the stage of beginning maturity (R7) for soybean biological material was reached 8 days earlier compared to the first sowing date (Figure 3).
If in the first experimental year the period required to reach the vegetation phase R7 was much lower for genotypes sown late compared to the first sowing date, the opposite happens in the last experimental year. If the very early soybean variety Perla had the shortest period from sowing to early maturity, the Raluca TD variety and the T26-6126 line, reached the R7 vegetation phase in a greater number of days.

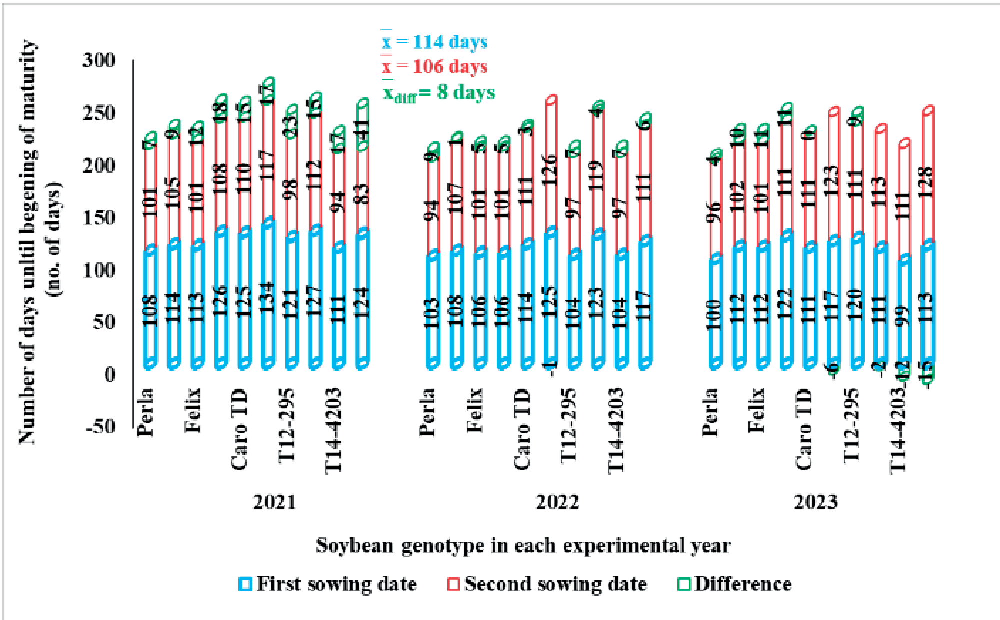

Figure 3. Number of days until beginning of maturity (R7) for each soybean genotype and sowing date experimented (Turda, 2021-2023)

In a study conducted by Serafin-Andrzejewska (2021), when 20 days of delayed sowing date was experimented in relation to the early date, a shortening of the growing period by 14 days was observed. Research by Księżak & Bojarszczuk (2022) showed that the 20-day delay in the sowing date resulted in shortening the growing season by 18 days, compared to soybeans sown at the optimal time, indicating that the growing season remains the same in number of days, even if sowing is done earlier.
In our experiment, by practicing the second sowing date, the growing season of the ten soybean varieties was 6 days shorter (Figure 4). The ten soybean cultivars experimented are characterized by different maturity, therefore, differences in their development were observed. As expected, for: T26-6126 line, Raluca TD and Avatar semi-early soybean genotypes a longer period of vegetation was identified in all three experimental years and two sowing dates.

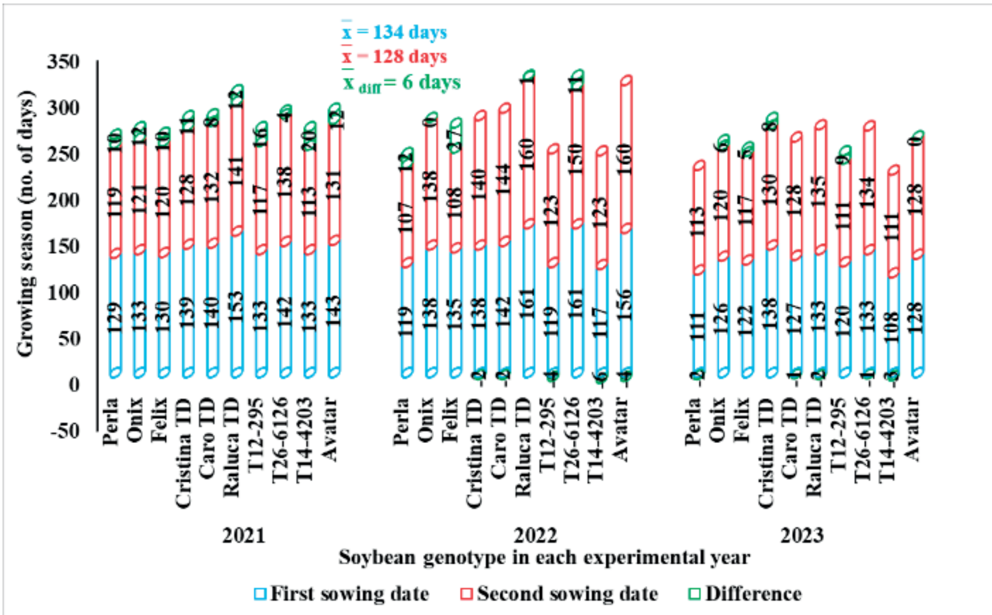

Figure 4. Number of days until the end of maturity (R8) for each soybean genotype and sowing date experimented (Turda, 2021-2023)

From the table with analysis of variance (ANOVA) it can be seen that grain yield and seed quality were greatly influenced by the three factors studied (Table 1).

In 2022, with very significantly negative differences compared to the average of years, the lowest values were obtained for: grain yield (1424 kg/ha), fat content (18.61%), oleic acid (23.35%) and linoleic acid (52.77%). In 2023, the highest grain yield of 3540 kg/ha was achieved. Also, in the same experimental year, the highest values of protein content were identified (38%), soybeans being also the oiliest (22.83%), similar results were obtained in a study by Popa et al. (2023). The research carried out by Şimon et al. (2023) shows the important contribution of the time of sowing the crop in the increase of soybean yield.

Regarding the sowing date factor, the practice of late sowing date resulted in a decrease of approximately 100 kg/ha in the grain yield. Also, the quality of the seeds was influenced by the sowing date. While there was an increase in protein content from 35.70% to 36.26%, these results are in line with those reported by Khan et al. (2001) which stated that the percentage of protein in soybeans sown later was higher than in soybeans sown earlier. The oil content decreased by 0.40% by practicing late sowing.

The highest seed yield was observed in semi early soybean genotypes (3540 kg/ha) followed by early soybean cultivars (2570 kg/ha) and very early soybean genotypes (2416 kg/ha). As stated by Suciu et al. (2020), yield and yield elements vary by maturity group and genotype. In terms of quality, the chemical composition of seeds varied between the three maturity groups studied. Generally, for late genotypes, a decrease in quality was observed compared to the early soybean cultivars.

The results obtained by Kane et al. (1997) showed that delayed seeding of soybeans led to an increase in the percentage of protein and linolenic acid, but also to a decrease in the percentage of fat and oleic acid, while the linoleic acid content was not affected.

Table 1. ANOVA test for yield, yield elements and main quality parameters in ten soybean genotypes sown at two different dates

FACTOR		Yield (kg ha-1)	Protein content (%)	Fat content (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
YEAR (Y)	2021	2821***	33.39^{000}	19.56^{000}	4.84**	23.55^{00}	55.17***	6.60^{ns}
	2022	1424^{000}	36.56***	18.61^{000}	5.00***	23.35^{000}	52.77^{000}	6.58^{ns}
	2023	3540***	38.00***	22.83***	4.42^{000}	24.70***	54.57*	6.04^{00}
	Average-Control	2595^{Ct}	35.98^{Ct}	20.33^{Ct}	4.75^{Ct}	23.87^{Ct}	54.17^{Ct}	6.41^{Ct}
SOWING DATE (S)	First sowing date (Control)	2639^{Ct}	35.70^{Ct}	20.52^{Ct}	4.88^{Ct}	23.75^{Ct}	54.35^{Ct}	6.23^{Ct}
	Late sowing date	2550^{00}	36.26***	20.15^{000}	4.62^{000}	23.99***	53.99^{00}	6.58***
SOYBEAN MATURITY GROUP (M)	Very early genotypes (000)	2416^{00}	36.94***	20.67^{ns}	4.88**	24.14^{ns}	53.82^{ns}	6.84***
	Early genotyeps (00)- Control	2507^{Ct}	35.89^{Ct}	20.50^{Ct}	4.83^{Ct}	23.99^{Ct}	53.94^{Ct}	6.07^{Ct}
	Semi-early genotypes (0)	3540***	35.11^{000}	19.83^{00}	4.54^{000}	23.47^{000}	54.75***	6.31*
ANOVA	Y			***	***	***	*** | *** | ***	***
			S				*** | *** | *** | *** | *** | **	***
			M				*** | *** | *** | *** | *** | ***	***

In other studies, different seed yield for late season and short season genotypes was obtained when delayed sowing was practiced (Chen et al., 2010; Salmerón et al., 2015; Vossenkemper et al., 2016).

Based on our experimental results, by analysing the interaction of sowing date and maturity group (Figure 5), it can be stated that the highest yield was obtained in the first sowing date. For late genotypes (MG 0), a decrease in yield, statistically assured as very significant (2755 kg/ha) was obtained when second date of sowing was analysed compared to the first sowing date (2968 kg/ha).

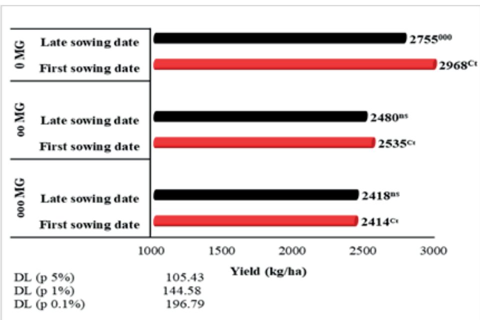

Figure 5. Interaction of sowing date and maturity group on soybean yield (Turda, 2021-2023)

Based on chemometric analysis obtained for yield and quality of ten soybean genotypes, sown at the recommended time, a high value for protein content in T12-295 perspective line and high yield in Avatar genotype are highlighted (Figure 6).

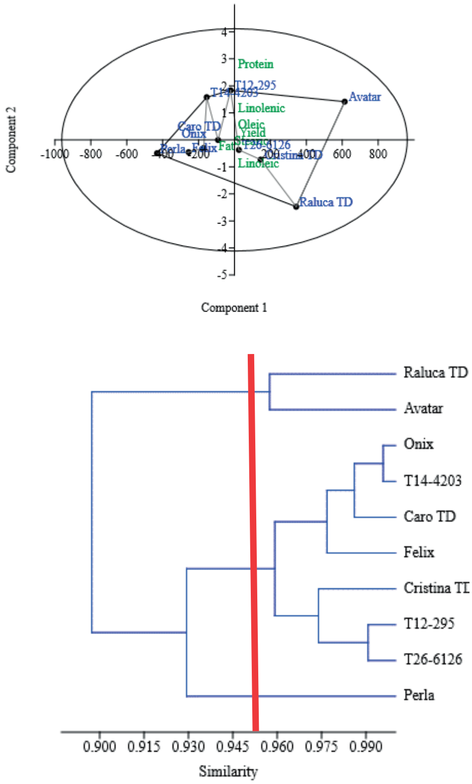

Figure 6. PCA and hierarchical clustering based on yield and quality in ten soybean genotypes obtained in the first sowing date

In terms of hierarchical clustering, while Perla variety presented an independent position related with maximum value obtained for fat content and minimum value for yield, 2 different clusters are observed. First cluster groups Avatar and Raluca TD varieties, with high yield and minimum values for almost all quality parameters. The second one consist in seven soybean genotypes with medium values obtained for studied traits.

When chemometric analyses was processed for second sowing date (Figure 7), high protein was related with T12-295 and T14-4203, while high yield was obtained in three genotypes: Avatar, Raluca TD and Caro TD.

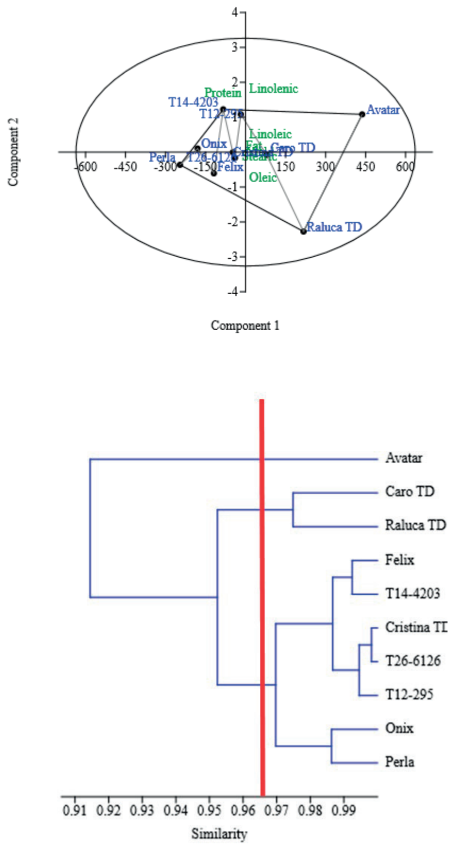

Figure 7. PCA and hierarchical clustering based on yield and quality in ten soybean genotypes obtained in second date

In this experimental variant, four different clusters are identified, while Avatar variety occupies an independent position based on obtained results. First cluster consist in Caro TD and Raluca TD varieties, with similar values obtained for yield, protein content, oleic and linoleic content. Second cluster groups Felix variety and T14-4203 line, with appropriate yield, protein content and oleic content. Cristina TD, T12-295 and T26-6126 form the third cluster with medium values for all studied parameters. With the smallest yield, highest fat content and stearic acid combined with good result for protein content, Onix and Perla varieties are grouped in the fourth cluster.

CONCLUSIONS

The period from emergence to the beginning of flowering stage (R1) was shortened by six days when delayed sowing date was experimented compared to the earliest sowing date.

The same trend was observed for the number of days from emergence to beginning of maturity (R7) and until the end of maturity (R8), respectively.

The most favourable for high soybean yield (2639 kg/ha) was the first sowing date.

In terms of quality, delayed sowing date contributed to an increase in protein content from 35.70% to 36.26%, while the oil content decreased by 0.40% when late sowing was practiced.

Fatty acid profile was different depending on sowing date, higher results for oleic and linolenic acid being obtained when late sowing was experimented.

Based on the experimental results, a decrease in yield was obtained when late genotypes were sown in the second date. For early soybean biological material, the results for production were similar in both sowing dates.

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MALT QUALITY PARAMETERS OF DIFFERENT BARLEY VARIETIES

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Abstract

The study presents the technological malt quality of the facultative six-row barley variety Smarald, winter six-row barley Ametist and Simbol varieties, and winter two-row barley Artemis and Wintmalt varieties. The Romanian genotypes were compared with one of the best varieties recommended for malt and beer in the Czech Republic, the winter two-row barley genotype Wintmalt. In the studied barley varieties, the protein content ranged between 9.3-12.5% and all the varieties (except the two-row Artemis variety) registered a starch content over the standard (60.0%). Regarding the extract, just one variety had a value of over 80% (six-row Smarald variety) with the highest malt extract, followed by Simbol and Wintmalt varieties (79.9% and 79.4% respectively). The values of diastatic power ranged from 189 ^{0}WK (Simbol variety) to 419 ^{0}WK (Wintmalt variety). The apparent final attenuation moved from 77.9 to 81.2 % in all varieties, the lowest value was registered by the Artemis barley variety. All the studied genotypes have presented an increased β-glucan content (479-1610 mg/l), except the Wintmalt variety which registered the lowest level of this quality index (216 mg/l).

***Key words**: barley, variety, quality indices, malt.*

INTRODUCTION

Winter barley varieties are valuable sources of characters usable within the breeding programs targeted at malting, feeding, or other specific qualities of new barley varieties (fodder).

Growth conditions of barley greatly affect the malting properties and many features are correlated with barley variety so determining the varietal influence on the malt quality is very important. Romania is one of the Brewers of Europe members and of the total beer consumed in the national market, 90% is produced by the members of the Romanian Brewers Association (www.berariiromaniei.ro).

During the malting process, several main criteria are used to describe the technological quality of the barley grain: the malt extract, Kolbach index, friability, viscosity, and diastatic power (Jamar et al., 2011). One of the main malt parameters is the extract, represented by the amount of soluble substances present in malt (Briggs, 1978; Ayoub, 2003).

A parameter important mainly in Central Europe (Psota and Kosař, 2002) is the relative extract at 45°C which indicates enzymatic degradation.

One of the most common parameters used for the malt evaluation, given by the ratio between the total content of nitrogenous substances in malt and the soluble nitrogen in wort is the Kolbach index (www.e-malt.com). The optimal value of this parameter ranges from 35% to 45% (a value >45% means excessive decomposition of the total protein content from malt and the total protein content from the wort, meanwhile the value >41% shows the best decomposition and values between 35%-41% shows a good response for the decomposition process. Value <35% highlights insufficient decomposition of the previously mentioned compounds according to www.homebrewersassociation.org.

Diastatic power represents the combination between starch and 4 types of enzymes namely α-amylase, β-amylase, limit dextrinase, and α-glucosidase (Fox and Bettenhausen, 2023).

The significance of this parameter is underlined by the conversion of starch to extract.
Friability represents the degree of endosperm hydrolysis (Briggs, 1987). β-glucan enzyme content in sweet wort emphasizes the level of cytolytic modification (Psota and Kosař, 2002). The high level of β-glucans content negatively affects the brewing quality due to the increase of wort viscosity and the beer filtration (Simic et al., 2008).
The main purpose of this paper is to characterize the technological quality of some winter barley varieties, to study the variation of the six-row varieties main traits compared with one of the recommended two-row winter barley varieties for brewing, and also to the relationship between the main grain quality and malt parameters.

MATERIALS AND METHODS

This study presents the experimental data obtained during one growing season by five winter barley varieties (4 registered in Romania during the 2012-2015 period and one recommended by the Czech Republic malting industry, namely Wintmalt variety released in Germany).
Seed samples (2015's harvest year) were obtained from the barley breeding experimental field (NARDI Fundulea) and shipped to the Research Institute of Barley Malting (Czech Republic).
Malting quality of the studied winter barley varieties (3 six-row and 2 two-row winter barley varieties) was assessed taking into consideration the analyses performed at NARDI Fundulea (yield, thousands grain weight, protein, and starch content) meanwhile the malt and wort analyses were carried out in the Czech Republic, at RIBM (Research Institute of Barley Malting), Malting Institute Brno.
The micro-malting analyses were performed according to the methodology presented by the European Brewery Convention (2010) and Mitteleuropäische Brautechnische Analysenkommission (2011).

1. Determination of technological quality

The yield level was determined for each barley variety by weighing after the seeds were cleaned into a thresher following to be counted for thousand-grain weight (TGW). The protein and starch content were measured using infrared technology (INFRATECH 1241). Seed fractions have been determined using Sortimat in three replications and then presented as mean values.

2. Malting and malt analysis

Samples of barley varieties (500 g) were malted in the automatic micro malting equipment of KVM (Uničov, Czech Republic).
The Research Institute for Barley Malting always uses the same regime of steeping, germination, and kilning for varietal testing.
Steeping was performed in the steeping box for 72 h, with wet stages and air rests alternating. The air temperatures were maintained at 14.0°C and the duration of wet stages and air rest are as follows: on the first day, the humid stage took 5 h and the air rest 19 h, on the second day, the humid stage took 4 h and was followed by 20 hours air rest.
By steeping or spraying on the third day, the water content of the germinating grains was adjusted to 45% and the germination was performed in the germination box. The total germination time was 72 h and the temperature during germination was maintained at 14.0°C.

Table 1a. Conditions and schedule of steeping and germination

Time (h)		Temperature of outgoing air (°C)
Steeping	*	14.0
Wet period	5.0	
Dry period	19.0	
Wet period	4.0	
Dry period	20.0	
Wet period	24 h	
Dry period		
Germination	72 h	14.0

*Water content was adjusted to 45% by steeping or spraying.

Table 1b. Conditions and schedule of kilning

Time	Temperature of ingoing air	Temperature of outgoing air	Fan speed	Air recirculation
h	°C	°C	%	%
1.0	14.0 / *to* 55.0	14.0 / *to* 25.0	70	0
11.0	55.0	25.0 / *to* 35.0		0
1.0	55.0 / *to* 60.0	40.0 / *to* 45.0		40
1.0	60.0 / *to* 65.0	45.0 / *to* 50.0		40
2.0	65.0 / *to* 70.0	50.0 / *to* 55.0		40
1.0	70.0 / *to* 75.0	55.0 / *to* 65.0		40
1.0	75.0 / *to* 80.0	65.0 / *to* 78.0		80
4.0	80	78		80

Kilning took place in a single-floor electrically heated kiln. The free-drying stage lasted 12 h at 55°C. During the forced drying stage, the

temperature was gradually increased for 6 h up to 75°C. The curing stage was carried out for 4 h at 80°C.
Conditions and procedure of malting are given in Tables 1a and 1b. For the micro malting test, only sieving fractions over 2.5 mm are used.
The following traits were determined in the unmalted barley grain and the malt and sweet wort produced: nitrogenous substances in the unmalted grain, extract in malt dry matter, relative extract at 45°C, Kolbach index, diastatic power, apparent final attenuation, friability, β-glucans in the sweet wort and turbidity of the sweet wort according to the methodologies presented in MEBAK (2011) and EBC Analysis committee (2010). Malt clarity determined visually was rated: 1-clear, 2-weakly opalizing, and 3-opalizing.
Malt quality and sweet wort produced from the tested varieties were assessed using the malt quality parameters (the malting quality index used in the Czech Republic and developed by Psota and Kosař, in 2002 at RIBM).
The obtained experimental results both from the field and laboratory were analyzed in EXCEL 2016 and interpreted according to the standard values established by the Analytica-European Brewery Commission Barley (EBC, 2010) and collection of Brewing Analysis Methods of the MEBAK (Mitteleuropäische Brautechnische Analysenkommission, 2011).

RESULTS AND DISCUSSIONS

The yield level (Figure 1) of the studied varieties ranged between 7.46 t/ha (Ametist six-row variety) and 9.19 t/ha Wintmalt two-row variety). Thousands grain weight (TGW, Figure 2) ranged between 49.9 g (Simbol variety) and 53.3 g (Artemis variety).
Barley grain samples of the tested varieties exhibited a content of nitrogenous substances (protein content exceeded in two cases, one case fulfilled the standard, and two cases under 9.5%) between 9.3% and 12.5% (Figure 3), according to a low quantity of nitrogen fertilizer applied (100 kg urea/ha commercial product with 46% a.s.) and rotation with pea (the best previous plant for barley rotation). All the varieties (except Artemis) registered a starch content (Figure 4) over the standard (60.0%).

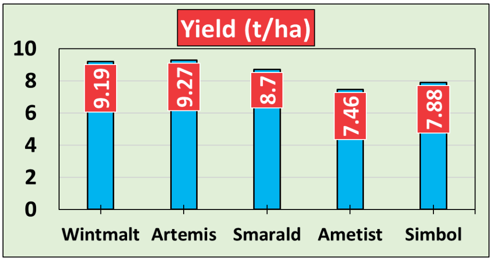

Figure 1. The average value of the yield

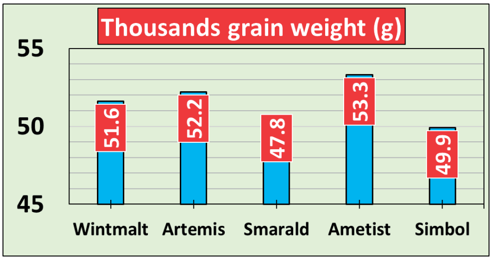

Figure 2. The average value of the TKW

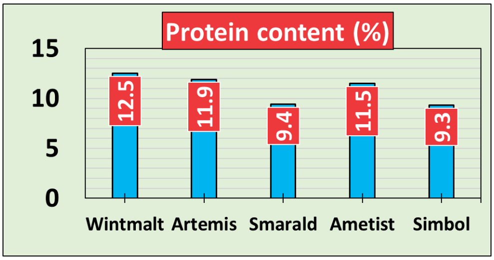

Figure 3. The average value of protein content

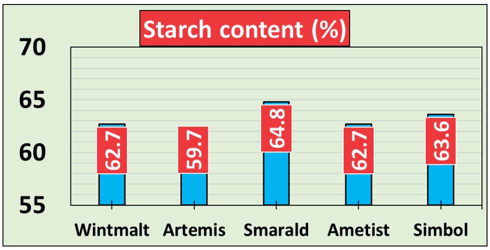

Figure 4. The average value of starch content

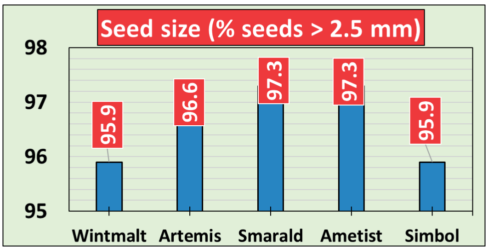

Figure 5. The average value of the seed size

Table 2. The methods used for malt analyses

No.	Methods	Units	References	Wintmalt	Artemis	Smarald	Ametist	Simbol
1	Bulk density	kg/hl	-	65.9	71.1	68.2	68.3	69.5
2	Moisture	%	-	11.7	11.7	11.5	11.2	11.6
3	Malt moisture	%	-	5.08	4.99	4.79	4.95	4.82
4	Saccharification rate	min	-	10	15	15	10	15
5	Extract	%	-	79.4	77.1	80.3	78.5	79.9
6	Relative extract at 45^{0}C	%	MEBAK 2011	30.3	30.2	31.6	33.8	31.2
7	Haze wort 15^{0}C	EBC	EBC 2010	6.35	9.96	10.97	4.91	21.59
8	Haze of wort 90^{0}C	EBC	EBC 2010	7.30	9.15	9.51	4.60	16.87
9	Wort Colour (Colorimeter)	-	-	3.2	3.1	4.2	3.1	4.3
10	Viscosity of wort	mPa.s	EBC 2010	1.493	2.061	1.661	1.826	1.716
11	Diastatic power	WK	EBC 2010	419	259	307	384	189
12	Friability	%	EBC 2010	72.4	51.1	72.2	51.4	70.1
13	Homogeneity by friabilimeter	%	EBC 2010	92.6	66.8	82.6	74.0	90.1
14	Partly unmodified grains	%	EBC 2010	7.4	33.2	7.4	26.0	9.9
15	Whole unmodified grain	%		0.6	4.6	0.2	0.4	0.1
16	β-glucans content	mg/l	EBC 2010	218	1610	479	993	606
17	Protein content of malt	%	EBC 2010	12.3	11.3	9.0	10.9	8.7
18	Total N of malt	%	EBC 2010	1.96	1.80	1.44	1.74	1.39
19	Soluble nitrogen in malt	%	EBC 2010	4.7	3.6	3.6	4.2	3.5
20	Kolbach index	%	EBC 2010	38.1	32.3	40.4	38.5	40.6
21	Apparent final attenuation	%	EBC 2010	79.9	77.9	81.2	80.0	80.1
22	Malting quality index	9-1	Psota et al., 2002	1.8	1.3	2.0	1.7	1.5

	Extract	Diastatic power	Homogeneity by friabilimeter	Beta-glucan content	Kolbach index	Malting quality index	Yield	Thousand grain weight	Protein content (%)	Starch content (%)	Seed size (> 2.5 mm diameter)
Extract	1										
Diastatic power	-0.02	1									
Homogeneity by friabilimeter	0.82	0.09	1								
Beta-glucan content	-0.89	-0.33	-0.93	1							
Kolbach index	0.94	0.01	0.73	-0.80	1						
Malting quality index	0.74	0.55	0.47	-0.76	0.68	1					
Yield (kg/ha)	-0.24	0.11	-0.03	0.04	-0.55	-0.06	1				
Thousand grain weight	-0.76	0.40	-0.44	0.49	-0.56	-0.50	-0.15	1			
Protein content (%)	-0.66	0.67	-0.26	0.25	-0.65	-0.23	0.41	0.79	1		
Starch content (%)	0.96	0.05	0.66	-0.80	0.99	0.81	-0.40	-0.70	-0.68	1	
Seed size (> 2.5 mm diameter)	-0.10	0.22	-0.60	0.30	0.01	0.37	-0.28	-0.05	-0.15	0.16	1

Figure 6. Correlation between studied quantity and quality parameters

The malt-analyzed parameters are presented in Table 2, including the methods used by the EBC Analysis Committee, 2010 and MEBAK, 2011. According to Pearson correlations (Figure 6), the yield was not correlated with other parameters. The thousands grain weight (TGW) was positively correlated with protein content and diastatic power.

Between protein and starch content, was shown a negative correlation (-0.68) and between seed size and grain friability (-0.60). The TGW was negatively correlated (-0.76) with the extract because a small seed shows a low extract. The highest correlations occurred between the extract and two of the malt parameters (friability and Kolbach index), which were 0.82 and 0.94, respectively. Also, it seems that the β-glucans content could be related to the TGW (0.49) and is negatively correlated with the Kolbach index and starch content (-0.80).

Wintmalt is a mid-early malting variety with good resistance to lodging (plants are medium to high), very high grain weight (over 51 g), and good assortment (sieving fractions above 2.5 mm was 95.9%). The variety is mid-resistant to powdery mildew and mid-resistant to net blotch. Under the 2015 climatic conditions, the yield was over 9 t/ha. Malt produced from the German variety Wintmalt had a low value of extract (around 79%), at 64.8% starch content and content of nitrogenous substances in non-malted grain at the level of 12.5%, with a low level of proteolytic modification (Kolback index 38.1%). The level of diastatic power was 419WK. Degradation of cell walls and β-glucans content in sweet wort were at the optimum level (218 mg/l) compared with the other varieties. This variety had the best homogeneity value by friabilimeter (92.6%). Apparent final attenuation moved around the value of 80%.

Evaluating the varieties for the malting quality index (from 9 highest to 1 lowest), the Wintmalt variety achieved 1.8.

Artemis is a mid-early malting variety with good resistance to lodging (plants are medium to high), very high grain weight (over 52 g), and good assortment (sieving fractions above 2.5 mm was 96.6%). The variety is mid-resistant to powdery mildew and mid-resistant to net blotch. Under the 2015 climatic conditions, the yield exceeded 9 t/ha. Malt of Romanian variety Artemis had the lowest level of extract (77.1%) at 11.9% seed nitrogen content, and 59.7% starch content with the lowest level of proteolytic modification (Kolback index 32.3%). The level of diastatic power was 259WK and b-glucan content in sweet wort was the highest level from all studied varieties (1392 g/ml over the Wintmalt variety). This content in β-glucans recommends another use of grain (food or forage). The homogeneity value by friabilimeter was under 70% and apparent final attenuation moved around the value of 78%.

The achieved values in the studied technological parameters, show the Artemis variety has a malting quality with the point evaluation 1.3.

Smarald is a mid-early malting variety with good resistance to lodging (plants are medium), good grain weight (over 47 g), and good assortment (sieving fractions above 2.5 mm was 97.3%). The variety is mid-resistant to powdery mildew and mid-resistant to net blotch. Under the 2015 climatic conditions, the yield was 8.7 t/ha. Malt of variety Smarald registered a slightly higher value of extract compared with two-row winter barley (80.3%), at 9.4% protein content, 64.8% starch content, and a value of 40.4% for Kolback index. The level of diastatic power was 307WK and β-glucans content was higher than the level of Wintmalt but the lowest from those three six-row winter barley studied (Table 2). The homogeneity value by friabilimeter was 82.6% and apparent final attenuation was around 81%. The achieved values in the studied technological parameters, show the Smarald variety has a malting quality with point evaluation 2.

Ametist is a mid-early malting variety with a good resistance to lodging (plants are medium to high), good grain weight (over 53 g) and good assortment (sieving fractions above 2.5 mm was 97.3%). The variety is mid-resistant to powdery mildew and mid-resistant to net blotch. Under the 2015 climatic conditions, the yield was 7.4 t/ha. Malt of variety Ametist gave an extract over 78%, at 11.5% protein content, 65.7% starch content, and a value of 38.5% for the Kolback index. The level of diastatic power was 384WK and b-glucan content was the highest level (993 g/l) compared with Smarald and Simbol. Also, this content in β-glucans recommends another use of grain (food or forage). The homogeneity value by friabilimeter was below 74% and apparent final attenuation moved around the value of 80%.

The achieved values in the studied technological parameters, show the Ametist variety has a malting quality with the point evaluation 1.7.

Simbol is a mid-early malting variety with good resistance to lodging (plants are high), good grain weight (over 49 g), and good assortment (sieving fractions above 2.5 mm were 95.9%). The variety is mid-resistant to powdery mildew and mid-resistant to net blotch. Under the 2015 climatic conditions, the yield was 7.8 t/ha.

Malt of variety Simbol gave a better value of extract (79.9%) at the content of nitrogenous substances in non-malted grain at the level of 9.3% protein and 63.6% starch content. The value of diastatic power was the lowest (189WK un) but the value of the Kolbach index was 40.6% (higher than two-row barley varieties). The level of β-glucans content was 606g/l, a mid-value between the Smarald and Ametist varieties. This genotype had a good homogeneity value by friabilimeter (90.1%) and a good value of apparent final attenuation (80.1%). The achieved values in the studied technological parameters, show the Simbol variety has a malting quality with the point evaluation 1.5.

CONCLUSIONS

The Smarald variety had the highest malt extract, followed by Simbol and Wintmalt varieties.

The values of diastatic power ranged from 189 WK (Simbol variety) to 419 WK (Wintmalt variety). All the studied genotypes have presented an increased β-glucans content (479-1610 mg/l), except the Wintmalt variety which registered the lowest level of these quality indices (218 mg/l).

The lowest b-glucan content was exhibited by the autochthonous barley variety Smarald.

New studies had to be initiated regarding the barley grain's β-glucans level content under different environments.

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EFFECTS OF DIFFERENT FOLIAR TREATMENTS AT MAIZE CROP

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Abstract

Foliar treatments are an important tool to enhance productivity of the maize plants and their ability to support stress factors, especially in the present when climate change has become the biggest global challenge to agriculture and food production. These treatments can be done with foliar fertilizers, biostimulator products or by using fungicide products with a stay-green effect on maize plants. Starting from these realities, the aim of this paper is to present the effect of different foliar treatments (foliar fertilisers, biostimulator products and the fungicide Retengo with the stay green effect) on maize under the specific growing conditions from South Romania. In this respect, field research was performed in the specific growing conditions of South Romania (44°12`55``N, 26°51`51``E) under rainfed conditions in the years 2022 and 2023. The experimental variants were represented by two foliar fertilizers (Microfert-U and Kingfol Zn), two biostimulator products (Terra Sorb and Atonik), and one fungicide (Rentengo) with a stay-green effect. The obtained results brought attention to the positive effects of fungicide Retengo as a fungicide with AgCelence effect to the maize grain yield. Among the tested biostimulators and foliar fertilizers, the highest grain yield were obtained when there was applied the biostimulator Atonik and the foliar fertilizer Kingfol Zn, Atonik being more effective in the less favourable climatic conditions and Kingfol Zn in the more favourable climatic conditions.

Key words: *maize, grain, yield, plant height, foliar fertilizer, biostimulator, stay green effect.*

INTRODUCTION

Maize is an important grain forage and food crop, this being of strategic importance for agriculture (Stoyanov & Kuneva, 2024). Due to its production potential, the highest among cereals (Ion et al., 2015), it is known worldwide as the queen of cereals (Tajamul et al., 2016). Together with wheat, maize represents the most important cereals in the world (Soare et al., 2018).

The great variety of genotypes and the high ecological plasticity in maize made it possible to cultivate maize in very varied conditions in terms of soil, climate, and relief conditions (Kusmec et al., 2018).

Maize responds favourably to various levels of agricultural technologies, both in subsistence conditions and in conditions of high-performance technologies (Gouse, 2012; Lana et al., 2018). Maize requires a specific crop technology, within which an important role is played by fertilization (Bășa et al., 2016; Ștefan et al., 2018). In fact, fertilization is a key component of any crop production system and it is one of the technological elements where improvement is always searched (Haraga & Ion, 2023).

Usually fertilization consists in applying nutrients to soil, this method being the most effective for nutrients required by plants in high amounts. But, foliar fertilization of crops can complement soil fertilization, this being an important crop management strategy in maximizing crop yields (Fageria et al., 2009). In this respect, most studies revealed a positive impact of foliar fertilization (Luţă et al., 2022).

In the present, in order to achieve high yields, cultivated plants cannot always get enough microelements from the soil, therefore the importance of foliar fertilization increased (Jakab et al., 2016). Nutrient uptake through leaf stomata is faster compared to root absorption, making it an efficient approach to promptly rectify plant nutrient deficiencies (Asare et al., 2023).

Foliar fertilization determines a great number of positive effects in the plant, principally at physiological level (respiration and photosynthesis), at morphological level, (root length and leaf area index), and upon the yield of various crops (Tejada & Gonzalez, 2003;

Tejada et al., 2016). Foliar application of fertilizers improves the absorption of nutrients that are immobile and difficult for plant root absorption, thus reducing the plant deficiency symptoms, and it can be used as a remedy for drought affecting maize at the vegetative stage to maximize yield (Asare et al., 2023).

Nowadays, more farmers use different bacterium preparations in nutrient supply of arable crops, which can improve the nutrient supply of plant (Jakab-Gábor et al., 2007). Also, foliar application of specific organic matters has demonstrated to be a powerful tool for stimulating the plant to a more intense but balanced vegetative development (Gheorghe et al., 2014). Biostimulants are increasingly being integrated into production systems with the goal of modifying physiological processes in plants to optimize productivity (Yakhin et al., 2017).

The aim of this paper is to present the effect of different foliar treatments (foliar fertilisers, biostimulator products and the fungicide Retengo with the stay green effect) on maize under the specific growing conditions from South Romania.

MATERIALS AND METHODS

Research was performed in field experiments located in South Romania, respectively at Agribest Mânăstirea Farm (44°12`55``N latitude, 26°51`51`` E longitude), in the area of Mânăstirea commune, Călărași county. The field experiments were performed under rainfed conditions in the years 2022 and 2023. The soil is a chernozem cambic with a humus content of 3.29% and a pH of 6.4.

The preceding crop was winter wheat in both experimental years.

Tillage consisted in a disc harrowing passage performed after harvesting the winter wheat, followed in October by ploughing at 25 cm depth. The seedbed preparation was done one day before sowing.

Fertilization was performed with 222.2 kg of DAP (18:46:0) complex fertilizer, which was applied at sowing to ensure a quantity of 40 kg/ha of nitrogen and 102.2 kg/ha of phosphorus (P_2O_5). In the growth stage of 6 leaves of maize plants, there was applied 200 kg/ha of Ammonium Nitrate (NH_4NO_3) with 33.5% nitrogen content, which means a nitrogen rate of 67 kg/ha. So, the total applied nitrogen rate was of 107 kg/ha.

Sowing was performed in first decade of April in both experimental years. The sowing density was of 70,000 germinal seeds/ha, the row spacing was 70 cm and the sowing depth was of 7 cm. The maize hybrid used into the experiments was KWS Kasmir from FAO group 370.

Weed control was performed by two herbicides. First herbicide was Adengo (Isoxaflutole 225 g/l + Thiencarbazone-methyl 90 g/l + Cyprosulfamide (safener) 150 g/l) applied immediately after sowing in a rate of 0.35 l/ha, and the second herbicide was Arigo (Nicosulfuron 12% + Rimsulfuron 3% + Mesotrione 36%) applied in a rate of 330 g/ha in the growing stage of maize plants of 6 leaves.

The field experiments were based on the method of subdivided plots into 3 replications. The experimental factor was the foliar treatment which the following graduations:

- Without foliar treatment – Control variant;
- Microfert-U;
- Kingfol-ZN;
- Terra Sorb Complex;
- Atonik;
- Retengo.

Microfert-U is a foliar fertilizer containing 90 g/l N, 30 g/l P_2O_5, 30 g/l K_2O, 12 g/l S, 0.5 g/l B, 0.3 g/l Mn, 0.5 g/l Zn, 0.25 g/l Cu, 0.15 g/l Mg, 0.2 g/l Fe. This product was applied in a rate of 5 l/ha in the growth stage of 6 leaves of the maize plants.

Kingfol Zn is a suspension liquid foliar fertilizer with a highly concentrated single element (70% Zn) designed to correct zinc deficiency. This product was applied in a rate of 0.5 l/ha in the growth stage of 6 leaves of the maize plants.

Terra-Sorb Complex is a biostimulator product based on L-α-amino acid from enzymatic hydrolysis, designed for foliar spray. The product has a high concentration of free amino acids (20%), a nitrogen content of 5.5%, magnesium content of 0.8% MgO, and a full and balanced proportion of micronutrients (1.5% B, 1% Fe, 0.1% Mn, 0.1% Zn, 0.001% Zn). This product was applied in a rate of 3 l/ha in the growth stage of 7 leaves of the maize plants.

Atonik is a plant growth regulator and biostimulator based on three nitrophenolates (3 g/l sodium p-nitrophenolate, 2 g/l sodium o-nitrophenolate, 1 g/l sodium 5-nitroguaiacolate), naturally occurring compounds in plant cells. This product was applied in a rate of 0.6 l/ha in the growth stage of 6 leaves of the maize plants.

Retengo® is a high-performance fungicide based on Pyraclostrobin (200 g/l), with AgCelence® effects, which improves the production and quality of maize crops. Due to the stay green effect, it reduces senescence and premature ripening of plants. This product was applied in a rate of 1 l/ha in the growth stage of 7 leaves of the maize plants.

Practically, the experiment included 2 foliar fertilizers, 2 biostimulator products and 1 fungicide with the effect of stay green.

Each experimental variant consisted in 12 lines of maize plants at 70 cm between rows, which means 8.4 m, with a length of 10 m along rows for each experimental variant.

In the present paper, there are presented the results regarding plant height and grain yield reported at moisture content of 14%.

The mean average temperature recorded for the period March-September in the year 2022 was of 17.8ºC, while for the same period in the year 2023, it was of 18.6ºC (Table 1).

The sum of rainfall recorded for the period March-September in the year 2022 was of 281.7 mm in the year 2022, while for the same period in the year 2023, it was of 238.4 mm (Table 1).

Comparing the two experimental years from a climatic point of view, one can conclude that the year 2023 was warmer and drier than the year 2022.

Table 1. Climatic conditions during maize plant's vegetative period at Mânăstirea, Călărași county, Romania

Month	**Temperature (ºC)**		**Rainfall (mm)**	
	2022	**2023**	**2022**	**2023**
March	3.7	8.3	15.8	7
April	11.9	10.8	68.8	25.6
May	17.7	16.2	31.9	97
June	22.3	21.6	69.2	52.8
July	25.1	25.9	17.3	14.2
August	25.1	25.7	13.5	30.7
September	18.6	21.4	65.2	11.1
Average/Sum	***17.8***	***18.6***	***281.7***	***238.4***

RESULTS AND DISCUSSIONS

The used foliar treatments, either foliar fertilizer or biostimulator or fungicide with the effect of stay green, they had strong impact on the vegetative growth of the maize plants. Regarding the maize plant height, comparing to control variant, all the treatments determined very significant differences in both experimental years except in 2023 for the product Microfert-U which determined a distinct significant difference compared to control (Table 2).

The highest height plant in 2022 were obtained in the case of the products Atonik and Kingfol Zn with 228 cm. In the year 2023, the highest height plant was registered in the case of the fungicide Retengo (222 cm), which is interesting because this product determined in the previous year (2022) the smallest plant height among the tested products. This means that in the conditions of high temperatures and low rainfall which characterised the year 2023 the fungicide Retengo with the stay green effect had the most important effect upon the vegetative growth of the maize plants, respectively on the plant height. In the better climatic conditions of 2022 compared to 2023, the most important effect upon the vegetative growth of the maize plants determined the biostimulator Atonik and the foliar fertilizer Kingfol Zn.

The foliar fertilizer Microfert-U determined the smallest value of the plant height in 2023 (210 cm) and one of the smallest value in 2022 (222.6 cm), this coming after the product Retengo.

As limits of variation, the maize plants had a height between 186 cm in the unfertilized version from the year 2023 and 228 cm in the case of Atonik biostimulator in the year 2022.

The less favourable climatic conditions of the year 2023 determined a smaller average plant height for the entire experiment (210.8 cm) compared to 2022 (218.6 cm).

Table 2. Maize plant height at different foliar treatments under different climatic conditions in South Romania

Foliar treatment	Plant height in 2022			Plant height in 2023		
	Plant height (cm)	Differences to control		Plant height (cm)	Differences to control	
		cm	%		cm	%
Untreated	187.0	Control	-	186.0	Control	-
Microfert-U	222.6	35.6 ***	19.0	210.0	24.0 **	12.9
Kingfol Zn	228.0	41.0 ***	21.9	214.7	28.7 ***	15.4
Terra Sorb Complex	224.3	37.3 ***	19.9	214.6	28.6 ***	15.4
Atonik	228.0	41.0 ***	21.9	217.6	31.6 ***	17.0
Retengo	222.0	35.0 ***	18.7	222.0	36.0 ***	19.4

$LSD_{5\%}$ = 14.61 cm
$LSD_{1\%}$ = 20.18 cm
$LSD_{0.1\%}$ = 28.95 cm

$LSD_{5\%}$ = 14.36 cm
$LSD_{5\%}$ = 20.13 cm
$LSD_{5\%}$ = 28.46 cm

If in the case of the maize plant height all the treatments determined differences statistically significant compared to control variant, in the case of maize grain yield in 2022 only the fungicide Retengo determined differences distinct significant compared to control variant (Table 3). In the year 2023, the fungicide Retengo determined differences very significant compared to control variant, and the biostimulator Atonik determined differences distinct significant and the biostimulator Terra Sorb Complex and the foliar fertilizer Kingfol Zn determined significant differences.
Compared to control variant, the fungicide Retengo determined the highest differences of 1441 kg/ha (14.6%) in the year 2022 and of 665 kg/ha (14.8%) in the year 2023. So, the highest grain yields were obtained in both experimental years in the condition of South Romania using Retengo as a fungicide with AgCelence effect, respectively 11,332 kg/ha in 2022 and 5,125 kg/ha in 2023.

Among the tested biostimulators and foliar fertilizers, as in the case of plant height, the highest grain yield were obtained when there was applied the biostimulator Atonik and the foliar fertilizer Kingfol Zn, Atonik being more effective in 2023 and Kingfol Zn in 2022.
In both experimental years, the smallest grain yield differences not statistically significant compared to control were obtained in the case of using the foliar fertilizer Microfert-U (459 kg/ha in 2022, respectively 158 kg in 2003).
The grain yields obtained in 2023 are much lower than those obtained in 2022, the year 2023 being warmer and drier not only than 2022 but also than the last decade in the experimented region. Thus, the average grain yield for the experiment was of 10,509 kg/ha in 2022 and of 4,785 kg/ha in 2023. The average grain yield obtained in 2023 represent less than half of those obtained in 2022, respectively 45.5%.

Table 3. Maize grain yields at different foliar treatments under different climatic conditions in South Romania

Foliar treatment	Grain yield in 2022			Grain yield in 2023		
	Grain yield (kg/ha)	Differences to control		Grain yield (kg/ha)	Differences to control	
		kg	%		kg	%
Untreated	9891	Control	-	4487	Control	-
Microfert-U	10350	459	4.6	4645	158	3.5
Kingfol Zn	10570	679	6.9	4798	311 *	6.9
Terra Sorb Complex	10400	509	5.1	4762	275 *	6.1
Atonik	10515	624	6.3	4866	379 **	8.4
Retengo	11332	1441 **	14.6	5152	665 ***	14.8

$LSD_{5\%}$ = 778.05 kg/ha
$LSD_{1\%}$ = 1090.84 kg/ha
$LSD_{0.1\%}$ = 1541.82 kg/ha

$LSD_{5\%}$ = 258.73 kg/ha
$LSD_{5\%}$ = 362.75 kg/ha
$LSD_{5\%}$ = 512.71 kg/ha

Despite the significant differences compared to control variant concerning the maize plant height obtained by applying the foliar treatments (very significant differences in both experimental years except in 2023 for the product Microfert-U which determined distinct significant differences), the differences obtained in the case of grain yield by applying foliar treatments were not so strong. The increased of plant height determined by applying different foliar products has correlated positively with the obtained grain yield, but this was a weak correlation (Figure 1).

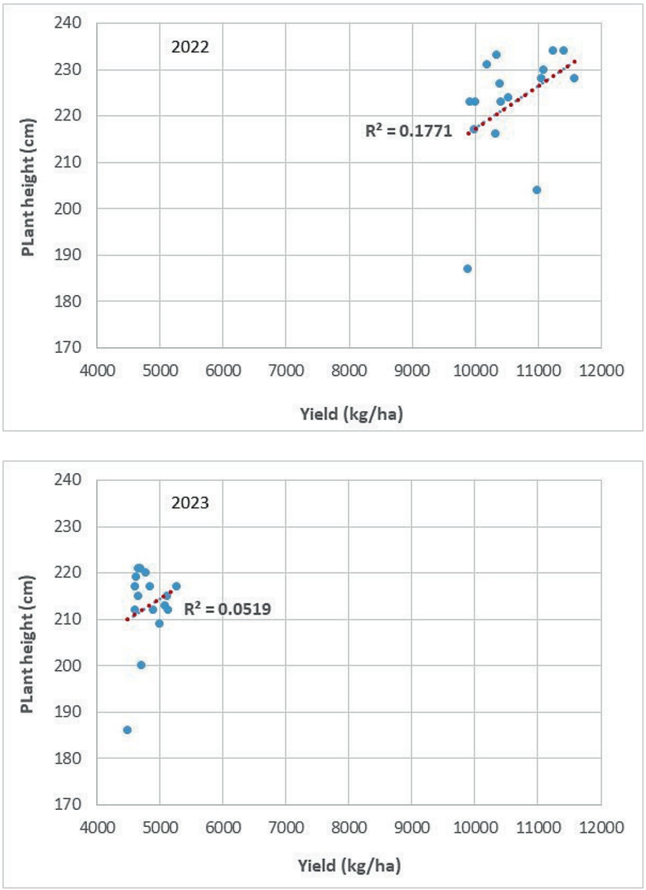

Figure 1. Correlation of the maize grain yield with the plant height

CONCLUSIONS

Following the research carried out under the specific conditions of South Romania, in the conditions of high temperatures and low rainfall the fungicide Retengo with the stay green effect had the most important effect upon the vegetative growth of the maize plants, respectively on the plant height. In better climatic conditions, the most important effect upon the vegetative growth of the maize plants determined the biostimulator Atonik and the foliar fertilizer Kingfol Zn.

The highest grain yields were obtained regardless of the climatic condition by using Retengo as a fungicide with AgCelence effect, respectively with the stay green effect. Among the tested biostimulators and foliar fertilizers, the highest grain yield were obtained when there was applied the biostimulator Atonik and the foliar fertilizer Kingfol Zn, Atonik being more effective in the less favourable climatic conditions and Kingfol Zn in the more favourable climatic conditions.

The increased of plant height determined by applying different foliar products has correlated positively with the obtained grain yield, but this was a weak correlation.

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EFFECTS OF DIFFERENT NITROGEN RATES AND FERTILIZERS ON MAIZE YIELD UNDER GROWING CONDITIONS OF SOUTH ROMANIA

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Abstract

The actual increasing concerns of our society about the adverse environmental impacts of nitrogen losses in the context of increasing cost of nitrogen fertilizers impose to the farmers the optimization of nitrogen fertilization according to specific growing conditions of their crops. Nowadays, the selection of the right fertilizer products, the appropriate rate and time of application are essential for farmers to make sound nitrogen management decisions. In this context, the aim of this paper is to present the obtained results regarding the effects of different nitrogen rates and nitrogen fertilizer products on maize grain yield under the specific growing conditions of South Romania. The research was carried out in field experiments located in South Romania (44º22' N latitude and 26º89' E longitude), under rainfed conditions in the years 2022 and 2023. The experimental factors were the following: Factor A – Nitrogen Rate, with 4 graduations, respectively 36 kg/ha, 80 kg/ha, 120 kg/ha, 160 kg/ha; Factor B – Nitrogen Fertilizer, with 4 graduations, respectively Ammonium Nitrate, Urea, Urea with NutriSphere-N, Urea Ammonium Nitrate (UAN). The obtained results brought attention to the positive effects of Urea with NutriSphere-N to the maize grain yield, as well as to the dependence of the nitrogen rate on the climatic conditions of the year. Under drought conditions, the nitrogen rate should not be high, as this could be even a limiting yielding factor.

Key words*: maize, nitrogen, rate, fertilizer, grain yield.*

INTRODUCTION

Maize (*Zea mays* L.) has a special importance at global level, this being given by its food and fodder values, by the several uses as raw material in various industrial sectors, as well as by the agronomic characteristics of the crop, such as: high ecological plasticity, high yielding potential, very good capacity to capitalize on fertilizers and irrigation water, full mechanization of the crop technology, high multiplication coefficient, small amounts of seed required for sowing, and it does not impose special restrictions in the crop rotation. Also, grain maize is an important cash crop for farms without livestock (Finke et al., 1999). Maize's ecological flexibility makes it "the plant of choice" for grain and feed in climates raging from temperate to tropical as long as there is no frost and mean temperatures are mostly above 10 degrees Celsius (Haraga & Ion, 2022). Therefore, maize is cultivated in many regions of the world (Erenstein et al., 2013).

As harvested area, maize ranks the second place in the world, after wheat, and the first place in Romania, which has the largest cultivated area with grain maize in the European Union.

Fertilizers are essential for providing the necessary nutrients to the soil and promoting plant growth, their efficient use being important to ensure that plants get the right amount of nutrients they need to produce a high yield (Zaib et al., 2023). Improving the nutritional status of plants through fertilizer application and maintaining soil fertility has been the critical step in food production since the beginning of the "Green Revolution" in both developed and developing countries (Huang et al., 2021).

Evaluation of long term field studies has shown that fertilizer input is critical to crop production, the average percentage of yield attributable to fertilizer generally ranging from about 40 to 60% in temperate climates and tends to be much higher in the tropics (Stewart & Roberts, 2012). Practically, in the modern agriculture, the importance of using chemical fertilizers is undeniable (Leonte et al., 2023).

Mineral nutrients are essential for increasing maize yields, maintaining soil productivity, and preventing soil degradation. Among nutrients, nitrogen (N) is a major one, this being needed in large amount to increase growth and yield of maize (EL-Guibali et al., 2015).

Maize is known to be a heavy feeder of nitrogen fertilizer (Muhamman et al., 2014). Nitrogen fertilizer is universally accepted as a key component to high maize grain yield and optimum economic return (Gehl et al., 2005).

Nitrogen is a very critical nutrient for maize production, but in the same time it is also the most difficult to manage. Optimization of nitrogen fertilization in maize crop cannot be done on a global scale, but must be done for specific soil and climatic conditions, in accordance with other agrotechnical activities (Matev & Minev, 2020).

In many situations, profitable maize production requires supplemental nitrogen, but sometimes the narrow profit margins impose to closely manage the costs. Excess nitrogen application, more than maize plants can use results in nitrogen loss to the environment, generating pollution problems and loss of money. Therefore, the selection of the right fertilizer products, the appropriate rate and time of application are essential for farmers to make sound nitrogen management decisions.

One of the important factors, which influence the yields of agricultural crops, is the weather condition (Pirttioja et al., 2015). It should be noted that not only crop yields (Ray et al., 2015), but also the efficiency of the use of resources, in particular nutrients from soil and fertilizers (Ryan et al., 2012) are influenced by the weather conditions. Several studies have evaluated the behavior of maize genotypes in various climatic conditions (Ramirez-Cabral et al., 2017) all over the world, and the results shows that crop yields were greatly influenced by the weather conditions of the year.

Drought is one of the main constraints in maize cultivation in South Romania, which is the most important Romanian growing area for maize (Ion et al., 2023). In the context of evident climate changes in the maize growing areas from South Romania, the farmers need to adapt and find the best solutions regarding the nitrogen fertilization. In this context, the aim of this paper is to present the obtained results regarding the effects of different nitrogen rates and nitrogen fertilizer products on maize grain yield under the specific growing conditions of South Romania.

MATERIALS AND METHODS

The research was carried out in field experiments located in South Romania, respectively at Agribest Mânăstirea Farm (44º22' N latitude and 26º89' E longitude) in the area of Mânăstirea commune, Călărași county. The field experiments were performed under rainfed conditions in the years 2022 and 2023.

In the studied area, the specific soil is chernozem cambic with a humus content of 3.29% and a pH varying from 6.4.

For the period March-September 2022, the average temperature was 17.8ºC, respectively 18.6ºC for 2023. For the same period (March - September), the sum of rainfall was 281.7 mm in 2022 and 238.4 mm in 2023 (Table 1). In both years, the months March, July and August were dry months. The highest rainfall was registered in April, June and September in 2022 and in May and June in 2023. As a conclusion, the year 2023 can be characterized as being warmer and drier than the year 2022.

The studied biological material was the maize hybrid KWS Kashmir from FAO group 370, which is a simple hybrid designed for intensive production with a fast release of moisture in the final stage of grain ripening. The kerner is quite large, having a high TGW (Thousand Grain Weight) value, which is one of the key elements of a high yield.

The preceding crop was winter wheat in both experimental years. Also, the crop technology was similar in both years. After harvesting the preceding crop, there was performed a harrowing work, and in Autumn (October) there was performed the ploughing at 25 cm depth. The preparation of the germination bed was made with a combinatory one day before sowing. The sowing was done in the first decade of April, with a sowing density of 70,000 germinal seeds/ha, at a depth of 7 cm and at 70 cm row spacing. The control of the weeds was performed by the application immediately after sowing of the herbicide Adengo (Isoxaflutole 225 g/l + Thiencarbazone-methyl 90 g/l + Cyprosulfamide (safener) 150 g/l), in a rate of 0.35 l/ha.

The field experiments were based on the method of subdivided plots into 3 replications, with the following factors:

- Factor A – Nitrogen Rate, with 4 graduations:
  - a1 - 36 kg/ha;
  - a2 - 80 kg/ha;
  - a3 - 120 kg/ha;
  - a4 - 160 kg/ha.
- Factor B – Nitrogen Fertilizer, with 4 graduations:
  - b1 - Ammonium Nitrate;
  - b2 - Urea;
  - b3 - Urea with NutriSphere-N;
  - b4 - Urea Ammonium Nitrate (UAN).

Ammonium Nitrate (NH_4NO_3) is a nitrogen fertilizer containing 50% NH_4^+ (the ammonium ion) and 50% NO_3^- (the nitrate ion), with a total nitrogen content of 33.5%.

Urea [$CO(NH_2)_2$] is an organic nitrogen fertilizer containing 46% nitrogen.

Urea with NutriSphere-N is a urea fertilizer coated with Maleic Itaconic Copolymer (Nutrisphere-N) designed to enhanced efficiency fertilizer product through reducing of urea volatilization, nitrification, and denitrification processes.

Urea Ammonium Nitrate (UAN) is a liquid fertilizer that contains a mixture of urea and ammonium nitrate, with a nitrogen content of 32%.

At sowing, there was used Diammonium Phosphate (DAP) 18:46:0 in a rate of 200 kg/ha of commercial product, which assured 36 kg/ha of nitrogen and 92 kg/ha of phosphorus. The difference of nitrogen for each rate and fertilizer product was applied before seedbed preparation. The experimental variant with 36 kg/ha of nitrogen, which was chosen as control variant, received only the nitrogen coming from the DAP fertilizer used on all the surface.

Each experimental variant consisted of 126 m^2 resulting from 18 plant rows at 70 cm row spacing, which means 12.6 m, and 10 m of row length.

The grain yield was calculated in kg/ha and it was reported at 14% moisture content.

Table 1. Climatic conditions during maize plant's vegetative period at Mânăstirea, Călărași county, Romania

Month	**Temperature (ºC)**		**Rainfall (mm)**	
	2022	**2023**	**2022**	**2023**
March	3.7	8.3	15.8	7
April	11.9	10.8	68.8	25.6
May	17.7	16.2	31.9	97
June	22.3	21.6	69.2	52.8
July	25.1	25.9	17.3	14.2
August	25.1	25.7	13.5	30.7
September	18.6	21.4	65.2	11.1
Average/Sum	***17.8***	***18.6***	***281.7***	***238.4***

RESULTS AND DISCUSSIONS

The grain yields obtained at maize under specific conditions of South Romania with a nitrogen rate of 36 kg/ha in the control variant are quite high despite the small amount of received nitrogen, respectively 7,616 kg/ha in 2022 and 4,496 in 2023 (Table 2). This is due to the soil good fertility, the most important limiting factor being the climatic conditions, firstly the rainfall and then the high temperatures especially in the flowering period. Thus, the yield obtained in 2023 is much lower than that obtained in 2022, the year 2023 being warmer and drier than the year 2022.

Fertilization with nitrogen no matter the rate, fertilizer product and climatic conditions of the year determined obtaining of differences assured statistically, except the application of ammonium nitrate at highest rate (160 kg/ha of nitrogen) in the year 2023 (Table 2).

The highest grain yields were obtained in both experimental years in the condition of using Urea with NutriSphere-N as fertilizer product at the highest rate, respectively at 160 kg/ha of nitrogen, respectively 11,231 kg/ha in 2022 and 5,305 kg/ha in 2023.

By applying nitrogen fertilizers at different rates, the smallest yield increase compared to the yield obtained in the control variant

(36 kg/ha on nitrogen) was obtained in the condition of using Urea at the rate of 80 kg/ha of nitrogen in 2022 (1508 kg/ha yield increase), and in the condition of using Ammonium Nitrate at the rate of 160 kg/ha of nitrogen in 2023 (276 kg/ha yield increase).

In 2022, at the nitrogen rate of 80 kg/ha, the highest grain yield was obtained in the conditions of using UAN, which being liquid is fast used by the maize plants, while at the rates of 120 and 160 kg/ha the highest grain yields were obtained in the conditions of using Urea with NutriSphere-N. In the same year, the smallest grain yield was obtained in the conditions of using Urea at the nitrogen rate of 80 kg/ha, and in the conditions of using Ammonium Nitrate at the rates of 120 and 160 kg/ha.

In 2023, respectively in the conditions of higher temperatures and lower rainfall than in 2022, the highest grain yields were obtained in the conditions of using Urea with NutriSphere-N at the rates of 80 and 160 kg/ha of nitrogen, and in the condition of using Urea at the nitrogen rate of 120 kg/ha. In the same year, the smallest grain yield was obtained in the conditions of using Ammonium Nitrate at the nitrogen rates of 80 and 160 kg/ha, and in the conditions of using UAN at the rate of 120 kg/ha.

As average values obtained at different nitrogen rates, in both experimental years the differences obtained by applying nitrogen compered to control variant were assured statistically (Table 3). But, if in 2022 the difference was just positive distinct significant at the nitrogen rate of 80 kg/ha and positive very significant at the nitrogen rates of 120 and 160 kg/ha, in 2023 the difference was positive very significant at the nitrogen rates of 80 and 120 kg/ha, and just positive distinct significant at the nitrogen rate of 160 kg/ha.

The highest average grain yield was obtained at the nitrogen rate of 160 kg/ha in 2022 and at the nitrogen rate of 120 kg/ha in 2023 (Table 3). In has to be highlighted that in the warm and dry year 2023, the average grain yield obtained at the nitrogen rate of 160 kg/ha is smaller than that one obtained at the nitrogen rate of 80 kg/ha. Moreover, even the highest average grain yield was obtained at the nitrogen rate of 120 kg/ha, the yield difference between that one and that obtained at the nitrogen rate of 80 kg/ha is very small, respectively of 59 kg/ha.

As average values obtained at different nitrogen fertilizers, in both experimental years the differences obtained compared to control Ammonium Nitrate fertilizer are not statistically significant (Table 4). The highest average grain yields in both experimental years were registered in the case of fertilizer Urea with NutriSphere-N. On the second place comes the fertilizer UAN, on the third place the fertilizer Urea, and on the last place is Ammonium Nitrate.

Table 2. Maize grain yields at different nitrogen rates and nitrogen fertilizers under different climatic conditions in South Romania

Experimental factors		Yields obtained in 2022			Yields obtained in 2023		
Nitrogen Rate (kg/ha)	Nitrogen Fertilizer	Yield (kg/ha)	Differences to control		Yield (kg/ha)	Differences to control	
			kg/ha	%		kg/ha	%
36	-	7616	Control	-	4496	Control	-
80	Ammonium Nitrate	9487	1871 *	24.6	4935	439 **	9.8
	Urea	9124	1508 *	19.8	5047	551 ***	12.3
	Urea + NutriSphere-N	9598	1982 **	26.0	5245	749 ***	16.7
	UAN	10118	2502 **	32.9	5210	714 ***	15.9
120	Ammonium Nitrate	9671	2055 **	27.0	5209	713 ***	15.9
	Urea	9757	2141 **	28.1	5280	784 ***	17.4
	Urea + NutriSphere-N	11045	3429 ***	45.0	5139	643 ***	14.3
	UAN	10645	3029 ***	39.8	5044	548 ***	12.2
160	Ammonium Nitrate	10022	2406 **	31.6	4772	276	6.1
	Urea	11132	3516 ***	46.2	4946	450 **	10.0
	Urea + NutriSphere-N	11231	3615 ***	47.5	5305	809 ***	18.0
	UAN	10211	2595 ***	34.1	5142	646 ***	14.4

$LSD_{5\%}$ = 1412.01 kg/ha
$LSD_{1\%}$ = 1901.59 kg/ha
$LSD_{0.1\%}$ = 2521.16 kg/ha

$LSD_{5\%}$ = 281.88 kg/ha
$LSD_{5\%}$ = 379.62 kg/ha
$LSD_{5\%}$ = 503.31 kg/ha

Table 3. Maize grain yields as average values at different nitrogen rates under different climatic conditions in South Romania

Experimental factor	Yields obtained in 2022			Yields obtained in 2023		
Nitrogen Rate (kg/ha)	Yield (kg/ha)	Differences to control		Yield (kg/ha)	Differences to control	
		kg/ha	%		kg/ha	%
36	7616	Control	-	4496	Control	-
80	9582	1966 **	25.8	5109	613 ***	13.6
120	10279	2663 ***	35.0	5168	672 ***	14.9
160	10649	3033 ***	39.8	5041	545 **	12.1

$LSD_{5\%}$ = 1412.01 kg/ha
$LSD_{1\%}$ = 1901.59 kg/ha
$LSD_{0.1\%}$ = 2521.16 kg/ha

$LSD_{5\%}$ = 321.25 kg/ha
$LSD_{5\%}$ = 429.08 kg/ha
$LSD_{5\%}$ = 561.47 kg/ha

Table 4. Maize grain yields as average values at different nitrogen fertilizers under different climatic conditions in South Romania

Experimental factor	Yields obtained in 2022			Yields obtained in 2023		
Nitrogen Fertilizer	Yield (kg/ha)	Differences to control		Yield (kg/ha)	Differences to control	
		kg/ha	%		kg/ha	%
Ammonium Nitrate	9727	Control	-	4972	Control	-
Urea	10026	299	3.1	5091	119	2.4
Urea + NutriSphere-N	10625	898	9.2	5230	258	5.2
UAN	10325	598	6.1	5132	160	3.2

$LSD_{5\%}$ = 1735.78 kg/ha
$LSD_{1\%}$ = 2337.60 kg/ha
$LSD_{0.1\%}$ = 3099.24 kg/ha

$LSD_{5\%}$ = 325.85 kg/ha
$LSD_{5\%}$ = 438.83 kg/ha
$LSD_{5\%}$ = 581.81 kg/ha

CONCLUSIONS

Following the research carried out on the chernozem cambic soil from South Romania, the tested fertilizer product with the best results in maize grain yield was Urea with NutriSphere-N, this being followed by fertilizers UAN and Urea, the last place being taken by Ammonium Nitrate. However, taking the Ammonium Nitrate fertilizer as control, the differences obtained by the other fertilizer products are not statistically significant.

The effect of the nitrogen rate on the maize grain yield is very depended by climatic conditions of the year. Thus, in the more climatic favorable year 2022, the highest yield was registered at the nitrogen rate of 160 kg/ha, while in the warm and dry year 2023, the highest yield was registered at the nitrogen rate of 120 kg/ha, but with a small difference compared to the nitrogen rate 80 kg/ha. So, under drought conditions, the nitrogen rate should not be high, as this could be even a limiting yielding factor.

In general, fertilization with nitrogen no matter the rate, fertilizer product and climatic conditions of the year determined obtaining of yield differences assured statistically.

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INFLUENCE OF SOWING DATES ON SEED YIELD AND HARVEST MOISTURE OF MAIZE HYBRID PARENTAL LINES

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Abstract

This study presents the findings of research aimed at determining the seed yield and harvesting moisture of inbred lines, parental components of maize hybrids of various FAO groups, depending on sowing dates in the agroecological conditions of the Forest-Steppe region of Ukraine. The research is based on the evaluation of different FAO group lines-parental components of Ukrainian breeding: early-maturing - OR26A (FAO 240), AV20B (FAO 260), OR28A (FAO 260); mid-maturing - OR32A (FAO 320), AV30B (FAO 320). The seed moisture content before harvest for the lines-parental components of hybrids varied within the FAO group and sowing dates. Yield calculation demonstrated that the realization of seed yield potential for each inbred line depends on the genotype and sowing dates. Improving the elements of variety agrotechnics for lines - parental components of maize hybrids of different FAO groups provides an opportunity to increase seed productivity of lines for timely seed provision of hybridization areas and the accelerated introduction of innovative hybrids into production.

Key words: *maize, lines - parental components, sowing time, collecting seed moisture, seed yield.*

INTRODUCTION

Due to global climate changes, characterized by rising air temperatures, decreasing relative humidity, fluctuating and irregular precipitation, and an increase in the deficit of quality irrigation water, there is an urgent need to develop new innovative lines, parental components, and hybrids of maize. Additionally, the development of agrotechnological elements for their cultivation is essential. To cultivate maize without irrigation, it is crucial to maximize the use of soil moisture reserves during the autumn-winter period. Hence, an early start to sowing becomes necessary, requiring the creation of maize lines, parental components, and hybrids with enhanced cold resistance for early planting.

On the other hand, organic grain production without herbicide use favors late maize sowing, enabling weed control through mechanical means. Late planting allows for additional cultivation, contributing to better weed control (Гадзало et al., 2020).

Thus, the development of technology for early, optimal, and late planting of maize lines, parental components, and hybrids in the conditions of the Central Steppe of Ukraine is pertinent.

An essential aspect of maize cultivation technology is the application of different sowing dates, impacting plant growth, development, productivity, and the immunological state of crops. Ongoing discussions regarding the sowing dates of maize lines, parental components, recommend both earlier and later sowing compared to optimal timing. Researchers highlight the close connection between sowing dates and weather conditions during seed germination and early plant development. Early sowing may be more effective than later sowing, but sowing in cold and unheated soil, followed by a return of cold weather, may be detrimental (Cantarero et al., 2000; Otegui et al., 1995; Shumway et al., 1992).

Considering the individual plant reactions, breeders create mid-early and early-maturing lines, parental components of maize hybrids belonging to the flint group, known for their increased cold resistance. This enables early planting since cold-resistant maize hybrids can germinate at a soil temperature of +6°C (Dang et al., 2016; Кирпа & Стюрко, 2014).

Delaying maize planting results in flowering occurring long after the summer solstice, and grain filling occurs closer to the end of the harvest season. As the sowing date is critical for grain setting and grain filling, any delay may negatively affect seed yield. It leads to a reduction in the number, size, and activity of germinating seeds, as well as a decrease in assimilation production through photosynthesis during the grain-filling period (Cirilo & Andrade, 1996; Tsimba et al., 2013).

Determining optimal planting dates is crucial for identifying critical processes in crop breeding modeling and developing strategies for creating new genetic material. Early-maturing lines often fail to fully utilize available solar radiation during favorable growth temperatures, thereby not realizing their full yield potential (Porter et al., 1998). Conversely, late-maturing lines may not ripen before the onset of the first frost. Late planting of early-maturing hybrids can sometimes equal or surpass late-maturing lines. Early-maturing lines may also be more profitable, as later hybrids may require additional artificial drying for safe storage (Saggenborg et al., 1999).

Analyzing literature review data leads to the conclusion that planting dates are a significant agronomic factor influencing seed yield in maize lines. There is no unanimous agreement among scientists regarding the optimal temperature to start planting maize. Therefore, studying the impact of planting dates on yield and harvesting moisture for new lines – parental components in Ukrainian breeding under the conditions of the Central Steppe of Ukraine is relevant.

The objective of this article is to determine the influence of planting dates on the yield and harvesting moisture of seed lines – parental components of maize hybrids from different FAO groups.

MATERIALS AND METHODS

Field experiments were conducted from 2019 to 2021 in the agricultural production cooperative "PEREMOHA" (Klepachi village, Khorol district, Poltava region) in the agro-ecological zone of the Central Forest-Steppe. The climate in the Central Forest-Steppe is moderately continental, with relatively mild, low-snow winters and warm, moderately humid summers. The soil of the research plot is typical chernozem. The cultivation practices for maize varieties in the experiments were generally accepted for the Forest-Steppe zone of Ukraine. The predecessor crop was soybean. The research was conducted following the field experiment methodology, and statistical analysis was carried out using variance analysis (Ушкаренко et al., 2009; Ушкаренко et al., 2014).

A two-factor study was set up using the method of randomized split blocks, with four repetitions. The planting area of the plots was 50.0 m², and the accounting area was 30.0 m². Factor A – planting date, with the following dates: 15.04, 25.04, 05.05, 15.05. Factor B – different FAO group lines, parental components of Ukrainian breeding: mid-early: OR–26A (FAO 240), AB–20B (FAO 260), OR–28A (FAO 260); mid-maturing: OR–32A (FAO 320), AB–30B (FAO 320).

RESULTS AND DISCUSSIONS

The conducted experimental studies revealed a significant impact of planting dates on the development of plants and the yield formation of seed lines – parental components of maize hybrids from different FAO groups. Depending on the experimental factors, the crops are subjected to varying agrometeorological conditions, resulting in diverse growth patterns and development, ultimately leading to variable productivity.

During the investigations from 2019 to 2021, the indicator "seed yield" for hybrids from different FAO groups varied depending on planting dates, ranging from 2.43 to 4.44 tons per hectare (Figure 1).

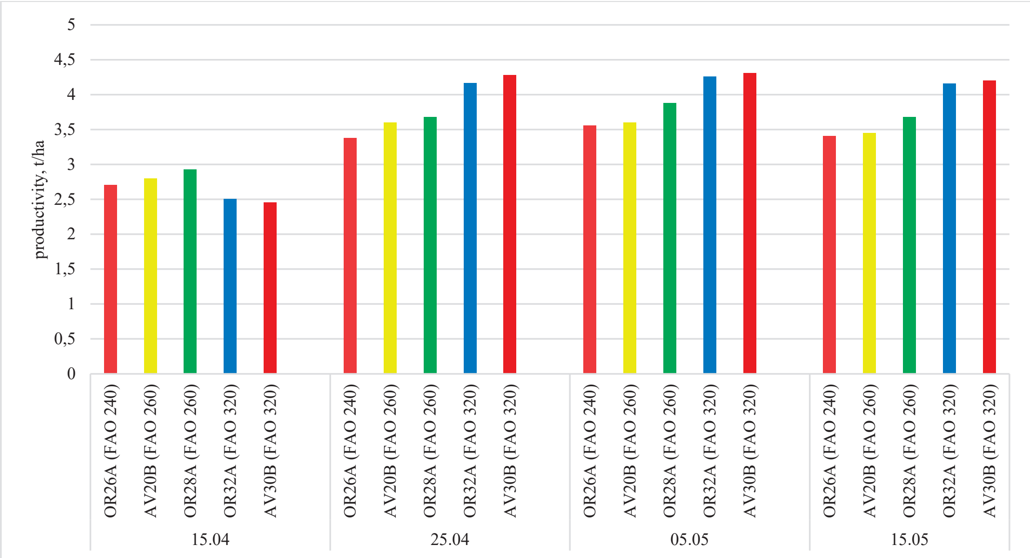

Figure 1. Seed Yield of Parental Lines of Maize Hybrids at 14% Moisture Content, Depending on Planting Dates, t/ha

According to the results of the conducted research, it has been determined that the parental lines of maize hybrids from different FAO groups demonstrated maximum yield for later planting dates.

Mid-early line OR–26A (FAO 240) showed maximum seed yield in 2019 and 2021 for planting on 05.05, with 3.61 and 3.65 t/ha, respectively. In 2020, for planting on 15.05, it recorded a seed yield of 3.59 t/ha. The minimum yield of 2.66 t/ha was observed for planting on 15.04, resulting in a yield reduction of 0.99 t/ha, or 26.4%.

Mid-early line AB–20B (FAO 260) demonstrated maximum seed yield in 2019 and 2021 for planting on 05.05, with 3.71 and 3.72 t/ha, respectively. In 2020, for planting on 15.05, it recorded a seed yield of 3.70 t/ha. The minimum yield of 2.77 t/ha was observed for planting on 15.04, resulting in a yield reduction of 0.95 t/ha, or 25.5%.

Mid-early line OR–28A (FAO 260) exhibited maximum seed yield in 2019 and 2021 for planting on 05.05, with 3.96 and 4.11 t/ha, respectively. In 2020, for planting on 15.05, it recorded a seed yield of 3.87 t/ha. The minimum yield of 2.89 t/ha was observed for planting on 15.04, resulting in a yield reduction of 1.22 t/ha, or 29.6%.

Mid-early line OR–32A (FAO 320) showed maximum seed yield in 2019 and 2021 for planting on 05.05, with 4.37 and 4.35 t/ha, respectively. In 2020, for planting on 15.05, it recorded a seed yield of 4.40 t/ha. The minimum yield of 2.47 t/ha was observed for planting on 15.04, resulting in a yield reduction of 1.93 t/ha, or 43.9%.

Mid-early line AB–30B (FAO 320) demonstrated maximum seed yield in 2019 and 2021 for planting on 05.05, with 4.40 and 4.42 t/ha, respectively. In 2020, for planting on 15.05, it recorded a seed yield of 4.44 t/ha. The minimum yield of 2.43 t/ha was observed for planting on 15.04, resulting in a yield reduction of 2.01 t/ha, or 45.3%.

In recent years, there has been an increased demand for simple maize hybrids in the Ukrainian market. Production practices indicate that these hybrids are characterized by high yields, technological efficiency, disease resistance, and uniformity in key morphological indicators. However, it is challenging to combine a complex of valuable economic traits with a high degree of stability in performance.

The low harvest moisture content of maize seeds is primarily determined by the duration of the vegetation period, with early maturity being the dominant factor.

Figure 2 presents data on seed moisture content before harvest. During the 2019–2021 research period, this indicator for the seeds of parent lines – components of hybrids from different FAO groups – varied within the FAO groups and planting dates.

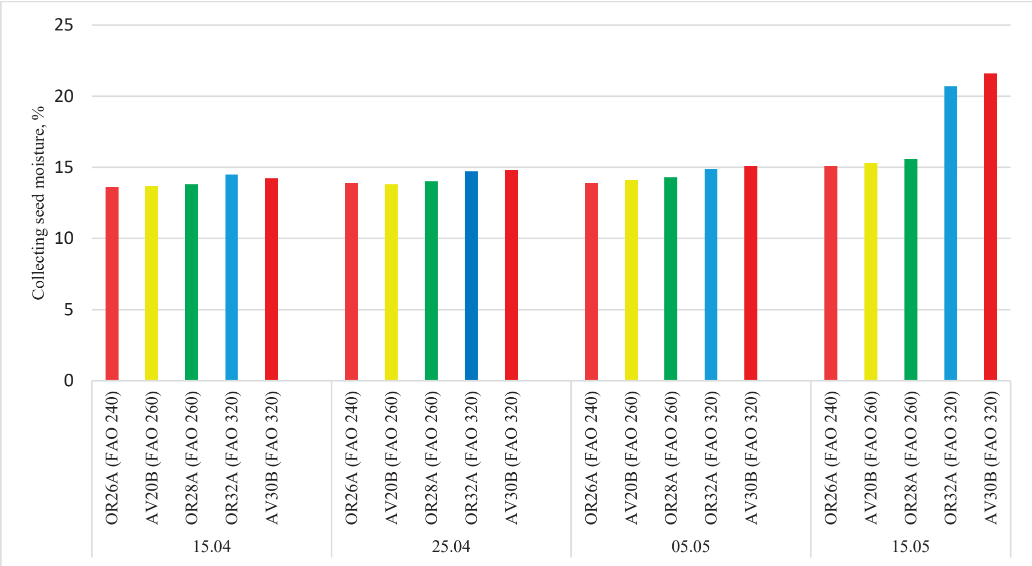

Figure 2. Harvest moisture content of parent lines - components depending on sowing dates, % (average for 2019-2021)

In addition to the main additional costs for grain drying, grain quality losses from *Fusarium rots* also directly depend on the moisture content of cobs. Therefore, production is extremely interested in low harvest moisture. Low harvest moisture also depends on harvesting dates, and delaying harvesting and postponing it to late autumn does not bring the expected natural grain drying due to low moisture release rates at low temperatures and secondary moisture absorption during autumn rains.

The seed moisture of all parent lines – components of maize hybrids from different FAO groups at the time of harvesting ranged from 12.9% to 22.9%, indicating the extraordinary importance of studying this indicator as a primary indicator of maize cultivation technology, high efficiency, and profitability. The variation in this indicator is explained by different sowing dates and FAO groups of lines.

The maximum data for the "grain moisture" indicator, 14.9–22.9%, for all maize lines were observed for sowing on 15.05, and the minimum moisture content of the grain, 12.9-15.1%, was observed for sowing on 15.04. Regarding the dependence of harvest moisture on the FAO groups of lines, a pattern was observed: minimal grain moisture was characteristic of the OR–26A line, 12.9-15.4%, while the maximum was observed for the OR–32A (FAO 320) and AV–30B (FAO 320) lines, 13.9–22.9%.

Thus, practically all lines, except for the FAO 320 hybrids, had baseline grain moisture at the time of harvesting, allowing for post-harvest drying. This is important in the process of forming energy-efficient technologies for growing agricultural crops.

Production is interested in maize harvesting dates that fall in the early third decade of September when the forecasted moisture is less dependent on weather fluctuations.

The difference in grain moisture depending on sowing dates was more clearly defined in lines with an extended vegetation period. These lines, such as OR–32A (FAO 320) and AV–

30B (FAO 320), had variations in seed moisture ranging from 6.6% to 7.9%, comparing early with late sowing dates. The difference in seed moisture between early and optimal dates in the OR–32A (FAO 320) and AV–30B (FAO 320) lines was significantly smaller (from 0.5 to 1.3%). This can be explained by the fact that the "sowing – emergence" period for the early sowing date was more extended, and the difference in calendar time for emergence was significantly less compared to calendar sowing dates.

The harvest moisture of lines FAO 240–260 for early, optimal, and late dates was almost at the same level. This indicates that the ripening period of these genotypes fell in August, when there is low relative air humidity, high day and night temperatures, promoting accelerated moisture release and a reduction in moisture to minimum levels, below which natural seed moisture practically does not decrease.

Improving the elements of variety agronomy for parent lines of maize hybrids from different FAO groups provides an opportunity to increase the seed productivity of the crop.

CONCLUSIONS

Each FAO group of parent lines of maize hybrids in the conditions of the Central Steppe of Ukraine has its optimal sowing date. The mid-ripening line OR–26A (FAO 240) showed the maximum seed yield in 2019 and 2021 for sowing on 05.05-3.61 and 3.65 t/ha, respectively, in 2020 for sowing on 15.05-3.59 t/ha. The minimum yield of 2.66 t/ha was recorded for sowing on 15.04, resulting in a yield decrease of 0.99 t/ha or 26.4%. The mid-ripening line AB–20B (FAO 260) showed the maximum seed yield in 2019 and 2021 for sowing on 05.05-3.71 and 3.72 t/ha, in 2020 for sowing on 15.05-3.70 t/ha. The minimum yield of 2.77 t/ha was recorded for sowing on 15.04, resulting in a yield decrease of 0.95 t/ha or 25.5%. The mid-ripening line OR–28A (FAO 260) showed the maximum seed yield in 2019 and 2021 for sowing on 05.05-3.96 and 4.11 t/ha, in 2020 for sowing on 15.05-3.87 t/ha. The minimum yield of 2.89 t/ha was recorded for sowing on 15.04, resulting in a yield decrease of 1.22 t/ha or 29.6%. The mid-ripening line OR–32A (FAO 320) showed the maximum seed yield in 2019 and 2021 for sowing on 05.05-4.37 and 4.35 t/ha, in 2020 for sowing on 15.05-4.40 t/ha. The minimum yield of 2.47 t/ha was recorded for sowing on 15.04, resulting in a yield decrease of 1.93 t/ha or 43.9%. The mid-ripening line AB–30B (FAO 320) showed the maximum seed yield in 2019 and 2021 for sowing on 05.05-4.40 and 4.42 t/ha, in 2020 for sowing on 15.05-4.44 t/ha. The minimum yield of 2.43 t/ha was recorded for sowing on 15.04, resulting in a yield decrease of 2.01 t/ha or 45.3%. Minimum grain moisture values of 12.9-15.1% were observed for sowing on 15.04, and the minimum seed moisture of 12.9-15.4% was charcteristic of the OR–26A line.

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BRASSICACEAE SPECIES (Brassicaceae Burnett) IN THE COLLECTION OF "ALEXANDRU CIUBOTARU" NATIONAL BOTANICAL GARDEN (INSTITUTE) AS POTENTIAL HONEY PLANTS

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Abstract

The collection of honey plants of the "Alexandru Ciubotaru" National Botanical Garden (Institute) includes species of different position in the taxonomic system. Thus, 5 species of the Brassicaceae Burnett family have been researched - flowering herbaceous annual and perennial plants. These species belong to 5 genera. The genus Brassica is represented in the collection by: B. napus L. subsp. oleifera DC. (rapeseed); Sinapis L. - Sinapis alba L. (white mustard); Isatis L. - Isatis tinctoria L. (woad); Bunias L.- Bunias orientalis L. (the Turkish wartycabbage, warty-cabbage, hill mustard); Crambe L. - Crambe cordifolia Steven. (greater sea-kale, colewort). All of these species start the growing season early and the flowering stage occurs in May-June, providing honey-producing and pollinating insects with food. Flowering is staggered, abundant, lasting about 20-30 days. The flowers are attractive for a wide range of insects, the most common belong to the order Hymenoptera: Apidae; Diptera: Syrphidae. Both cultivated and wild Brassicaceae can bring a significant contribution to the diversification and use of the potential sources of nectar and pollen in the Republic of Moldova.

***Key words**: Brassicaceae Burnett, diversity, honey plants, insects.*

INTRODUCTION

The representatives of the Brassicaceae Burnett family (Cruciferae Adans.), both cultivated species and those from the wild flora, contribute significantly to making use of the nectar-pollen producing potential in the Republic of Moldova. Considering that they are valuable plant species, used in different fields of the national agro-economy, their research is of topical interest. Brassicaceae are distributed in various geographical areas, and their centers of diversity include the Irano-Turanian and the Mediterranean floristic regions, as well as the Himalayas, the Cape, the Andes, Western USA, Australia and New Zealend (Appel & Al-Shehbaz, 2003; Nikolov et al., 2019). The Brassicaceae Burnett. family is very large, comprising about 3000-4000 herbaceous species of annual or perennial plants, rarely suffrutescent and woody, glabrous or with diverse indumentum. In the spontaneous flora of Bessarabia, Brassicaceae are represented by 48 genera and 97 species. Plants with alternate leaves, simple, entire or variously divided, actinomorphic flowers grouped in racemose inflorescences, without bracts. At the base of the stamens there are 4 or 2 nectariferous glands of various shapes. Fruits glabrous or pubescent, smooth or reticulate-rough. Seeds relatively small, different in shape and color (Comanici & Palancean, 2002; Flora Bassarabiei, vol. 3, 2020). Due to the position of nectar secreting glands, floral insect visitors collecting nectar often inadvertently pick up and disperse pollen. Floral nectaries of crucifers vary in their morphology, size and distribution. There are four nectary types based on number and distribution of the organs: (1) annular, a continuous zone of nectarial tissue around the receptacle; (2) two nectary type - two opposing nectaries at the flower base; (3) four-nectary type - made up of two pairs of nectaries classified as lateral and median; (4) eight-nectary type - two pairs of lateral and two pairs of median nectaries (Davis et al., 1996; Davis et al., 1998).

Brassicaceae flowers are tetramerous, with four sepals arranged in medial and lateral positions, alternating with four petals in diagonal position (Ronse de Craene, 2010). The androceium consists of six stamens, two outer and four inner stamens with longer filaments opposite the medial sepals and shifted toward the median line. The gynoecium consists of two carpels and has a false septum dividing the ovary into two compartments (Appel & Al-Shehbaz, 2003).

Rapeseed inflorescences are umbelliform racemes with hermaphrodite flowers, with long pedicel, regular corolla, bright yellow, with 4 oblong ovate petals that reach 10-15 mm long, 4 sepals, 6 stamens, of which 4 long and 2 short, fused unicarpellate genioceum. Pollination is predominantly allogamous, entomophilous, honey potential productivity – 50-80 kg/ha honey (Țîței & Roșca, 2021).

White mustard has raceme inflorescences with flowers of 8-14 mm in diameter, attached to long, pubescent petiole. The length of the sepals is 4-6 mm, when blooming - patent, later retracted. Petals yellow, obovate, 7-9 mm long, tapering into a 6-8 mm long unguicule (Ghendov, 2020). Honey bees, solitary bees, bumblebees, flies etc. frequently visit the flowers of *Sinapis alba* to collect nectar and pollen, and serve as agents of cross pollination. Expert beekeepers could manage to get about 10-50 kg honey per hive during a season or 50-60 kg/ha (Ion et al., 2018).

Tatarican colewort is known for its voluminous, multi-branched inflorescences. The flowers are numerous, 7-8 mm in diameter, glabrous sepals 2.5 x 1.5 mm, elongated white petals, staminal filaments 2.8-3.0 mm. This species is researched for its honey, medicinal, fodder and food qualities (Vergum et al., 2019).

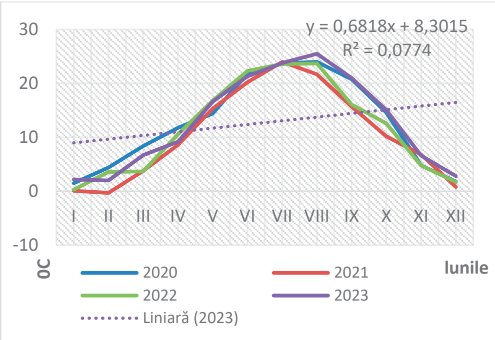
Woad is distinguished by the abundance of yellow flowers, gathered in raceme inflorescences, it has tetradynamous androecium, consisting of six stamens with two shorter filaments, actinomorphic, hermaphrodite. Honey potential productivity is around 70-100 kg/ha and fresh mass productivity reaches 20-35 t/ha and 50-800 kg/ha fruits (Țîței & Roșca, 2021). In the specialized literature these plants are described as useful plants of high economic importance. They are used in food, as valuable sources of oil, spices, as ornamental plants etc. (Al-Shehbaz, 2011; Warwick, 2011). According to the data obtained by Țîței (2022) regarding the evaluation of the quality of silage prepared from plants of the Brassicaceae family, the researched species are of interest in diversifying the assortment of agricultural crops of high potential as forage and energy biomass. The harvested biomass mixed with other traditional crops can be ensiled and fed to domestic ruminants, but also can be used as a substrate to produce biomethane. The biochemical biomethane potential from plant substrate of Brassicaceae species varied from 349 to 379 L/kg of organic matter (Ababii et al., 2023).

MATERIALS AND METHODS

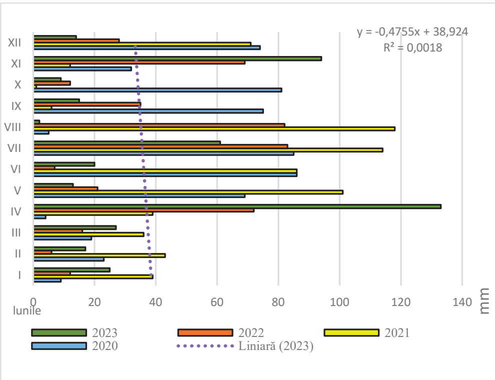
Research activities on potential honey plants have been conducted over the years at the "Alexandru Ciubotaru" National Botanical Garden (Institute), State University of Moldova, based on plants of different taxonomical position included in the families: Asteraceae, Brassicáceae, Fabaceae, Hydrophylaceae, Lamiaceae, Linaceae, Malvaceae, Papaveraceae, Pedaliaceae, Polygonaceae, Salicaceae, Rutaceae (Cîrlig et al., 2020). The National Botanical Garden is located in the South-Eastern area of the city of Chisinau, Republic of Moldova, on an area of 104 ha, divided into sectors, based on ecological-systemic principles. The climatic conditions are favorable for the growth and development of plants, with some exceptions, when high temperatures and extended periods of drought are recorded, a fact that has been proven by the systematization of the data provided by the State Hydrometeorological Service (Figure 1 A, B) (meteo.md).

The year 2022 was characterized by high temperatures and deficiency in precipitation (inconsistent weather temperatures in spring in terms of temperatures, hot summer). As compared with 2021, the air temperature was higher by 1.0-1.5 C and the amount of precipitation – lower, and compared with 2020, the air temperature was lower by 0.5-1.5°C and there was more precipitation. The year 2023 was characterized by higher temperatures and a significant precipitation deficit in the August-October period. The years 2023 and 2020 were ranked 1st among the years with high average annual temperature. The spring of the previous year was characterized by inconsistent weather.

The average temperature during the growing season was 9.7-11.2°C, 0.5-1.1°C higher than the norm. But in April, the highest amount was recorded (amount of 85-175 mm). The summer was hot and dry. The average temperature of the season was 21.3-23.9°C, by 2.1-2.7°C higher than the norm.



A



B

Figure 1. Main meteorological indices during the research period 2020-2023: A - average monthly temperature; B - monthly precipitation total

The research described in this article refers to 5 species of plants in the family Brassicaceae Burnett – *B. napus* L. subsp. *oleifera* DC., (rapeseed); *Sinapis alba* L. (white mustard); *Isatis tinctoria* L. (woad); *Bunias orientalis* L. (the Turkish wartycabbage, warty-cabbage, hill mustard); *Crambe cordifolia* Steven (greater sea-kale, colewort). The plants are native to various geographical areas, being received by us through international seed exchange from abroad (Humboldt-Universitat zu Berlin, Germany; Botanical Garden, Marie Curie-Sklodowska University; Botanical Garden of the University of Medicine and Pharmacy of Târgul Mureș, Romania) and from research institutes of our country (Institute of Genetics, Physiology and Plant Protection).

The study was carried out in the period 2020-2023, during the active growth season of the plants, with special attention focused on the generative periods. The methodical indications in force were used to research the biological peculiarities of plant growth and development and phenological observations (Beideman, 1974; Metodiceskie ucazania po semenovedeniu introduțentov, 1980). The seed material of the researched taxa was tested, under laboratory conditions, to determine the germination capacity. The experiments were made according to the recommendations form Metodiceskie ucazania po semenovedeniiu introduțentov (Методические указания по семеноведению интродуцентов) (1980). To determine the spectrum of entomofauna associated with brassicaceae plants, surveys were conducted during the growing season, with intervals of 2-4 days, direct observations in the field were made, specimens were collected and pictures of the insects were taken. Later, under laboratory conditions, the insects were subjected to taxonomic determination with the help of entomological species determination guides (Talmaciu & Talmaciu, 2014; Plavilsciov, 1994; Mamaev, 1985).

The goal of the current study has been to highlight the species of useful plants of the Brassicaceae family with high potential for honey production; to achieve it, the following objectives were set: determining the germination capacity of the seeds, obtaining the vegetative material for multiplication as necessary, establishing the phenological stages, determining the spectrum of associated entomofauna.

RESULTS AND DISCUSSIONS

The research on the resources of plants with high melliferous potential started at the "Alexandru Ciubotaru" National Botanical Garden has been aimed at expanding the range of useful plants, in order to make use of the available potential honey crops and introduce new crops from other geographical areas, organizing an uninterrupted conveyor of blooming honey plants that will serve as a source of nectar and pollen for useful entomofauna. In this context, plant species from the Brassicaceae family were also included in the research. The germination capacity of the seeds was tested, obtaining the following values: *I. tictoria* - 57.57±4.88%; *S. alba* - 94.7±9.98%; *B. napus* - 66±1.92%; *C. cordifolia* - 1.67±0.52%; *B. orientalis* - 2±0.82%. The seeds of *B. orientalis* and *C. cordifolia* that germinated were incorporated into the soil in cell trays and stored under greenhouse conditions. As a result, planting material was obtained, which was later transplanted in open ground. The seedlings were moved to the experimental plots, when they reached 12-16 cm in height and developed 4-5 true leaves, they had a healthy appearance and were not affected by diseases or parasites.

Brassica napus L. subsp. *oleifera* DC. - rapeseed (Figure 2 A) is a plant with many uses, particularly in the food industry, cosmetics, medicine, animal feed, biofuel production, all parts of the plant being useful - flowers, seeds, leaves, stem and roots.

A B

Figure 2. A - *Brassica napus* L.;
B - *Bunias orientalis* L.

In the Republic of Moldova, rapeseed is a profitable crop and undemanding to pedoclimatic conditions. According to the National Bureau of Statistics, the agricultural production of rapeseed in 2021 was 92.4 thousand tons, and in 2022 it was 77.3 thousand tons (Statistica.md). Rapeseed flowers can be used to prepare herbal teas (Lim et al., 2017), and the consumption of pollen helps strengthening the immune system, due to the presence of steroids (Feng et al., 2011).

Rapeseed culture, in general terms, provides nectar and pollen to bees at a time when the few other melliferous plants are blooming; therefore, it contributes to the strong development of bee families in the early spring period. In the experimental sectors in the Botanical Garden, the flowering period of rapeseed depends on the period of incorporation of the seeds into the soil. Its productivity reaches 35-100 kg of honey per hectare. Honey has yellow color, specific taste, crystallizes fast (Heroica, 1986).

Pollinating insects, especially the honey bee, are attracted to the light yellow pollen of rapeseed, available for a period of about 15-25 days. Insects are active on flowers since the morning hours (7:30-8:00), when the air temperature reaches values of 8-12°C. Bees are active all day, but the maximum intensity was recorded between 12:00 and 14:00. The amount of nectar generated by a rapeseed flower is 0.3-0.8 mg, and the daily honey production is 2-3 kg (lumeasatului.ro).

Bunias orientalis L., the Turkish wartycabbage, is a biennial or perennial plant, with an erect, branched stem, the leaves on branches attached to long petioles, and those from the stem – to short petioles. The flowers are small, yellow, produced in multifloral raceme inflorescences. It is a common species for the Republic of Moldova (Figure 2 B), widespread in the Atlantic, Central and Eastern Europe, the Mediterranean Region, Asia Minor, Caucasus, Western Siberia, Central Asia, where it is used as a food, honeydew and medicinal plant (Flora Basarabiei, 2020). Total nectar carbohydrate production per ten *B. orientalis* flowers averaged 0.3 mg, sugar concentration of nectar, which was 28%, was in the range reported for other crucifers (Denisov et al., 2016). Nectar production in *B. orientalis* flowers is relatively low, but as a result of extremely high flower display, this species may be a valuable food source for visiting insects and hence compete for pollinators with native flora and depress the visits of pollinators to other plants (Hochkirch et al., 2012.)

Sinapis alba L. (=*Brassica hirta* Moench, *Brassica alba* (L.) Rabenh.), white mustard, is an annual plant, with a short growing season, cultivated for seeds and green mass, recommended in beekeeping as a honey plant.

The yellow flowers, opening in sequence, attract pollinators and honeybees throughout the flowering stage. Honey productivity reaches 50-100 kg/honey/ha (Burmistrov, 1990).

A B

Figure 3. *Sinapis alba* L.: A - inflorescences developed on a shoot; B - the total number of fruits, flowers and floral buds in an inflorescence

The biometric study of mustard plants revealed that by the end of May - mid-June (flowering stage) the plants reach a height of 68-85 cm, there can be about 8-18 inflorescences on one shoot (Figure 3 A). In one inflorescence, there can be 6-15 buds, 8-18 flowers and 4-6 developing fruits at a time (Figure 3 B). Leaf size varies between 18-20 cm long and 8-11 cm wide. There are usually 10-12 leaves per shoot.

Crambe cordifolia Steven (=*Crambe glabrata* DC.) - the greater sea-kale. Under the climatic conditions of the Republic of Moldova, it reaches up to 180 cm in height. The stem is glabrous with basal leaves - petiolate, entire and with irregularly toothed margin. The inflorescences are very branched, with numerous, small, white flowers. It has gained popularity as a fodder, medicinal and food plant, and its abundant flowers attract many pollinating insects. The number of seeds depends on the pollination process. Bulk seed density is 273.75±0.73, and weight of 1000 seeds is 4.30±0.12 g (Ababii et al., 2023).

Isatis tinctoria L. - woad - perennial plant, with simple, erect stems, the basal leaves are petiolate, and those in the middle and apical area of the stem are sessile. The growing season starts early (in March) and the plants grow rapidly and abundantly. It is a source of food for insects in May-June (Figure 4). Honey productivity is about 60 kg/honey/ha (Burmistrov, 1990). Flowering and fruiting are staggered and last 25-30 days. In May, there can be simultaneously 7-8 flower buds, 148 flowers and 192 fruits on a shoot. The weight of 1000 seeds is 5.4±0.19, bulk seed density is 88.3±0.13 g (Ababii et al., 2023).

Figure 4. *Isatis tictoria* L. in the collection of the National Botanical Garden

Brassicaceae species are plants with a relatively short growing season. They bloom among the first in the collection of forage-honey plants. The flowering stage occurs in May-June. Forecasting the flowering period of honey plants is based on the flowering stage of each plant species, from the accumulation of the effective amount of temperature during the period from the beginning of active growth to the full flowering of each crop. Determining the honey base, classifying plants according to phenological criteria - the length of the flowering stage and the period when it occurs - spring, summer or autumn, are essential data to be taken into account while building a honey conveyor. The phenological study of the researched species allowed the identification of the vegetative and generative phenological phases (Table 1), as well as their duration in correlation with the climatic conditions recorded in the Republic of Moldova.

The researched Brassicaceae have been able to complete the entire biological cycle, producing viable seeds. The species *I. tinctoria, B. orientalis* and *C. cordifolia*, being perennial plants, in the first year form only plant mass (rosette of leaves) and do not go through the cycle of generative phases. Starting with the second year of vegetation, the rate of growth and development of the shoots intensifies, the plants develop generative organs, bloom and bear fruit.

Table 1. The phenological spectrum of Brassicaceae species with high potential for honey production

Species	Month	March		April			May			June			July		
	Year	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Isatis tinctoria	2020	-	v	v	B	B	B / F	F	F	F	x	x	x	x	x
	2021	-	v	v	B	B	B / F	B / F	F	F	F	x	x	x	x
	2022	v	v	v	B	B	B	B / F	F	F	x	x	x	x	x
	2023	-	v	v	B	B / F	B / F	B / F	F	F	x	x	x	x	x
Sinapis alba	2020	-	-	-	-	- / v	v	v / v	B / F	B / F	B / F	F	x	x	x
	2021	-	-	-	-	- / v	v	v / B	B	B / F	F	F	F	x	x
	2022	-	-	-	v	v	v / B	B	B / F	F	F	x	x	x	x
	2023	-	-	-	v	v	v	B	B / F	F	F	x	x	x	x
Bunias orientalis	2021	-	-	-	v	v	B	B / F	F	F	x	x	x	x	x
	2022	-	-	v	v	v	B	B / F	F	F	x	x	x	x	x
	2023	-	v	v	v	v	v / B	B	B / F	F	F	x	x	x	x
Brassica napus	2020	-	-	-	v	v	v	B	B	B / F	F	F	F	x	x
	2021		-	-	v	v	v	B	B	B / F	F	F	F	x	x
Crambe cordifolia	2022	-	-	-	v	v	v	B	B	B / F	F	F	x	x	x
	2023	-	-		v	v	v	B	B	B / F	F	F	F	x	x

NOTE: V –vegetative phases; B – bud development; F – flowering; X – fruiting/seed ripening; "-" – dormancy

The studied species begin the growing season in early spring - usually in the middle of March (*I. tinctoria* came out of dormancy on 12. 03. 2022). Starting with the middle of April, Brassicaceae progress through the generative stages: bud development, flowering, fruiting, which can be staggered or overlapping (bud development, flowering and fruiting occurring at the same time). In the experimental sectors of the Botanical Garden, the flowering period of the annual species (*S. alba, B. napus*) depends on the period of incorporation of the seeds into the soil. The seeds were incorporated into the soil later than usual, depending on the weather conditions, by the end of March – the middle of April, a reason for that was to create diversified and successive sources of food for beneficial insects, available throughout the growing season.

One of the objectives of the current study was to research, determine and compile the list of useful entomofauna detected on the flowers of Brassicaceae plants, being known as high-potential honey plants and providing food for insects starting in May. The insects were monitored during the flowering stage, throughout the day. Nectar composition, concentration and accessibility are important in determining pollinators. In Brassicaceae species, the nectar is produced by the glands located at the base of the androecium, between the sepals and the petals. The structure of nectar in these plant species, which is hexose-dominated, makes it accessible and easily collected by butterflies and short-tongued bees (Davis et al., 1998; Baker & Baker, 1983). The optimal temperature for nectar secretion in most honey crops is considered to be 16-25°C, with air humidity of 60-80% (Cerevko, 2001).

The spectrum of insects visiting the generative organs of the studied plants was determined, as well as their classification according to the systematic position. Woad, being among the first species in the collection to bloom, was visited by a larger number of insects - 14 species (*Apis mellifera, Lasioglossum malachurus, Arge ustulate, Eristalis tenax, Epicometis hirta, Chrysomela limbate, Oedemera mobilis, Cantharis pellucida, Trichodes alvearius, Entomoscelis adonidis, Coccinella septempunctata, Eurydema dominulus, Cercopis arcuate, Polyommatus icarus*), which belong to 6 orders (Hymenoptera, Diptera, Coleoptera, Hemiptera, Homoptera, Lepidoptera), 14 families (Apidae, Holictidae, Argidae, Syrphidae, Scarabaeidae, Chrysomelidae, Oedemiridae, Cantharidae, Cleridae, Chrysomelidae, Coccinellidae, Pentatomidae, Cercopidae, Lycaenidae) and 14 genera.

The white mustard flowers were visited by 11 insect species (*Apis mellifera, Bombus lapidaries, Lasioglossum malachurus, Lasius niger, Scolia maculata, Eristalis tenax, Epicometis hirta, Coccinella septempunctata, Eurydema oleracea, Dolycoris baccarum, Pieris brassicae*) of 5 orders (Hymenoptera, Diptera, Coleoptera, Hemiptera, Lepidoptera), 9 families (Apidae, Holictidae, Formicidae, Scoliidae, Syrphidae, Scarabaeidae, Coccinellidae, Pentatomidae, Pieridae) and 11 genera.

On *B. orientalis* plants, 6 insect species were detected (*Apis mellifera, Lasioglossum malachurus, Arge ustulata, Lasius niger, Eristalis tenax, Epicometis hirta*) of 3 orders (Hymenoptera, Diptera, Coleoptera) and 6 families (Apidae, Holictidae, Argidae, Formicidae, Syrphidae, Scarabaeidae), these species play a major role in the plant pollination process by visiting the flowers where they find their source of food during the flowering period, which occurs in May.

The flowering stage of rapeseed, under the conditions of the Botanical Garden, was recorded in June, nectar and pollen serving as food for the 6 insect species identified during this period (*Apis mellifera, Lasioglossum malachurus, Lasius niger, Eristalis tenax, Epicometis hirta, Coccinella septempunctata*), belonging to 3 orders (Hymenoptera, Diptera, Coleoptera), 6 families (Apidae, Holictidae, Formicidae, Syrphidae, Scarabaeidae, Coccinellidae).

The species *Apis mellifera* and *Eristalis tenax* were most frequently detected on the flowers of the researched brassicaceae plants. A bee collects nectar for 6-8 seconds on a flower, actively participating in the pollination process.

CONCLUSIONS

The research carried out in the "Alexandru Ciubotaru" National Botanical Garden (Institute) on plants of the family Brassicaceae Burnett (*B. napus* L. subsp. *oleifera* DC.*; Sinapis alba* L.*; Isatis tinctoria* L.*; Bunias orientalis* L.*; Crambe cordifolia* Steven) highlighted their melliferous potential, proved by the interest exhibited by pollinating insects, especially *Apis mellifera*, towards these plant species. The researched species have been recognized as economically important plants, as valuable honey and fodder crops. All of these species start the growing season early and the flowering stage occurs in May-June, providing honey-producing and pollinating insects with food. Flowering is staggered, abundant, lasting about 20-30 days. The flowers are attractive for a wide range of insects, the most common belong to the order Hymenoptera: Apidae; Diptera: Syrphidae. Both cultivated and wild Brassicaceae can bring a significant contribution to the diversification and use of the potential sources of nectar and pollen in the Republic of Moldova.

Making use of the available plant resources with high-potential for honey production and expanding the assortment of valuable plants could significantly contribute to the diversification and creation of an uninterrupted chain of nectar and pollen sources for honey production, besides, it would help beekeepers to preventively plan their activities throughout the entire season.

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INCIDENCE OF *Nezara viridula* L. ATTACK ON SOME HOST PLANTS

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Abstract

Nezara viridula L. is a cosmopolitan, invasive species with a highly polyphagous. Some host plants are used as a food source and others for reproduction and food. The species attacks all aerial parts of herbaceous plants, but also the green leaves, the growth tips and the harvest of fruit trees and bushes. They are recorded considerable damages at the fruiting stage. This paper presents the attack of Nezara viridula L. species on several host plants (tomato, pepper, broccoli, peach, raspberry, rose, mallow, artichoke) by assessing the anatomical, morphological and organoleptic changes. Through optical microscopy studies, some changes are highlighted in the pericarp, mesocarp and vacuolar juice of affected pepper fruits, compared to healthy ones. The analyzed fruits show significant defects in shape, color, smell and taste which are confirmed by optical microscopy studies. That means these fruits are unsuitable for consumption, marketing and storage.

***Key words**: Nezara viridula L., invasive species, host plants, polyphagous, microscopy studies.*

INTRODUCTION

In the last decade, the green bug *Nezara viridula* L. has caused considerable damage to horticultural and agricultural harvest. The species is noticeable by its pronounced polyphagism (Panizzi, 2008; Kurzeluk et al., 2015; Marcu, 2018). The host plants are used for food or reproduction and they are represented by cultivated plants and wild flora species. Their favourites food sources are flower buds, flowers, fruits and seeds. The damages are produced both in the adult and in the larval instar (Grozea et al., 2012; Ciceoi et al., 2017). The produced destructions differ according to plant species, variety and its phenological stage. The main cause of damage is the way insects feed: they penetrate and inject saliva into plant tissues, perforating them. The cells content is emptied, the fruits are deprived of nutrients and remain small. The fruits show little discoloration spots, which gradually become brown and blackish. Horticultural products are depreciated regarding their quality, so they are unsuitable for consumption and marketing. Also, the fruits which are improperly developed fall prematurely. Ornamental plants are affected at the level of the leaves, by the appearance of some spots, by the flower buds falling and the presence of an unpleasant smell (Grozea et al., 2015). In addition to these facts, the insect can mechanically convey spores of some pathogenic fungi and bacteria from one plant to another (Kaiser and Vakili, 1978; Rusin et al., 1988). In this regard, *Nezara viridula* L. has been observed to carry the spores of *Nematospora* spp., which causes internal rot of cotton, soybeans, tomatoes, citrus fruits, beans and other crops. The insect is also considered a potential vector for *Xanthomonas phaseoli* pv. *phaseoli* in beans (EPPO, 2023) and *Pantoea agglomerans* at cotton (Esquivel & Medrano, 2020). As a defense response of the plant to the attack produced by *Nezara viridula* L., Giacometti et al. (2020) show that a large amount of lignin is synthesized in attacked soybean seeds and the cell wall thickens compared to the mechanically damaged cells.
Observations on tissue and cellular changes, produced by bugs from Pentatomidae family, were initiated in Romania by Ciochina (2021).
The species *Nezara viridula* L. is a cause for concern for horticulturists, with difficulties in control and high ecological plasticity.

MATERIALS AND METHODS

Visual observations were made in different crops near to Bucharest, in the period 2021-2023, in order to detect and identify the attack produced by the green bug *Nezara viridula* L. The attacked material was described and photographed for each affected plant species. There were established samples of attacked fruits for laboratory study, by optical microscopy techniques. At the same time, there was collected healthy material for comparison in microscopy studies of the tissues and cellular level. The collected material was also analysed regarding organoleptic features. In the beginning, the harvested fruits were used for visual analysis in connection to the changes of their aspect, the pericarp colour, the caused deformations, and also for observations regarding the changes in taste and smell. Then some incisions were made in the pericarp of pepper fruits, obtaining fragments of 3-4 cm in length which were used to determine the changes about the pericarp thickness, the colour and consistency of affected fruits in contrast to the healthy pepper fruits (Figure 1).

Figure 1. Material preparation in order to obtain microscopic sections

Utilizing a blade, there were made transversal cuts in the pericarp of the healthy pepper fruit, respectively in the pericarp of the attacked fruit. Based on these microscopic preparations (Figure 2), observations were made with Optika microscope regarding the occurred changes at the cellular and tissue level as a result of *Nezara viridula* L. attack.

Figure 2. Sample preparation for microscopy technique

RESULTS AND DISCUSSIONS

Following these observations, the species *Nezara viridula* L. was detected on 9 host plants, a fact that is confirmed by specialized literature (Grozea et al., 2012). The pest was present on tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) plants from the field area and protected spaces, which weren't equipped with protective nets. Tomato and pepper fruits showed obvious changes regarding shape, colour and size (Figures 3 and 4).

Figure 3. Tomato fruits attacked by *Nezara viridula* L.

Figure 4. Pepper fruits attacked by *Nezara viridula* L.

Nectarine fruits (*Prunus persica* L.) show numerous discoloration spots. They are small and unsuitable for consumption (Figure 5).

Figure 5. Nectarine fruits attacked by *Nezara viridula* L.

Raspberry plants (*Rubus idaeus* L.) were used for both food and reproduction. The images highlight hatch larvae, which cluster and then spread, feed and continue their development (Figure 6).

Figure 6. *Nezara viridula* L. larvae on raspberry plants

The roses (*Rosa* spp.) display different larvae phases that feed on the growth tips, flower buds and flowers (Figure 7). The flower bugs stop opening or are transformed into small, asymmetrical and misshaped flowers.

Figure 7. Different larval instars on the rose plant

The mallow plants (*Althaea officinalis* L.) are used as a food source for larvae (Figure 8). The flower buds are preferred by larvae in their development process. Also, artichoke plants (*Cynara scolymus* L.) are attacked by both larvae and adult buds at the inflorescence level (Figure 9).

Figure 8. Different larval instars on the mallow plant

Figure 9. *Nezara viridula* L. on artichoke plant

Cauliflower plants (*Brassica oleracea* var. *botrytis* L.) are used as a food source for larvae (Figure 10), but also for reproduction.

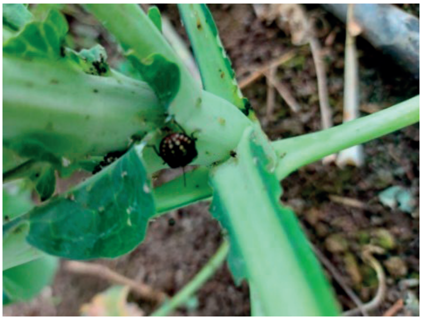

Figure 10. *Nezara viridula* L. on the cauliflower plant

Different plant species are used as food sources for larvae and adult bugs or for reproduction. Among the plant organs attacked, the fruits are the most affected.

There were highlighted colour and structure changes in the observations regarding the external and internal appearance of pepper and nectarine fruits.

The pulp of nectarine fruits shows white spongy areas under to the spots which are localized on the fruit skin (Figure 11).

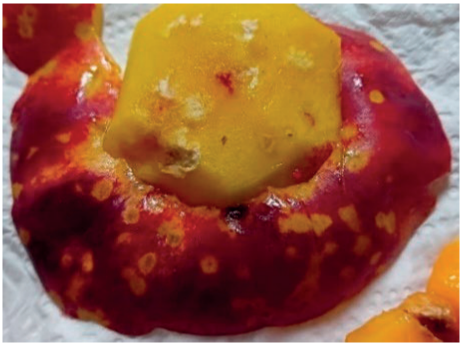

Figure 11. Spongy areas in the nectarine fruits pulp

Attacked apple fruits (*Malus domestica* Borkh.) are depreciated (Figure 12). The lesions produced by larvae and adults are "gateways" for penetration and installation of various microorganisms.

Figure 12. Apple fruits attacked by *Nezara viridula* L.

Tomato fruits show discoloured areas on the skin and spongy tissue inside the fruit.

There are remarked areas with deep tissues, known as cloudy spots (Figure 13), on which different microorganisms settle and continue the deterioration of the fruits (Jones and Caprio, 1994).

Figure 13. Tomato fruits with discoloured and cloudy spots

In addition to these observations regarding the external appearance of the horticultural products, the taste of the fruits, which is generally sweet and slightly acidic in tomatoes, is quite affected in the attacked areas, being atypical, undergoing changes, and the smell is non-specific and unpleasant. The disturbing taste and aroma can be attributed to the enzymes introduced by the pest into the specific area via saliva. In that manner, the olfactory and gustatory properties are no longer the characteristic ones, but distorted because of the attack by which the pest injects its saliva into the fruit.

Attack on tomato fruit is described by Lye et al. (1988). Researchers sustain that in the bugs feeding process they inject enzymes that help to decomposing and liquefaction of the fruit cells, which are emptied of their contents. The tissues become spongy with a cork aspect.

Pepper fruits show spongy, whitish-coloured changes in their tissues, as a result of the emptying of cell contents by pricking and sucking (Figure 14).

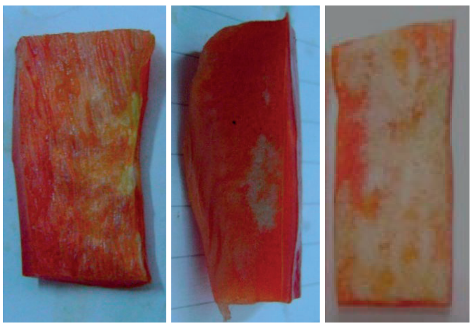

Figure 14. Spongy-looking tissues in pepper fruits

In the organoleptic analysis, the healthy fruit has a smooth aspect, being uniformly coloured, having characteristic red colour, with a specific and crunchy taste. The attacked fruit is discoloured, slightly deformed, yellowish, having an orange or off-white colour. The pericarp thickness of the attacked fruit has a reduced size compared to the healthy one. Also, it has a bland taste, unpleasant smell and spongy consistency (Table 1).

Table 1. Organoleptic, morphological and anatomical changes induced by *Nezara viridula* L. in pepper fruits

Characteristics		Healthy fruit	Attacked fruit
Organoleptic changes	External aspect	smooth, glossy, characteristic red colour	deformations, discolorations, slightly deep areas
	Taste	specific	unpleasant
	Smell	pleasant	unpleasant, repulsive
Internal changes	Consistency	crispy	porous, spongy
	The average thickness of the pericarp	0.65 mm	0.3 mm
	Mesocarp cells	normally hydrated	atrophied
	Vacuolar fluid	in normal quantity	low quantity

Transverse sections made in the pericarp of healthy pepper fruits and peppers attacked by *Nezara viridula* L. show anatomical changes, at the histological and cellular level. Analysing the epidermis of the healthy fruit and the attacked one, it is observed that the epidermis of the healthy fruit has a normal aspect (Figure 15).

Figure 15. Healthy pepper fruit epidermis

The epidermis of the attacked fruit contains necrosis in the intercellular spaces (Figure 16).

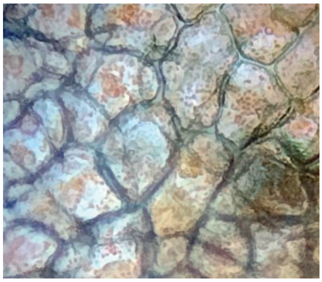

Figure 16. The epidermis of pepper fruit attacked by *Nezara viridula* L.

The mesocarp of the healthy pepper fruit has a normal appearance, being composed by polygonal cells which present vacuolar fluid, with normal chromoplasts (Figure 17).

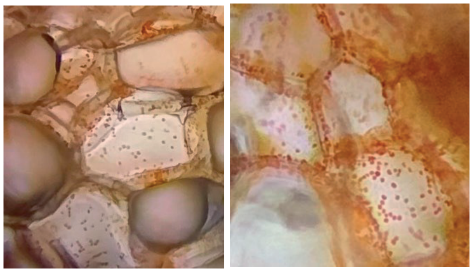

Figure 17. The mesocarp of the healthy pepper fruit

At the same time, the mesocarp cells of the target areas from attacked fruits, are atrophied, dehydrated, lacking vacuolar fluid, with necrotic chromoplasts. These facts are a result of the bugs feeding method. After stinging the fruit, they suck the vacuolar content, which remains poor in nutrients, air enters through the fruit wall and necrosis occurs, including at the level of the epidermis (Figure 18).

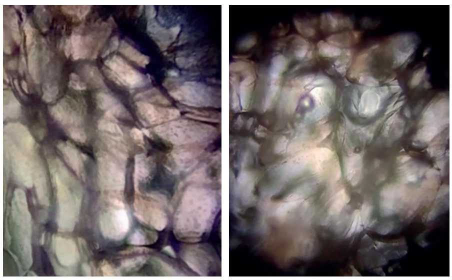

Figure 18. The mesocarp of the pepper fruit attacked by *Nezara viridula* L.

CONCLUSIONS

The green bug *Nezara viridula L.* was detected on 9 species of horticultural host plants such as: *Solanum lycopersicum* L., *Capsicum annuum* L., *Prunus persica* L., *Rubus idaeus* L., *Rosa* spp., *Althaea officinalis* L., *Cynara scolymus* L., *Brassica oleracea* var. *botrytis* L. and *Malus domestica* Borkh. There was recorded significant damage following the attack on pepper, tomato and nectarine fruits.

Optical microscopy studies, carried out on pepper fruits attacked by *Nezara viridula* L., reveal changes at the tissue and cellular level.

The highlighted anatomical-morphological changes are reflected by organoleptic damage and qualitative deterioration of the attacked fruits.

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THE EFFECT OF THE USE OF COMPOST AS A FERTILIZER MATERIAL FOR THE CROP OF LETTUCE (*Lactuca sativa* L.)

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Abstract

The increase of the amount of biodegradable waste that has occurred in the last decades is worrying and composting is a sustainable practice for managing these types of waste. Compost is generally used to build soil quality, water-holding capacity of the soil, and to encourage vigorous plant root system development. This article presents a study that included four types of compost produced from different biodegradable wastes, namely: wood ash + wheat straw; food scraps + eggshells; poultry litter + wheat straw and poultry litter + food scraps. An study was organized in the greenhouse, in pots, where lettuce (Lactuca sativa L.) seedlings were planted using soil-compost mixtures as follows: 30% compost + 70% soil; 50% compost + 50% soil; 70% compost + 30% soil. A control with 100% soil was also made. In this paper, the effects of the compost used as fertilizing material are presented in relation with the macronutrients (N; P; K; Ca; Mg) content of the lettuce plant and the pigments in the lettuce leaves (chlorophyll a, chlorophyll b and carotenoid pigments).

Key words: *compost, organic fertilizers, sustainable agriculture, lettuce, chlorophyll pigments.*

INTRODUCTION

The global context in recent years has led to an increase in the demand and use of organic fertilizers and soil improvers as an alternative to conventional fertilization. By adding organic matter from the compost, the quality of the soil can be improved from a chemical and physical point of view. The use of waste in agriculture is an economical way of disposal of these materials and is interesting from an ecological point of view because it reduces their negative effects on the environment (Ruangcharus et al., 2021; Li et al., 2021; Hargreaves et al., 2008). Also, studies have shown that the use of organic fertilizers can lead to an increase in agricultural production in crops such as tomatoes, stevia, potatoes or even in fruit orchards (Khan et al., 2017; Coelho et al., 2018; Minin et al., 2020; Jindo et al., 2016).

Regarding the introduction into the circular economy of organic wastes through composting and the use of compost as a fertilizing material for agriculture, existing data show that of the amount of compost produced, approximately 85% is used as fertilizer or soil improver in agriculture, gardening, horticulture and landscaping (EC, 2019). This may suggest that part of the problems exposed by the UN through the objectives of sustainable development can be achieved with the reintroduction of biodegradable waste into the circuit of nature, through composting. But in order to be used in agriculture, the compost must meet certain criteria regarding the maturity of the final compost and the content in heavy metals (Brinton, 2001; CR 2029/91 EEC).

Comparative studies on the effects of organic and chemical fertilization have shown that the use of compost has a positive impact on increasing the yield of lettuce plants, as well as the nitrogen and potassium content in the plants (Reis et al., 2014). More recently, other studies have shown that compost can provide the necessary amount of nutrients for the growth of lettuce plants and with the correct use of organic fertilizers, maximum lettuce production can be obtained (Garcia-Lopez, 2022; Bhatta et al., 2022). Regarding the impact of the use of organic compost on the pigments in the lettuce leaves, recent studies show that the amount of compost does not significantly affect the pigment content in the leaves and the compost can be sufficient for the growth of lettuce plants (Vasileva et al., 2023; Kumngen et al., 2023).

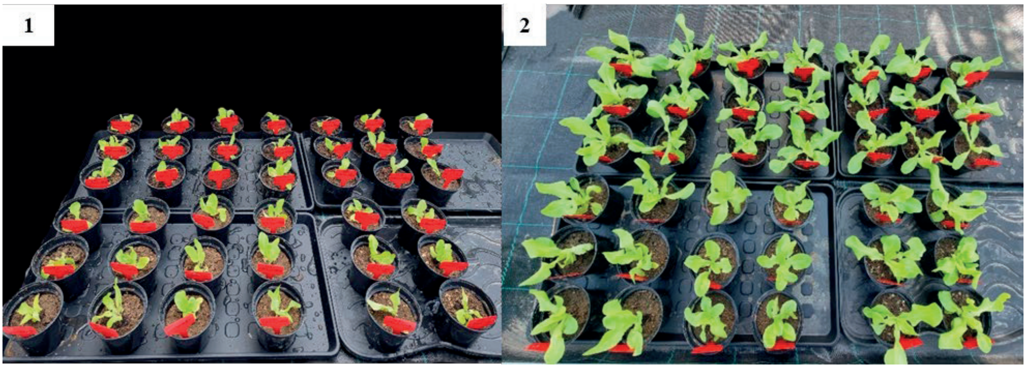
In this work we have proposed to present the results obtained in a study in which we tested four types of compost obtained from different mixtures of biodegradable waste using our own recipes. The effects of these composts, used in different proportions in mixtures with soil, on lettuce plants (*Lactuca sativa* L.) were monitored, from the point of view of the plants' content in macronutrients and pigments in the leaves.

MATERIALS AND METHODS

The experiment was organized in the greenhouse between March 15, 2023 and May 6, 2023.

Soil mixtures and four types of compost, obtained from various organic wastes, were used in different proportions.

Individual pots were used in which lettuce seedlings (*Lactuca sativa* L.) were planted (Photos 1 and 2).



Photos 1 and 2. Lettuce after planting and 2 weeks later

All composts were produced domestically using composting containers with a volume of 320L each. The soil used for this experiment is reddish preluvosol type coming from Ilfov County, Romania. Soil-compost mixtures were used in proportions of 30%, 50% and 70%, as well as a control variant with 100% soil content, resulting in 13 variants, each one in 3 replicates.

The types of compost and the mixtures used within the experiment are presented in Table 1. The soil and the composts physico-chemical properties were also analyzed.

The data obtained in the experiment were analyzed as averages of the replicates within each variant. The graphs were made in Excel.

Table 1. Composts type and studied soil and compost mixtures

Type of compost	Soi land compost mixtures	
C1 – Wood ash and wheat straw (ratio 4:1, 10 months old) compost;	V1 - Control (100% soil)	V7 (70% C2+ 30% soil)
C2 – Food scraps and egg shells (ratio 4:1, 10 months old) compost;	V2 (30% C1+ 70% soil)	V8 (30% C3+ 70% soil)
C3 – Poultry manure and wheat straw (ratio 3:1, 24 months old) compost;	V3 (50% C1+ 50% soil)	V9 (50% C3+ 50% soil)
C4 – Poultry manure and food residues (ratio 3:1, 24 months old) compost.	V4 (70% C1+ 30% soil)	V10 (70% C3+ 30% soil)
	V5 (30% C2+ 70% soil)	V11 (30% C4+ 70% soil)
	V6 (50% C2+ 50% soil)	V12 (50% C4+ 50% soil)
		V13 (70% C4+ 30% soil)

RESULTS AND DISCUSSIONS

Compost and soil physico-chemical properties

Soil and the composts physico-chemical properties are presented in Table 2. The pH of composts ranged from 7.1 to 9.46. Compost made from wood ash and wheat straw had an alkaline pH of 9.46, and the other composts had a neutral pH of around 7. The soil also had a neutral pH of 7.11. The total organic carbon content of composts ranged from 4.95% (C2) to 14.92% (C3), well above the soil reserve (1.25%).

Nitrogen is the most important nutrient for plants, being needed in large quantities for their growth, development, and reproduction (Leghari et al., 2016). Nitrogen content was higher in two of our composts, C3 (1.31%) and C4 (1.27%) respectively, which have been made from mixtures that included poultry manure. Total N normally ranges from less than 1% to 2.5% in finished composts (Wilden et al., 2001; Coelho et al., 2018; Jalili et al., 2019; Peng et Pivato, 2019).

Table 2. Physico-chemical characterization of composts and soil used within the study

Composts/Soil	pH	Umd (%)	Corg (%)	N (%)	$N\text{-}NO_3$ (mg/kg)	$N\text{-}NH_4$ (mg/kg)	P (%)	K (%)
Soil	7.11	12.33	1.25	0.11	6.13	3.78	0.08	1.17
C1	9.46	12.55	6.61	0.19	2.11	5.31	0.84	1.68
C2	7.91	25.01	4.95	0.56	105.19	5.68	0.27	1.86
C3	7.14	42.69	14.92	1.31	229	59.33	1.47	1.23
C4	7.1	22.22	13.62	1.27	136	53.67	1.62	1.48

At the end of the composting process, a higher concentration of nitrates (NO_3^-) is usually observed compared to the concentration of ammonium (NH_4^+) (Addai et al., 2023). The higher values of nitrates ($N\text{-}NO_3^-$) content were recorded in C3 compost (229 mg/kg DM), C4 compost (136 mg/kg DM) and C2 compost (105.19 mg/kg DM). The $N\text{-}NO_3^-$ content of C1 compost was the lowest (2.11 mg/kg DM). The raw material of this compost could influence the evolution of nitrogen in this case, but this topic will be studied further. The soil also had a very low content in $N\text{-}NO_3^-$ (6.13 mg/kg DM). The greatest content in ammonium ($N\text{-}NH_4^+$) was recorded in C3 compost (59.33 mg/kg DM) and in C4 compost (53.67 mg/kg DM). The composts C1 and C2 had a very low content in $N\text{-}NH_4^+$ (5.31 and 5.68 mg/kg DM respectively). The soil content in $N\text{-}NH_4^+$ was 3.78 mg/kg DM.

The phosphorus reserve in the soil is 0.08%. The C3 and C4 composts recorded te highest content in phosphorus (1.47% and 1.62% P). The lowest phosphorus content was observed in C2 compost which was made from food scraps and eggshells. The potassium content in composts and soil exceeded 1%.

Lettuce biomass production

Figure 1 shows the results regarding the mass of lettuce plants, as the average of the repelications of each variant (in grams).

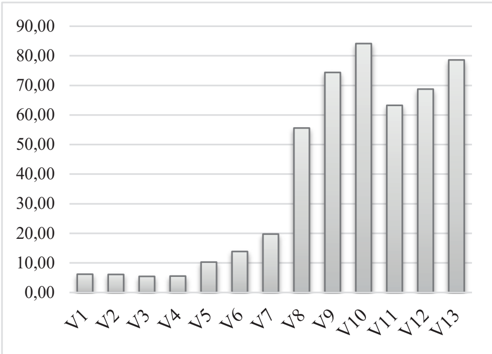


Figure 1. Production of lettuce biomass obtained in different soil mixtures and compost

Except for the variants (V2, V3 and V4) in which compost C1 (obtained from wood ash and wheat straw) was used, all other variants with compost and soil mixtures, regardless of the proportion of compost in the mixture, led to productions of biomass over those achieved in the control variant. In the case of the other 3 types of compost, it can be seen that the increase in biomass production was almost proportional to the increase in the amount of compost in each variant. Thus, the differences recorded in the production of lettuce biomass in the variants where composts were used, compared to the control (100% soil), look like below:

C1 compost: (V3 < V4) < V1; V2 < V1;
C2 compost: (V5 < V6 < V7) > V1;
C3 compost: (V8 < V9 < V10) > V1;
C4 compost: (V11 < V12 < V13) > V1;

In the variants with C2 compost, the letuce biomass production was: 66.18% (V5), 124.27% (V6) and 219.42% (V7) higher than the control (V1 - control).

The same trend of increasing production, compared to the increase in the proportion of compost in mixtures, was observed in the variants where C3 compost (poultry manure and wheat straw) and C4 compost (poultry manure and food scraps) were used. Therefore the lettuce biomass production was 800% (V8), 1103% (V9), 1261% (V10 wich had the highest production was obtained, namely 84.13 g). The variants V11, V12 and V13 achieved higher biomass production than control by 924%, 1012% and 1172%, respectively .

The results obtained are consistent with other studies conducted by other authors that have demonstrated the ability of compost to substitute chemical fertilizers and the benefits it brings to lettuce production (Jaza Folefack, 2008; Gimenez el al, 2019; Bhatta, 2022).

Macronutrients in lettuce plants

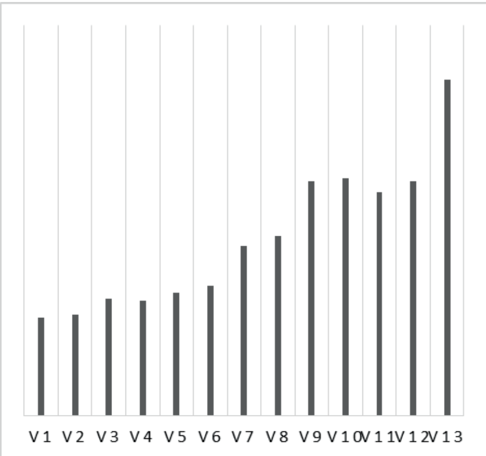


Figure 2. Nitrogen content of lettuce plants (*Lactuca sativa*)

Lettuce plants in all variants with compost recorded a higher nitrogen content (Figure 2) compared to control (Reis et al., 2014; Garcia-Lopez et al., 2022). The variants where C1 compost (wood ash and wheat straw) was used exceeded the control variant by 4% (V2), 20% (V3) and 17.33% (V4) nitrogen content. In variants where C2 compost (food scraps and eggshells) was used, the nitrogen content was higher than in the control variant as the proportion of compost increased. Thus, the increase was as follows: V5 < V6 < V7.

Regarding the variants with C3 compost (poultry manure and wheat straw), the nitrogen content was higher in all variants where compost was used than control, with 84% (V8), 141.33% (V9) and 144% (V10).

The variants V11 (30% C4), V12 (50% C4) and V13 (70% C4), with mixtures of C4 compost (poultry manure and food debris), recorded the biggest content of nitrogen in lettuce plants compared to control by 130%, 140% and 244%, respectively. Therefore, as it can be seen in the graph presented above, the N content of lettuce plants was growing proportionaly to the compost quantity added within mixtures. Also, in the variants where the composts C3 and C4 were used the nitrogen content of plants followed the nitrogen content of composts (1.31 and 1.27% total nitrogen respectively - Table 2) which were biggest.

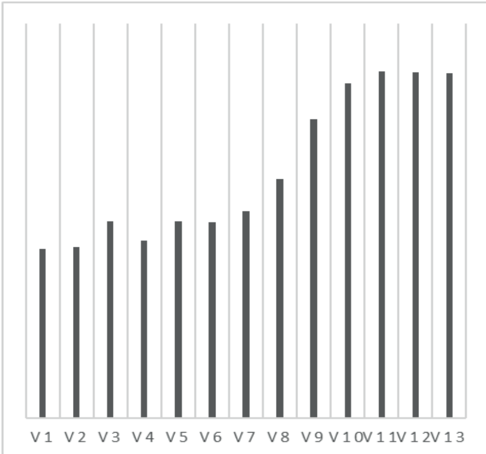


Figure 3. Phosphorus content in lettuce plants (*Lactuca sativa)*

The phosphorus content of lettuce plants is shown in Figure 3. Compared to the control variant resulting in 0.30% phosphorus content, plants that were grown using C1 and C3 resulted in phosphorus contents close to the control variant, the phosphorus content oscillating between 0.30% (V2) and 0.37% (V7). The phosphorus content of lettuce plants grown in the variants in which C3 compost (poultry manure and wheat straw) was used, gradually increased with the increase in the amount of compost used and resulted in a higher phosphorus content than the control variant, thus : with 40% (V8), 76.67% (V9) and 96.67% (V10). The highest phosphorus content in lettuce plants resulted from the use of C4 compost (poultry manure and food scraps), which regardless of the soil-compost ratio (30%, 50%, 70%) the lettuce plants had double phosphorus contents compared to control.

The potassium content (Figure 4) of lettuce grown in all variants that contained compost was higher than V1-control (Reis et al., 2014). In V2 (30% C1) content was 18.81% higher than V1, 3.67% in V3 (50% C1) and 33.49% in V4 (70% C1). In variants where C2 (food scraps and eggshells) was used, an increase in phosphorus content was observed as the compost content was higher in the mixtures. Thus, compared to the control, V5 resulted in a higher content by 0.46%, V6 by 23.39% and V7 by 37.16%. In fact, the variants where C3 and C4 were used resulted in the highest values of potassium content in lettuce plants, oscillating between 3.59% K and 4.59% K, over 65% more than the control, although C1 and C2 composts had a higher potassium content than C3 and C4 (Table 2).

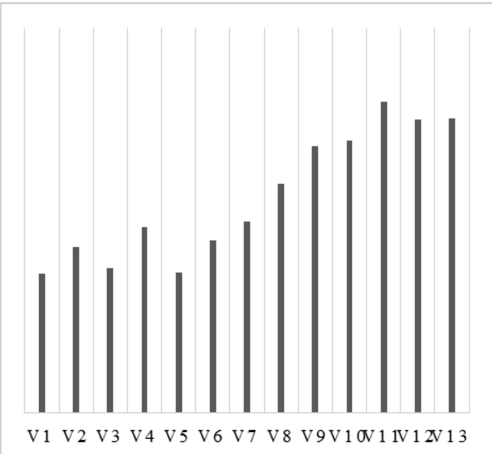


Figure 4. Potassium content in lettuce plants (*Lactuca sativa*)

The calcium and magnesium content of lettuce plants is shown in Figure 5. In variants where C1 was used, calcium content was higher in V2 (30% C1) and V4 (70% C1), compared to variants containing C2 and having a higher calcium content, relative to the control, by 2.63% (V5), 19.47% (V6) and 25.75% (V7). In variants where C3 and C4 were used, the calcium content of lettuce was lower than the control by 20% (V8) to 42% (V13). The magnesium content of lettuce plants, harvested from pots containing C1, was higher than the control by 25% in V2 and 33.33% in V4, while V3 (50% C1) had a similar content of magnesium as the control. Except for V3, all variants containing compost, regardless of compost type, had a higher Mg content compared to the control, with the highest being detected in V9.

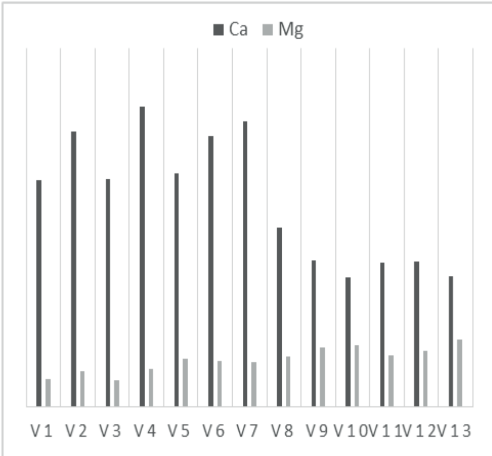


Figure 5. Calcium and magnesium content of lettuce plants (*Lactuca sativa*)

Pigments in lettuce leaves

The amounts of chlorophyll a and b and carotenoid pigments in lettuce leaves were determined shortly after harvest and the results, expressed in mg/100 g by mass of fresh lettuce leaves, are shown in Figures 6 and 7. The determinations were made for each repetition, then the average per variant was made. Chlorophyll A is directly related to the photosynthetic process of plants and consequently to photoassimilated production while chlorophyll B does not play such an important role in leaf pigmentation and photosynthetic processes and is mainly important in amplifying the light absorption spectrum, considered as an accessory pigment (Taiz and Zeiger, 2010).

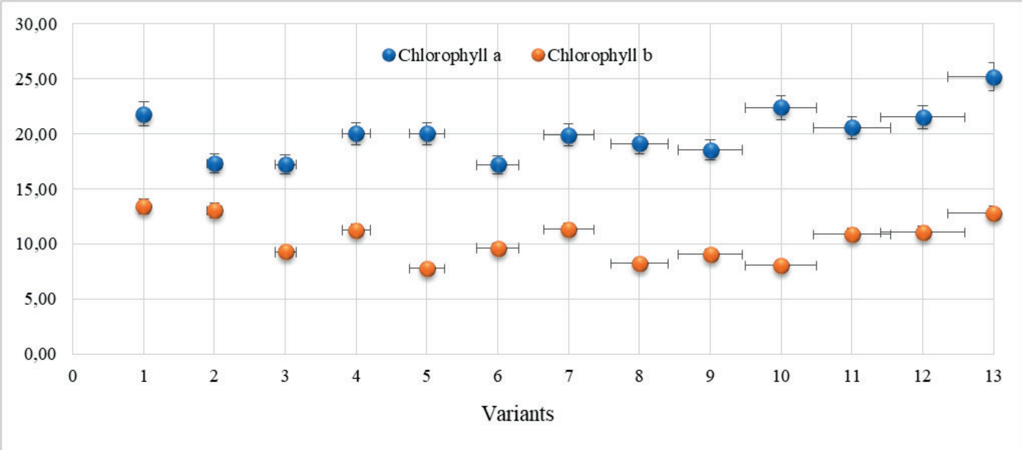


Figure 6. Chlorophyll a and b in lettuce leaves (mg/100 g)

The chlorophyll a content in variants that contained C1 compost was lower than in the control variant, by 20.6% in V2, 21.1% in V3 and 8.2% in V4. Similar results were found for variants where compost C2 compost was used while in V13 where 70% C4 compost (poultry manure and food scraps) was used, had the highest chlorophyll a content, respectively 15.6% more than the control variant, although V10, with a content of 70% C3 compost approached the value of the control, chlorophyll a content being 2.7% higher than the control variant.

The chlorophyll b content had the highest value in leaves harvested in V1, the control variant. Thus, significantly low differences were recorded in the variants where all types of compost were used, regardless of the proportion used. The closest chlorophyll b content to the control variant being V13 (70% C4) wich was lower by 4.7% than the control. In the variants containing compost C3 the chlorophyll b content was lower than the control, respectively with 32.6% in V9, 38.7% in V8 and 40% in V10. Data reported in other studies have shown that the amount of compost applied does not significantly affect the pigment content of lettuce (Hernandez et al., 2015; Vasileva et al., 2023). Carotenoids are tetraterpenic pigments, exhibiting yellow, orange, red, and purple colors (Maoka, 2020).

Regarding the content of cartoenoid pigments in fresh lettuce leaves, the values obtained from the variants where composts were used were close to control resulting in 6.63 mg/100 g biomass. V5 and V12 had a higher content of cartoenoid pigments than the control by 8.74% and 2.11%, respectively.

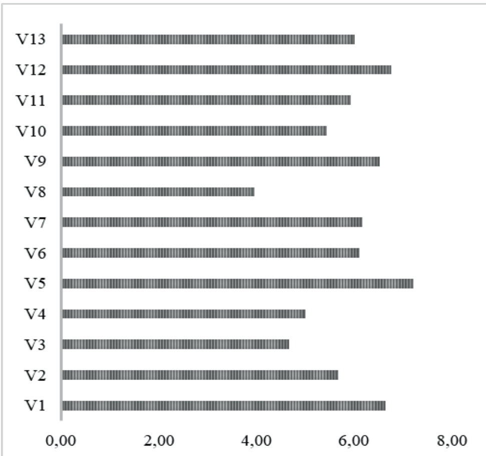


Figure 7. Carotenoid pigments in lettuce leaves (mg/100 g)

CONCLUSIONS

This study was carried out to verify the hypothesis that compost from biological waste can be an alternative source of nutrients for plants, which is confirmed by the results obtained, both related to biomass production and nutrient uptake by plants. lettuce (nitrogen, phosphorus, potassium, calcium and magnesium), which shows that, on the one hand, it is very important to know the physicochemical properties of composts used for soil cultivation.

Regarding the content of chlorophyll a and b and carotenoid pigments, there were differences between the variants that contained compost in the mixture and the control variant, depending on the type of compost used.

These results have important implications for sustainable agriculture and it is necessary to carry out more experiments in the field to have a clearer overview of the types of compost that can be used as fertilizing material for growing plants.

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POLLINATORS DIVERSITY IN RAPESEED CROPS OF SOUTH ROMANIA

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Abstract

The study on pollinators diversity in rapeseed crops was carried out in the spring season of 2024, in four sites located in South Romania. Rapeseed (Brassica napus L. ssp. oleifera (DC.) Metzg.) is an important oil crop in Romania reaching approximately one third of the total area of oilseed crops. The obtained data show that the observed pollinators belonged to four orders (i.e., Hymenoptera, Diptera, Lepidoptera, and Coleoptera), but Hymenoptera insects were the most numerous pollinators. The obtained results revealed that the major floral visitors of the Hymenoptera order belonged to Apidae family. Undoubtedly, the honey bee (Apis mellifera) was the most important visitors (about. 60%) followed by some species of solitary bees and bumblebees. The wild bees ranked a secondary place, they being known to have a significant contribution to increasing agricultural production. A high number of non-syrphid flies were also observed on flowers in rapeseed crops.

Key words*: rapeseed, pollination, insect pollinators.*

INTRODUCTION

Insects play a considerable role in providing services for human well-being (Jactel et al., 2021) since the world's agricultural crops depend, at least in part, on pollinators, mainly honey bees. Food crops such as apples, almonds, strawberries, onions, pumpkins, coffee, cocoa, watermelons and mangoes could be most at risk due to declining insect populations. With 70% of agricultural crops being pollinator dependent, and with the ongoing global decline in wild and managed pollinators, there is a growing concern that the negative impact of crop pollinators' dependence on global food production and stability will become even more pronounced (Adamidis et al., 2019).

Rapeseed (*Brassica napus* L. ssp. *oleifera* (DC.) Metzg.) is a globally important crop, benefitting by a mixed pollination system (self-pollination and cross-pollination). Although rapeseed is mainly considered a self-pollinating plant (Williams et al., 1986), cross-pollination is mediated by wind, insects and plants movement. The sugar content and flower morphology play an important role in attracting different pollinators (Shakeel et al., 2018). The floral physiology of rapeseed (i.e., bilaterally symmetrical yellow flowers with sticky pollen) and the high volume of nectar found in rapeseed flowers that is food resource to bees and other insects (Ion et al., 2012) implies rather the insects for the dispersion of pollen, and more less the wind (Adamidis et al., 2019).

Many authors have hypothesized that a good nectar secretion of the rapeseed flowers is associated with a better pollination due to the increased number of bees that visit the flowers. From this point of view, rapeseed is one of the main melliferous plants due to its nectar secretion and important number of flowers per plant (Ion et al., 2012). Even if the self-fertility of the rapeseed flowers is an important characteristic, the seed yields obtained without entomophilic pollination are significantly lower (Bommarco et al., 2012). Many studies conducted globally show the benefits of insect visitation in rapeseed crops production and quality (Bommarco et al., 2012; Stanley et al., 2013; Jauker & Wolters, 2008). Differences among rape seed varieties regarding the volume of nectar could explain the results regarding the effect of insect pollination on oilseed rape production (Ion et al., 2012). Rapeseed plants show variation in their ability to compensate for a pollination deficit, which may depend on the cultivar, climatic conditions and the pollination efficiency of flower visiting insects (Holzschuh et al., 2012; Hoyle et al., 2007, Stanley et al., 2013; Ion, 2021). Many researches have shown a positive relationship between crop yield, the diversity and abundance of insect pollinators and the amount of native vegetation in the area around the crop. Maybe it worth a more thorough examination of pollinator dependence (i.e. the extent that a rapeseed plant/variety depends on pollinator visitation for the fruit set) and its associated traits among rape seed varieties (Adamidis et al., 2019). On-going agricultural intensification threatens wild pollinator communities with negative consequences for crop productivity (Klein et al., 2007). Wild pollinator abundance and diversity contribute to oilseed rape yield by mediating increased allocation to seeds rather than above-ground biomass. The maintenance of large and varied pollinator habitats near crops is guaranteeing such beneficial effects from wild bees.

Although rapeseed has become an important crop in Romania in recent years, reaching approximately one third of the total area of oilseed crops, studies on the abundance and diversity of pollinating insects in rapeseed crops in Romania are missing or insufficient. Furthermore, the contribution of pollinating insects to crop yield has rarely been estimated. It is important to note that the yield of rapeseed crops is generally influenced by three components: the number of plants per unit area, the number of seeds per plant, and the seed weight. Considering that one of the main components of yield, namely the number of seeds per plant, depends on the quality of the pollination process, the correct management of the pollination process can influence the seed yield. Insects are widely used as indicators to evaluate ecosystem biodiversity and for environmental assessment, mainly because they are relatively small in size, widely distributed, inhabit complex and diverse environments, and are very sensitive to environmental changes (Zhao et al., 2023).

The present study started from the idea that there is a strong need to identify pollinators in rapeseed crops and to determine whether these insects visit the rapeseed flowers which could help the cross pollination. Therefore, understanding the current state in insect community composition is helpful to reveal the current status of insects' biodiversity in rapeseed fields and its maintenance mechanisms (Zhao et al., 2023). Our study is all the more necessary as there is an increasingly accentuated trend of the pollinator decline. The honey bee population losses in Romania (Căuia & Ion, 2023) could contribute to understand some difficulties in providing the necessary number of insects/area unit to assure quality pollination. Following these aspects, this study is focused on a preliminary evaluation the diversity and abundance of bees and other insect species present in comercial rapeseed crops from South Romania.

MATERIALS AND METHODS

The study was conducted in South of Romania, in Ilfov County (43°37'07" N, 25°23'32" E). The region lies in a favourable area for rapeseed (*Brassica napus* L. ssp. *oleifera* (DC.) Metzg.) cultivation in Romania. The region includes the main commercial rapeseed crops in intensive system.

The surveys of insects were undertaken in four commercial rapeesed crops, respectively located in Balotești - Dumbraveni (44.632284, 26.092778) (site 1), Balotești - Dimieni (44.604816, 26.133809) (site 2), Grădiștea (44.632492, 26.303770) (site 3) and Moara Domnească (44.496743, 26.232794) (site 4).

The rapeseed crops were chosen based on the presence of similar microtopography conditions, namely the presence in their immediate vicinity of some dispersed large patches of woody vegetation or proximal groups of smaller patches of woody vegetation such as forest edges, forest curtains, hedges, uncultivated lands and also household gardens. In these conditions, the insect species richness, diversity, and abundance can be expected to show a great level because of the relatively high richness and coverage of vegetation species from study areas.

Data gathering was performed in April 2024 during the flowering of the rapeseed crops. The year specific weather conditions led to an early blooming (1th April) as a result of high temperature values before the start of flowering, but also to an extension of the flowering period (15th May) as a result of temperatures falling below the specific average of the month and of the precipitations that occurred during flowering.

The transect method was used to evaluate the insects. Transects were laid in each rapeseed crop at least 50 m from the road to reduce border effects. Along each transect, 4 surveillance areas were established in each study site. At the start of each transect all insects seen on or near flowers were counted in an imaginary quadrat extending 1.5 m either side and 1.5 m out in front of the observer. The recording of insect species from each transect were for 10 minutes, between 11:00 and 13:00, and this was performed by two operators. Surveillance areas were established every 10 m along each transect. Local temperature and wind speed were recorded at the start of each transect as these can affect insects behaviour, although counting was conducted only at temperatures above 15°C to eliminate the impact of low temperatures on counts. These factors were included in initial analyses of data.

To assess the diversity and abundance of pollinating insects a **protocol** was used (Vaissière et al., 2011). In each rapeseed crop, the all observed insects both on flowers and leaves, in the surveillance areas, as well those in flight, were recorded and registered into six main categories (honey bees, wild bees, bumblebees, syrphids, butterflies, and other insects). Additionally, where possible, images of observed insects under rapeseed crops conditions were taken and then processed. Based on these, insects were classified as accurately as possible into genera and species according to some relevant reference materials (Packer, 2023). Insect specimens' identifications were confirmed with the aid of open-access galleries and databases of insect taxa (along with the references provided therein). Websites were also excellent sources for insect identification.

In order to simplify the identification and classification of bee insects (honey bees, wild bees and bumblebees) under field conditions, some observations were made on their behaviour of collecting pollen. Taking into account especially their various forms of adaptation for pollen collection, one grouped bee species into three categories, respectively bees that have the whole body covered with dense hairs and retain a large amount of pollen in the baskets, bees which have their hind legs covered with such a dense brush of bristles that the pollen is easily retained, and bees that collect and transport pollen on their abdomens.

In order to highlight the differences between the assessed groups of pollinators in the surveillance areas, the obtained results were analysed using the statistical software (NCSS, 2021) for descriptive statistic and ANOVA one-way tests. For a better view of differences, the data were presented by boxplot quartiles.

RESULTS AND DISCUSSIONS

A total of 678 individual insects were recorded in the surveillance areas of rapeseed crops, in the studied locations and throughout the evaluation period. They were distributed over six main insect categories, respectively honey bees, wild bees, bumblebees, syrphids, butterflies, and other insects. The abundance variability of the obtained data on different categories of insects is presented in the Figure 1 and Table 1. The average percentage of all recorded insects was the following: 61.1% honey bees, 9.4% wild bees, 4.1% bumblebees, 4.6% syrphids, 8.4% butterflies, and 12.4% other insects. Of these, dominant insect categories were honey bees and wild bees. Other insect categories accounted a little over 12% of the total individual insects.

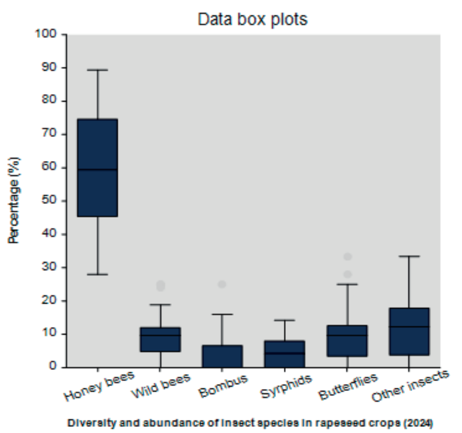


Figure 1. The abundance variability of insects in the categories evaluated in rapeseed crops

The results of our study showed that pollination of rapeseed crops have clearly been dominated by honey bees.

Table 1. A summary descriptive statistic for data gathered on all sampled sites during the study

Short descriptive statistics	Honey bees (n)	Wild bees (n)	Bombus (n)	Syrphids (n)	Butterflies (n)	Other insects (n)	Total insects (n)
Counts	28	28	28	28	28	28	28
Sum	414	64	28	31	57	84	678
Mean	14.79	2.28	1.00	1.11	2.04	3.00	24.21
St. Error	1.48	0.32	0.25	0.23	0.31	0.47	1.62
Lower 95% CL mean	11.75	1.63	0.49	0.64	1.39	2.04	20.88
Upper 95% CL mean	17.82	2.94	1.51	1.57	2.68	3.96	27.54
Min.	4	0	0	0	0	0	9
Max.	36	7	4	4	7	10	44

Although wild bees and bumblebees were less visible compared to honey bees, they are important and efficient as pollinators. It is known that worldwide, social bees such as the honey bee make up about 90% of the effective pollinating insects, several species of solitary bees (Andrenidae, Halictidae, Megachilidae and lower Apidae) and bumblebees (*Bombus* spp.) are as efficient (Mesquida et al., 1988) but they are less known and appreciated by farmers. Species of Diptera, Lepidoptera, Hemiptera and Coleoptera are less abundant, and less efficient in the rapeseed crop pollination.

Bee pollination of oilseed rape has been studied by numerous researchers who have demonstrated more or less appreciable effects, and sometimes drawn contradictory conclusions (Mesquida et al., 1988).

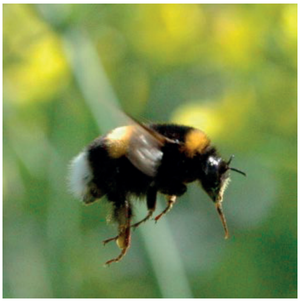
A possible explanation why bumblebees, as a semi-social species, had a relatively low number in rapeseed crops would be that this crop blooms early in season and the biologic cycles of insects does not allow an abundance in crops. In addition, in 2024 conditions, rapeseed crops had a much earlier flowering (early April). However, early rapeseed crops appear to be important for these species as they can contribute to increased populations for subsequent crops such as sunflower or other species of interest in neighbouring areas, thus leading to higher productivity and a better yield quality.

Variability of insects collecting pollen

Within the studied rapeseed crops it was found that solitary bees can often be confused with honey bees, wasps or even flies, as they show an extraordinary diversity in terms of morphology and foraging behaviour. The differences mainly concern the disposition and coloring of the hair that cover their bodies, the conformation of the legs etc. To determine the mechanism underlying the differentiation of them, and taking into account the various forms of adaptation for pollen collection (Figure 2), the following results were found:

- Bees that have the whole body covered with dense hairs and retain a large amount of pollen in the baskets: two insects species were identified in this category, namely honey bee (*Apis mellifera* - fam. Apidae) and the bumble bee (*Bombus terestris* - fam. Apidae).
- Bees which have their hind legs covered with a dense brush of bristles as the pollen to be very easy retained. In this category we have identified three insect species from wild bees' category, namely *Andrena flavipes* (fam. Andrenidae), *Andrena cineraria* (fam. Andrenidae) and *Lasioglossium* sp. (fam. Halictidae).
- Bees that collect and transport pollen on their abdomens covered with hairs. Members of this group were difficult or impossible to identify from field images. According with literature Osmia bees could be included in this category. Further researches would be necessary to make easier their identification and to inform farmers about the presence of Osmia into rapeseed crops. The solitary blue orchard bee (*Osmia lignaria*), compared with honeybees, flies early in the spring when it might still be too cold for honey bees, which means that they could appear in rapeseed crops even before flowering to improve the crops pollination.

Apis mellifera (Apidae)



Bombus terestris (Apidae)

Andrena flavipes (Andrenidae)

Andrena cineraria (Andrenidae)

Lasioglossium sp. (Halictidae)

Figure 2. Representatives of bees collecting pollen in rapeseed crops (Ilfov County, Romania, 2024)

A relatively high number of non-syrphid flies were observed on rapeseed flowers in all study sites (Figure 3). Non-syrphid flies have also been recorded in other studies on rapeseed crop (Garrat et al., 2014). The obtained data are similarly with other studies that registered non-syrphid Diptera as made up the majority of the flower-visiting Diptera in the agricultural studies. Although there are many records of non-syrphid Diptera visiting flowers, they are generally not regarded as important pollinators (Orford et al., 2015). Further research is necessary to understand their contribution on the rapeseed pollination.

Anthomyiidae

Chrysopidae

Lepidoptera

Figure 3. Some representatives of non-syrphid flies and butterflies category in the studied rapeseed crops (Ilfov County, Romania, 2024)

In the category "other insects" there were included various species of insects which are not specialised in pollination (known without specialized organs for the transport of nectar and pollen) as Coleoptera, Hemipterae, Orthoptera or Nemopterae. In the same category were recorded also the insects considered harmful to rapeseed crops, such as aphids (Aphididae), thrips (Thysanoptera), or the hairy beetle (Coleoptera) (Figure 4). Some of them lay their eggs in the flower, from which the larvae develop and spread the accumulated nectar, but also the pollen, thus causing damage to the pollination process (Figure 4). It has to be also mentioned the presence in this group of some useful insect species for rapeseed crops, which perform sanitary functions, such as the lacewing insect (from the Chrysopidae family) or the ladybug (from Coccinellidae family). The diversity of insect visitors to rapeseed crops has also been noticed in other studies, some showing an impact of seasonality and local landscape (Garrat et al., 2014).

Scarabaeidae | Scarabaeidae | *Brassicogethes aeneus* larvae

Figure 4. Some representatives of other insects' category in the studied rapeseed crops (Ilfov County, Romania, 2024)

Spatial variability of insect species

Regarding spatial variability of insect species, the obtained results of this study showed that the insect community had some different characteristics along the sites at the individual levels and insect categories, despite the fact that all study sites were selected to have similary microtopography conditions. The number of individual insects from one site was higher compared to the other 3 locations, even double (i.e. 247 individual insects in site 1 - Balotești-Dumbraveni, compared with 120 in site 3 - Grădistea) (Figure 5).

Honey bees and wild bees were dominant insect categories in all sites. In addition, these two insect categories seemed to be the most balanced. Bumblebees recorded the lowest value or even were missing in site 2 Balotesti-Dimieni. The obtained data confirm also that the morphology of the rapeseed flower allows insects belonging to many orders (Hymenoptera, Diptera and Lepidoptera) to collect pollen and nectar. There are studies showing that along with honey bees, many non-*Apis* bee species across multiple genera, including *Andrena*, *Agapostemon*, *Bombus*, *Lasioglossum*, *Halictus, Melissodes* have been observed visiting flowers in both conventional and organic canola fields (Esquivel et al., 2021). Authors as Pearson (1932) cited by McGregory (1976) considered that bees from various families as Andrenidae, Megachilidae and Nomadidae [= *Nomada* spp. of Anthophoridae] are more important than honey bees in the pollination of Brassica species, but they did not offered any information regarding the relative populations, either on the plants or in the area. Sneep (1952) cited by McGregory (1976) mentioned the presence of *Bombus* and *Psithyrus* in the area of rapeseed crops but only incidentally.

A commonality of rapeseed crops is that they produce large number of flowers that producing copious amounts of nectar, making them highly attractive to pollinators. Rapeseed is a mass-flowering crop which represents for approximately 4 weeks an important source of nectar for bees as well as for other insects. Rapeseed crops often exceed and surpass semi-natural habitats in the sheer quantity of floral resources they offer at a given time (Esquivel et al., 2021). A characteristic of rapeseed crops is that they bloom in the spring, sometimes in conditions of temperatures below the minimum 12ºC, the threshold temperature for honey bee flight, in windy or rainy conditions. Under these conditions, some wild bee populations, which search for food even at lower temperatures, could be effective pollinators of rapeseed crops.

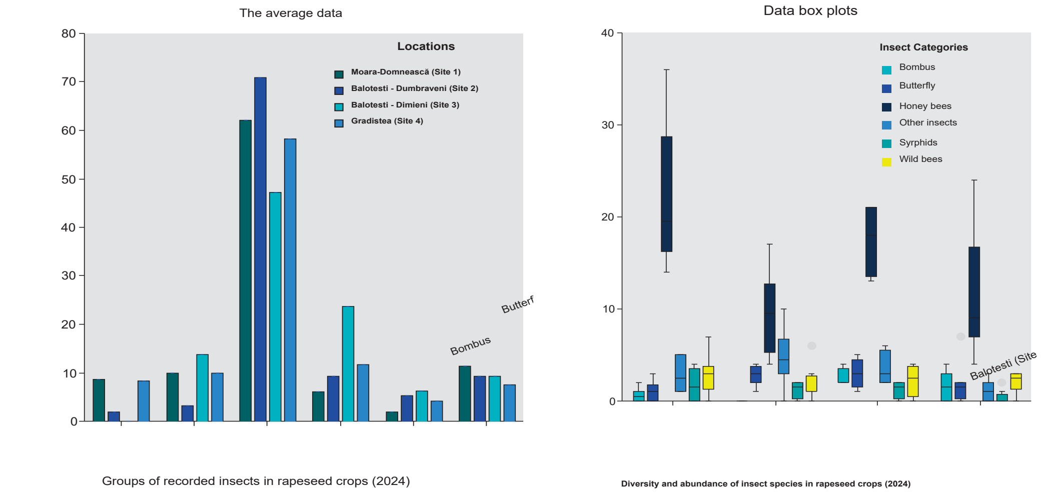


Figure 5. The variability of data regarding the insects' groups registered in the four studied locations

Westphal et al. (2003) cited by (Esquivel et al., 2021) demonstrated that densities of bumblebees were not related to the proportion of semi-natural habitat within an agricultural landscape, but were driven by the availability of mass-flowering crops in the landscape that were highly rewarding to foragers.

The abundance of insect species found on our study is particularly interesting not only in terms of biodiversity and biological resources, but also from a purely ecological point of view. The abundance of species reflects a diversity of both micro-habitats and resources, and may indicate a relative 'good health' of these micro-habitats cultivated with rapeseed (Aissat et al., 2023).

CONCLUSIONS

The obtained preliminary data highlighted that the structure of insect species as visitors in rapeseed crops was unequally distributed to the six main insect categories, respectively honey bees, wild bees, bumblebees, syrphids, butterflies and other insects.

Rapeseed in south of Romania (Ilfov county) was visited by almost 10 species of insect pollinators, but the dominant insect category was honey bee. The wild bee species, as specialised pollinators, were represented by *Andrena flavipes*, *Andrena cineraria* and *Lasioglossium* sp.

Among bees' category that have the whole body covered with dense hairs and retain a large amount of pollen in the baskets, two insects' species were identified, namely honey bee (*Apis mellifera* - fam. Apidae) and the bumble bee (*Bombus terestris* - fam. Apidae).

Among bees which have their hind legs covered with a dense brush of bristles for the pollen to be easily retained, three insect species were identified, namely *Andrena flavipes* (fam. Andrenidae), *Andrena cineraria* (fam. Andrenidae) *and Lasioglossium* sp. (fam. Halictidae).

The preliminary results showed also that the insect community registered some differences in the four studied loctions, at the insect category and species levels, despite the fact that all sites were selected to have similarly microtopography conditions.

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POLYCYCLIC AROMATIC HYDROCARBONS OCCURRENCE IN CEREAL BASED-PRODUCTS - A REVIEW

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants known to have toxic properties, carcinogenic and mutagenic potential, cereals and derivative products being the most important sources of PAH exposure to humans as a result of the high intake of this kind of products. The aim of this study was to investigate the occurrence of PAH in cereals and cereal-based products and the effect of different factors on the content of these compounds. The factors that influence the PAH content of cereal-based products were the raw material used in the recipe, the category of processed product, the baking parameters (time and temperature), the type of fuel used. The maximum level for benzo(a)pyrene (BaP) and sum of BaP, benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbF), and chrysene (ChR) in processed cereal-based food and baby foods for infants and young children was established by regulation no. 835/2011. This study can provide an overview of the PAH content of different cereal-based products commercialized on the market.

***Key words**: benzo(a)pyrene, bread, cereals, contamination, PAH.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) represent a class of organic, colourless, white or pale-yellow solid compounds (Abdel-Shafy and Mansour, 2016). These compounds are semi-volatile or non-volatile and ubiquitous. PAHs have two or more linear or angular condensed aromatic rings, the number varying from two to ten (Huang and Penning, 2014). Based on the number of fused aromatic rings, PAHs can be divided into light molecular PAHs (2-4 rings) and high molecular PAHs (> 5 rings).

PAHs can result from the incomplete combustion of organic material or heat-induced decomposition, being omnipresent pollutants in the environment (EFSA, 2008). The contamination sources can be natural or anthropogenic, PAHs being present in water, soil, air, and implicitly in cereals (Abdel-Shafy and Mansour, 2016).

PAHs have been classified by IARC as possible carcinogens (Group 2B), probable carcinogens (Group 2A), and carcinogens for humans (Group 1) (IARC, 2010). The United States Environmental Protection Agency (US EPA) have been listed 16 priority PAHs that poses carcinogenic properties as follows: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene (BaA), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), benzo (g,h,i)perylene, benzo(k)fluoranthene, chrysene (ChR), dibenzo(a,h)anthracene, fluoranthene, fluorene, indene(1,2,3-cd)pyrene, naphthalene, phenanthrene, and pyrene (US EPA, 2010).

In Europe, based on the risk potential EFSA (2008) selected 4 PAHs to be indicators for the carcinogenic PAHs: BaP, BaA, BbF, and ChR. Among all PAHs, BaP is considered as the most carcinogenic compound, being classified as a carcinogen (Group 1) (Joint FAO/WHO, 2005), while BbF, BaA and ChR were included in the class of possible carcinogens (Group 2B) (IARC, 2010).

Cereals are important sources for both human and animal nutrition, being widely consumed. All over the world, grains such as wheat, rice, corn and cereal derived products such as bread, biscuits, pasta and other cereal-based products are very high consumed. Cereals provide energy due to the vitamin, protein, lipid and mineral content (Kamalabadi et al., 2019; Rozentale et al., 2017).

Although processed cereals are in general characterized by low levels of PAH

contamination, according to EFSA, cereals, bread and cereal-based products are major sources of human exposure to PAHs (SCF, 2002; EFSA, 2008) mainly because of their high consumption volume (Ibáñez et al., 2005; Ciecierska and Obiedziński, 2013; Abramsson-Zetterberg et al., 2014; Escarrone et al., 2014).
Additionally, PAH contamination is inevitable in bread and cereal-based products due to the high temperature of the baking process. A maximum content of PAHs is thought to form when materials are heated to temperatures ranging between 500-550°C, while the bread baking temperature is around 250°C (McGrath et al., 2007).
The Commission Regulation (EU) no. 835/2011 does not establish the maximum level for PAHs present in cereals, it only set the maximum level of 1 μg/kg for BaP, and sum of BaP, BaA, ChR, and BfF in processed cereals and baby foods for infants and young children.
Research on the PAH content of cereals, flour, bread and cereal-based products is limited. However, when examining the existing studies, some conclusions can be reached regarding the occurrence and level of PAHs in cereals and cereal-based products, and the factors that influence the content of these compounds.
In this study we realized a literature review to analyse the content of PAHs in cereals and cereal derived products, and we also evaluated the influence of different factors on the level of these contaminants.

MATERIALS AND METHODS

Data search and selection criteria

The present literature review was performed in order to evaluate the PAH contamination of cereals and cereal-based products by performing a compressive search during 2010 until 2024 of the articles indexed in Web of Science, and also from the databases of Google Academic, and Scopus. Original research articles and reviews published in English were included in the search and the following terms were used: "*polycyclic aromatic hydrocarbons*", "*PAH*", "*benzo(a) pyrene*", "*BaP*", "*cereals*", "*flours*", "*bread*", "*pasta*", "*biscuits*", "*cereal products*", and "*cereal-based products*".
Once the search was realized, the articles were analysed and the most relevant manuscripts for this study were selected. Based on the selection, this review was synthesized and divided on the category of cereal derived products: cereals and flours, bread and other cereal-based products.

RESULTS AND DISCUSSIONS

PAH content in cereals and flours

The concentration of PAHs in cereals must be monitored, the content being influenced by the degree of contamination of the region in which the grains were cultivated, the degree of industrialization of the area, the harvest year, and the way of drying the cereals. The PAH content of cereals and flours reported in literature are presented in Table 1.
The PAH contamination of wheat was monitored in several countries, like Poland (Ciercierska and Obiedziński, 2013; Roszko et al., 2020; Thabit et al., 2022), Russia, Germany, France, Ukraine, Latvia, Lithuania, Estonia (Thabit et al., 2022), Romania (Muntean et al., 2019a; Thabit et al., 2022), California (Kobayashi et al., 2008), Pakistan (Aamir et al., 2021) and results showed that the content varies depending on the country of origin of the grains.
The PAH content of wheat samples is influenced by the geographical area where it was cultivated and the degree of pollution. As it can be noticed in Table 1, BaP was detected only in wheat samples from Poland (Roszko et al., 2020).
Muntean et al. (2019a) studied the PAH content in winter wheat cultivated in three different regions in Romania with different pollution levels, in the period 2012-2014, and determined the presence of BaA, naphthalene and anthracene. In this study, ChR was not detected, although when Thabit et al. (2022) studied the contamination level of wheat from Romania, it was determined a content of ChR of 3.70 μg/kg, with a degree of sample contamination of 90%. As expected, pollution had an effect on the PAH contamination, the most contaminated wheat samples being those cultivated in the high traffic area, with 15 PAH content of 7.83-8.67 μg/kg, followed by those from the experimental field (4.8-5.37 μg/kg),

and those in the area with historical contamination (3.06-3.80 µg/kg) (Muntean et al., 2019a). Contrary, in the study realized by Roszko et al. (2020) it was shown that the PAH content was not influenced by the region of cultivation of wheat sample from Poland.

Table 1. PAH content of cereals and flours from different regions

Cereal/ flour	**Country**	**BaP**	**BaA**	**ChR**	**BbF**	**Other PAHs in high proportion, µg/kg**	**4 PAH, µg/kg**	**Sum PAH, µg/kg**	**Reference**
		µg/kg							
Wheat	Poland	0.09	0.08	0.18	0.08	2.24 NAP	0.43	1.03 (16)	Roszko, 2020
	Poland	nd	0.05±0.00	nd	nd	1.14±0.09 PHN 0.58±0.14 FLT	*	2.35±0.15 (19)	Ciercierska, Obiedziński, 2013
	Poland	*	*	*	4.10	*	*	*	Thabit, 2022
	Russia	*	*	*	4.15	1.92 PHN	*	*	Thabit, 2022
	Germany	*	*	3.22	*	2.40 ANT	*	*	Thabit, 2022
	France	*	3.82	*	*	*	*	*	Thabit, 2022
	Ukraine	*	*	*	3.92	*	*	*	Thabit, 2022
	Latvia	*	4.20	*	*	*	*	*	Thabit, 2022
	Lithuania	*	*	*	*	5.86 FLT	*	*	Thabit, 2022
	Estonia	*	2.85	*	*	*	*	*	Thabit, 2022
	Romania	*		3.70	*	*	*	*	Thabit, 2022
	Romania	*	0.03-0.16		*	1.39÷3.60 NAP; 1.32÷2.93 FLR	*	*	Munteanu, 2019a
	Pakistan	*	*	*	*	*	*	169 (16)	Aamir et al., 2020
Maize	Pakistan	*	*	*	*	*	*	159 (16)	Aamir et al., 2020
Rice	Pakistan	*	*	*	*	*	*	53 (16)	Aamir et al., 2020
Rice	Spain	-	-	-	-	0.097±0.006 ÷ 5.5±0.3 ANT - ÷ 4.9±0.3 FLR	-	0.18÷10.54 (16)	Rascón et al., 2018
Corn	Romania	*	nd-0.07	*	*	1.23÷4.59 NAP 0.02÷0.25 ANT	*	*	Munteanu, 2019b
Barley	Poland	-	-	-	-	-	-	-	Thabit, 2022
	Russia		3.41	*	5.81	*	*	*	Thabit, 2022
	Germany	-	-	-	-	-	*	*	Thabit, 2022
	France	-	-	-	-	*	*	*	Thabit, 2022
	Ukraine		4.55	*	*	*	*	*	Thabit, 2022
	Latvia	*	*	5.61	*	*	*	*	Thabit, 2022
	Lithuania	*	*	*	*	6.21 FLR	*	*	Thabit, 2022
	Estonia	-	-	-	-	*	*	*	Thabit, 2022
	Romania	-	-	-	-	*	*	*	Thabit, 2022
Rye	Poland	nd	0.06±0.00	nd	nd	1.45±0.23 PHN 0.19±0.04 FLT	*	2.93±0.22 (19)	Ciercierska, Obiedziński, 2013
Cereals	Latvia	0.061	0.121	0.288	0.139	*	0.61	*	Rozentale et al., 2017
Wheat flour	Poland	nd	nd	nd	nd	0.64±0.09 PHN 0.19±0.01 FLT	*	1.07±0.14 (19)	Ciercierska, Obiedziński, 2013
Wheat flour	Spain	-	-	-	-	0.013±0.001 NAP 0.79±0.04 ANT	-	0.803 (16)	Rascón et al., 2018
Corn flour	Spain	-	-	-	-	0.086±0.005 NAP 2.4±0.2 ANT	-	2.486 (16)	Rascón et al., 2018
Rye flour	Poland	nd	nd	nd	nd	0.59±0.11 PHN 0.20±0.01 FLT	*	1.25±0.15 (19)	Ciercierska, Obiedziński, 2013
Wholemeal rye flour	Poland	nd	nd	nd	nd	0.51±0.11 PHN 0.21±0.01 FLT	*	1.35±0.20 (19)	Ciercierska, Obiedziński, 2013
Wheat bran	Poland	nd	0.05	nd	0.11	0.79±0.05 PHN 0.17±0.04 FLT	*	1.87±0.18 (19)	Ciercierska, Obiedziński, 2013
Rye bran	Poland	nd	0.05	nd	nd	0.76±0.09 PHN 0.59±0.01 FLT	*	2.65±0.17 (19)	Ciercierska, Obiedziński, 2013
Bran from rye grinding-wholemeal rye flour	Poland	nd	0.08	0.16	nd	1.19±0.11 PHN 0.55±0.03 FLT	*	3.65±0.23 (19)	Ciercierska, Obiedziński, 2013
White flour	Kuwait	nd	nd	nd	nd	4.33 NAP; 31.4 PHN; 5.04 FLT	*	43.1 (16)	Al-Rashdan et al., 2010
Wholewheat flour	Kuwait	nd	nd	nd	nd	12.9 NAP; 32.0 PHN; 8.14 FLT	*	56.4 (16)	Al-Rashdan et al., 2010
White rice dried by LPG, in oven and by wood	Brazil	nd	nd	nd	nd	7.0 NAP 1.4-2 PHE	*	*	Escarrone et al., 2014
Parboiled rice dried by LPG, in oven and by wood	Brazil	nd	nd	nd	nd	1.0-6.0 NAP 1.0 PHE 1.0 FLT	*	*	Escarrone et al., 2014
Brown rice, parboiled	Brazil	nd	nd÷1.5±0.7	0.9±0.1 ÷	nd	3.5±0.7 ÷ 81.4±0.0 PHE 6.2±0.8 ÷ 35.6±0.5 NAP	*	12.7÷131.6 (16)	Bertinetti et al., 2018

Cereal/	Country	BaP	BaA	ChR	BbF	Other PAHs in high	4 PAH,	Sum PAH,	Reference
brown rice, polished rice dried by wood				3.3±0.7					
Brown rice, parboiled brown rice, polished rice dried by rice husk	Brazil	nd	nd÷1.1±0.2	0.9±0.3 ÷ 4.9±0.7	nd	0.4±0.2 ÷ 44.4±1.9 PHE 4.7±0.1 ÷ 14.1±1.5 NAP	*	6.6÷120.8 (16)	Bertinetti et al., 2018
Brown rice, parboiled brown rice, polished rice dried by LPG	Brazil	nd	nd	nd÷ 2.4±0.2	nd	nd ÷ 22.9±1.3 PHE nd ÷ 9.9±1.8 NAP	*	nd÷63.9 (16)	Bertinetti et al., 2018
Brown rice, parboiled brown rice, polished rice dried by electric heating	Brazil	nd	nd	nd	nd	nd ÷ 4.0±0.9 PHE 2.9±0.3 ÷ 5.5±0.6 NAP	*	2.9÷9.1 (16)	Bertinetti et al., 2018

BaP- benzo(a)pyrene, BaA- benzo(a)anthracene, ChR- chrysene, BkF- benzo(k)fluoranthene, NAP- naphthalene, PHN-phenanthrene, FLT-fluoranthene, ANT- anthracene, FLR- fluorene, nd- not detectable, LPG- liquefied petroleum gas,
"-" compound not found, *- compounds were not studied or reported. PAHs are presented as range or as mean, as reported in references. The number in parentheses indicates the number of compounds included in the reported sum.

The harvest year had no influence on the PAH content of wheat samples cultivated in the same country (Munteanu et al., 2019a; Roszko et al., 2020).

Low molecular weight PAHs were mainly found in wheat samples cultivated in different European countries (Ciecierska and Obiedziński, 2013; Muntean et al., 2019a; Roszko et al., 2020; Thabit et al., 2022), phenanthrene, anthracene, fluoranthene, fluorene, naphthalene, BaA, ChR and BbF being detected, their concentration differing according to the area of origin, the degree of positivity of the samples being over 50% (Thabit et al., 2022).

Also, in a study conducted in California, the predominant PAHs in wheat cultivated in the same season and harvested from different geographical areas were the low molecular weight ones, with 2 to 4 rings, with naphthalene being the most abundant PAH (Kobayashi et al., 2008). In general, PAH profiles suggest contamination of wheat from emission sources resulting from combustion (liquid fossil fuel, coal, biomass).

When it comes to barley samples collected from different European countries, fluoranthene, BaA, ChR and BbF contaminated the samples in a proportion greater than 70% (Thabit et al., 2022).

Additionally, Muntean et al. (2019b) determined the degree of contamination of corn cultivated in regions from Romania with different pollution degrees, and the most contaminated samples were those from the area with heavy traffic, the PAH content varying between 6.34-9.28 µg/kg.

Compared to the results reported in European countries, in Pakistan, Aamir et al. (2021) determined a higher content for 16 PAHs in cereals (maize, rice and corn), probably as a consequence of pollution. A much lower content of 16 PAH was determined for the rice cultivated in Spain (Rascón et al., 2018) compared to the results reported by Aamir et al. (2021).

Besides cereals, flours were also analysed to determine the PAH contamination. The wheat, corn, rye, and wholemeal rye flours didn't present the 4 carcinogenic PAHs (Al-Rashdan et al., 2010; Ciecierska and Obiedziński, 2013; Rascón et al., 2018). BaA was detected in the bran of wheat, rye, and the mixture of rye bran and wholemeal rye flour brans, BbF was detected in wheat bran, and ChR was detected in the bran flours mixture. The PAH content of wheat and rye flours from the European countries (0.803-1.25 µg/kg) was lower than the PAH content of brans (1.35-3.65 µg/kg). The wheat, rye and wholemeal flours analysed by Ciecierska and Obiedziński (2013) were characterized by a high content of light PAH (phenanthrene, anthracene, fluoranthene and pyrene) of 93, 80 and 61% of all PAHs. The brans obtained from these cereals presented a

content of light PAH of 70, 63 and 74%, respectively.
The 16 PAH content of white and wholewheat flours from Kuwait was much higher (43.1-56.4 μg/kg) than the content of 19 HAP of wheat flours from Europe (Al-Rashdan et al., 2010). This contamination might be the result of pollution of the region in which the grains were cultivated (Kobayashi et al., 2008; Abdel-Shafy and Mansour, 2016; Muntean et al., 2019a).
A common practice for maintaining the quality of grain is to dry it to a specific moisture content and maintain it. Reducing the moisture content is achieved in most countries by burning gasses and wood, hence exposure to smoke. It was observed that by drying white rice by burning wood and by using the oven, phenanthrene was accumulated at a concentration of 1.4 and 2 μg/kg, respectively (Escarrone et al., 2014). By drying white rice with liquefied petroleum gas (LPG) the formation of naphthalene (7 μg/kg) resulted, which was reduced by 14% when parboiled rice was used. In parboiled rice dried in oven, a 4 μg/kg naphthalene content was determined, while when this rice was dried by wood, besides naphthalene (5 μg/kg), phenanthrene (1 μg/kg), and fluoranthene (1 μg/kg) were found (Escarrone et al., 2014).
Contrary, when Bertinetti et al. (2018) studied the influence of drying method on the 16 PAH content of rice, the highest concentration of 16 PAHs (131.6 μg/kg) resulted when wood was used for drying which was 2.9-fold lower for rice husk, 8.3-fold lower for LPG, and 17.1-fold lower when electric heating was used. The concentration of PAHs was found to increase by 120.6-663.3% in the case of rice that was parboiled and dried with different fuels. Increasing the drying temperature from 40°C to 60°C increased PAH contamination by 64.39% (Bertinetti et al., 2018). As in the study realized by Escarrone et al. (2014), the predominant PAHs were naphthalene and phenanthrene.

PAH content in bread products

The occurrence and the PAH formation in the bakery chain, from raw materials to finished products, have been studied by several authors (Ciecierska and Obiedziński, 2013; Rozentale et al., 2017).
Contamination of bread with PAHs can depend both on the contamination of the raw bakery materials (Ciecierska and Obiedziński, 2013), mainly flour, and on the baking process (Chawda et al., 2017; Karşi et al., 2021). An important issue is also the temperature of the heat treatment, taking into account its impact on the level of contamination of the bread.
The PAH content of bread made with wheat flour (Rozentale et al., 2017; Al-Rashdan et al., 2010; Kacmaz, 2016b), rye flour (Ciecierska and Obiedzinski, 2013; Rozentale et al., 2017), and wholemeal rye flour (Ciecierska and Obiedzinski, 2013) commercialized in different countries is synthesized in Table 2. Results showed that the contamination with PAHs of breads was influenced by the flour used, but also by the country of production and assortment.
Rascón et al. (2018) studied the contamination level with the EPA's 16 PAH content of breads (breadcrumbs, breadsticks, wheat cakes and flat bread) marketed in Spain and determined a content ranging between 1.55 and 8.77 μg/kg. Samples were characterized by a high content of anthracene of 0.07-4.8 μg/kg. From the 4 priority PAH only ChR was found in bread.
Additionally, the highest percentage of 4 HAPs for wheat and rye breads sold on the Latvian market, was represented by ChR, with a content ranging between 0.108-0.611 μg/kg (Rozentale et al., 2017). The mean concentration of BaP in bread made with wheat flour was 0.064 μg/kg, while the level of 4 PAHs was 0.49 μg/kg. Among the analysed samples marketed in Latvia, rye bread was the most contaminated with PAHs, with an average content of 0.084 μg/kg for BaP, and 0.71 μg/kg for 4 PAHs, respectively. A total of 14% of the analysed bread samples from Latvia (35) exceeded the 4 PAH level of 1 μg/kg settled by the EU regulation (Rozentale et al., 2017).
The contamination with PAH was also investigated for bread marketed in Kuwait (Al-Rashdan et al., 2010). The content of 16 PAHs in bread sold in this country varied between 1.06 and 287.5 μg/kg, higher than the ones reported in Spain (Rascón et al., 2018), Latvia (Rozentale et al., 2017), and Iran (Kamalabdai et al., 2019) for the wheat bread. Among the 16 analysed bread samples from Kuwait, 5 samples exceeded the BaP level (5.57-16.5 μg/kg) imposed by the European legislation;

for the rest of the samples BaP was not detected. Ciecierska and Obiedzinski (2013) determined the amount of 19 PAH in raw materials, doughs and breads baked at different conditions (temperature 230-260°C, time 40 min).

Table 2. PAH content of bread commercialized in different regions

Bread	Country	BaP	BaA	ChR	BbF	Other PAHs in high proportion, µg/kg	4 PAH, µg/kg	Sum PAH, µg/kg	Reference
		µg/kg							
Bread (breadcrumb, breadsticks, wheat cakes and flat bread)	Spain	-	-	- ÷ 0.67±0.04	-	0.07±0.004 ÷ 4.8±0.3 ANT - ÷ 3.2±0.2 FLU	- ÷ 0.67	1.55÷8.77 (16)	Rascón et al., 2018
Commercial bread	Spain	-	-	- ÷ 0.48±0.03	-	0.041±0.002 ÷ 0.59±0.03 NAP 0.42±0.02 ÷ 2.4±0.1 ANT	- ÷ 0.48	1.29÷4.8 (16)	Rascón et al., 2018
Commercial bread home-toasted	Spain	- ÷ 0.98±0.05	-	0.24±0.01 ÷ 1.8±0.1	-	1.5±0.1 ÷ 3.8±0.2 NAP 0.97±0.05 ÷ 7.7±0.4 ANT	0.24 ÷ 3.45	10.31÷17.82 (16)	Rascón et al., 2018
White bread	Kuwait	nd÷16.5	nd÷2.31	nd÷1.53	nd÷7.27	*	*	1.06÷287.5 (16)	Al-Rashdan et al., 2010
Sandwich white bread	Kuwait	nd÷9.04	nd÷6.22	nd÷4.19	nd÷5.58	*	*	28.3÷149.3 (16)	Al-Rashdan et al., 2010
Bread	Belgium	<0.03÷0.20	*	*	*	*	0.11÷0.22	*	Kacmaz et al., 2016a
Bread	Turkey	0.17±0.05	0.03±0.02	0.05±0.03	0.05±0.02	*	0.28±0.09	*	Kacmaz, 2016b
Wheat bread	Latvia	0.064	0.094	0.202	0.133	*	0.49	*	Rozentale et al., 2017
Rye bread	Latvia	0.084	0.178	0.279	0.166	*	0.71	*	Rozentale et al., 2017
Wheat-rye bread baked at different temp	Poland	nd	0.05÷0.09	nd÷0.23	nd÷0.15	0.65÷2.28 PHN 0.49÷1.96 FLT	*	1.59÷7.37 (19)	Ciercierska, Obiedziński, 2013
Rye bread baked at different temp	Poland	nd	0.07÷0.18	0.15÷0.27	nd	2.57÷5.99 PHN 1.58÷3.21 FLT	*	5.62÷13.04 (19)	Ciercierska, Obiedziński, 2013
Wholemeal rye bread baked at different temp	Poland	nd÷0.24	0.06÷0.29	0.15÷0.53	nd÷0.23	1.67÷5.65 PHN 0.49÷3.62 FLT	*	2.67÷13.55 (19)	Ciercierska, Obiedziński, 2013
Brown bread	Kuwait	nd÷11.1	nd÷1.79	nd÷0.7	nd÷3.32	*	*	29.2÷56.4 (16)	Al-Rashdan et al., 2010
Senan bread-industrial	Iran	nd	-	-	-	*	*	*	Eslamizad et al., 2016
Sangak bread-traditional	Iran	1.97	-	-	-	*	*	*	Eslamizad et al., 2016
Sangak bread	Iran	-	-	-	-	1.7± - PYR 0.59±0.31 ANT 0.40±0.37 FLT	*	2.25±2.05 (8)	Khaniki et al., 2021
Sangak bread	Iran	-	-	-	-	5.57±5.21 PHN 14.59±5.52 ANT	*	10.08±6.38 (10)	Peiravian et al., 2021
Lavash bread-traditional	Iran	nd	nd÷2.12 (0.14)	nd÷2.58 (0.17)	nd÷2.58 (0.33)	50.10 NAP; 4.50 ACE; 0.68 PHN	*	64.87 (16)	Kamalabdai et al., 2019
Taftoon bread-traditional	Iran	nd	nd÷9.20	nd÷11.22	nd	60.62 NAP; 1.76 PHN; 1.73 ANT	*	68.97 (16)	Kamalabdai et al., 2019
Baguette - industrial	Iran	nd	nd÷5.15	nd÷7.16	nd	61.51 NAP 4.48 PYR 3.35 ACE	*	118.66 (16)	Kamalabdai et al., 2019
Taftoon bread	Iran	-	-	-	-	0.88±1.1 FLT 0.59±0.34 ANT 0.54±0.17 PYR	*	1.15±1.03 (8)	Khaniki et al., 2021
Barbari bread	Iran	-	-	-	-	1.06±1.15 FLT 0.99±0.75 PYR 0.62±0.25 ANT	*	2.64±2.15 (8)	Khaniki et al., 2021
Lavash bread	Iran	-	-	-	-	0.9±- PYR 0.73±0.3 ANT 0.48±0.24 ACY	*	2.25±1.67 (8)	Khaniki et al., 2021
Industrial bread	Iran	-	-	-	-	1.23± - PYR 0.43±0.17 ANT 0.35±0.18 NAP	*	1.66±1.49 (8)	Khaniki et al., 2021
Tandoor bread	India	1.18÷17.41	4.12÷14.51	0.24÷ 12.08	0.52÷8.92	3.14÷51.88 PHN 5.22÷31.8 ANT	*	113.36÷211.19 (16)	Chawda et al., 2017

Bread	Country	BaP	BaA	ChR	BbF	Other PAHs in high proportion, µg/kg	4 PAH, µg/kg	Sum PAH, µg/kg	Reference
		µg/kg							
						15.52÷49.83 FLT			
Tawa bread	India	0.19÷2.62	0.44÷3.47	15.37÷20.09	0.18÷2.67	7.31÷11.15 PHN 0.35÷5.34 ACE 3.41÷10.17 FLT	*	59.64÷77.12 (16)	Chawda et al., 2017
Bread baked at household electricity	Turkey	nd**	0.02±0.004**	0.013±0.003**	nd**	7.63±1.85 ACE** 3.12±0.35 NAP**	*	2.68±3.33**(16)	Karşi et al., 2023
Bread countryside firewood	Turkey	0.013±0.014**	0.035±0.021**	0.32±0.30**	nd**	3.57±2.74 ACE* 2.58±1.24 NAP*	*	2.71±3.23** (16)	Karşi et al., 2023
Bread-commercial firewood	Turkey	0.059±0.052**	0.092±0.040**	0.125±0.034**	nd**	1.08±0.387 NAP* 1.68±0.358 FLR* 1.64±0.552 PHE*	*	4.40±1.52**(16)	Karşi et al., 2023
Bread – commercial natural gas	Turkey	0.023±0.020**	0.078±0.045**	0.059±0.021**	nd**	1.19±0.702 NAP* 1.31±0.255 FLR*	*	3.58±1.74** (16)	Karşi et al., 2023

BaP- benzo(a)pyrene, BaA- benzo(a)anthracene, ChR- chrysene, BkF- benzo(k)fluoranthene, NAP- naphthalene, PHN-phenanthrene, FLT- fluoranthene, ANT- anthracene, FLR- fluorene, PYR- pyrene, ACE- acenaphthene, ACY- acenaphthylene, nd- not detectable, "-" compound not found, *- compounds were not studied or reported, **- expressed in µg/kg d.w. PAH are presented as range or as mean, as reported in references. The number in parentheses indicates the number of compounds included in the reported sum.

For all types of bread obtained, the PAH content was higher after baking than in the dough, increasing as the baking temperature elevated, confirming the fact that PAHs are formed during heat treatment. Among all 19 PAHs determined, 4 low molecular weight PAHs (phenanthrene, fluorene, pyrene and anthracene), account for about 90% of the total PAHs. The 4 EU marker PAHs were also reported to be detected at low levels. BaP was detected only in the crust of wholemeal rye bread (0.24 µg/kg) baked at the highest temperature (260°C). Regardless of bread type and baking temperatures, the concentration of 19 PAHs in bread portions followed the trend: crumb < loaf of bread < crust. For the bread obtained from a mixture of wheat and rye flour, the content of 19 PAH varied between 1.59-7.37 µg/kg, for that obtained from rye between 5.62-13.04 µg/kg, and for that from whole rye flour it varied between 2.67-13.55 µg/kg (Ciecierska and Obiedzinski, 2013), showing that the contamination is dependent on the type of flour used.

Kacmaz et al. (2016a) studied the level of the 4 EU priority PAHs in bread samples traded on the Belgium market and determined an average content ranging between 0.11-0.22 µg/kg. BaP content ranges from below the LOQ (0.03 µg/kg) to 0.20 µg/kg. In another study realized by the same author, it was found that the total content of 4 PAHs in bread commercialized in Turkey varied between 0.16-0.46 µg/kg with an average of 0.28 µg/kg. The largest share in the sum of 4 PAHs is represented by BaP with an average content of 0.17 µg/kg (Kacmaz, 2016b).

The level of BaP in traditional Sangak bread traded in the Iranian market was determined by several authors (Eslamizad et al., 2016; Khaniki et al., 2021; Peiravian et al., 2021). The BaP content determined ranged between undetectable and 1.97 µg/kg. In addition to BaP, Peiravian et al. (2021) detected the presence of phenanthrene and anthracene in the analysed breads, while Khaniki et al. (2021) determined the presence of pyrene, anthracene and fluoranthene.

In the study realized by Eslamizad et al. (2016) it was also analysed the bread obtained under industrial conditions (Senan), and BaP was not detected in these samples. Also, Kamalabadi et al. (2019) determined the content of 16 PAHs in 117 assortments of traditional bread (62 Lavash and 55 Taftoon) and 52 assortments of industrial bread (baguette) sold in the Iranian market. Lavash bread is 2-3 mm thickness and baked at 332°C, 80 s, while Taftoon bread is 3-5 mm thickness and baked at 315°C, 2.5 min. The baguette, on the other hand, is 5-6 cm thickness and is baked at a lower temperature (245°C), for a longer time (14 min). The most abundant PAH detected was naphthalene (average 57.41 µg/kg). BaP content was below the LOD value (0.2 µg/kg). The bread obtained under industrial conditions presented the highest average content of 16 PAH (118.66 µg/kg) as a result of the greater thickness of the bread and the longer baking time compared to the other analysed breads. Taftoon bread had higher PAH content compared to Lavash bread due to longer baking time and baking temperature (Kamalabadi et al., 2019).

In another study carried out in Iran, four traditional bread assortments (Taftoon, Barbari, Lavash, and Sangak) and one industrial assortment sold on the local market were analysed from the point of view of the content of 8 PAHs (Khaniki et al., 2021). With the exception of Taftoon bread which had a PAH content of 1.15 μg/kg, the other assortments of traditional bread had a content of 2.25-2.64 μg/kg, higher than the one obtained for industrial bread (1.66 μg/kg). Contrary to the study of Kamalabadi et al. (2019), in general the level of PAHs was higher in traditional breads, compared to those obtained industrially. From the analysed bread, Barbari bread, which is a barley bread, had the highest PAH content.

In the assortments of breads commercialized in Iran, naphthalene, phenanthrene, fluoranthene, anthracene, acenaphthylene, and pyrene were also determined (Khaniki et al., 2021).

In addition to the grain harvesting region, the type of flour used, and the bread assortments specific to each country, the PAH content of these products is also influenced by the baking method, the type of oven and fuel used for burning.

The effect of baking method on PAH concentration in bread was reported by Chawda et al. (2017) who studied the influence of two ovens, with coal and with gas on the Tandoori and tawa breads, respectively, sold in the Indian market. The authors obtained a concentration of 16 PAHs ranging between 113.36-211.19 μg/kg in the first mode of baking for Tandoori bread, and for the second mode (tawa bread), the level was between 59.64-77.12 μg/kg. The level of 16 PAHs in bread baked with gas oven was about 2.5-3.5-fold lower than for bread baked with coal ovens. In both assortments of breads, 3- and 4-ring PAHs were predominant.

By using the gas as a heating source for the oven, bread presented a higher content of ChR than when the coal was used as a thermal agent. Additionally, Karşi et al. (2021) investigated the effect of different fuel ovens on the 16 EPA priority PAHs in bread samples baked in Turkey. The bread was made in 6 commercial bakeries using different oven fuels (2 using firewood and 4 of them using natural gas), 5 traditional countryside bakeries (firewood) and 2 ovens of household (electricity). PAHs with 2 to 4 rings were predominant in the bread samples. By using electric ovens, the lowest content of 16 HAP was obtained (2.68 ± 3.33 μg/kg). When wood-fired ovens were used, the content of 16 PAHs was higher for breads made in commercial bakeries (4.40 ± 1.52 μg/kg) compared to those made in traditional bakeries (2.71 ± 3.23 μg/kg). For the samples baked in natural gas ovens, a PAH content of 3.58 ± 1.74 μg/kg was obtained. In conclusion, for samples baked in wood-fired ovens (rural and commercial ovens) the level of 4 PAHs found was 2 to 10-fold higher than when ovens with natural gas or with electricity were used (Karşi et al., 2021).

Bread can be subjected to toasting, a common method by which slices of bread are heated before consumption, and like this being contaminated with PAHs. Rey-Salgueiro et al. (2008) studied the effect of sandwich bread toasting treatment on PAH levels using several conditions: direct toasting (with flame, coal-grill or gas oven) or indirect toasting (with electric oven). By using the electric oven and the toaster with gas the formation of PAHs in toasted bread was not reported, while when using the grill and flame the PAHs were detected. These differences may occur as a result of the deposition of PAHs from the smoke resulting from roasting. During electric oven toasting, the contamination with PAH can be the result of macronutrients pyrolysis (Rey-Salgueiro et al., 2008).

Also, Rascón et al. (2018) analysed the effect of toasting in an electric home toaster on the level of 16 PAHs in wholegrain, multiseed, black bread, sliced wheat bread, and white bread. BaP was determined only in samples subjected to the toasting treatment. For the non-toasted samples, the highest content of 16 PAH was determined for the black bread (4.8 μg/kg) and for whole grains breads (4.49 μg/kg). By subjecting the samples to heat treatment, the level of 16 PAH increased considerably being 3.7-, 5.61-, 2.71-, 7.99-, and 5.07-fold higher for wholegrain, multiseed, black bread, sliced bread and white bread, respectively, compared to untoasted bread. All samples presented naphthalene and anthracene in high concentrations. By toasting, the level of 4 HAP exceeded the legal limit allowed.

Thus, it can be concluded that by subjecting bread samples to thermal treatments, the level of PAH contamination increases considerably, being influenced by temperature, time and type of fuel used.

PAH content in other cereal-based products

In addition to bread, other cereal products such as cookies, breakfast cereals, biscuits and pasta sold in different markets have been analysed for the PAH content and the reported results are presented in Table 3.

The PAH contamination of these samples is influenced by the raw materials used (Rascón et al., 2018), the ingredients added (Kacmaz, 2016b), and also by the technological process and the fuel used for the thermal treatment.

The content of 4 PAHs in breakfast cereal samples traded on the Turkish market varied between 0.07-0.87 µg/kg, with an average of 0.37 µg/kg (Kacmaz, 2016b). The breakfast cereals analysed in this study included major ingredients, either alone or in a mixture: rice, oats, bran and fruits, wheat, maize, and chocolate powder. The largest share in the sum of 4 PAHs was represented by BaP with an average concentration of 0.22 µg/kg. The breakfast cereals (granola, chocolate granola and milk-filled cereal) commercialized on the Spanish market, presented a higher content of BaP, ranging between 3.1-4.2 µg/kg (Rascón et al., 2018). This can be the result of the high temperatures used in their production, the fat presence and also the ingredients drying process. The content of 4 PAHs for these samples exceeded the limit of 1 µg/kg imposed by the Commission Regulation (EU) no. 835/2011.

Table 3. PAH content of processed cereals from different regions

Processed cereals	Country	BaP	BaA	ChR	BbF	Other PAHs in high proportion, µg/kg	4 PAH, µg/kg	Sum PAH, µg/kg	Reference
		µg/kg							
Breakfast cereals	Turkey	0.22±0.07	0.09±0.06	0.11±0.06	0.08±0.04	*	0.37± 0.26	*	Kacmaz, 2016b
Breakfast cereals	Spain	3.1±0.2 ÷ 4.2±0.2	-	- ÷1.9±0.1	-÷3.5±0.2	- ÷ 3.9±0.2 NAP 0.08±0.004 ÷ 3.3±0.2 ANT	- ÷ 7.7	3.5÷22.3 (16)	Rascón et al., 2018
Cookies	Spain	-	-	- ÷ 0.35±0.02	-	0.26±0.01÷0.72±0.04 NAP 1.4±0.1÷2.9±0.2 ANT	-	1.66÷3.97 (16)	Rascón et al., 2018
Short cake	Nigeria	6.8±10.6	8.3±16.7	6.8±13.3	36.7±86.6	36.35±88.9 NAP 74.8±187.0 ACY 53.4±95.7 ACE	-	303.9±237.7 (16)	Iwegbue et al., 2015
Digestive biscuits	Nigeria	1.1 ± 2.3	2.4±3.4	10.9±16.9	9.8±11.6	43.0±44.6 ACY 39.2±65.0 PHE 78.3±113.3 ANT	-	271.4±192.4 (16)	Iwegbue et al., 2015
Cookies	Nigeria	nd	1.8±1.6	14.2±27.1	70.1±124.9	47.5±33.6 ACY 54.5±88.1 ANT	-	219.6±212.7 (16)	Iwegbue et al., 2015
Shortbread	Nigeria	0.1±0.1	28.7±55.6	22.0±30.1	53.1±41.9	69.3±45.3 ANT 37.9±27.4 PYR	-	340.9±227.9 (16)	Iwegbue et al., 2015
Wafers	Nigeria	10.3±22.2	7.7±12.7	16.8±11.4	39.2±68.2	96.2±146.7 NAP 16.8±21.2 PYR	-	236.8±121.3 (16)	Iwegbue et al., 2015
Cream crackers	Nigeria	nd	0.4±0.5	2.9±3.7	3.3±4.4	49.9±57.4 ANT 105.5±116.2 ACY	-	276.9±275.1 (16)	Iwegbue et al., 2015
Pringles	Nigeria	14.2±18.8	2.0±1.9	17.5±24.1	3.9±3.2	61.2±92.3 NAP 82.1±53.6 ACY	-	224.7±90.1 (16)	Iwegbue et al., 2015
Pasta	Spain	-	-	- ÷ 0.92±0.06	-	0.018±0.001 ÷ 0.24±0.01 NAP 0.15±0.01 ÷ 0.99±0.05 ANT	- ÷ 0.9	0.168÷1.98 (16)	Rascón et al., 2018
Pasta/ noodles	Nigeria	357±580 ÷ 834±1050	- ÷ 1270±590	- ÷ 871±140	-÷ 1490±240**	- ÷ 1008±950 PHN	564±610 ÷ 5615±930 **	564±610 ÷ 7889±730	Charles et al., 2017
Pasta locally produced	Nigeria	0.2±0.0 ÷ 3.0±2.0	0.3±0.3 ÷ 7.0±1.0	0.1±0.0 ÷ 2.0±1.0	0.1±0.0 ÷ 2.0±3.0	nd – 300.0±20.0 ANT nd – 300.0±30.0 PHE	1.0±0.0 ÷ 10.0±0.0	9.0±2.0 ÷ 800.0±30.0	Ihedioha et al., 2019
Pasta imported	Nigeria	0.2±0.1 ÷ 0.7±0.3	nd ÷ 1.0±0.0	nd ÷ 0.3±0.0	0.2±0.1÷ 0.7±0.2	nd – 4.0±0.7 NAP	0.4±0.1 ÷ 2.0±0.0	2.0±3.0 ÷ 7.0±2.0	Ihedioha et al., 2019

BaP- benzo(a)pyrene, BaA- benzo(a)anthracene, ChR- chrysene, BkF- benzo(k)fluoranthene, NAP- naphthalene, PHN-phenanthrene, ANT-anthracene, ACE- acenaphthene, ACY- acenaphthylene, PYR- pyrene, **- sum of benzo(b,j,k) fluoranthene, sum of benzo(b,j,k)fluoranthene is used in calculation, nd- not detectable, "-" compound not found, *- compounds were not studied or reported. PAH are presented as range or as mean, as reported in references. The number in parentheses indicates the number of compounds included in the reported sum.

Cookies commercialized on the Spanish market presented a 16 PAH content ranging between 1.66 and 3.97 µg/kg (Rascón et al., 2018). These cookies presented naphthalene and anthracene in a high proportion (0.26-2.9 µg/kg).
The content of 16 PAHs in different assortments of biscuits and cookies, commercially available in the Nigerian market was studied by Iwegbue et al. (2015), determining a PAH content ranging between 18.4-880.3 µg/kg, with an average of 219.6 µg/kg. The authors stated that variations in PAH content in different assortments of biscuits can be the result of the raw materials, baking method, type of fuel used in baking and oven thermal conditions. Biscuit samples from China presented higher concentrations of 16 PAHs than those commercialized on the European and the United States markets, showing that the area of production influences the PAH content. In general, the biscuits presented PAHs in relation to the number of aromatic rings in the following order: 3-rings > 4-rings > 5-rings > 2-rings > 6-rings. Phenanthrene, fluoranthene, and pyrene, which are low molecular PAHs, tend to form during the baking process at temperatures above 220°C (Houessou et al., 2007). By increasing the baking temperature (260°C), a significant level of pyrene, ChR and BaA was formed.
Another cereal-based product, quite popular worldwide, where PAHs can form is pasta: noodles, spaghetti, macaroni, etc. Ihedioha et al. (2016) assessed the PAHs content in Nigerian and imported brands of pasta and found 16 PAHs ranging between 9-800 µg/kg and for BaP between 0.2-3 µg/kg in Nigerian brands, and a content much lower in imported brands, of 2-7 µg/kg, and 0.2-0.7 µg/kg, respectively. PAH content varied between pasta brands, but also within the same brand due to heat treatment applied (temperature, time), distance to the energy source, oxygen availability, fat content and type of fuel used.
When Charles et al. (2018) determined the concentrations of 16 PAHs in different brands of noodles available on the same market, very high concentration of 16 PAHs were found, ranging between 564-7.889 µg/kg, while the sum of carcinogenic PAHs was found to be 564-5.615 µg/kg. BaP was detected at a concentration of 357-834 µg/kg, demonstrating serious health problems, especially since this type of product is widely consumed by children.
In Europe, the contamination with 16 PAHs in different cereal based-products purchased from various supermarkets was studied by Rascón et al. (2018), and for pasta it was determined a much lower content (0.168-1.98 µg/kg) than the one reported in Nigeria (Ihedioha et al., 2016; Charles et al., 2018) but nevertheless, the content was higher than the limit imposed by European regulations. BaP was not detected in the analysed pasta.
For most cereal-derived products, the predominant PAHs were naphthalene, anthracene and phenanthrene.

CONCLUSIONS

This study can provide an overview of the degree of PAH contamination of cereals and cereal derived products, and the factors that influence the formation of these contaminants.
Results of the study showed that cereals tend to be contaminated with low molecular PAH, with 2 to 4 rings.
The level of contamination with PAHs of cereals is influenced by the degree of pollution of the area where it is cultivated, the harvest year and also the way of drying the grains.
When it comes to cereal derived products, the PAH content is dependent of the flour type used in the manufacturing recipe, the region of production and assortment specific to each country, the baking process which implies baking time and temperature, but also the type of fuel used as a thermal agent.
The PAH content is higher in bread than in the dough from which it was obtained, showing that during baking PAH contamination can occur. When using natural gas or electric ovens, the PAH content in bread samples was lower than when using wood or grill for baking it.
In cereal-based products such as cookies, biscuits, pasta, the content of PAH is affected by the raw materials and ingredients used, the fat content of samples, and the heat treatment applied.
The presence of these pollutant environment contaminants in cereals and cereal-based products should be monitored and measures should be taken when the maximum level of 1

µg/kg for BaP, and sum of BaP, BaA, ChR, and BfF in processed cereals and baby foods for infants and young children is exceeded.

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APPROACHES TO THE ASSESSMENT OF SOME HABITATS OF COMMUNITY IMPORTANCE IN ROMANIA

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Abstract

All European member states must assess the conservation status of the habitats targeted by Habitats Directive 92/43/EEC based on the information on their status and trends and the main pressures and threats affecting them. Our paper presents the assessment of the Romanian community importance habitats (excepting forests) for all five biogeographical regions of Romania on coherence with the member states and with a special emphasis on the Sites of Community Importance (SCIs) of Natura 2000 network. The conservation status of the habitats is determined based on assessment on the parameters and their future prospects. The assessment of the scope and influence of the threat is realized for the evaluation of future prospects.

Key words: *Habitats Directive 92/43/EEC, community importance habitats, Romania, characteristics of habitats.*

INTRODUCTION

In the European Union (EU, the 'Community'), all member states use the Habitats Directive (92/43/ECC) on the conservation of natural habitats and of wild fauna and flora as a key instrument for biodiversity conservation (Mehtälä & Vuorisalo, 2007) and for maintaining and improving the supply of ecosystem services (Maes et al., 2012), directing their efforts toward designation of protected areas (Tucker et al., 2019). Thus, the Natura 2000 network was created, designating areas comprising natural habitat types and species (listed in Annex I and II of the Directive) which occur within the territory of each EU member state and for the conservation of which the Community has special responsibility. It is mandatory for member states to monitor and assess the conservation status of these natural habitat types and species and to report their findings to the Community (Pihl et al., 2001; Evans, 2006; Morris, 2011).

The goal of the Habitats Directive is to halt degradation of natural habitats and species, and to maintain or restore them in favourable conservation status through the implementation of conservation and restoration measures (Art. 2) (Louette et al., 2015), taking account of climate change (Normand et al., 2007) and other anthropogenic pressures (intensive agriculture, forestry and fisheries, urban sprawl, pollution, etc.) (Mihoub et al., 2017; Geldmann et al., 2019). For all EU countries, implementation of the Directive has been a high priority (Brown et al., 1997; Loidi, 1999; Dimopoulos et al., 2005; McLeod et al., 2005; Schneider & Drăgulescu, 2005; Costa et al., 2007; Zingstra et al., 2009; Jeanmougin et al., 2017; Silan et al., 2017). Environmental assessment procedures under the Directive represent a major tool for controlling activities affecting Natura 2000 sites (Garcia-Ureta, 2018).

Many valuable natural areas and the species that live in them have been greatly degraded or lost, over a quarter of Europe's animal species are at risk of extinction. Biodiversity is also vital for human economy and well-being (Habitats Directive, 1992).

The EU biodiversity strategy for 2030 (B.S. 2023) prioritises the preservation and restoration of Europe's biodiversity (Mammola et al., 2020; Hermoso et al., 2022; B.S., 2023). Even with expansion of the Natura 2000 network, loss of biodiversity continues (Spiliopoulou et al., 2023). To implement the goals of the strategy, biodiversity monitoring represents a central instrument for conservation (Harding et al., 2001; Hlásny et al., 2021), through achieving accountability and progress. Effective policy-making demands sufficient evidence, but the data are fragmented and disconnected because monitoring in Europe suffers from gaps and variation in: taxonomy, spatial coverage, and temporal resolution, regulating mechanisms of biodiversity and the relationship of biodiversity to ecosystem services (De Meester et al., 2011; Maes et al., 2012; Maes, 2013; Moersberger et al., 2023a).

The EU implements policies and legislative frameworks for nature protection through the Habitats and Birds Directives, and has developed habitat (or biotope) classifications: Palaearctic (Devilliers & Devilliers-Terschuren, 1996; Janišová et al., 2016), CORINE (Moss & Wyatt, 1994; Romao, 1996), EUNIS (Davies et al., 2004; Moss, 2008; Chytrý et al., 2020). These provide typologies with definitions of habitat types intended to aid their recognition, mapping, monitoring, and protection (Loidi, 1999; Evans, 2010; 2012; Morris, 2011; Bunce et al., 2013).

Before accession to the European Union in 2007, scientists in Romania defined the habitats/biotopes that had major importance for nature conservation in EU and accession countries, and starting with the CORINE programme in 1985 and EMERALD network, investigated protected areas that could be candidates for integration into international programmes (Mihăilescu et al., 2003). Strategies were developed for implementation of the Natura 2000 network for protected areas (Munteanu & Mihăilescu, 2005), and habitats identified for proposing Natura 2000 site (Mihăilescu, 2006). During accession, partly through an EU Phare project (EuropeAid/12/12160/D/SV/RO), habitats and sites were described from Annex I of Habitats Directive whose conservation requires designation of special areas of conservation (SAC, SCIs), so that Romanian habitat types could be integrated into the Natura 2000 network (Doniţă et al., 2005; 2006; Schneider & Drăgulescu, 2005). Description of the European habitats (EUR27, 2007) was further enhanced with characterisation of their physical-biogeographic context in Romania and information to facilitate their identification (Combroux et al., 2007; Gafta & Mountford, 2008). Criteria for designation of Natura 2000 sites were delineated (Mihăilescu, 2010).

Classification of habitat types was based partly on phytosociology (defined plant communities or syntaxa), but this is considered complex and sometimes unclear (Angelini et al., 2018; Rodwell et al., 2018). Thus, assessment of the conservation status of habitat types is based partly on structure (typical/characteristic species), function and future habitat trends (Noss, 1990; Bendali & Nellas, 2016; Müller-Kroehling, 2019). In the Habitats Directive, monitoring of Annex 1 habitats and species from Annexes II, IV and V is required in Article 11, with reporting to the Commission every 6 years of their assessed conservation status required by Article 17 (DG Environment, 2017).

Such methods were developed in all European countries both for habitat types (Angelini & Casella, 2015; Chen, 2021) and their conservation status (Mehtälä & Vuorisalo, 2007; Zingstra et al., 2009; Sipkova et al., 2010; Silan et al., 2017; Ellwanger et al., 2018; Strat et al., 2018; Tsiripidis et al., 2018; Velagic-Hajrudinovic, 2019a; 2019b; Prisco et al., 2020; Delbosc et al., 2021; Melart, 2022; Santangelo et al., 2022).

Our aim has been to develop and apply approaches for assessment of some habitats of community importance in Romania, describing evaluation of their conservation status and their value under article 17 of Habitats Directive.

MATERIALS AND METHODS

In response to requests from the European Community, Romanian academic institutions, coordinated by the Ministry of Environment, Water and Forests, developed the POS project "Monitoring of the conservation status of species and habitats from Romania in the framework of article 17 of Habitats Directive" (2011-2015) which was the basis for Romania to report to the European Commission. The next stage of reporting will be based on POIM project "Completing knowledge level of biodiversity through implementing the monitoring system of conservation status of species and habitats from Romania in the framework of article 17 of Habitats Directive 92/43/CEE" (2019-2023). The Article 17 report for Romania has a section with assessments of habitat conservation status in the whole national territory, not only those within Natura 2000 sites (Zaharia, 2013; INCDPM, 2014; Mihăilescu et al., 2015; Trif et al., 2015; Ursu et al., 2020).

We used information on the European Community sites as well as other publications and reports, and expertise developed within the POS and POIM projects, to focus, as required by the Commission, on assessment of the status and trends, with main pressures and threats, for some habitats occurring in the five biogeographic regions present in Romania (Figure 1).

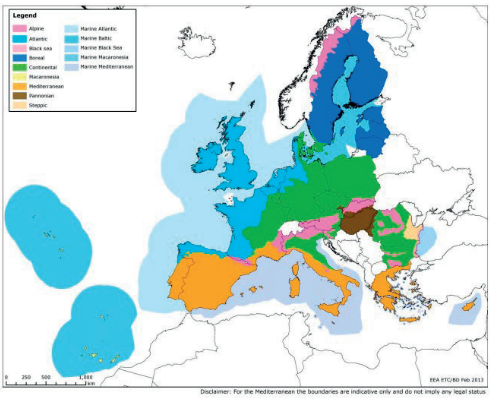

Figure 1. European biogeographical and marine regions for reporting under Article 17 of the Habitats Directive (after ETC/BD, 2014)

Conservation status is assessed using a standard methodology as being either 'favourable', 'unfavourable-inadequate' or 'unfavourable-bad' (Table 1), based on four parameters as defined in Article 1 of the Habitats Directive: 1. range (within the biogeographical region concerned); 2. area covered by habitat type within range; 3. specific structures and functions (including typical species); 4. future prospects (as regards range, area covered and specific structures and functions).

Table 1. Abbreviations and colour codes for Conservation Status classes (after ETC/BD, 2014)

Conservation Status	Colour	Abbreviation
Favourable	Green	FV
Unfavourable-inadequate	Amber	U1
Unfavourable-bad	Red	U2
Unknown	Grey	XX

The definition of 'favourable' conservation status of a habitat is given in Article 1(e) of the Habitats Directive as: a) its natural range and areas are stable or increasing; b) the specific structure and functions which are necessary for its long-term maintenance exist and are likely to continue to exist for the foreseeable future; and c) the conservation status of its typical species is favourable as defined in (i).

We have used the Article 17 Reference Portal for the technical specifications required for reporting to the European Commission. Romania reported to the Commission on all habitats listed in Annex I of the Habitats Directive and their conservation status by biogeographical or marine region, mapping their distribution. The distribution maps provide information about the actual occurrences of the habitats, based on their inventory. Where field data on actual occurrences of the habitat were insufficient, modelling and extrapolation have been used. The distribution map consists of 10x10 km ETRS89 grid cells in the ETRS LAEA 5210 projection.

RESULTS AND DISCUSSIONS

To explain the assessment of habitats of community importance, we highlight attributes defined in the Explanatory Notes and Guidelines of the Habitats Directive (DG Environment, 2017) i.e. **Range (and Surface Area), Habitat structure, Pressures (and**

threats and conservation measures) and **Future Prospects**, all assessed separately for each biogeographical region. In Romania, the five biogeographical regions are Alpine, Continental, Pannonian, Steppic and Black Sea (formerly 'Pontic') and marine region (Marine Black Sea) (Figure 2).

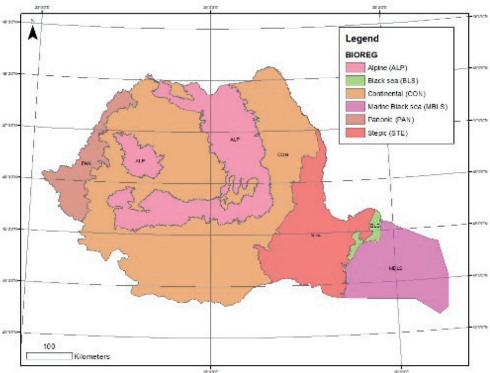

Figure 2. The map of the biogeographic regions

Range is defined as 'the outer limits of the overall area in which a habitat is found at present'. Based on the map of actual distribution, the range is calculated using a standardised process that ensures repeatability for different reporting rounds. Thus, the area covered by the habitat type within the range is assessed including all significant ecological variation for each biogeographical region and testing whether the area is sufficiently large to allow its long-term survival. The 'favourable reference value' must be at least the range (size and configuration) when the Directive was first applied. If this value was not acquired, then the reference for favourable range should be larger or based on information on historic distribution. In the absence of other data, 'best expert judgement' may be used.

The area covered by a habitat type within this range (the '**surface area'**) is based on all sites where it is present in the region, and represents the total area (in km^2) currently occupied. This attribute is calculated and reported as a range (minimum and maximum) and/or as a best available single value. The total surface area of the habitat in a biogeographical region is defined as the minimum necessary to ensure its long-term viability. This attribute should include any necessary areas for restoration or development for which its present coverage is insufficient to ensure long-term viability. As with **range**, the favourable reference value must be at least the surface area when the Directive came into force, and is assessed in the same way.

The short-term trend of habitat area within the Natura 2000 network is categorised as either: stable, increasing, decreasing, uncertain or unknown. It is a measure of directional change over time and should reveal changes within the network. The short-term trend should be evaluated over a period of 12 years (two reporting cycles) and is used in the evaluation matrix to assess the conservation status.

Habitat structure is formed by species, but can also include abiotic features. The typical species (or groups of species) are those occurring regularly in the habitat type and are those which are good indicators of favourable habitat quality. For assessing conservation status, the list of typical species should ideally remain stable across reporting periods, but need not be restricted to those listed in Annexes II, IV and V of the Habitats Directive.

Pressures are factors acting now and/or during the current reporting period (six-year), and have an impact on the long-term viability of the habitat and its typical species. Continuation of such pressures may lead to threats. High pressures have important direct or immediate influence on one or several parameters of conservation status at the biogeographical scale (causing significant decline or deterioration or preventing habitat from reaching favourable status).

Threats are factors expected to act in the future after the current reporting period, with future/foreseeable impacts (within the next two reporting periods) and are likely to affect the long-term viability of the habitat and its typical species.

Conservation measures are taken inside or outside Natura 2000 sites for each habitat type: a) maintaining its current range, surface area or structure and functions; b) expanding its current range; c) increase its surface area; and d) restore the structure and functions, including the status of typical species.

Future Prospects indicate the expected direction of change in conservation status in the near future based on a consideration of current status, reported pressures and threats, and measures being taken for each of the other three parameters (Range, Area, and Structure and functions).

The concept of favourable reference values is derived from definitions in the Directive, and relates to the "long term natural distribution, structure and functions as well as the long-term survival of its typical species" (Article 1(e)) in their natural range. Overall assessment of conservation status uses four categories: 'favourable', 'unfavourable-inadequate', 'unfavourable-bad' and 'unknown', based on the evaluation matrix for assessing conservation status for a habitat (Table 2).

Table 2. Overall assessment of the conservation status (CS)

Status of parameters	All favourable or few favourable + one unknown	One or more inadequate, but no bad	One or more bad	Two or more unknown + favourable or all unknown
Overall assessment of CS	favourable	unfavourable-inadequate	unfavourable-bad	unknown

If the overall conservation status is 'favourable', 'inadequate' or 'bad', a qualifier is added i.e.: improving or deteriorating or stable or unknown. The qualifier should be based on trends (for range, area covered by habitat, and structure and functions) over the reporting period i.e. a cycle of six years.

Trends are essential in assessing all conservation status parameters except future prospects. Short-term trends are assessed for two reporting cycles. Long-term trends are assessed for four reporting cycles and are likely to be more statistically robust.

In order to set favourable reference values for habitat types, data and information should ideally be gathered on nine factors: a) current situation and assessment of deficiencies (pressures/problems); b) trends (historical, short-term, long-term); c) natural, ecological and geographical variation (including variation in: species composition, conditions in which habitats occur, ecosystems); e) ecological potential (potential extent of range, taking into account physical and ecological conditions, contemporary potential natural vegetation); f) natural range, historical distribution and abundance and causes of change, including trends; g) connectivity and fragmentation; h) dynamics of the habitat type; and i) requirements of its typical species.

For the reporting period 2007-12, the conservation status of the habitats of community interest indicated that, from 743 assessed habitats, the conservation status was: unfavourable-bad (42 habitats), unfavourable-inadequate (433), favourable (216), and unknown (52) (Mihăilescu et al., 2015). For the identification of habitats, the experts used the manuals that placed Romanian habitats in their EU context (Doniţă et al., 2005; 2006; Gafta & Mountford, 2008).

For each proposed main objective, those characteristics (attributes) of each targeted habitat were identified that reflect its properties and can be quantified. Where the existing information allows, a range of values for each attribute was defined within which the properties of the analysed habitat do not alter, thus facilitating the interpretation of the results. The monitoring plan used the main general indicators for the levels of research shown in Table 3.

Table 3. The indicators used in the monitoring plan

Levels	Indicators/Composition
Habitat	- the proportion of the habitat within the analysed range - types of the component plant associations - identification, distribution, diversity, abundance
Community/ ecosystem	- identification of species and their relative abundance - Frequency, abundance, species diversity inside the communities, proportion of endemic, threatened and near extinct species - Proportion of dominance – diversity
Population/ species	- Relative and absolute abundance - Density

Data aggregation of the information for a monitoring plot is done in two stages: 1) spatial aggregation, map generation according to the reporting format starting from the primary data/field data; and 2) non-spatial aggregation that involves the generation of specific files, according to the reporting format, starting from the primary data. Distribution maps have been created following the standard reporting format. This process is illustrated by an example mapping the range of the habitat 1310 *Salicornia* and other annuals colonising mud and sand (Figure 3).

In order to assess the distribution of the habitats in Romania, we identified the location of each habitat from historical data (literature or phytosociological records).

To establish conservation/management measures, we gathered supplementary information about the type and intensity of the impacts of every activity on species and habitats. These impacts were further assessed in terms of the entire nature protected area, species of conservative interest, and variation in the habitat types of conservative interest.

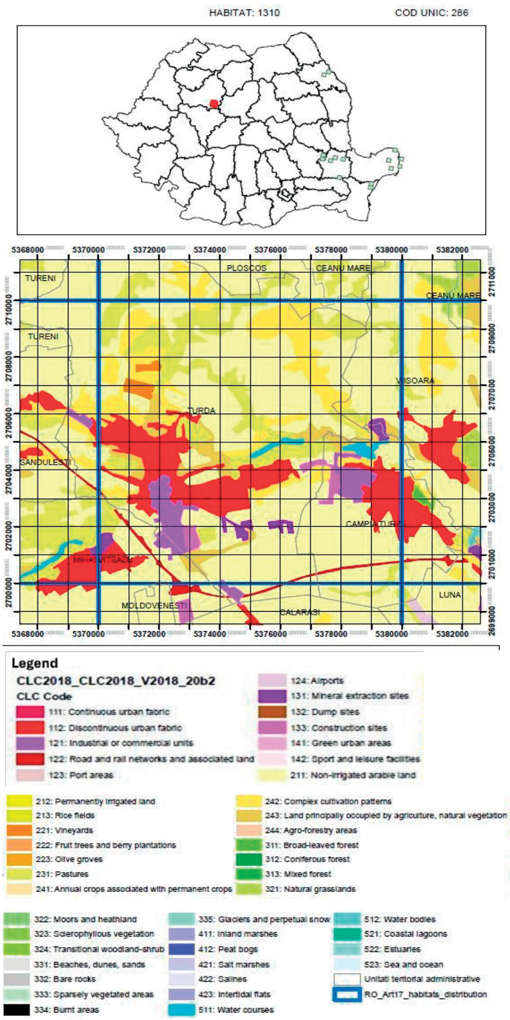

Figure 3. The range of habitat 1310 on Romanian territory (after E.W.F.M.O, 2023)

Monitoring plots were established inside or outside Natura 2000 sites - *Sites of Community Importance (SCIs)* - according to the requirements of EC reporting, and the range and surface area of each habitat. For instance, saline habitats present in Romania have a large distribution in more than one biogeographic region (Table 4).

To assess short-term trends, we used the observed trend for major parameters, such as distribution area and structure and functions, observed over 2 reporting cycles (total 12 years).

Table 4. The establishment of the monitoring plots according with the range of saline habitats

Habitat Cod	ALP	CON	PAN	STE	BLS	Total plots	Plots in two different biogeographic regions
1310	0	3	0	11	7	16	5
1410	0	0	0	0	7	7	0
1530*	0	17	9	11	5	39	3

Legend:
1310 Salicornia and other annuals colonising mud and sand.
1410 Mediterranean salt meadows (*Juncetalia maritimi*).
1530* Pannonic salt-steppes and salt-marshes.
ALP - Alpine bioregion
CON - Continental bioregion
PAN - Pannonian bioregion
STE - Steppic bioregion
BLS - Black Sea (formerly Pontic) bioregion

The trend is described using qualitative indicators such as: stable, recovering, declining and unknown. Evaluation of short-term trends is also the key control for the quality analysis of successive reporting results. In order to establish pressures and threats, we used the nomenclature standardly applied at the European level.

Pressures observed in the field study shall be assessed by determining the specific intensity of each one exerted on the target habitat. The categories of intensity assessment are also qualitative i.e. low intensity, medium intensity, high intensity, unknown intensity.

Following analysis of the existing pressures observed in the field, the expected threats are evaluated, again establishing the specific intensity of each exerted on the target habitat. The intensity assessment categories are precisely similar to those for pressures.

At bioregion level, the information in the 10 x 10 km (plots) grid is summarised for each category of the parameters using a weighted average to provide the final evaluation for the habitat. For example, species composition (one of the essential parameters in assessing habitat structure) will vary from one plot to another, requiring that they be grouped by classes of different species diversity (large, medium, small), and weighting of each class will lead to the specific evaluation for each bioregion. Thus, if for habitat 1310 we obtain 20 plots within the large specific composition class (12 characteristic species), just 7 plots in the class of average composition (7 characteristic species), and none in the class of small composition (3 characteristic species), we can infer that, for the target bioregion, the habitat is in a favourable state with regard to evaluation of structure. The third level of aggregation is of the national attributes resulting from the aggregation of data at the bioregion level.

The methodology and monitoring plan for the collection of field data and for the assessment of the conservation status are described in Order no. 3352 (28 December 2023) for eight habitat groups: saltmarshes and salt meadows, inland dunes, freshwater, meadows, other grasslands, swamps and peatlands, groves and cliffs.

The field recording sheets for monitoring are completed after establishment of representative samples of phytocoenoses, and components of habitats of community interest. These sheets will contain information on relief, biotope condition, species listed in the sample area, etc.

Monitoring of habitats and the plant species that define them is done using the plots/quadrats method on transects or by permanent plots/quadrats (relevés) method, which has advantages when conducting comparative studies. The plots shall be chosen according to the vegetation gradient present in the habitat of community interest. The minimum number of quadrats (relevés) depends upon the available resources, statistical analysis and habitat surface area. The data and observations gathered from the field represent not only the basis for all analyses but also interpretation in order to obtain objective results of high scientific value.

Threats and pressures for all habitats can be selected from the Reference portal for reporting under Article 17 of the Habitats Directive, usually the most relevant and important ≤10.

At both national and international level, Habitats Directive (92/43/EEC) is the foundation of nature conservation in Europe and the development of EU Biodiversity Strategy for 2030. Bonari et al. (2023) believe that, with the knowledge gained after so many years working on habitats, Annex I of the Habitats Directive should be updated to resolve ambiguities in the definition of Annex 1 habitat types, decrease uncertainties in classification and improve conservation success. These updates would include new habitat types, new subtypes within pre-existing habitat types, and involve preparation of expert systems for automatic classification based on the list of typical species.

To achieve accountability and progress in conservation, in the future biodiversity assessment/ monitoring will be the foundation for achieving the goals of the 2030 Global Biodiversity Framework (Miu et al., 2020), the European Biodiversity Strategy, and the EU Green Deal (Moersberger et al., 2023b). For implementing these goals, one target is to protect 30% of European land by 2030 through a resilient transnational conservation network creating key hubs of the network that might host extensive natural areas and biodiversity hotspots in Europe (Chauvier-Mendes et al., 2024). Furthermore, Toivanen et al. (2024) present European geodiversity data at resolutions of 1 km and 10 km, incorporating aspects of geological, pedological, geomorphological and hydrological diversity, and have demonstrated their potential use in correlating geo-richness with vascular plant species richness (exemplified by two contrasting areas: Finland and Switzerland). So as not to lose biodiversity entirely, scientists believe that it is vital to designate Key Biodiversity Areas (KBAs) which serve as essential habitats for the world's threatened species; the extent and severity of human disturbance in these KBAs must also be assessed (Yang et al., 2024).

CONCLUSIONS

Assessment of conservation status is developed using standard methodology based on four parameters as defined in Article 1 of the Habitats Directive. For reporting to the EC, Romania followed the Reference portal for reporting under Article 17 of the Habitats Directive and created the guide regarding the protocols and unitary methodologies for monitoring the conservation status of community interest habitats. The guide has been recently introduced in Romanian legislation (E.W.F.M.O, 2023) and must be followed by all scientists reporting the conservations status of habitats to the European Union.

Data resulting from the monitoring should capture where the main objective of the conservation action need to take place. Therefore, in conjunction with the reporting format, one of three options may be chosen: (a) if all, or the vast majority, of the conservation measures are limited to Natura 2000; (b) where there is a proportionate investment in implementation of the measures inside and outside Natura 2000; and (c) if all, or the vast majority, of, of the measures are taken outside Natura 2000 land. At the current stage, most of the habitats that are not in the Natura 2000 network are neither mapped at bioregion level or national level in Romania.

Romania is presently at making its second report under Article 17 of the Habitats Directive.

The first report provided the reference level for later reports, including assessment of short-term conservation trends over a single reporting cycle. The short-term nature of the data so far available means that the full appraisal recommended by the standard EU methodology cannot yet be carried out.

The methodology outlined above is not only consistent with that required by the European Commission, allowing fuller appraisals of the condition of Romanian biodiversity in the future, but also should provide data and condition assessments that can be applied by the national government in determining policy. Romania is among the most diverse countries in Europe for its habitats, species and overall environmental value, especially within the Natura 2000 network of sites, but also generally within the wider country. Effective management and conservation of this internationally important resource will demand a robust methodology, which both the EU and Romanian Ministry of Environment, Water and Forests can use.

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TENDENCIES IN WET ZONES VEGETATION EVOLUTION UNDER ANTHROPIC DISTURBANCES IN THE ROMANIAN BANAT

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Abstract

Wet zones have always been of great importance to humanity. The historical province of the Banat was known as a huge expanse of swamps in the past, but the current map of the region (its Romanian part) is fundamentally different, as a result of hydro-relief works. Our study is a synthesis about the evolution of the vegetation of some wet zones. Observations refer to a significant period of time. We processed and compared several hundred species and phytosociological rélevés from many location. Correlations were made between the nucleus of common species, the compared groups, the dendrogram with the grouping of the rélevés, the diversity profile of the parameters. From the processing of the data, it appears that there are significant losses in the current structure and composition of the vegetation. These are caused by a series of factors such as: reduction of the areas occupied by wet habitats, hydro-relief works, climate changes, expansion of invasive species, fragmentation, other agricultural activities.

Key words: *Romanian Banat, wet zones, vegetation, anthropic disturbance.*

INTRODUCTION

Wetlands, defined by the Ramsar Convention as “areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres” or by the EPA (Environmental Protection Agency) as “areas where water covers the soil, or is present either at or near the surface of the soil all year or for varying periods of time during the year, including during the growing season”, are very vulnerable, Gomoiu et al. (2009) considering them among the most endangered ecosystems on the globe. In the synthesis on the restoration of wetlands in Central Europe, Pfadenhauer & Grootjans (1999) consider essential the in-depth knowledge of ecological processes, Rittenhouse & Peterman (2018) and Stengård et al. (2020) explain the role of connectivity in the conservation of these dynamic ecosystems.
The importance of wetlands, translated by the term ecosystem services, is well known, from supporting the needs of food and water, to mitigating climate change, controlling floods, soil erosion, the circuit of substances, up to multiple cultural services. The causes of wetland degradation and loss are equally well known: agriculture, industries, transportation, excessive urbanization, global warming, pollution, expansion of invasive species etc.
In Europe, there is a wide range of wetlands, estimated at several thousand, as an expression of the climatic peculiarities of our continent. On account of some forecasts, Okruszko et al. (2011) draw attention to the possibility of losing an important number of the services brought by these areas (up to 46% in 2050) and consider that water management is crucial.
In Romania, there are 345 wetland sites covering 10.5% of the country's surface (Tanislav, 2014). In terms of the ecosystem services provided and the biodiversity they conserve, wetlands in Romania are very important, although throughout history they have been underestimated and considered wastelands (Ciobotaru et al., 2014; 2019). Speaking about the value of wetlands, Gomoiu et al. (2009) emphasize the importance of the genetic resources they hold, considering them huge reservoirs of biological diversity. Against the background of the same problems identified globally (Giosan et al., 2014; Davidson, 2018; Walpole & Davidson, 2018; Li et al., 2018; Finlayson, 2019; Cadier et al., 2020; Ballut-Dajud et al., 2022; Beranek et al., 2022 etc.),

the loss of wetland services is a major problem here as well.

The vast majority of wetlands in Romania (96%) are located in the plain area with a dry or semi-arid climate, distributed over a wide variety of soils (Ciobotaru et al., 2014). The Habitat Quality (HQ) index indicates their high susceptibility to pressure exposure state, outside the Danube Delta and Lower Danube Floodplain (Ciobotaru et al., 2019).

Over time, large-scale land reclamation and hydraulic improvement works were carried out in Banat (watercourses regularization and swamps drainage, the latter considered the source of multiple epidemics, from the 18th century), in order to use the land for agriculture and transport development. The current map of the region is fundamentally different from what was described by the Italian scientist Francesco Griselini, on the occasion of his trip to the Banat of Timișoara, in 1774. Few natural wetlands were preserved, so that over time artificial ones were created (for tourism, fish farming, irrigation, flood mitigation, energy generation, as water reservoirs etc.). The only natural wetland that has survived is the Mlaștinile Satchinez area, nicknamed "the Banat delta", an area declared a nature reserve in 1942 with the involvement of the famous Romanian ornithologist, Dionisie Linția, and which is today a Natura 2000 site (ROSCI0115 Mlastina Satchinez, ROSPA0078 Mlastina Satchinez). Unfortunately, its state of preservation is not the best. Several other areas that protect habitats and important species should be mentioned, such as: Lunca Mureșului Natural Park (Ramsar site), Lunca Timișului (ROSCI0109, ROSPA0128), Lunca Mureșului Inferior (ROSCI0108, ROSPA0069), Lunca Bârzavei (ROSPA0127), Mlaştinile Murani (ROSPA 0079), Lacul Surduc (mixed nature reserve).

Gomoiu et al. (2009) show how the changes undergone over time by some wetlands in the Western Plain of Romania influence the flora and vegetation. Among the extinct species, these authors mention: *Hippuris vulgaris, Acorus calamus, Pedicularis palustris, Salix aurita* and among those in expansion, *Phalaris arundinacea, Glyceria maxima, Lythrum salicaria, Lycopus europaeus, Oenanthe aquatica, Rorippa amphibia, Agrostis stolonifera*. Under the aspect of vegetation evolution, attention is paid to the impoverishment of some characteristic phytocenoses and the expansion of ruderalized ones in which the problem of invasive species appears, such as *Reynoutria japonica*. From the list of neophytes from Romanian wet zones, made by Anastasiu et al. (2008), the most invasive are considered: *Amorpha fruticosa, Azolla filiculoides, Elodea nuttallii, Helianthus tuberosus, Impatiens glandulifera, Paspalum paspalodes, Reynoutria japonica, Rudbeckia laciniata, Solidago canadensis, Solidago gigantea* subsp. *serotina, Xanthium strumarium* subsp. *strumarium (X. italicum)*.

Pătruț & Coste (2006) mention that the hydro-ameliorative works carried out throughout history in the Banat Plain have determined the lowering of the water table level, so that the aquatic and palustrine biotopes have decreased their surfaces, the tendency of the evolution of the vegetation being predominantly hydrophilic and paludicolous, towards xerophilic and meso-xerophilic vegetation.

MATERIALS AND METHODS

Within our research we considered phytocenological surveys from some wet zones from Romanian Banat. The samples were made in an interval of over 60 years (1956 - present) by: Soran (1956), Boșcaiu (1966), Grigore (1971), Vicol (1974), Oprea et al. (1974), Arsene et al. (2005), Neacșu et al. (2008 - 2022). The relevés were carried out in the following wet zones: the Timiș floodplain, Liebling (Soran, 1956), the surroundings of Lugoj (Boșcaiu, 1966), the Timiș-Bega interfluve area (Grigore, 1971), Oprea, I.V., Stratul, E., Iacob, M. (1974), the Lugoj piedmont (Vicol, 1974), Satchinez lowland (Arsene et al., 2005), Surduc, Pișchia, Liebling and Sânandrei lakes (Neacșu et al., 2008-2022). The processed variables for own data are: the number of relevés per association, the number of species per relevés, the number of species per association, general vegetation coverage, sample area size, vegetation height.

RESULTS AND DISCUSSIONS

In the synthetic table, we gathered 292 relevés. Of these samples, 124 belong to us and 168 are studied from the scientific literature (other authors). The table contains 386 species. The plant associations analyzed are: *Lemnetum minoris* (Oberd. 1957) Müller et Görs 1960, *Lemno-Spirodeletum* W. Koch 1954, *Eleocharidetum acicularis* W. Koch 1926 em. Oberd. 1957, *Myriophyllo-Potametum* Soó 1934, *Trapetum natantis* Müller et Görs 1960, *Polygono-Potametum natantis* Soó 1964, *Scirpo-Phragmitetum* W. Koch 1926, *Typhaetum angustifoliae* Pignatti 1953, *Typhaetum latifoliae* G. Lang 1973, *Glycerietum maximae* Hueck 1931, *Schoenoplectetum lacustris* Eggler 1933, *Iretum pseudacori* Eggler 1933, *Eleocharidetum palustris* Schennikow 1919, *Leersietum oryzoides* Krause 1955 em. Pass. 1957, *Phalaridetum arundinaceae* (Horvatič 1931) Libbert 1931, *Cariecetum ripariae* Knapp et Stoffer 1962, *Polygono hydropiperi-Bidentetum* Lohm. 1950, *Echinochloo-Polygonetum lapathifolii* (Ujvárosi 1940) Soó et Csűrös (1944) 1947, *Conietum maculati* I. Pop 1968, *Sambucetum ebuli* (Kaiser 1926) Felföldy 1942, *Salicetum albae* Issler 1924 s.l., *Rubi-Salicetum cinereae* Sonasak 1963, *Pruno spinosae-Crataegetum* Heuck 1931.

Data series analysis using bar chart - box plot, led to the diagram in Figure 1. It presents the variation ranges of the data series for the AG (general vegetation coverage), IV (vegetation higth), and SN (species number) parameters, the standard error, and the outlier values (in the case of the IV parameter, R110, come from a *Salicetum albae* phytocoenosis from Surduc).

The multivariate analysis (PCA) led to the diagram presented in Figure 2 (correlation between groups diagram) and Figure 3 (Var-covar disregard diagram). It was found the formation of some groups of study points with common areas, but also with the polarization of some points in relation to the indices considered (AG, IV, SN). In the case of the diagram in Figure 2, axis 1 explains 77.979% of variance and axis 2 explains 16.211% of variance). In the case of the diagram in figure 3, PC1 explained 99.126% of variance and PC2 explained 0.7714% of variance.

On the diagram in Figure 2, it can be seen that on axis 1, which explains 77.979% of the variance, there are mainly marsh associations, slightly flooded up to associations with *Typha angustifolia* and *Typha latifolia*. Most of the other associations, including the aquatic ones, are grouped in the central area and a few are eccentric. Axis 2 has little explanatory value. On the diagram in Figure 3 it can be seen that axis 1, which explains 99.126% of the variance, is influenced by the presence of some abnormal relevés (R110 *Salicetum albae*, R93 *Polygono hydropiperi-Bidentetum*, R117 *Rubi-Salicetum cinereae*, R43 *Typhaetum angustifoliae*, R30 *Scirpo-Phragmitetum*). These relevés are from the Surduc and Pişchia area. Their positioning is mainly due to the large differences in the height of the vegetation (R110), compared to most relevés and the appearance of some species that are missing in the others.

The cluster analysis led to the dendrogram in Figure 4 under conditions of statistical safety (Coph.corr = 0.924). The dendrogram reconfirms the eccentric position of R110 and the separation into 7 clusters. Examining these clusters, it is observed that each brings together relevés with very diverse phytocenoses; we find phytocenoses from the same plant association distributed in different clusters. This can be explained by the fact that the list of species is consistent, that most relevés include few species, so that the accidental presence of a species greatly influences the cluster analysis.

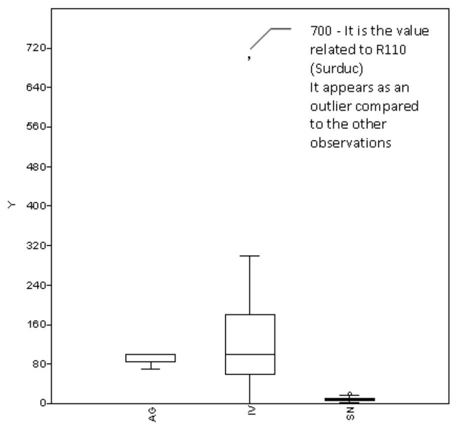

Figure 1. Boxplot diagram for the parameters AG (general vegetation coverage), IV (vegetation height) and SN (species number)

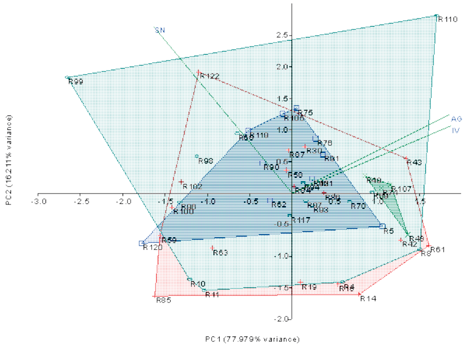

Figure 2. Principal Component Analysis - Correlation between groups

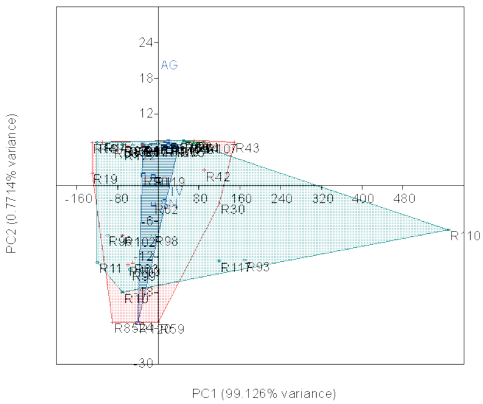

Figure 3. Principal Component Analysis Var-covar Disregard

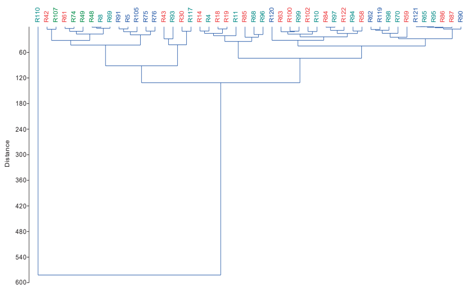

Figure 4. Dendrogram regarding the grouping of relevés (RN) in relation to the general vegetation coverage (AG), vegetation height (IV) and the number of species (SN)

CONCLUSIONS

From the comparative analysis of the data, the following trends can be observed:

- the significant trend of decrease in specific diversity in the wet zones;
- the high presence of adventive species, in recent years studies: *Ambrosia artemisiifolia, Erigeron annuus, Phytolacca americana, Robinia pseudacacia, Xanthium italicum, Xanthium spinosum, Amaranthus retroflexus, Juncus tenuis, Eriochloa villosa*, *Lindernia dubia* (the last two reported in our phytocenoses at Pişchia and Surduc). Also, *Amorpha fruticosa* and *Echinocystis lobata* with sporadic presence in the past (Grigore, 1971, Vicol, 1974), are now among the worst invasive species in the Romanian wet zones;
- the sporadic presence or the disappearance of some important species (*Elatine alsinastrum* - Grigore, 1971: Uliuc, Urseni, Albina, Utvin / Near Threatened IUCN Red List, *Marsilea quadrifolia* - Grigore, 1971: Uliuc, Urseni, Albina, Utvin, Moşniţa Veche, Ghiroda, Sînmihaiul Român / EU Habitats Directive, Bern Convention, *Salvinia natans* - Boşcaiu, 1966: Lugoj surriundings; Grigore, 1971: Albina, Uliuc, Urseni, Ghiroda, Sînmihaiul Român, Moşniţa, Utvin, Peciu Nou, Otelec, Ionel, Livanda, Diniaş, frequent in the interfluve, Timişoara; Vicol, 1974: Lugoj / Bern Convention);
- the decrease of the areas with aquatic and marsh vegetation (e.g. Neacşu et al. 2018);
- the tendency of ruderalization in wet zones.

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COMPARISON OF SOME EXTRACTION TECHNIQUES FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) FROM OILSEEDS BY GC-MS/MS

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic pollutants with potential risk to human health and can contaminate the oilseeds crops during the growth period and/or during the drying/roasting processes. In order to determine the 4 PAHs regulated (Commission Regulation (EU) No 835/2011) in sunflower seeds by GC-MS/MS, two preparation techniques were evaluated: QuEChERS extraction with modifications and saponification with liquid-liquid extraction. Different factors were studied to isolate PAHs: type of solvent/salt, quantity of reagents/solvents, stirring mode, etc. Acetonitrile extracts were purified by freeze-combined with d-SPE QuEChERS. The comparison of the preparation techniques was evaluated in terms of recovery (50-120%) and co-extract residue values (≤ 2 mg/mL). QuEChERS extraction was selected as the optimal variant, obtaining the lowest co-extract residue values (≤ 0.5 mg/mL) and recoveries between 94.62-102.41%. This methodology was also verified on other samples: sunflower seeds with different fat content, sunflower seed core, pumpkin seeds, flaxseeds, rapeseeds, sesame etc. No PAHs were detected in the analysed samples.

Key words*: gas chromatography, oleaginous seeds, polycyclic aromatic hydrocarbons, QuEChERS extraction, sunflower seeds.*

INTRODUCTION

Vegetable oils are widely used, both for direct human consumption (salads), but also as a medium used for frying food or are incorporated into various food products (cakes, bread, biscuits, etc.) or to obtain products such as: margarine, mayonnaise, etc., thus becoming a major source of dietary PAH exposure. Due to their lipophilic character and their widespread distribution in the natural environment, the level of PAH contamination in oilseeds and their derived processed products has become a serious concern, as up to 15-50% of the absorption and dietary exposure to PAHs from food is attributed to these groups of contaminated foods (Veyrand et al., 2013).
Aware of the toxic effects of PAHs on human health, since 2002 the European Commission (EC) has introduced recommendations and regulations (SCF, 2002; EFSA, 2008) that requires to monitor and to establish maximum permissible limits of these toxic compounds. PAHs represent a major group of chemicals being considered cancer-inducing agents. Several PAHs have been assessed as a potential carcinogenic by the International Agency for Research on Cancer (IARC, 2010). European legislation (Regulation no 835/2011 with subsequent amendments 1327/2014; 1993/2015; 1125/2015; 1255/2020) established maximum limits for 4 PAHs: benzo(a)pyrene (BaP), chrysene (Chr), benzo(a)anthracene (BaA), benzo(b) fluoranthene (BbF) and a separate maximum level for benzo(a)pyrene (BaP), which is the most studied PAH and it is used as a marker of toxicity and occurrence of PAHs in food. Hundreds of PAHs consist of at least two aromatic rings linked together. The chemical structures of the 4 PAHs are shown in Figure 1.

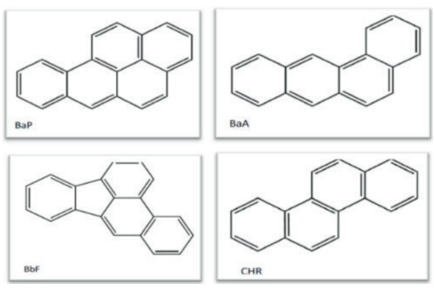

Figure 1. Chemical structure of the 4 PAHs for which maximum limits are set (EFSA, 2008)

PAHs contain several isomers that refer to the same molecular formula, but to distinct configurations. For example, BaP and BbF, like BaA and ChR have identical molecular masses but different structures (Table 1).

Table 1. PAH identification
(IARC, 2010; Sampaio et al., 2021)

HAP abbreviation	Chemical formula	Molecular weight (g/mol)	Boiling point (°C)	Melting point (°C)	Classification IARC*
BaP	$C_{20}H_{12}$	252.3	495	179	1
BaA	$C_{18}H_{12}$	228.3	438	158	2B
CHR	$C_{18}H_{12}$	228.3	448	254	2B
BbF	$C_{20}H_{12}$	252.3	481	168	2B

*IARC: 1 - carcinogenic to humans; 2B - possible carcinogenic to humans

The quantification of PAHs in food is a difficult task, not only because of the complexity and diversity of food matrices, but especially because of their physicochemical properties (Sun et al., 2019). The samples differ widely in both composition and PAH contamination, from trace amounts to thousands of µg/kg in product (Sun and Wu, 2020). Extraction and purification steps play a critical role in the success of an assay to improve selectivity and sensitivity. In these steps, the analytes are transferred from the matrix into an extract, usually in an organic solvent, as free as possible from interfering compounds and compatible with the chromatographic system and the detection method. The selection of the most suitable analytical protocol should be based on the type of food product (type of matrix), the structure and the physicochemical properties of the PAHs (Sun et al., 2019; Wu et al., 2020).
The current trend is to develop new concepts in sample preparation in order to provide a faster and more efficient way to process samples.
In this context, the present study considered to establish the optimal conditions for two preparation techniques of oilseed extracts: method I- QuEChERS (Quick, Easy, Cheap, Efficient, Robust and Safe) extraction and method II- saponification by liquid-liquid extraction (LL), followed by purification by d-SPE QuEChERS. Different factors were studied for each preparation method: type of extraction salt, volume of solvent/purified extract, type of solvent, etc. (method I) and quantity of reagents/ solvents, stirring method, freezing time (method II), respectively.

We aim to find an optimal protocol for extracting and purifying extracts from oilseeds, using low amounts of chemical reagents, eliminating or replacing highly toxic reagents, obtaining minimal amounts of analytical waste, and in agreement with the principles of green chemistry.

MATERIALS AND METHODS

Experimental materials

In this study, three varieties of sunflower seeds with different fat content: S1- 46.39%, S2- 42.06%, S5- 48.44% (Figure 2), and other oilseeds purchased from the Romanian market: mixture of sunflower and pumpkin seeds (MSP), hulled sunflower seeds (HS), white sesame seeds (WS), linseed (L), hulled pumpkin seeds (HP) and rapeseed (R) (Figure 3) were used.

Figure 2. Sunflower seeds, natural and ground, with different fat content (S1, S2, S5)

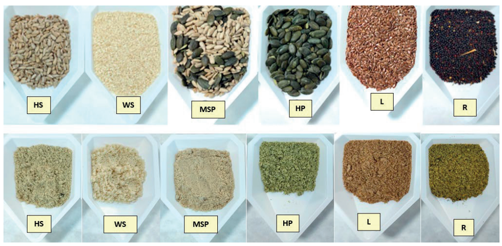

Figure 3. Oilseeds (natural and ground) sold on the Bucharest market

The samples used were processed unspiked or spiked with PAHs solution in different concentrations (2, 5, 10 µg/kg).

Standards, solvents and reagents

The following standards were used: reference standard of native PAHs (mixture of 4 PAHs) in isooctane (EFSA-4 Native PAH, ES-5542, 5 µg/mL); reference standard for labelled PAHs (mixture of 4 ^{13}C-labelled PAHs) in nonane (EFSA-4 ^{13}PAH, ES-5540, 5 µg/mL), both from Cambridge Isotope Laboratories, Inc, USA.

As injection standard (ISI), solution of 9-fluorobenzo[k]fluoranthene (FBkF) in toluene of 100 µg/mL concentration was used which was added to the sample extract prior to injection into the GC-MS/MS.

Solvents of chromatographic purity were used: acetonitrile, n-hexane, methanol, purchased from VWR Chemicals, Belgium. QuEChERS extraction salt kits were used: citrate salts (EN)- 4 g $MgSO_4$ + 1 g NaCl + 0.5 g Na_2H-citrate x 1.5 H_2O- disodium hydrogen citrate sesqui-hydrate rate + 1 g Na_3-citrate x 2 H_2O- trisodium citrate dihydrate; acetate salts (AOAC) - 6 g $MgSO_4$ + 1.5 g CH_3COONa; 4 g $MgSO_4$ + 1 g NaCl (ORIGINAL), purchased from Macherey-Nagel GmbH & Co, Germany/Agilent Technologies, USA/Thermo-Scientific, USA.

In order to purify the extracts, d-SPE-QuEChERS purification kits from Agilent were used: 0.15 g PSA + 0.9 g $MgSO_4$ (5982-5056CH); Enhanced Matrix Removal-Lipid (EMR-Lipid 5982-1010 + EMR-Lipid 5982-0101). Z-Sep$^+$ (Supel QuE Z-Sep$^+$ Bulk, 55418-U, Sigma Aldrich St Louis, USA) was used as well as sorbent for purification. Anhydrous magnesium sulphate ($MgSO_4$) of analytical grade was purchased from Merck, KGaA, Germany.

Disposable ceramic homogenizers (Thermo Fisher Scientific) for 15- and 50-ml tubes were used to homogenize the samples.

Sample preparation

Two extraction methods for the PAH determination from oilseed matrices, were carried out, for both d-SPE QuEChERS purification being used.

Method I - QuEChERS extraction with modifications

For this method 15 experimental variants were performed (Table 2) with the variation of extraction salts, volume of solvent/extract, the type of solvent in combination with the type of sorbent and the weight of sample used.

Table 2. Experimental variants - Method I

Crt. No.	Variable factor			Variant	QuEChERS method with modification (Method I)
1.	QuEChERS extraction salt	EN		V1	5 g sample weighed in a 50 mL centrifuge tube + 10 mL water + 10 mL hexane (H) + QuEChERS salts → vortexing/homogenization → centrifugation → hexane evaporation → collecting the residue with 10 mL acetonitrile (ACN) → vortexing/homogenization → freezing 1-3 h → filtration through quartz wool → 3.5 mL extract purified by EMR-Lipid
		AOAC		V2	
		ORIGINAL		V3	
2.	Volume of ACN/purified extract	10 mL/3.5 mL		V4	5 g sample weighed in a 50 mL centrifuge tube + 10 mL water + 10 mL H + QuEChERS citrate salts → vortexing/homogenization → centrifugation → hexane evaporation → collecting residue with 5-10 mL ACN → vortexing/homogenization → freezing 1-3 h → filtration by quartz wool → 3.5/5 mL extract purified by EMR-Lipid
		10 mL/5 mL		V5	
		5 mL/3.5 mL		V6	
		5 mL/5 mL		V7	
3.	d-SPE QuEChERS Solvent type/sorbent type	H	EMR	V8	5 g sample weighed in a 50 mL centrifuge tube + 10 mL water + 10 mL H/ACN + QuEChERS citrate salts → vortexing/homogenization → centrifugation → hexane evaporation → collecting the residue with 10 mL ACN → vortexing/homogenization → freezing 1-3 h → filtration through quartz wool → 3.5 mL extract purified by EMR-Lipid/500 mg Z-Sep$^+$
			Z-Sep$^+$	V9	
		ACN	EMR	V10	
			Z-Sep$^+$	V11	
4.	Weight of sample	2 g		V12	2/3/4/5 g sample weighed in a 50 mL centrifuge tube + 10 mL water + 10 mL H + QuEChERS citrate salts → vortexing/homogenization → centrifugation → hexane evaporation → collecting the residue with 10 mL ACN → vortexing/homogenization → freezing 24 h → filtration through quartz wool → 3.5 mL extract purified by 500 mg Z-Sep$^+$ + 300 mg $MgSO_4$
		3 g		V13	
		4 g		V14	
		5 g		V15	

Method II - Saponification and liquid-liquid extraction (LL)

The variants carried out (Table 3) in this method were aimed at optimizing the saponification and LL extraction steps with the reduction of the volume of organic reagents/solvents, with the variation of the shaking method of the extracts during the saponification and with the variation of the freezing time of the extracts before d-SPE QuEChERS purification.

To evaluate the performance of each method, the following factors were considered: obtaining colourless and clean extracts, with values of co-extract residues as low as possible (≤ 2 mg/mL), increasing the extraction efficiency by obtaining good recoveries of the 4 PAHs, which should fall within the limits imposed by European regulations no 836/2011 (50-120%).

To investigate the removal of the compounds from the matrix, after the sample extraction and purification procedure, the co-extractive residue of the sample was determined gravimetrically. The co-extraction residue weight was determined by evaporating 1 mL of the final sample extract under a nitrogen atmosphere.

PAHs analysis by GC-MS/MS was performed using a gas chromatograph (Trace GC Ultra) coupled with tandem triple quadrupole mass spectrometer (TSQ Quantum XLS), both from Thermo Fisher Scientific (USA).

Table 3. Experimental variants - Method II

Crt. No.	Variable factor		Variant	Working method (Method II)
1.	Quantities of solvents used in the saponification stage	200 mL CH_3ONa 200 mL hexane 200 mL methanol + water	V1	2 g sample weighed in 250 mL Erlenmeyer flask + 200/100 mL methanolic solution of sodium methoxide (CH_3ONa) → bath stirring (V3/30 min, 60°C) → cooling to room temperature → extraction with hexane (H) (2 x 100/50 mL) → extract washing with a mixture of methanol + water (4:1, v/v) (2 x 100/50 mL) → concentration to a volume of 6 mL → purification by d-SPE QuEChERS (0.15 g PSA + 0.9 g $MgSO_4$)
		100 mL CH_3ONa 100 mL hexane 100 mL methanol + water	V2	
2.	Stirring method of the extract during saponification	Water bath with heating and ultrasonic stirring	V3	2 g sample weighed in 250 mL Erlenmeyer flask + 100 mL CH_3ONa solution → bath stirring (30 min, 60°C) → cooling to room temperature → extraction with H (2 x 50 mL) → extract washing with a mixture of methanol + water (4:1, v/v) (2 x 50 mL) → concentration to a volume of 6 mL → purification by d-SPE QuEChERS (0.15 g PSA + 0.9 g $MgSO_4$)
		Bath with heating and magnetic stirring	V4	
		Water bath with heating and mechanical stirring	V5	
3.	Freezing time	No freezing + EMR-Lipid	V6	2 g sample weighed in 250 mL Erlenmeyer flask + 100 mL CH_3ONa solution → bath stirring (V5/30 min, 60°C) → cooling to room temperature → extraction with H (2 x 50 mL) → extract washing with mixture of methanol + water (4:1, v/v) (2 x 50 mL) → concentration until H evaporation → collecting residue with 2 x 5 mL ACN → vigorous vortexing → purification with and without freezing → filtration through quartz wool → purification by EMR-Lipid (5 mL)
		Freezing 24 h + EMR-Lipid	V7	

Injection of extracts was performed with an autosampler (Thermo Fisher Scientific, USA), and analytical separation was achieved on a TraceGOLD TG-PAH capillary column (30 m × 0.25 mm I.D. x 0.10 μm) from Thermo Fisher Scientific (USA). GC separation was initiated by volatilizing the sample in an injector (Right PTV), heated to 70°C, in splitless mode. The oven temperature program was: initial temperature 80°C, held for 1 min, an increased with 15°C/min on ramp 1 to 210°C, a 25°C/min increase on ramp 2 to 260°C, a 5°C/min increase on ramp 3 to 305°C (held 2 min), then an increase with 25°C/min on ramp 4 to 350°C (held 5 min). The temperatures of the transfer line and for the ion source were 340°C and 300°C, respectively. Total acquisition time was 30.67 min. The MS/MS operated using the electron ionization (EI) technique, in SRM mode, with the precursor ions fragmentation into product ions. Table 4 lists precursor-to-product ion transitions (MS/MS transitions).

Table 4. Parameters for the analysis of PAHs from oilseeds, by GC-MS/MS

PAH/labelled PAH/ISI	Precursor ion (*m/z*)	Product ion (*m/z*) and quantification ions (*m/z*) with bold
BaA	228	202, **226**
$^{13}C_6$ (BaA-IS)	234	208, **232**
CHR	228	202, **226**
$^{13}C_6$(CHR- IS)	234	208, **232**
FBkF	270	249, **268**
BbF	252	226, **250**
$^{13}C_6$ (BbF-IS)	258	232, **256**
BaP	252	226, **250**
$^{13}C_4$ (BaP-IS)	256	228, **254**

As collision gas Argon was used. Xcalibur software was used for data processing. To each sample, labelled PAHs were added from the beginning, therefore it compensates the analyte losses that might occur during preparation.

PAHs were quantified based on a calibration curve (PAH/PAH-IS area ratio as a function of PAH/PAH-IS concentration ratio), with peaks identified based on MS response and GC retention time. Each sample was analysed using the same analytical conditions.

Statistical analysis

Results for the co-extract residues were expressed as mean ± standard deviation (sd) and were statistically analysed by using Minitab statistical software version 20. One-way analysis of variance (ANOVA test), followed by Tukey's test were used to evaluate the statistical significance between results. The chosen level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSIONS

The higher amount of fat in oilseeds and their derivative products has become one of the major challenges in the laboratory analysis of PAHs. Inefficient separation of these substances may adversely affect the identification and/or quantification of PAHs. The separation of PAHs from high-fat food samples (Alomirah et al., 2010; Dost & Ideli, 2012; Mohammadi et al., 2020; Sánchez-Arévalo et al., 2020) before the further steps of the analytical process is a current and difficult problem for which efforts are being made to develop new preparation methods. Even with the use of advanced mass spectrometry-based techniques, extensive steps are required to extract and purify PAHs from complex fatty matrices (Parrilla Vázquez et al., 2016). To establish the optimal method for PAHs determination, the S1 sunflower seeds were used in experiments.

Method I - QuEChERS extraction with modifications

QuEChERS extraction salt type

In these variants (V1-V3), three extraction salt kits were used: EN, AOAC and original salts and the results are presented in Table 5.

From a visual point of view, the extracts from the proposed variants were clean and colourless and from a gravimetric point of view, the co-extract residues varied between 0.77-0.91 mg/mL, fulfilling the imposed criterion (≤ 2 mg co-extract/mL). The lowest values were obtained in the case of V1 with the use of citrate salts. No PAHs were detected in the sunflower seeds (S1) studied.

The average recoveries obtained for the samples spiked with 5 µg/kg PAHs solution ranged between 98.23-118.61%, fulfilling the criterion imposed by the European regulation.

Table 5. Results obtained in V1, V2, V3 (Method I)

Variant/ co-extract, mg/mL	PAH, µg/kg (mean ± sd)		Recovery, % (mean ± sd)	RSD (%)
	Spiked – 5 µg/kg			
V1 (EN)/ 0.77 ± 0.02[b]	BaA	5.27 ± 0.15	105.44 ± 3.09	2.93
	Chr	5.40 ± 0.11	108.05 ± 2.13	1.97
	BbF	5.16 ± 0.26	103.13 ± 5.17	5.01
	BaP	4.91 ± 0.12	98.23 ± 2.37	2.42
V2 (AOAC)/ 0.91 ± 0.03[a]	BaA	5.93 ± 0.12	118.61 ± 2.40	2.03
	Chr	5.12 ± 0.05	102.44 ± 0.97	0.94
	BbF	5.41 ± 0.13	108.17 ± 2.60	2.40
	BaP	5.88 ± 0.17	117.67 ± 3.42	2.91
V3 (ORIG.) 0.79 ± 0.01[b]	BaA	4.99 ± 0.08	98.77 ± 0.77	1.53
	Chr	5.65 ± 0.35	113.06 ± 6.93	6.13
	BbF	5.13 ± 0.15	102.64 ± 2.99	2.91
	BaP	5.32 ± 0.43	106.31 ± 8.57	8.06

Values followed by different letters are statistically different ($p < 0.05$)

The highest co-extract residue value was determined for V2, when acetate salts were used, but the values for all variants were withing the criterion settle by EC. Since no significant difference ($p > 0.05$) was registered between the values of co-extract residues obtained with V1 and V3, when choosing the optimal variant to continue the experiments the relative standard deviation (RSD) was considered. In the case of citrate salts (V1) it was obtained lower RSD values (<5.01%) than in the case of original method (V3) (1.53-8.06%), therefore this variant was selected as optimal for an efficient extraction and used in further proposed experiments.

Volume of ACN/purified extract

Taking into account the protocol from the previous variants and aiming to obtain clean extracts, with an amount of co-extract residues as low as possible, it is observed (Figure 4) that collecting the extract after hexane evaporation with a larger volume of solvent (10 mL ACN/V4/V5) the lowest co-extract residue values were obtained compared to displacing a smaller volume (5 mL/V6/V7). ACN is known to be a medium-polarity solvent which extract PAHs with weak polarity and are capable to dissolve it (Payanan et al., 2013; Sun and Wu, 2020).

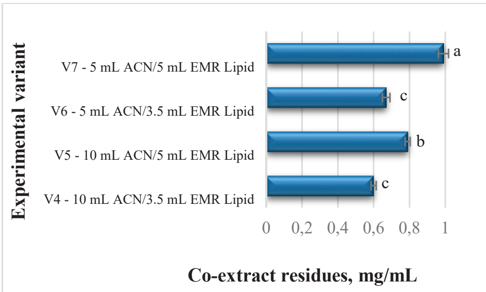

Figure 4. Co-extract residues from V4-V7 (Method I)
Columns followed by different letters are significantly different ($p < 0.05$)

By comparing V4 with V5 co-extract residues it was observed that when a smaller volume of extract (3.5 mL) was used for purification a significant lower value of co-extract residues (0.60 mg/mL) was determined (Figure 5). The results obtained in V4 are also confirmed by the areas, S/N, the higher chromatographic peak intensities obtained in this variant, which were higher than the ones obtained in the variants V5, V6, and V7.

Also, when it comes to recoveries (Figure 6), the use of a larger volume of ACN (10 mL/V4) and a lower volume of extract (3.5 mL) led to good results for samples spiked with 5 µg/kg PAH (96.82-112.50%).

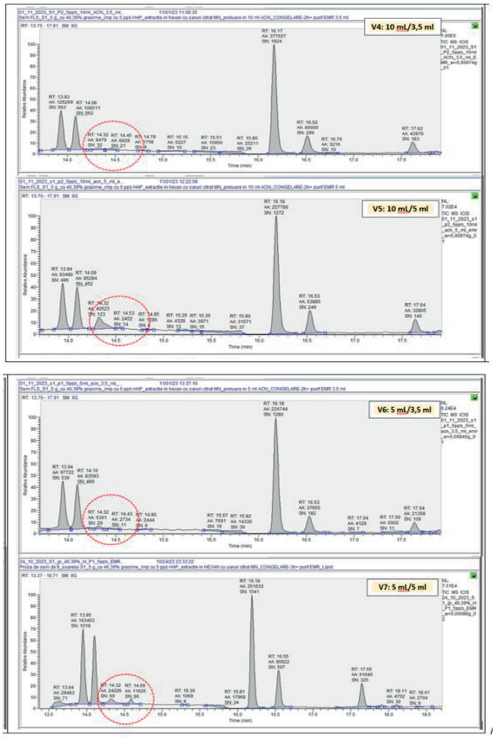

Figure 5. Chromatograms of extracts from V4-V7 (method I)

The recovery obtained for Chr did not fall within the required range (50-120%) in the case of V6. A sufficiently cleaned-up extract was obtained for V4 variant, and based on these results and the obtained recoveries, this variant was selected as optimal and it was used in the following experiments.

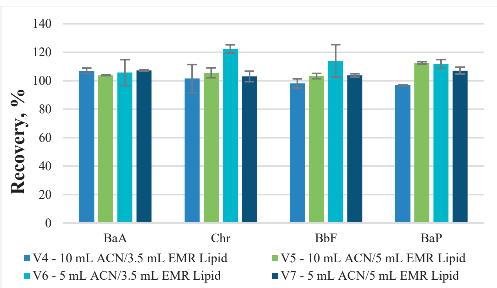

Figure 6. PAH recoveries for V4-V7 (method I)

Payanan et al. (2013) also showed that when a higher volume of ACN (8-10 mL) was used for extraction, the PAHs recoveries were better than when using 4 mL ACN.

d-SPE QuEChERS solvent type/sorbent type

For the QuEChERS extractions the following solvents were used: hexane (H) and acetonitrile (ACN), and for the d-SPE QuEChERS purification two types of sorbents were used: EMR-Lipid (V8, V10) and Z-Sep$^+$ (V9, V11). EMR-Lipid is a new adsorbent salt which has the purpose to remove lipids from the food matrix, while Z-Sep$^+$ is a silica support coated with zirconium dioxide sorbent used as well for lipids adsorption (Sun and Wu, 2020; Belarbi et al., 2021).

Unspiked samples and spiked samples with 5 and 10 µg/kg PAH solution were analysed. The co-extract residues and recoveries results obtained in the experimental variants are presented in Figures 7 and 8.

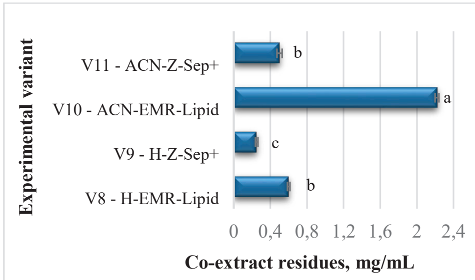

Figure 7. Co-extract residues for V8-V11 (method I)
Columns followed by different letters are significantly different ($p < 0.05$)

In all variants except V10, the extracts were clear, colourless. In V10, the final extract showed a yellowish colour, which is also reflected by the higher value of the determined co-extract residue (2.22 mg/mL), exceeding the imposed criterion (≤ 2 mg/mL).

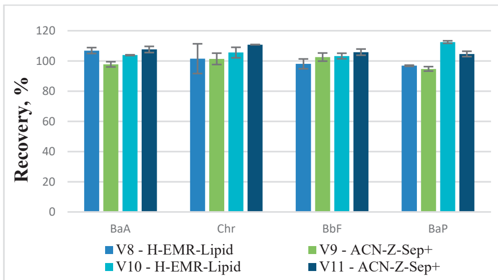

Figure 8. PAH recoveries for V8-V11 (method I)

Comparing the results of the variants with H extraction (V8, V9) with those with ACN extraction (V10, V11) it was observed that by using H the lowest amounts of co-extract residues were determined for both types of sorbents (0.25 mg/mL/Z-Sep$^+$/V9; 0.60 mg/mL/ EMR-Lipid/V8). The lower values of co-extract residues in the case of H extraction determined cleaner extracts. These results were correlated with the higher values of the chromatographic peak intensities of the 4 PAHs (areas, S/N, etc.). The recoveries determined for the spiked samples ranged between 94.75-106.88%, falling within the required range of 50-120% (Figure 8). Based on these results, the variant with H and Z-Sep$^+$ was consider optimal and was used in the following experiments.

Weight of sample

In order to investigate the influence of sample weight on the co-extract residue and recoveries, different weights of S1 sample (2, 3, 4 and 5 g) were studied, maintaining the previously established optimal parameters. The extracts were subjected to a longer freezing time (24 h) and during the d-SPE QuEChERS purification with Z-Sep$^+$ sorbent, $MgSO_4$ was added in order to remove any water traces from the final extract.

Regardless of the sample weight used, it is found that the values of the co-extract residues determined are similar (0.21-0.22 mg/mL) ($p > 0.05$) (Table 6).

Table 6. Results obtained for V12-V15

Variant/ co-extract, mg/mL	PAH, µg/kg (mean ± sd) Spiked - 5 µg/kg		Recovery, % (mean ± sd)	Ion ratio status
V12/ 0.21 ± 0.02^a	BaA	2.12 ± 0.09	84.89 ± 3.70	Failled/Not used
	Chr	2.11 ± 0.36	84.50 ± 14.43	Passed/Failled
	BbF	2.52 ± 0.02	100.99 ± 0.83	Failled/Coelution Failure
	BaP	2.27 ± 0.01	90.78 ± 0.51	Passed/Passed
V13/ 0.22 ± 0.01^a	BaA	2.63 ± 0.01	105.09 ± 0.39	Passed/Not used
	Chr	2.24 ± 0.07	89.79 ± 2.64	Passed/Passed
	BbF	2.51 ± 0.01	100.36 ± 0.56	Failled/Coelution Failure
	BaP	2.65 ± 0.06	106.16 ± 2.30	Passed/Passed
V14/ 0.21 ± 0.01^a	BaA	2.39 ± 0.00	95.65 ± 0.07	Passed/Passed
	Chr	2.44 ± 0.03	97.54 ± 1.25	Passed/Passed
	BbF	2.50 ± 0.07	100.06 ± 2.76	Coelution/Failure/Passed
	BaP	2.47 ± 0.08	98.71 ± 3.18	Passed/Passed
V15/ 0.21 ± 0.01^a	BaA	2.56 ± 0.00	102.41 ± 0.07	Passed/Passed
	Chr	2.45 ± 0.01	98.05 ± 0.23	Passed/Passed
	BbF	2.50 ± 0.01	99.96 ± 0.35	Passed/Passed
	BaP	2.37 ± 0.01	94.62 ± 0.55	Passed/Passed

Values followed by different letters are statistically different ($p < 0.05$)

The recovery values of the 4 PAHs, in the case of the experimental variants, ranged between 84.50-106.16%, falling within the imposed criterion (50-120%).

By using a lower amount of sample (< 5 g) the criteria specified in the processing method for qualifier ions, specific to BbF, Chr and BaA compounds (ion ratio confirmation, target ratio, qualifier ion coelution, etc.) were not confirmed. The status obtained for these compounds during quantification was "Failed/Coelution Failure/ Not used". In the case of V4 in which 5 g of sample were used, the criteria imposed for the qualification ions specific to the 4 PAHs were met, resulting the "Passed" status. In the case of this variant, the best results were obtained for the determined concentration (2.37-2.56 µg/kg), recovery (94.62-102.41%), as well as the lowest values of standard deviations. Additionally, comparing V15 (24 h freezing) with V9 (1-3 h freezing) from previous experiments, it is observed that the application of a longer freezing time caused a reduction of co-extracts by 16% and therefore V15 was considered as the optimal procedure for Method I. Similarly, Payanan et al. (2013) showed that a 24 h freezing time eliminates the fat from the extract, without influencing the PAH content of sample.

Functionality of the method I/V15 on other types of oilseeds

Based on V15/Method I, the oilseeds shown in Figure 3 were processed.

These seeds were processed unspiked and spiked with 10 µg/kg. No PAHs were quantified in the analysed oilseeds. The average values (n=4) of co-extract residues determined from oilseeds varied between 0.14-1.35 mg/mL, falling within the imposed criterion (Figure 9). A significant higher value was obtained for white sesame seeds.

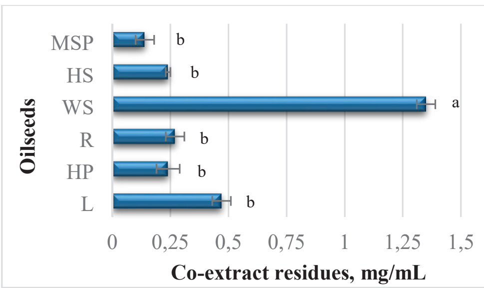

Figure 9. Co-extract residues (mean ± sd) for different oilseeds (V15/method I). Columns followed by different letters are significantly different ($p < 0.05$)

From the point of view of extraction efficiency, the obtained results showed that very good recoveries were determined for all 4 PAHs, in the case of HP seeds (100.32-100.97%) and MSP seeds (97.28-101.23%). For the other seeds (L, R, WS, HS), the recoveries ranged between 93.25-110.71% in the case of BaA, Chr and BaP compounds, and for BbF, the recovery values exceeded the value of 120%.

Method II – Saponification and liquid-liquid extraction (LL)

Similar to Method I, the variable factors presented in Table 3 were evaluated, from the point of view of co-extractive residues and recoveries by using the S1 sunflower seed sample spiked with 5 and/or 10 µg/kg PAH solutions. The results for co-extractive residue, mean content and recoveries are presented in Table 7.

Table 7. Results obtained for V1-V7 variants/Method II

Variant/ co-extract, mg/mL	PAH, µg/kg (mean ± sd)		Recovery, % (mean ± sd)
V1/ 66.53 ± 5.53^{a}	Spiked - 5 µg/kg		
	BaA	5.18 ± 0.11	103.66 ± 2.18
	Chr	5.00 ± 0.00	100.00 ± 0.09
	BaA	5.43 ± 0.29	108.57 ± 5.88
	BaP	5.14 ± 0.02	102.83 ± 0.43
	Spiked - 10 µg/kg		
	BaA	10.69 ± 0.17	106.94 ± 1.69
	Chr	10.83 ± 0.04	108.31 ± 0.37
	BaA	10.52 ± 0.58	105.24 ± 5.78
	BaP	10.78 ± 0.18	107.78 ± 1.77
V2/V3 47.18 ± 6.50^{b}	Spiked - 5 µg/kg		
	BaA	5.26 ± 0.04	105.10 ± 0.85
	Chr	5.09 ± 0.06	101.74 ± 1.12
	BaA	4.94 ± 0.28	98.87 ± 5.54
	BaP	5.16 ± 0.18	103.11 ± 3.53
	Spiked - 10 µg/kg		
	BaA	10.30 ± 0.18	103.02 ± 1.82
	Chr	10.34 ± 0.09	103.43 ± 0.93
	BaA	9.76 ± 0.11	97.56 ± 1.06
	BaP	9.96 ± 0.01	99.59 ± 0.10
V4/ 39.02 ± 0.08^{b}	Spiked - 5 µg/kg		
	BaA	4.92 ± 0.60	98.34 ± 12
	Chr	4.78 ± 0.07	95.68 ± 1.48
	BaA	5.11 ± 0.13	102.17 ± 2.58
	BaP	5.03 ± 0.17	100.65 ± 3.48
	Spiked - 10 µg/kg		
	BaA	10.42 ± 0.17	104.15 ± 1.69
	Chr	10.15 ± 0.35	101.51 ± 3.47
	BaA	10.69 ± 0.15	106.93 ± 1.49
	BaP	10.13 ± 0.30	101.32 ± 3.05
V5/ 38.24 ± 0.30^{b}	Spiked - 5 µg/kg		
	BaA	5.20 ± 0.02	104.09 ± 0.46
	Chr	5.40 ± 0.34	107.98 ± 6.73
	BaA	5.49 ± 0.09	109.73 ± 1.90
	BaP	5.09 ± 0.14	101.72 ± 2.82
	Spiked - 10 µg/kg		
	BaA	9.95 ± 0.22	99.47 ± 2.21
	Chr	9.41 ± 0.00	94.15 ± 0.01
	BaA	10.08 ± 0.06	100.77 ± 0.58
	BaP	10.39 ± 0.35	103.89 ± 3.50
V6/ 2.42 ± 0.13^{c}	Spiked - 10 µg/kg		
	BaA	10.26 ± 0.37	102.61 ± 3.69
	Chr	8.62 ± 0.58	86.19 ± 5.80
	BaA	10.37 ± 0.63	103.73 ± 6.33
	BaP	8.06 ± 0.21	80.60 ± 2.11
V7/ 1.10 ± 0.04^{c}	Spiked - 10 µg/kg		
	BaA	9.27 ± 0.01	92.66 ± 0.14
	Chr	10.01 ± 0.05	100.07 ± 0.50
	BaA	10.51 ± 0.37	105.09 ± 3.72
	BaP	9.75 ± 0.67	97.52 ± 6.70

Values followed by different letters are statistically different ($p < 0.05$)

Although the final extracts from V1-V7 were clean, colourless, the determined values of co-extract residues varied on average between 1.10-66.53 mg/mL, exceeding, in the case of V1-V6, the criterion imposed for co-extracts.

Comparing V6 with V5 it was observed that when extract is obtained with ACN and EMR-Lipid (V6) in the d-SPE QuECHERS purification, a reduction of co-extracts by about 93% resulted compared to V5 in which the extracts were obtained in hexane and were purified with PSA. This could be an explanation for the higher amounts of co-extract residues in V1-V5. The fats are solubilized in hexane and their existence in the extract determines the inefficiency of the purification (Sánchez-Arévalo et al., 2020).

After running the extracts from these variants to GC-MS/MS, a significant loss of sensitivity was observed after a small number of injections, as well as a build-up of matrix co-extraction compound residues in the GC liner, causing the repeated maintenance of the GC-MS, with the change of the liner and the chromatographic column.

Figure 10 shows images of the liner before (a) and after its change (b), after a number of 40-50 injections.

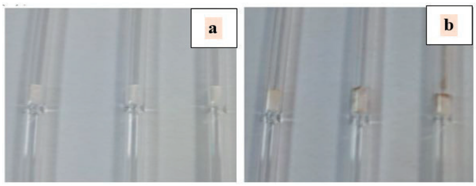

Figure 10. Liners before (a) and after (b) injection of extracts

It was also observed that when EMR-Lipid sorbent combined with extract freezing in ACN (V7) were used in d-SPE QuEChERS purification it led to a much lower co-extract residues (1.10 mg/mL) compared to the non-freezing variant V6 (2.51 mg/mL). The reduction of the co-extract amount in V7 could be explained by the fact that the fat present in ACN extracts subjected to low temperature (freezing), solidifies/ precipitates and can later be separated by centrifugation or filtration. Freezing removes most of the lipids, waxes and sugars, as well as other components with low solubility in ACN, which can adversely affect the robustness of the GC-MS/MS analysis. Highly lipophilic compounds such as tri-, di-, mono-glycerides and free fatty acids could be removed by freezing, some lipidic compounds being able to precipitate (Payanan et al., 2013; Parrilla Vázquez et al., 2016).

Taking into account all the inconveniences that appeared in variants V1-V6 within Method II, variant V7 was selected as optimal.

Functionality of the method II/V7 on other types of oilseeds

Based on V7 conditions, 3 types of sunflower seeds (S1, S2, S5) with different fat content (Figure 2) and three other types of oilseeds, L, R, HP (Figure 3) were processed. The mean values (n=2) of co-extract residues determined from oilseeds ranged between 1.07-2.04 mg/mL (Figure 11).

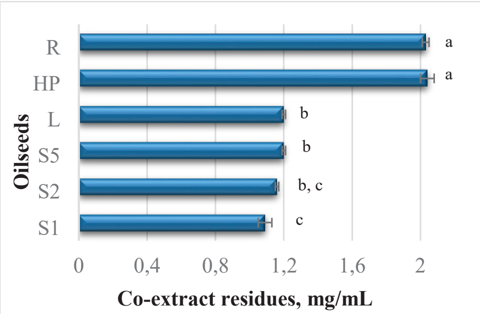

Figure 11. Co-extract residues (mean ± sd) for different oilseeds (V7/Method II). Columns followed by different letters are significantly different ($p < 0.05$)

No PAHs were identified in the analysed oilseeds. Recoveries obtained for samples spiked with 10 μg/kg PAH solution (sunflower seeds) were very good for all 4 PAHs, S1: 92.66-105.09%; S2: 93.92-105.47%; S5: 96.72-111.74%, meeting the criterion for recovery according to Reg. EU 836/2011.

CONCLUSIONS

Comparing the results, from the point of view of co-extractive residues and recoveries obtained, V15/Method I was selected as the optimal protocol for oilseeds processing for the determination of PAHs by GC-MS/MS.

The recoveries (50-120%) and co-extract residue values (≤ 2 mg/mL) determined by this variant fell within the required criteria. The final processing protocol consists of using 5 g of sample, 10 mL of water, 10 mL of hexane as extraction solvent and QuEChERS citrate salts; hexane evaporation followed by collecting the residue with 10 mL ACN and then the purification is performed by freezing the extract for 24 h and afterwards 3.5 mL of extract is purified by d-SPE QuEChERS, using the Z-Sep$^+$ sorbent. Also, this selected variant (QuEChERS extraction and purification) is easier to apply, causes minimal amounts of analytical waste, requires low amounts of chemical reagents, eliminates highly toxic reagents, and is much faster compared to V7/Method II (saponification with LL extraction).

Further research will be carried out in order to obtain better recoveries for BbF and to validate the method.

ACKNOWLEDGEMENTS

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STUDIES ON THE DISTRIBUTION, ECOLOGY AND PHYTOSOCIOLOGY OF *Ligularia sibirica* L. POPULATIONS IN THE CĂPĂȚÂNII MOUNTAINS, ROMANIA

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Abstract

Ligularia sibirica (L.) Cass. popularly called Siberian Leopard Plant is a species of community interest with a small area in the mountainous region of southwestern Romania. This rare species of Eurasian origin is found in Appendices II and IV of the Habitats Directive and the IUCN Red List of Threatened Species. L. sibirica was cited until the start of these studies from 2 locations in the Capăţănii Mountains: the basin of the Luncavăț river and the Buila Mountain, being mentioned as a single population with very few individuals. The populations identified in the studied territory are not stable and not well preserved. The populations in that area have a relatively small number of individuals, their size and state of preservation being closely related to soil moisture, nitrogen availability, temperature and lighting are the ecological factors that influence the morphological characteristics of L. sibirica populations.

***Key words**: Ligularia sibirica, populations, corology, ecology, plant communities, Căpățânii Mountains.*

INTRODUCTION

Ligularia sibirica (L.) Cass. popularly called Siberian Leopard Plant is a species of community interest with a small area in the mountainous region of southwestern Romania.
This rare species of Eurasian origin is found in Appendices II and IV of the Habitats Directive (https://environment.ec.europa.eu/topics/nature-and-biodiversity/habitats-directive) and the IUCN Red List of Threatened Species (https://www.iucnredlist.org).
In Romania, it is considered a rare species (Oltean et al.,1994).
Taxonomic classification:
Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Ligularia
In Europe, the species occupies the most areas in France and Romania, then in Bulgaria, Poland, Austria, the Czech Republic, Croatia, Latvia and Slovakia (Hultén and Fries, 1986; Hegi, 1987; Lannie and Sammul, 2013; Niculescu, 2019). According to some authors, its main distribution area is the European part of Russia and the Siberian taiga area (Hultén and Fries, 1986; Lannie and Sammul, 2013). Until the beginning of these studies in the Capatanii Mountains, only one population of *Ligularia sibirica* from the Luncavăț river basin and one from Cheia Valley, part of Buila Mountain, were cited. In Romania, it is found in depressions, clearings, marshes, weeds, in marshy places in forests, in mountainous and subalpine areas. From Romania it was mentioned from the following counties: Maramureș, Bistrița Năsăud, Cluj, Harghita, Covasna, Brașov, Vâlcea, Prahova, Neamț, Suceava, Bacău, Botoșani (Gh. Groza, 2008, M. Niculescu, 2006; 2019; 2020). Following the field research that we carried out between 2007-2023, we found that the most important populations in terms of the occupied surfaces and the number of individuals are found in the counties of Harghita, Bacău and Covasna. The Siberian Leopard plant is a hemicryptophyte species, with a height that can reach up to 130 cm, spread especially in the mountain area through depressions, clearings, marshes, forest edges, weedy forests along valleys, generally in places with high humidity and fairly high acidity.

MATERIALS AND METHODS

Study area: The thematic area is situated in the Căpățânii, Mountains (Figure 1). After the physical-geographical division into sectors, the Căpățânii Mountains belong to the Central European province, the Carpathian subprovince, the land of the Southern Carpathians, the Western District (Godeanu-Parâng). From an orographic point of view, the Căpăţânii Mountains present a high, main, bare ridge (crest) oriented in the east-west direction, from which there are numerous ramifications, to the north, as well as to the south, which have the shape of edges and crests and which locals call "plaiuri". These mountains stand out by their deeply fragmented and well developed massive relief (Popescu, 1968). The most important crests and ridges of these mountains, are the following: *Ursu Peak* (2,124 m), *Căpăţâna Peak* (2,113 m), *Balota Peak* (2,094 m), *Coşana Peak* (2,080 m), *Curmătura Funicelul* (1,820 m), *Mt. Piatra Roşie* (1,820 m), *Roman's Peak* (1,810 m), *Mt. Dârjala* (1,900 m), *Mt. Petriceaua* (1,650 m), *Muchia Căşăriei* (1,721 m) (Niculescu, 2006).

Figure 1. Aspect from the researched territory, Căpăţâna Peak (photo: Mariana Niculescu)

For the study of the plant community, we have used methods of phyto-sociologic research characteristic to the Central European phyto-sociologic School. In order to identify the plant communities in which the species *Ligularia sibirica* grows, the characteristic and dominant species were taken into account and the constant of the species was noted for each individual within the phytocoenosis. For the population and phytosociological studies of the species *Ligularia sibirica*, determinations were made in the field regarding the number of individuals within a phytocoenosis, the abundance-dominance of species within the phytocoenosis within the plant communities where it is found, the specific seasonal conditions in which it develops the species, variables related to the behavior of the species as well as data on the chorology of the species develops the species, variables related to the behavior of the species as well as data on the chorology of the species.

RESULTS AND DISCUSSIONS

According to the European Nature Information System species Natura 2000 data base (http://natura2000.eea.europa.eu), *Ligularia sibirica* is a species of community importance reported from 32 Natura 2000 Sites from Romania. Following field research between 2007-2023 in the Căpățânii Mountains, few populations of *Ligularia sibirica* were identified. The number of individuals varies from 2 to a maximum of 30 individuals, this being closely consistent with the substrate and ph-value as well as the degree of humidity in the soil, taking into account that it is a mesohygrophilous to hygrophilous species (Figures 2, 3).

Figure 2. *Ligularia sibirica* in the Căpățânii Mountains - Govora Valley
(photo: Mariana Niculescu)

Figure 3. *Ligularia sibirica* in the Căpățânii Mountains - Costești Valley (photo: Mariana Niculescu)

Thus, in addition to the populations identified in previous years in the Luncavăț Valley (in the upper basin) and Cheia Valley, following the studies carried out, populations were also identified in the Blajului Valley, Polovrăgenilor Valley, Costești Valley, Stan's Valley, Curpenilor Valley, Cacova Valley and Govora Valley (in the upper basin).

The plant communities in which these populations were identified vary greatly depending on the existing eco-pedo-climatic conditions in these valleys in the Căpățânii Mountains.

The plant communities in which the species *Ligularia sibirica* is found in the Căpățânii mountains are: *Telekio speciosae-Petasitetum hybridi* (Morariu 1967 n.n.) Resmeriţă et Raţiu, *Scirpetum sylvatici* Ralski 1931 em. Schwich 1944, *Carici remotae -Calthetum laetae* Coldea (1972) 1978 (Syn.: *Carici remotae Cardaminetum amarae* Dihoru 1964; *Caltheto-Ranunculetum* (Resmeriţă et al. 1971) Resmeriţă et O. Raţiu 1978; *Lythro salicariae-Juncetum effusi-inflexi* Todor et al 1971; *Deschampsietum caespitosae* Hayek ex Horvatić 1930 (Syn. *Agrostio stoloniferae Deschampsietum caespitosae* Ujvárosi 1947); *Cirsio waldsteinii-Heracleetum palmati* Pawl. et Walas 1940 (Syn. *Cardueto-Heracleetum palmati* Beldie 1967; *Heracleetum palmati* auct. roman.); *Hieracio rotundati-Piceetum* Pawl. et Br.-Bl. 1939 and *Salicetum fragilis* Passarge 1957 (Niculescu, M., 2006; 2020; Niculescu, M. et al., 2015; 2016; 2019).

It should be noted that compared to other locations in the country, especially compared to those in Moldova (Covasna, Harghita, Bacău), the populations identified in Oltenia, in the Căpățânii Mountains have much fewer individuals, the vigor of the plants is low and also the fruiting is not always at the expected level. In dry years, the population of *Ligularia sibirica* in the Căpățânii Mountains is low.

In Blajului Valley, in 2020, populations with only 2 maximum 3 individuals were identified (Figure 4).

Some of the populations with a small number of individuals are found at the edge of the spruce forests or in their clearings (Figure 5).

Cășăriei Mountain road, which has been widened and rebuilt in recent years, allowing the access of a large number of tourists to the area and the intensification of traffic with road vehicles, ATVs or other means of transportation.

All of these exert a very large anthropogenic impact on the entire biodiversity in the area.

There is no control and no involvement of the local authorities, municipality, forestry personnel in this area regarding the conservation of biodiversity, of Natura 2000 species, both flora and fauna. In this area there are also many cow sheds, with a large number of animals and a lot of household waste resulting from tourist activities (with the development of this road, tourism exploded), forestry and pastoral.

Figure 4. *Ligularia sibirica* in the Căpăţânii, Blaj Valley (photo: Mariana Niculescu)

The Luncavăț basin with its tributaries is the limit of the protected area North of the East Gorge, Costești Valley and Cacova Valley are integral parts of the Buila-Vânturarița National Park, so conservation measures for the entire biodiversity should exist and their application should work .

In the Căpăţânii Mountains this species grows in the following Natura 2000 habitats: 6430 - Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels, CLAS. PAL.: 37.7 şi 37.8; 6440 - Alluvial meadows of river valleys of the Cnidion dubii, CLAS. PAL.: 37.23; 3220 - Alpine rivers and the herbaceous vegetation along their banks, CLAS. PAL.: 24.221 and 24.222; 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 9410 - Acidophilous *Picea* forests of the montane to alpine levels (Vaccinio-Piceetea), CLAS. PAL.: 42.21 up to 42.23, 42.25. (Gafta and Mountford, 2008; Niculescu M., 2006).

Figure 5. *Ligularia sibirica* in the Căpățânii, Curpenilor Valley (photo: Mariana Niculescu)

In the researched territory, populations with a smaller number of individuals were identified in the floristic composition of some phytocoenoses of meso-hygrophilous meadows. In these phytocoenoses, the individuals grow more in height and the vigor of the plants is low, the stems are thinner, the inflorescences have a much smaller number of flowers (Figure 6).

Figure 6. *Ligularia sibirica* in the Căpățânii, in the meso-hygrophilous meadows (photo: Mariana Niculescu)

From the point of view of the habitat, in the Căpățânii Mountains the species *Ligularia sibirica* was most often identified in the weed plant communities along the valleys, in the mountain ridge. Most populations were identified on the southern slopes of the Căpățânii Mountains, less often on the northern ones. In meadows, through swampy places in meadows or forests, especially of spruce, the number of *Ligularia sibirica* populations is lower.

The most stable populations with the highest number of individuals were identified in the following plant community: *Telekio speciosae-Petasitetum hybridi* (Morariu 1967 n.n.) Resmeriţă et Raţiu 1978 (Table 1). This plant community is frequently found along streams and springs in the mountain floor. The phytocoenoses of the association develop on flat or slightly inclined lands, with high humidity, more or less shaded.

In the Căpățânii Mountains, such phytocoenoses are found on Polovrăgenilor Valley, Luncavăţ Valley, Blajului Valley, Costești Valley, Stan's Valley, Curpenilor Valley, Cacova Valley and Govora Valley (in the upper basin) at altitudes between 700 m and 1200 m, preferring alluvial soils. Phytocoenoses are mainly mesophilic, micro-mesothermic and euriionic towards acid-neutrophilic (Niculescu, 2006). Following the analyzes carried out, a rich floristic composition is found, the 10 phytocoenoses totaling more 40 species of cormophytes. They are well-cohesed phytocoenoses, with vegetation coverage between 90% and 100%.

Along with the edifying species, high constancy presents some tall species: *Cirsium oleraceum, Urtica dioica*, *Leucanthemum waldsteinii, Festuca gigantea, Ranunculus repens, Agrostis stolonifera, Rumex obtusifolius, Mentha longifolia, Cirsium waldsteinii, Myosotis sylvatica, Impatiens noli-tangere, Lysimachia vulgaris, Chaerophyllum hirsutum, Festuca gigantea*, which edifies the physiognomy of the plant community. Following the studies carried out regarding the bioforms that dominate in these phytocoenoses, it was found that hemicryptophytes dominate, and that the floristic elements, with the largest weight, are Eurasian species. From a karyological point of view, it was found that in these phytocoenoses the highest percentage is polyploid species.

Table 1. *Telekio speciosae-Petasitetum hybridi* (Morariu 1967 n.n.) Resmeriţă et Raţiu 1978 plant community

No. of relevée	1	2	3	4	5	6	7	8	9	10	K
Altitude m.o.s. (x 10 m)	110	100	90	95	115	115	95	110	95	80	
Exposure	SE	E	SE	SV	-	SV	E	-	V	SE	
Inclination (in grades)	7	5	10	10	-	5	10	-	10	5	
Canopy (%)	90	90	100	100	90	100	90	100	100	90	
Area (m^2)	100	30	50	100	50	30	100	50	50	100	
Char. Ass.											
Telekia speciosa	+	+	2	2	1-2	1-2	1	+	+	1	V
Petasites hybridus	5	5	4	4	4	4	4	5	5	4-5	V
Petasition officinalis											
Filipendula ulmaria	+	+	+	-	-	-	-	+	-	-	II
Carduus personatus	-	-	+	+	+	+	-	+	+	-	III
Cruciata laevipes	-	+	+	+	-	-	-	-	+	-	II
Chaerophyllum hirsutum	-	-	+	+	-	-	+	-	-	+	II
Lamium maculatum	+	+	+	+	-	-	+	+	-	-	III
Cirsium oleraceum	+	-	+	+	+	1	1	+	+	1	V
Heracleum sphondylium	+	+	-	+	-	+	-	-	-	-	II
Adenostyletalia											
Senecio germanicus	-	-	+	+	-	+	+	-	+	+	III
Stellaria nemorum	-	-	+	+	+	+	-	+	+	-	III
Leucanthemum waldsteinii	-	-	+	+	-	-	+	-	-	+	II
Cirsium waldsteinii	+	+	+	+	-	-	+	+	-	-	III
Molinio-Arrenatheretea et Molinietalia											
Agrostis stolonifera	+	-	-	+	-	+	+	-	+	-	III
Trifolium pratense	+	+	+	+	-	+	-	+	-	-	III
Holcus lanatus	+	+	+	+	-	+	-	+	-	+	IV
Ranunculus repens	1	1	+	+	+	+	1	+	+	+	V
Mentha longifolia	+	1	1	1	1	1	1	+	1	+	V
Poa pratensis	+	+	+	+	+	-	-	+	+	-	IV
Centaurea phrygia	+	+	+	+	-	+	+	-	-	-	III
Lotus corniculatus	-	-	+	+	+	+	-	+	+	-	III
Prunella vulgaris	-	+	+	+	-	-	-	-	+	-	II
Dactylis glomerata	-	-	+	+	-	-	+	-	-	+	II
Lysimachia nummularia	+	+	+	+	-	-	+	+	-	-	III
Artemisietea											
Rumex obtusifolius	-	+	+	+	-	-	+	-	+	+	III
Urtica dioica	+	+	+	+	+	+	-	+	-	+	IV
Arctium lappa	+	-	-	-	+	-	-	-	-	-	I
Querco-Fagetea											
Festuca gigantea	+	-	+	+	+	+	-	+	+	+	IV
Myosotis sylvatica	+	+	+	+	+	+	+	+	+	+	V
Salvia glutinosa	-	+	+	+	-	-	+	+	+	+	II
Impatiens noli-tangere	+	-	+	+	+	-	-	-	+	+	III
Brachypodium sylvyticum	+	+	+	+	-	+	+	-	-	+	IV
Poa nemoralis	+	+	+	+	+	+	+	+	+	+	V
Epilobietalia											
Chamerion angustifolium	+	-	+	+	-	-	-	-	-	+	II
Rubus idaeus	-	-	-	+	-	-	-	+	-	+	II
Magnocaricion elatae											
Ligularia sibirica	**+-1**	**1**	**+**	**+**	**1**	**1**	**1**	**1-2**	**1-2**	**+-1**	**V**
Lysimachia vulgaris	+	-	+	+	-	-	-	+	+	+	III
Variae Syntaxa											
Cardamine amara	+	+	+	+	-	-	+	+	-	+	IV
Carex remota	-	+	+	+	-	-	-	-	+	-	II
Equisetum telmateia	-	-	+	+	-	-	+	-	-	+	II
Hypericum maculatum	-	-	+	+	+	+	-	+	+	-	III

Place and data of the relevés: 1, 2 - Blaj Valley, 10.VI.2014, 18.VII.2022; 3, 4 - Luncavăț Valley, 23.VII. 2007, 21.VI.2018; 5, 6, 7 - Curpenilor Valley, 9.VII.2011, 15.IV.2015, 2.VIII.2021; 8 - Govora Valley, 22.V.2017; 9, 10 - Costești Valley, 25.VI.2019, 10.VII.2023.

CONCLUSIONS

Following field research in the Căpățânii Mountains, few populations of *Ligularia sibirica* were identified. The number of individuals varies from 2 to a maximum of 30 individuals, this being closely consistent with the substrate and ph-value as well as the degree of humidity in the soil, taking into account that it is a mesohygrophilous to hygrophilous species

From the point of view of the habitat, in the Căpățânii Mountains the species *Ligularia sibirica* was most often identified in the weed plant communities along the valleys, in the mountain ridge. Most populations were identified on the southern slopes of the Căpățânii Mountains, less often on the northern ones. The most stable populations with the highest number of individuals were identified in the following plant community: *Telekio speciosae-Petasitetum hybridi* (Morariu 1967 n.n.) Resmeriţă et Raţiu 1978. The populations identified in the studied territory are not stable and not well preserved. There is a very large anthropogenic impact on the entire biodiversity in the area. There is no control and no involvement of the local authorities, the municipality, the forestry staff in this area regarding the conservation of biodiversity, Natura 2000 species, both flora and fauna and implicitly on the protection of *Ligularia sibirica populations.*

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CHOROLOGY, ECOLOGY AND PHYTOSOCIOLOGY OF THE *Iris variegata* L. IN FOREST HABITATS FROM THE SOUTH OF OLTENIA, ROMANIA

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Abstract

Iris variegata L. (I. lepida Heuffel) (Iridaceae), is usually in the area from the oak forest zone to the beech floor, through meadows, thickets and forest clearings, in open forests, at the edges of forests. It can be easily recognized by its flowers with yellow inner perianth segments and yellow-white outer perianth segments mottled with brown to purple. This species is cited from few places in Oltenia. Following field research in the forest habitats of southern Oltenia, important populations of this species were identified. Such populations were identified in the lower Jiului basin, in the forest of the Segarcea and Perişor Forestry Districts. The species is found especially in the forests of the Quercus cerris and Q. frainetto, in the natural habitat - 91M0 Pannonian-Balkan oak - Oak forest.

Key words: *Iris variegata, populations, corology, ecology, plant communities, habitats.*

INTRODUCTION

Iris variegata L. (*I. lepida* Heuffel) (Iridaceae), is usually found in the area from the oak forest zone to the beech floor, through glades, thickets and clearings, in open forests, at the edges of forests. The species is found from the steppe area to the beech floor.

The species is often found on arid, sunny moors, on deep soils of the leached chernozem or typical chernozem.

The popular name of the species is Hungarian Iris the variegated iris.

Iris variegata L. which can be found in:

- Central Europe - Hungary, Southeast of the Czech Republic, Slovakia, Austria, South-West of Germany, Croatia and Serbia;
- Eastern Europe - Ukraine and Russia;
- South-East of Europe - Romania, Bulgaria and Albania.

This species is a perennial plant, prefers a sunny site and moderate temperature. Hungarian Iris is an endangered and protected species in Czechia and Slovakia.

Iris variegata has a 10-12 cm long rhizome, from which quite vigorous stems branch out in the upper part or in the middle, with about 6 flowers at the top. It can be easily recognized by its flowers with yellow inner perianth segments and yellow-white outer perianth segments mottled with brown to purple.

It is found in steppe meadows, in forests and forest edges, in sunny areas from the plains to the mountainous floor. It is a summer species, in the southern part of the country blooming in May, and in cooler summers in June.

In Romania, the species is found in the counties: Suceava, Bacău, Vrancea, Iași, Vaslui, Galați, Cluj, Mureș, Brașov, Sibiu, Giurgiu, Constanța, Tulcea, Arad, Caraș-Severin, Vâlcea, Prahova, Argeș, Bucharest, Dolj, Călărași. From the mountain region, the species was cited from: Trașcaului Mountains, Metalici Mountains, Cernei Mountains, Godeanu Mountains, Foarfeca Mountains, Coziei Mountains, Măcin Mountains.

According to the latest studies carried out on the photo-sociology of the species, in the Republic of Moldova the species is frequently found in some areas, having a high abundance-dominant in some forests so that it builds a new plant association *Iridio variegatae-quercetum pubescentis* (Pînzaru et al., 2022) grouped in the alliance *Quercion pubescenti-petraeae* Br.-Bl. 1932, *order Quercetalia pubescenti-petraeae* Klika 1933, class *QUERCO-FAGETEA* Br.-Bl. et Vlieger in Vlieger (1937) (Pînzaru et al., 2022).

Figure 1. The map of the studied territory

MATERIALS AND METHODS

Study area

The territory under study is located in the South part of Romania, the South of Oltenia in the lower Jiului basin, in the forest of the Segarcea and Perișor Forestry Districts.

The forest habitats where populations of this species were studied are part of the Silvostepa of Oltenia protected area.

The field research regarding the species *Iris variegata* was carried out in the forest habitats around the towns: Mărăcine, Tencănău, Perișor, Ciutura, Tîrnava, Fântânele, Criva, Vârvoru de Jos, Drăgoia, Căruia, Bujor, Cetățuia, Plenița, Verbița, Verbicioara, Mârza, Izvoare, Lipovu de Sus, Dâlga, and Radovan (Dolj County) (Figure 1).

Methods

To identify the species we looked into: *Romanian Flora*, vol. XI (1966) and *Flora Europaea*, (1964-1980), Flora Alpina (2004).

For the phytosociological studies, the phyto-sociological research methodology of the Central-European Phyto-Sociological School was used, which is based on the principles and methods developed by Braun-Blanquet & Jenny (1939).

The phytocoenoses in which the species *Iris variaegata* was identified were analyzed taking into account the characteristic, edifying, dominant aspects, the degree of coverage, altitude, slope, appearance and soil properties.

Chorological studies were carried out and the aim was to achieve a distribution as precise as possible in the South-Western part of Oltenia, especially in Dolj County. Population studies were also carried out, especially regarding population density.

RESULTS AND DISCUSSIONS

This paper presents chorological, ecological and phytosociological studies regarding the populations of the species *Iris variegata*, identified in the Southwest of Oltenia (Figure 2).

This species is cited from few places in Oltenia, from Radovan and Prisaca (Dolj County).

Figure 2. *Iris variegata* in Tencănău Forest
(photo: Mariana Niculescu)

Following the research carried out 42 populations were identified in the lower Jiului basin, in the forest of the Segarcea and Perișor Forestry Districts. The most important populations with a large number of individuals, increased vitality and good conservation status are found in the Mărăcine, Tencănău, Perișor, Ciutura, Tîrnava, Fântânele, Cetățuia, Criva, Vârvoru de Jos, Drăgoia, Căruia, Bujor, Cetățuia, Plenița, Verbița, Verbicioara, Mârza, Izvoare, Lipovu de Sus, Dâlga, and Radovan, (Dolj County) in the forest habitats. The species is found especially in the forests of the *Quercus* cerris and *Q. frainetto*, in the natural habitat - 91M0 Pannonian-Balkan oak - Oak forest.

The number of individuals within these populations varies from 4 to 21 individuals. The conservation status of the populations is good, the individuals are well developed, especially those that develop in the forest clearings where they form populations of 10-21 individuals (Figure 3).

In the less sunny places, through thickets, forest edges, the populations have a reduced number of individuals, reaching a maximum of 2-6 individuals and their behavior being less vigorous, with the stems usually.

Figure 3. *Iris variegata* in Marăcine Forest (photo: Mariana Niculescu)

Ecological and cenological characterization of the species *Iris variegata* L. in the investigated territory

Research carried out by us in habitats foresters highlighted the presence of some well-structured forest phytocoenoses floristically and cenotic, which belong to the plant community: *Quercetum frainetto-cerris* (Georgescu, 1945; Rudski, 1949) (Table 1).

Quercetum frainetto-cerris plant community develop on compact, forest-brown soils, strongly podzolite, with a high percentage of carbonates. Both the codominant species of the arborescent layer *Quercus cerris* and *Q. farnetto* are acidophilic elements.

They sometimes dominate one another in the tree layer, a fact that led the author of the association to call it "*Quercus farnetti-cerris*" or "*Quercus cerris-frainetti*" (Georgescu, 1945).

In some of the phytocoenoses analyzed, the *Iris variegata* species can achieve a coverage between 5-10%. The greatest coverage is found in phytocoenoses on flat, sunny lands.

The compaction of the canopy of the analyzed phytocenoses in this forest is between 0.7-0.8.

The shrub layer is quite well developed and is represented by the following species: *Crataegus monogyna, C. pentagyna, Acer tataricum, Cornus mas, Evonymus europaea, Sorbus domestica, Prunus spinosa, Ligustrum vulgare*.

The most common species of the grass layer are: *Lychnis coronaria, Carex michelii, Lithospermum purpureocaeruleum, Carex tomentosa, Festuca valesiaca, Euphorbia cyparissias, Vincetoxium hirundinaria, Carex caryophyllea, Stachys officinali, Ajuga reptans, Viola odorata, Viola alba, Helleborus odorus, Asparagus tenuifolius* etc. (Niculescu et al., 2009). Also, the following species participate with high constancy in making up the floristic composition of the grass blanket: *Helleborus odorus, Anemone nemorosa, A. ranunculoides, Asperula taurina, Viola suavis* etc.

In phytocoenoses *Quercetum farnetto-cerris* plant community from Oltenia still sporadically grows some xerothermic species characteristic of the *Quercetalia pubescenti-petraeae* order: *Paeonia peregrina, Euphorbia lingulata, Vicia sparsiflora, Physocaulis nodosus, Acanthus balcanicus, Ruscus aculetaus*.

A series of species that enter the floristic composition of the grassy layer within the

phytocoenoses in which the *Iris variegata* was identified are rare Natura 2000 species that are included in the national red lists.
From a conservative point of view, it was observed that the highest abundance-dominance of the species is within the Natura 2000 habitat - 91M0 Pannonian-Balkanic turkey oak - sessile oak forests; CLASS. PAL.: 41.76. (Gafta & Mountford, 2008) (Figures 4, 5).

Figure 4. 91M0 forest habitat

Ecological, phytosociological and population studies were done during the entire growing season to cover all morphological and phenological aspects.
Iris variegata populations show large numbers of individuals. Population density varies depending on the eco-pedo-climatic conditions in each forest where the studies were carried out.
Given that it is a xeromesophyte, subthermophile species in the south and Southwest of Oltenia, it finds favorable conditions for development and fruiting, thus the populations have a fairly large number of individuals.
The populations with the largest number of individuals are found in Mărăcine Forest, Tencănău Forest, Radovan Forest, Verbicioara Forest and Plenița Forest.
Although the *Iris variegata* species can also be found in meadow habitats, in the researched territory the species was found in forest habitats, with the maximum development in forest glades, where especially the temperature and light conditions are favorable.
In all the forests where the chorological, phytosociological and population studies were carried out, *Iris variegata* was found, the species was identified in the same type of habitat.
The abundance-dominance (+-2) of the *Iris variegata* species in the phytocoenoses also varies with the eco-pedoclimatic conditions and at the same time it is closely related to the climatic changes in Oltenia in recent years, following the studies carried out observing a decrease in the number of individuals compared to 2019, when the studies started.

Figure 5. *Iris variegata* in the 91M0 forest habitat

Table 1. *Quercetum frainetto-cerris* (Georgescu, 1945; Rudski, 1949) plant community

No. of relevée	1	2	3	4	5	6	7	K
Altitude m.o.s. (x 10 m)	120	110	90	120	125	125	120	
Exposure	-	-	-	-	-	S	E	
Inclination (in grades)	-	-	-	-	-	10	7	
Canopy (%)	0.7	0.8	0.8	0.7	0.7	0.7	0.7	
Coverage of herbacaeous layer (%)	60	70	70	70	70	60	60	60
Area	400	400	400	400	400	400	400	400
Char. ass.								
Quercus frainetto	3-4	3-4	4-5	4	4	2	2	V
Quercus cerris	2	2	1	+-1	+-1	4	4	V
Quercetalia pubescentis et Quercion petraeae								
Lathyrus niger	-	-	-	+	-	-	+	II
Cruciata glabra	+	+	+	+	+	+	+	V
Potentilla micrantha	+	+	+	+	+	+	+	V
Acer tataricum	+	+	+	+	+	+	+	V
Campanula persicifolia	+	+	+	+	+	+	+	V
Polygonatum latifolium	+	+	+	+	+	+	+	V
Paeonia peregrina	-	2	-	-	-	-	-	I
Quercion farnetto								
Lychnis coronaria	-	+	-	+	-	-	+	III
Tamus communis	+	+	+	+	+	+	+	V
Aremonia agrimonioides	+	+	+	+	+	+	+	V
Helleborus odorus	+-1	+	+-1	+-1	+-1	+-1	1	V
Queretea pubescenti-petraeae								
Crysanthemum corimbosum	-	+	+	+	-	-	+	III
Lithospermum purpureocaeruleum	1	1	1	1	1	1	1	V
Cornus mas	-	-	-	+	-	-	+	III
Viola hirta	+	+	+	+	+	+	+	V
Hieracium bauhini	+	-	+	-	+	+	+	III
Euonymus verrucosus	+	+	+	+	+	+	+	V
Asparagus tenuifolius	+	1	+	1	+	+	+-1	V
Doronicum hungaricum	+	-	-	+	+	+	+	IV
Polygonatum odoratum	+-1	+	+-1	+	+-1	+-1	+-1	V
Carex tomentosa	+	+	+	+	+	+	+	V
Ruscus aculeatus	-	+	-	+	-	-	+	III
Oryzopsis virescens	-	+	-	+	-	-	+	III
Iris variegata	**2**	**1**	**1**	**1**	**1-2**	**+-1**	**+**	**V**
Euphorbia lingulata	+	+	+	+	+	+	+	V
Vicia sparsiflora	+	+	+	+	+	+	+	V
Physocaulis nodosus	+	+	+	+	+	+	+	V
Acanthus balcanicus	+	+	+	+	+	+	+	V
Fagetalia et Querco-Fagetea	+	+	+	+	+	+	+	V
Euphorbia amygdaloides	+	+	+	+	+	+	+	V
Veronica officinalis	+	+	+	+	+	+	+	V
Corydalis solida	+	+	+	+	+	+	+	V
Corydalis cava ssp. *marchalliana*	+	+	+	+	+	+	+	V
Festuca heterophylla	-	+	+	-	-	-	+	III
Acer campestre	+	+	+	+	+	+	-	V
Anemone nemorosa	1	1	1	1	1	1	1	V
Anemone ranunculoides	1-2	1	1	1	1-2	1-2	+-1	V
Poa nemoralis	+	+	+	+	+	+	+	V
Brachypodium silvaticum	+	+	+	+	+	+	1	V
Euonymus europaeus	-	+	-	-	-	-	+	IV
Ghechoma hirsuta	+	-	+	-	+	+	+	III
Arum maculatum	-	-	-	+	-	-	-	II
Veronica chamaedrys	+	+	+	+	+	+	+	V
Primula vulgaris	2	2	1	1	1-2	1-2	1	V
Asperula taurina	-	+	+	-	-	-	+	III
Melissa officinalis	-	+	-	+	-	-	+	III

Poa sylvatica	-	+	-	+	-	-	-	V
Melica uniflora	+	-	-	+	-	+	-	III
Calamagrostis epigeios	+-1	1	+	1	1	+-1	+-1	V
Urtica dioica	+	+	+	+	+	+	+	V
Silene alba	+	+	+	+	+	+	-	IV
Rumex sanguinea	+	+	+	+	+	+	+	V
Potentilla argentea	+	+	+	+	+	+	-	IV
Geum urbanum	+	+	+	+	+	+	+	V
Agrimonia eupatoria	+	+	+	+	+	+	+	V
Anthriscus sylvesris	+	+	1	+	+	+	+	V
Alliaria petiolata	+	+	+	+	+	+	+	V
Hypericum hirsutum	+	-	-	+	+	+	-	III
Viola odorata	+	+	+	+	+	+	+	V
Viola suavis	1	+	+-1	+-1	+-1	+-1	1	V
Cruciata laevipes	+	+	+	+	+	+	+	V
Trifolium pratense	-	+	+	-	-	-	+	III
Trifolium pannonicum	+	+	-	+	+	+	-	IV
Lathyrus nissolia	-	+	+	-	-	-	+	III
Lathyrus sylvestris	+	-	+	-	+	+	+	IV
Lathyrus venetus	-	+	-	+	-	-	-	II
Lathyrus vernus	+	+	+	+	+	+	+	V
Lotus corniculatus	-	-	+	+	-	-	-	II
Astragalus glycyphyllos	+	+	+	+	+	+	+	V
Prunetalia et Prunion spinosae								
Prunus spinosa	+	-	+	+	+	+	-	IV
Cornus sanguinea	+	+	+	+	+	+	+	V
Ligustum vulgare	1	+	+-1	+-1	+-1	+-1	1	V
Evonymus europaea	+	+	+	+	+	+	+	V
Crataegus monogyna	+	+	+	+	+	+	+	V
Rosa canina	+	+	+	+	+	+	+	V
Geum urbanum	+	+	+	+	+	+	+	V
Carex silvatica	+	-	-	+	+	+	-	III
Mercurialis perennis	+	-	-	+	+	+	+	IV
Carex pillosa	+	+	-	+	+	+	-	III
Variae Syntaxa								
Stachys silvatica	-	+	-	+	-	-	+	III
Galeopsis speciosa	-	-	+	-	-	-	-	I
Veronica hederifolia	+	+	-	+	+	+	+	IV
Lapsana communis	+	+	+	+	+	+	-	IV
Verbascum phaeniceum	+	-	+	+	+	+	-	III
Myosotis sylvatica	-	+	-	+	-	-	+	III
Campanula bononiensis	+	-	+	+	+	+	+	IV
Leonurus cardiaca	+	+	-	+	+	+	+	IV
Campanula persicifolia	-	+	+	+	-	-	-	III
Ballota nigra	-	-	-	+	-	+	+	III

Place and data of the relevés: 1 - Tencănău Forest, 20.VI.2019, 2- Plenița Forest, 7.VI.2020, 3 - Mărăcine Forest 15.VII. 2022, 4 - Dâlga Forest, 20.VI.2023, 5 - Verbița Forest, 25.VI.2021, 6 - Mârza Forest 5.VI.2023, 7 - Radovan Forest, 10.VI.2023.

CONCLUSIONS

Iris variegata is a xeromesophilic and subthermophilic species that grows spontaneously in the South of Oltenia, especially in thickets and forest glades or in rarer and sunny forests.

From a phytosociological point of view, this species is found in the floristic composition of *Quercetum frainetto-cerris* (Georgescu, 1945; Rudski, 1949) plant community.

The species is found especially in the forests of the *Quercus cerris* and *Q. frainetto*, in the natural habitat - 91M0 Pannonian-Balkan oak - Oak forest. Population density varies depending on the eco-pedo-climatic conditions in each forest where the studies were carried out. Given that it is a xeromesophyte, subthermophile species in the South and South of Oltenia, it finds favorable conditions for development and fruiting, thus the populations have a fairly large number of individuals. The populations with the largest number of individuals are found in Marăcine Forest, Tencănău Forest, Radovan Forest, Verbicioara Forest and Plenița Forest. The abundance-dominance of the *Iris variegata* species in the phytocoenoses also varies with the eco-pedoclimatic conditions and at the same time it is closely related to the climatic changes in Oltenia in recent years.

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COMPOST QUALITY AND NITROGEN MINERALIZATION DYNAMICS DURING THE MATURITY STAGE OF LAVENDER WASTE COMPOST

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Abstract

The interest of Romanian small farmers in lavender cropping is increasing. Many of them have also developed lavender processing lines so that the small industry around this species is growing. Additionally, the amounts of generated waste have also increased, but many arguments support the composting of this waste: good management of organic matter in the circular economy, identification of beneficial effects of compost, other than those known about composts obtained from biodegradable waste, etc. In our studies we composted lavender waste together with manure from small ruminants (sheep and goats). Both lavender waste and manure were collected from several small farms in Southern Romania, and the composting process was carried out in a system of 3 paddocks within also a small ecological farm. In addition to the evolution of the composting process and its parameters, the dynamics of nitrogen mineralization were analyzed. The compost reached the stage of maturity after 4 months of composting, and during the maturation the dynamics of nitrogen (N) mineralization were evaluated, and the physicochemical properties and the phytotoxicity of the resulting compost were also analyzed.

Key words: *compost, compost maturity, lavender, nitrogen mineralization dynamics.*

INTRODUCTION

In general, the production of organic waste is growing and the need to dispose it ecologically is increasing. Transforming this waste into organic fertilizer through composting is the most successful system used until now (de Bertoldi et al., 1983).

Composting can be viewed as the sum of complex metabolic processes carried out by different microorganisms that, in the presence of oxygen, use available nitrogen (N) and carbon (C) to produce their own biomass. In this process, additionally, the microorganisms generate heat and a solid substrate, with less carbon and nitrogen, but more stable, which is called compost (Roman et al., 2015; Azim et al., 2018). Mature composts obtained from organic waste can be used to increase soil fertility by improving their physical, chemical and biological properties, making it possible to reduce the consumption of synthetic fertilizers (Reimer et al., 2023). Besides, composts can be applied without potential danger for soils (González-Prieto, 1993).

Studies have also shown that repeated application of composted organic matter to cropland led to an increase of the microbial biomass and enzymatic activity. Long-lasting application of organic fertilizers increased organic carbon by up to 90% versus unfertilized soil, and up to 100% versus chemical fertilizer treatments. Repeated application of composted materials enhances soil organic nitrogen content by up to 90%, storing it for mineralization in future cropping seasons, often without inducing nitrate leaching to groundwater (Diacono et al., 2011).

Some authors have shown that compost application to soil resulted in an overall low nitrogen efficiency, partially due to a lack of synchronization between plant nitrogen demand and soil nitrogen release (Erhart et al., 2005; Lehtinen et al., 2017; Reimer et al., 2023). However, it has been also shown that, in the spring, the soils fertilized with compost have a higher mineral N content (Erhart et al., 2005; Tits et al., 2014; Reimer et al., 2023). Therefore, it is necessary to know the availability of nutrients, and especially N in compost, to come up with strategies to meet the nutrient needs of crops, and at the same time to protect the environment (Hartl et al., 2001).

There are many studies on nitrogen mineralization in e compost-fertilized soils, but fewer on nitrogen mineralization during the composting process. Thus, in this paper we will present some results obtained in a first study that we proposed to carry out in order to follow the mineralization of nitrogen during the maturity period of the compost.

MATERIALS AND METHODS

Composting materials and experimental design

The experimental site was located in Pelinu village, in Călărași county, which is in the South-East part of Romania (44°27′46″N 27°0′48″E). The climate of the region is temperate continental with a homogeneous regime, characterized by very hot summers and relatively cold winters. In 2021, the average annual temperature was 12.5°C, and annual precipitation was 712.8 mm (INS, 2023).

The raw materials for composting were undistilled lavender flower stalks (*Lavandula angustifolia* Mill.) and biomass resulting from the distillation process, and sheep and goat manure. Lavender waste was collected from several farms in Ialomița County and Călărași County, Romania, and sheep and goat manure was collected from two different farms in Călărași County, Romania. The experiment was established in November 2023. Three compost piles were made inside some paddocks (Photos 1, 2 and 3), each one with different mixture. The composting process took place for 4 months. The compost recipes were: C1: ±33% (kg/kg) sheep manure, ±33% (kg/kg) goat manure, and ±33% (kg/kg) wheat straws; C2: ±33% (kg/kg) sheep manure, ±33% (kg/kg) goat manure, and ±33% (kg/kg) distilled lavender stalks, and C3: ±33% (kg/kg) sheep manure, ±33% (kg/kg) goat manure, and ±33% (kg/kg) undistilled lavender biomass.

The composts temperature was measured every 3 days during the composting, and the piles were manually turned periodically during which the moisture was evaluated and completed to.

Photo 1. C1 – sheep and goat manure and wheat straws

Photo 2. C2 – sheep and goat manure and distilled lavender stalks

Photo 3. C3 – sheep and goat manure and undistilled lavender biomass

Phytotoxicity evaluation assay

There are several methods (chemical, physical and biological) that have been developed, by different authors, for compost maturity assessment (de Bertoldi et al., 1983; Peña et al.; 2020). Phytotoxicity test is one of the sensible methods used to evaluate the compost that will be used as soil fertilizer (de Bertoldi et al., 1983).

In order to assess the maturity of our composts, a laboratory incubation experiment was designed following the methodology slightly adapted from that proposed by some authors. The phytotoxicity level for all 3 compost recipes was evaluated in day 138 and the day 156 from the initiation of the composting process.

Compost samples were mixed with distilled water (1:10 weight/volume ratio - 10 g sample of compost and 100 mL distilled water) (Tiquia et al., 1997; Walter et al., 2006; Barral & Paradelo, 2011), the mixture was homogenized for 10 min and left to rest for 24 h. The aqueous extract was passed through a filter paper, and from each filtrate were extracted 3 tubes of 12 mL which were centrifuged at 4000 rpm for 20 min. In Petri dishes (94 mm diameter) with filter paper as support laid previously, it was added 4 ml of each compost solution obtained (Charles et al., 2011) and 20 seeds of garden cress (*Lepidium sativum* L.) (Tiquia et al., 1997; Barral & Paradelo, 2011; Cesaro et al., 2019) were spread (Photo 4). The experiment was done in 3 replicates for each compost (Tiquia et al., 1997; Barral & Paradelo, 2011; Cesaro et al., 2019). For control, another set of 3 replicates were made (Charles et al., 2011), applying distilled water instead of the filtrate. All 12 plates were placed for 48 h at 25°C in the incubator (Walter et al., 2006; Miaomiao et al., 2009; Barral & Paradelo, 2011; Cesaro et al., 2019). Finally, the number of germinated seeds was recorded, and the root length was measured (Photo 5).

Germination index (GI%) was determined by counting the average root length (RL) as well as the average number of germinated seeds (GR) in every sample and comparing with the control treatment. The GI% was calculated using the equation (Cesaro et al., 2015):

$$GI\% = 100\,x\,\frac{number\ of\ germinated\ seeds\ in\ extract\ x\ root\ length\ in\ extract}{number\ of\ germinated\ seeds\ in\ control\ x\ root\ length\ in\ control}$$

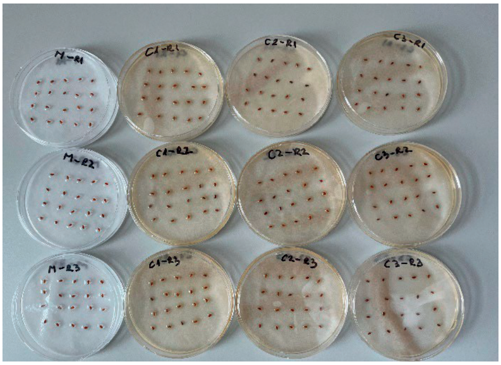

Photo 4. Garden cress (*Lepidium sativum* L.) seeds before incubation

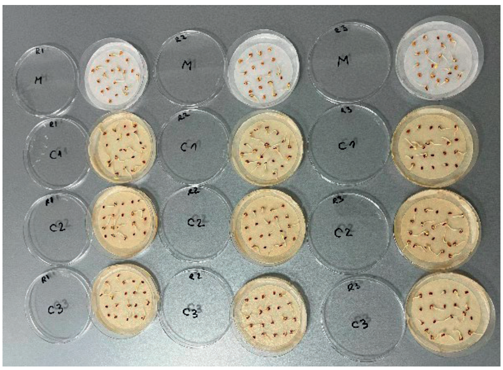
Photo 5. Garden cress (*Lepidium sativum* L.) seeds after incubation

Laboratory analysis of nitrogen

After 78 days since the compost piles were made, over a period of 3 months, samples were taken from all 3 piles, in days 78 - 92 - 104 - 118 - 132 - 146 - 174. From each pile were taken 3 samples of 1 kg each (total of 9 samples) which were analyzed for humidity, mineral N (NO_3^--N and NH_4^+-N), total nitrogen (N_t) and total carbon (C_t). The NO_3^--N was determined potentiometrically according to ICPA (1983), NH_4^+-N was determined through distillation according to ICPA (1981), and N_t and C_t were determined using a Vario MACRO Cube elemental analyzer.

The data were processed as the average of the 3 replicates, and the graphs were developed in Excel/MS Office.

RESULTS AND DISCUSSIONS

Composting process

The temperature of the four composts raised significantly in the first week (Figure 1), especially for C2 (sheep and goat manure and distilled lavender stalks) reaching 41°C, standing out the beginning of the thermophilic phase, which was followed by the cooling phase, when the temperature decreased rapidly until 4°C for C1 (sheep and goat manure and wheat straws) and the maturation phase, with temperature among 20 and 10°C (values similar with the outside temperature) (ICPA, 2016).

A standard composting process can be divided into four phases: the mesophilic phase, the thermophilic phase, the cooling phase, and the maturity phase (Ishii et al., 2000; Waszkielis et al., 2023). The duration of each phase is determined by process conditions, the composition of the raw materials, moisture content, temperature, aeration rate and oxygen availability (Waszkielis et al., 2023). In this experiment, the variation in temperature were observed and used as an indicator of transition between the composting phases.

Composting temperature is the result of the microbial activity, which can directly affect the decomposition of the organic matter (Kaiser, 1996; Tang et al., 2011; Peng et al., 2022). Generally, the temperature in compost rises immediately after piling and decreases drastically by turning (Zucconi et al., 1981; de Bertoldi et al., 1983; Kato et al., 2005; Qian et al., 2014) with a continuous variation until the composts reaches the maturity phase. Even though studies shown that high thermophilic temperature can be difficult to reach without adding composting additive (Liao et at., 2017; Peng et al., 2022; Ansari et al., 2023), no additional materials were used for this experiment.

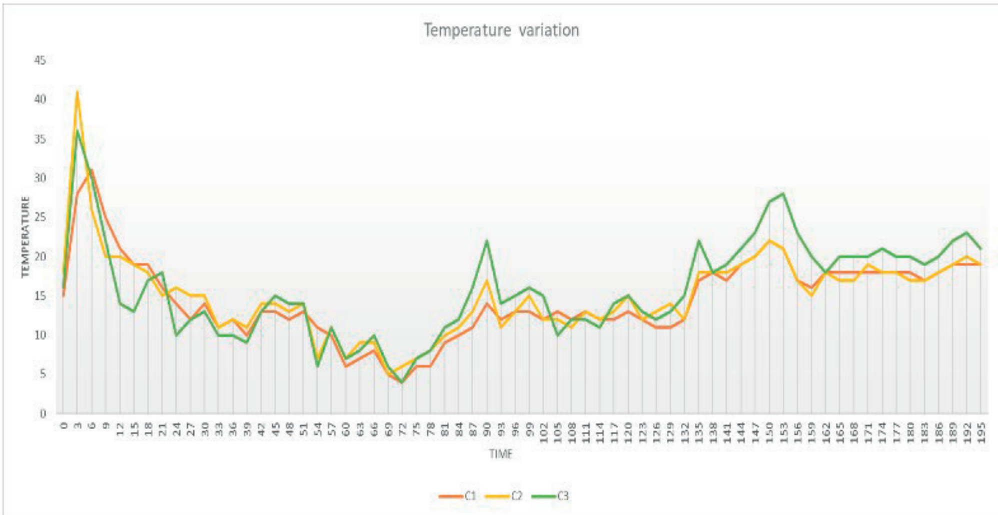

Figure 1. Temperature of C1, C2, C3 during the evaluation period

The humidity of our piles, during the analysis period, varied in the optimum range of 45% and 50% (Razmjoo et al., 2015; Azim et al., 2018), as followed: 51% and 57% for C1, 50% and 55% for C2, and 42% and 52% for C3 (Figure 2).

This is an indicator that the humidity was not a limiting factor for the composting process in this case, considering that the bacterial activity is limited when humidity is less than 30%, and humidity above 65% is decreasing the porosity of the compost resulting in an anaerobic growth and unpleasant odor emissions (Razmjoo et al., 2015; Azim et al., 2018).

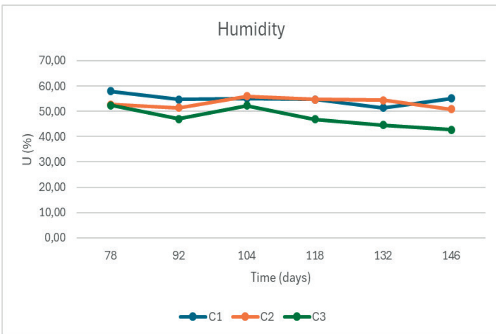

Figure 2. Humidity of C1, C2, C3 during the evaluation period

Optimum humidity during composting is hard to obtain, especially when composting outdoors. In practice, this issue is often solved by monitoring the temperature which can be a good indicator of the proper moment for the turning and watering the piles (Tiquia & Tam, 1998; Azim et al., 2018).

Phytotoxicity evaluation of compost extracts

The GI% for all 3 composts are shown in Table 1, and it can be seen that there were variations of the GI% both between the 3 types of compost, as well as between the 2 tests performed 18 days apart, however none of the composts presented phytotoxicity. As it is shown by Ravindran et al. (2017) and Milon et al. (2022), a GI% level of less than 50% indicates mild phytotoxicity, 50-80% indicates modest phytotoxicity, and >80% demonstrates no phytotoxicity.

Table 1. Germination index (GI %) for all 3 composts

	C1	C2	C3
Day 138	101%	86%	91%
Day 156	81%	80%	103%

The lowest values registered in all 3 composts were in C2 (80% and 86%), but these values reveal, however, the absence of phytotoxicity. During a peak of temperature of the compost, the toxicity of C3 increased and the toxicity of C1 and C2 decreased. This could be attributed to the biological degradation processes during the maturity stage, as there was still a residual amount of organic matter susceptible to decomposition (Cesaro et al., 2019).

Nitrogen dynamics

The N_t varies between 1.86% and 2.79% for all 3 composts (Figure 3), which is accordance with what was found by other authors who stated that the N_t can vary between 1% and 4% of the total dry matter weight of compost (Hirai et al., 1986; Willson, 1989; Kapetanios et al., 1993; Canet & Pomares, 1995; Bernal et al., 1998, Brinton & Evans, 2000; Azim et al., 2018).

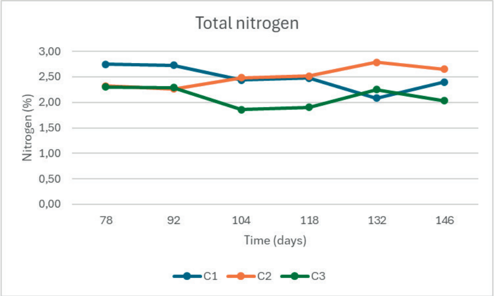

Figure 3. Changes in total nitrogen for different compost recipes

The total nitrogen in C1 and C3 showed a decrease in the last weeks of observation and only C2 showed a constant increase during that period.

Usually, the N content decreases during composting mostly because of ammonium volatilization, however it is recovered due to the mineralization of the OM and due to the activity of the nitrogen-fixing bacteria (de Bertoldi et al., 1983).

In the Glossary of Soil Science Terms published by the Soil Science Society of America (1997) mineralization is defined as "The conversion of an element from an organic form to an inorganic state as a result of microbial activity". For N, the first step is the conversion of organic N from organic matter (OM) to ammonium (NH_4^+-N) by a process called ammonification. Often, the NH_4^+-N is rapidly converted to nitrite (NO_2^-) and after that to nitrate (NO_3^--N) by the microbial process of nitrification. The amount of inorganic N (NH_4^+-N and NO_3^--N) originating from OM is named mineralized nitrogen. Nitrogen mineralization is a product of the amount of organic N and the N mineralization rate (NMR) (Gilmour, 2011).

In day 92 it can be seen an increase of ammonium, especially in C2 and C3 (Figure 4), which can be related to the rise of the temperature of the piles (Figure 1).

After day 104 the NH_4^+-N produced was either immobilized, as the piles were not turned anymore after this date (Michel & Reddy, 1998; Azim et al., 2018), either the NH_4^+-N was rapidly converted to nitrite (NO_2^-) (Müller et al., 1968; Gilmour, 2011).

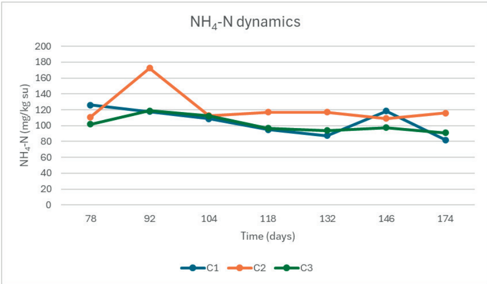

Figure 4. Changes in ammonium-N
for different compost recipes

In all 3 composts the content of NO_3^--N decreased in the first part of the observation period (Figure 5). For C1, the drop from day 104 was more abrupt compared with C2 and C3, possibly, due to the lack of air. The piles were turned and aerated after day 104, therefore the nitrification process was restarted (Müller et al., 1968).

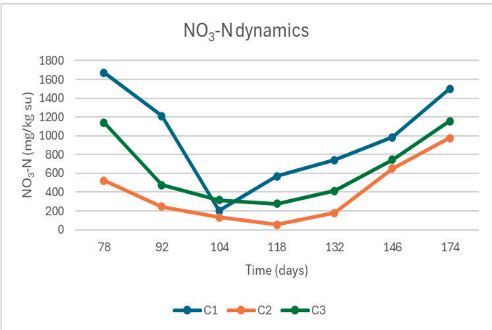

Figure 5. Changes in nitrate-N
for different compost recipes

The NO_3^--N dynamics can serve as an indicator of N mineralization, immobilization, and leaching (Hartl et al., 2001), and an increase of NO_3^--N content can be a sign of the compost maturity as it was observed by other authors (Sánchez-Monedero et al., 2001; Azim et al., 2018), and it is also viewed as a way of conserving N in compost (Cáceres et al., 2018). During composting, a part of the mineral nitrogen is reincorporated into the active microbial metabolism, another part is incorporated into organic matter compost in their humification, and another one is released in the form of inorganic nitrogen matrix (Larsen & McCartney, 2000; Azim et al., 2018).

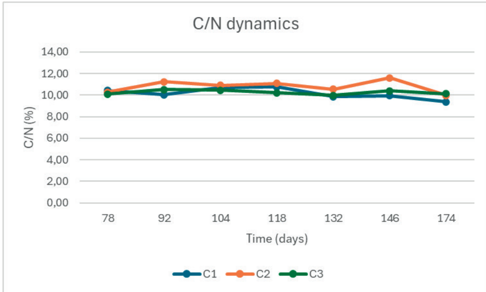

Figure 6. Changes in C/N ratio
for different compost recipes

Microorganisms are known to use 30 parts of carbon for each part of nitrogen in the decomposition process (Choi, 1999; Azim et al., 2018). Carbon is serving both, as a source of energy and elemental component for microorganisms, and nitrogen is essential for the synthesis of amino acids, proteins and nucleic acids. This is why, a C/N ratio of 25-30:1 at the beginning of the composting process is ideal (Azim et al., 2018).
All 3 composts from our study showed small variations of the C/N ratio (Figure 6). The C/N ratio of C1 varied between 10.76 and 9.39, the C2 C/N ratio varied between 11.60 and 9.97, and the C3 C/N ratio was between 10.51 and 9.99. The compost C2 showed a constant growth rate both of C_t and N_t during the evaluation period (Figure 7 and Figure 3).

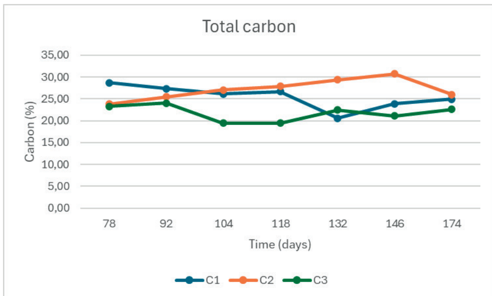

Figure 7. Changes in total carbon
for different compost recipes

The C/N ratio is a parameter used mostly to assess the maturity of the compost (Albrecht, 2007; Azim et al., 2018), and the findings show that a C/N ratio around 10 is the value for a mature compost produced from organic matter and/or manure (Forster et al., 1993; Brinton & Evans, 2002; Azim et al., 2018).

CONCLUSIONS

The present study shows that co-composting lavender wastes and manure from small ruminants (sheep and goats) can lead to a good quality compost.

A major issue identified by researchers regarding the supply of the N to the plants on compost fertilized soils, is the synchronization of plant demand and N supply through mineralization, which usually occurs outside the cropping period (Reimer et al., 2023), therefore it is necessary to predict N mineralization to synchronize its release with plant demand (Ros et al., 2011). The results of our study indicate that the compost resulted from lavender waste can help conserving N and release it slowly, making it easily available for the plants.

However, our research will continue in this sense in order to increase knowledge and for a more accurate understanding of the processes, especially in relation to lavender waste composting and the effects of such compost.

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DIVERSE MULTISPECIES INTERCROPPING OF ANNUAL PLANTS FOR ORGANIC FARMERS IN SOUTH-EAST ROMANIA

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Abstract

Cropping system diversification is a key factor in developing more sustainable crops and increase food security. A two-year field experiment was conducted at South-East part of Romania, to emphasize the right plant types, both legumes and non-legumes which can be used in intercropping system for organic agriculture. Intercropping pattern with two (Peas + Camelina; Peas + Flax; Peas + Oats; Flax + Oats; Spring Wheat + Camelina and Spring Wheat + Flax) and three species (Peas + Camelina + Spring Wheat; Peas + Flax + Spring wheat; Peas + Flax + Oats; Peas + Camelina + Oats) was used. Sole cropping of peas, camelina, flax, oats and spring wheat were also used. Two sowing rates were tested, at 50% and 100% of the recommended amount of seed. Results showed that averaged across years and intercropping patterns, yields were significantly more increased in mixtures crops compared to sole cropping ones. Through the land equalization ratio (LER) with values greater than 1, the mixtures of crops (of two or three species) that use environmental resources more efficiently and productively were identified.

Key words: *participatory research, crop mixtures, legumes, cereals, field pea, oat, flax, camelina.*

INTRODUCTION

Intercropping is a system that results in two or more species being sown and harvested together (Parvin et al., 2023). Although they have been known for a very long time and have been commonly used throughout the world, in Europe, intercropping significantly reduced its area in the 20th century with the increase in mechanization and chemical intensification of agricultural production. Today, intercropping is most commonly found in organic and low-input farming systems (Ergon et al., 2016). In recent years, the concept of ecological intensification has been developed. This concept seeks to increase the contribution of natural cycles and resource flow in agricultural production through the widespread use of ecosystem support and regulation services provided mainly by beneficial organisms. Cultivation of mixed crops, especially with leguminous, appears to be an option worthy of support for this intensification. Potential benefits of these systems include reduced input costs, rotational benefits and soil improvement (Fletcher, 2020; Iannetta et al., 2021). Due to the symbiotic nitrogen fixation and protein content of legumes, intercropping is of particular interest to legume-based mixtures, such as annual legume-cereal mixtures, in terms of production, yield stability and environmental services (Raseduzzaman & Jensen, 2017; Martin et al., 2020). Intercropping requires compatibility when it comes to management (eg. maturity and harvest time of cereal crops) and species interactions (eg. facilitation, competition). Therefore, it is important to identify optimal combinations of species and varieties within species for different regions (Lizarazo et al., 2020). Identifying suitable plant types for intercropping is particularly important for species at a competitive disadvantage, as is often the case with cereal legumes associated with cereals (Annicchiarico et al., 2019). In Romania, there are current studies of different annual crop mixtures of peas and cereals as cover crops (Petcu et al., 2022a), perennial mixtures with alfalfa or trifolium and grasses, in order to prove their productivity (Naie et al., 2024) or mixtures with medicinal plants used for their efficacy in repealing pests (Petcu et al., 2022b).

To be successful, it is recommended that mixtures to be composed of crops that have complementary rather than competing traits, as they use resources more efficiently than single crops.

In Romania, multispecies crops are used quite a bit, but different mixtures of species have started to be used to intercropping. Some examples of intercropping (mixes) with two and three species are presented in this paper, in order to identify the best combinations for production and the degree of utilization of the land.

MATERIALS AND METHODS

With organic farmers involvement has been designed and implemented different crop mixtures cultivation trials in organic agriculture conditions in Fundulea, Călărași, Romania (Field GPS coordinates: 44.446430, 26.514995) on chambic chernozem soil in 2022 and 2023.

The crop structure was composed of grain pea (cultivar Lavinia), spring cereals were: naked oat, spring wheat (cultivar T4068-19), camelina (cultivar Camelia), oil flax (cultivar Lirina). Two sowing rates were tested, at 50% and 100% of the recommended amount of seed. 100% seed rate was considered for wheat and oat - 500 germinable seeds/m^2; pea - 125 germinable seeds/m^2; camelina and flax - 450 germinable seeds/m^2. The intercropping variants were:

1. Pea+ spring wheat
2. Pea + oat
3. Pea + flax
4. Pea + camelina
5. Flax + spring wheat
6. Flax + oat
7. Camelina + spring oat
8. Camelina + oat
9. Pea + camelina + spring wheat
10. Pea + camelina + oat
11. Pea + flax + spring wheat
12. Pea + flax + oat

Along with these mixtures, variants of pure cultures were also used.

All experimental variants were analysed for production (kg/ha) and land equivalent ratio (LER).

Land equivalent ratios were calculated to measure the relative productivity of intercropping compared to pure/single cropping (monocrop). The calculation formula was the following LER = (Production of crop A from the intercrop system / production of crop A from the pure crop system) + (Production of crop B from the intercrop system / production of crop B from the pure crop system).

A value for LER of 1.0 means that productivity in intercropping is equivalent to that in pure cropping. The values of LER value of > 1.0 mean that in intercropped culture it is more productive than in pure culture, it is so 'over-yielding' as shown by Willey and Osiru (1972).

RESULTS AND DISCUSSIONS

From a climatic point of view, both years of experimentation in the Fundulea area were a very dry and hot year. The amount of precipitation that fell between March and July 2022 was 178.8 mm and in 2023 was 203.6 mm below the multi-year average for this period (291 mm), registering a deficit of 112.2 respectively 87.4 mm and the average monthly air temperatures were above the multi-year average of the period. The distribution of precipitation was unfavourable in 2023 because most of it was in April. The average monthly air temperatures were above the multi-year average of the period in 2022 but April and May were below multi-year average (Table 1).

Table 1. Average air temperature (°C) and monthly distribution of precipitation (mm) during the crop vegetation period. Fundulea, 2022-2023

Month	March	April	May	June	July	Sum
Temperature 2022	4.4	12.1	17.9	22.6	25.0	
Temperature 2023	8.2	10.8	16.9	22.3	26.1	
Multi-annual average	4.9	11.3	17	20.8	22.7	
Precipitation 2022	12.3	47.6	30.1	59.6	29.2	178.8
Precipitation 2023	10.0	77.2	32.4	40.2	43.8	203.6
Multi-annual average	37.4	45.1	62.5	74.9	71.1	291

The moisture deficit from March and May cumulated with cold from March to April in 2023 created unfavourable condition during early stage of vegetation, determining relatively low yield in 2023 as compared cu 2022. On the other hand, lower plant emergence in 2023 may have been due to dry conditions during tillage in 2022-2023 season, resulting in a course seedbed due to soil clods on the soil surface. So, yield of pea in pure culture was 700 kg (year 2023) and 854 kg/ha (year 2022), spring wheat produced 2232 and 3083 kg/ha respectively, camelina produced between 203 and 282 kg/ha, oat yield was over 3000 kg/ha and the yield of oil flax was 1800 kg/ha in 2023 and 3112 kg/ha in 2022, respectively (Figure 1). The lower productions in 2023 are due to the very strong drought this year.

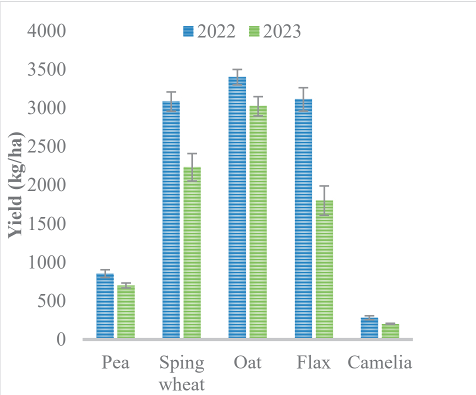

Figure 1. Mean grain yield in pure culture for pea, spring wheat, oat, flax and camelina obtained in 2022 and 2023

The variance analysis showed that in the year 2022 the seed rate per hectare had a very significant effect on the productions obtained in the mixtures of two species compared to the year 2023 (Table 2).

Table 2. Analyses of variance for the yield in intercrops with two species and two seed rate per hectare

Source of variance	FD	Factor F and significance	Factor F and significance
		2022	2023
Factor A (seed rate per hectar)	1	29.60***	5.01
Error A	2		
Factor B (Intercrop)	7	47.68***	10.61***
Interaction AxB	7	7.03	0.37
Error B	28		

In the mixtures of two species, the yields were superior to those obtained in pure culture in both years of experimentation. The studied seed sowing rate (50 and 100% of the recommended sowing rate) of pea intercropped with wheat, oat, flax as well as flax intercropped with wheat or oat exhibit significant positive effect on yield in 2022 and insignificant effects in 2023 (Figure 2). Sowing pea at the recommended or reduced seed rate in intercropping system with camelina resulted the same values of yield during condition of 2022. In the same time, we can see that during condition of 2023 year the highest yields were obtained at reduced seed rate. These results may be due the fact that in the dry year 2023 at the reduced sowing rate was a reduced competition among adjacent plants, which led to an increase in the amount of solar radiations intercepted by plants, as well as increment aeration and light distribution among plants, which led to increased photosynthetic activities and dry matter accumulation per individual plants, and therefore increasing seed yields/ha) (Figure 2).

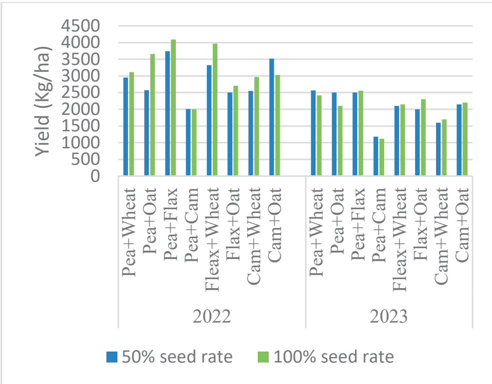

Figure 2. The yields of pea intercropped with spring wheat/oat/flax/camelina, flax intercropped with spring wheat/oat and camelina intercropped with spring wheat/oat obtained during 2022 and 2023 seasons

The analysis of the relative productivity of the intercropped crop compared to the pure crop, based on the land equivalent ratio (LER) is presented in figure three. Thus, in 2022, the superiority of using a reduced sowing rate compared to the recommended seed rate per hectare was highlighted. The pea and oat mixture has LER values of 1 at 100% sowing rate and over 2 at reduced sowing rate (50%) (Figure 3).

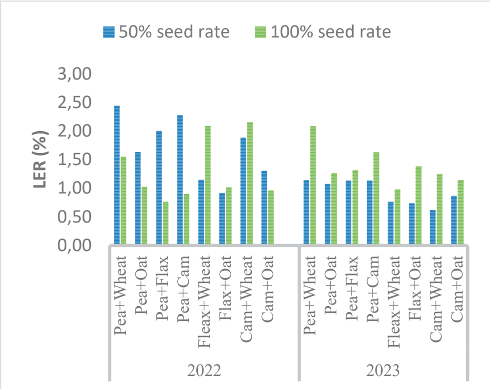

Figure 3. Land equivalent ratio (LER) for intercropping with two species

In the intercropping peas and flax, the land equalization ratio (LER) had a value below 1 at the recommended sowing rate compared to 50% of the recommended sowing rate, where the LER had a value of 2 (Figure 3) during 2022 season. Safina (2017) reported that land equivalent ratio (LER) ranged from 1.63 to 1.86 for intercropping flax with faba bean Giza-2 or Giza-843 varieties. Klimek-Kopyra et al. (2018) stated that intercropping pea with flax caused a significant increase in the number of seeds per pod and number of pods per plant.

Abd-Rabboh et al. (2021) found that when sugar beet and flax were intercropped, the maximum sugar beet root production and economic return of both crops were achieved by sowing flax at 12.5% of the recommended seed rate after 21 or 35 days of sowing sugar beet at 100% of the recommended seed rate (second or third sowing date).

Mixtures consisting of three species were also studied.

The variance analysis showed that in the year 2022 the seed rate per hectare had a very significant effect on the productions obtained in the mixtures of three species compared to the year 2023 (Table 3) when only component of mixture had a very significant effect.

Thus, in the mixture of peas, camelina and oats, the level of production was between 2000 and 3500 kg/ha, the differences being due to the climatic conditions. The year 2023 generated a higher degree of weeding, which led to lower productions. Similar results were obtained with the mixture of peas, flax and oats (Figure 4). The lower level of production in the mixture of peas, flax and oats could be due to the presence of the cuscuta (*Cuscuta campestris*) more in 2023 compared to 2022 (Figure 5).

Table 3. Analyses of variance for the yield in intercrops with three species and two seed rate per hectare

Source of variance	FD	Factor F and significance	Factor F and significance
		2022	2023
Factor A (seed rate per hectar)	1	14.70***	2.98
Error A	2		
Factor B (Intercrop)	7	119.27***	15.06***
Interaction AxB	7	26.98***	0.26
Error B	28		

Figure 4. The yields of pea intercropped with camelina and oat/spring wheat, pea intercropping with flax and spring wheat/oat obtained during 2022 and 2023 seasons

Figure 5. Aspect of the flax crop with cuscuta attack (*Cuscuta campestris*), 2023

The mixture of three species consisting of peas + spring wheat/oats are not always profitable in the mixed culture at the rate of seed recommended for sowing (Figure 6).

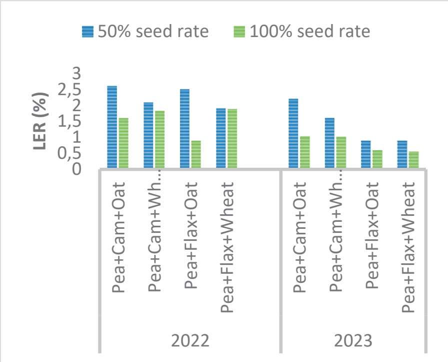

Figure 6. The land equivalent ratio (LER) for mixtures of three species

The mixture of three species in 2023 had LER values ranging from 0.5 to 1.04 at reduced seed rate, indicated that grain yield was reduced when three species were grown together, compared to their pure crops. The only pea intercrop with camelina and oat/spring wheat at recommended seed rate had LER values over 1 (Figure 6).

The reason for lower LER values at Fundulea in 2023 compared to 2022 could be beside the soils and climate of a region, factors including row intercrop planting configurations and the compatibility of the cultivars grown can affect yield outcomes.

Other studies examined the potential for reductions in nitrogen fertiliser use or fungicide use in intercropping systems, and any soil legacy effects that may improve subsequent cereal crop yields (Roberts & Dowling, 2020).

CONCLUSIONS

Good results in terms of the yield were obtained with the mixtures between peas and cereals.

In the mixture of three species, oats came with a production increase

Through the land equalization ratio (LER) with values greater than 1, the mixtures of crops that use environmental resources more efficiently and productively were identified.

It could be concluded that to obtain the best land usage the best option was to use intercropping with two species as compared to intercropping with three species.

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THE QUALITATIVE CHARACTERIZATION OF POLYFLORAL HONEY AND THE INFLUENCE OF THERMAL PROCESSING ON HYDROXYMETHYLFURFURAL CONTENT

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Abstract

Due to the low pH, water activity and high sugar content, honey is one of the healthiest food products. Few biological hazards are associated with the product, due to its composition. The hydroxymethylfurfural (HMF) content is recognized as a parameter that affects the freshness of honey. The content tends to increase during processing or due to long storage. The aim of this paper is to assess the physico-chemical quality of polyfloral honey from Alba County, Romania. The research was carried out on a sample of honey whose initial HMF values was 2.1 mg HMF/kg honey. The simulation of honey processing was carried out at temperatures between 30 and 100°C, and the treatment time varied between 30 and 300 min. The statistical correlation between the thermal treatment applied to the honey and time, calculated from the results obtained, represents an argument for a unique direction of hydroxymethylfurfural content.

Key words: *honey bee, HMF, processing, HACCP, statistics.*

INTRODUCTION

Honey, a natural traditional sweetener, is considered a functional food, and many studies have demonstrated its beneficial health properties (Rasad et al., 2018). Honey is used in the food industry, cosmetics, medicine due to its nutritional, therapeutic and dietary quality. Honey is obtained by processing flower nectar or plant manna (Mărghitaş, 2008).

Honey contains water, glucose, fructose, sucrose, dextrin, vitamins, minerals and small amounts of trace elements and proteins (Eteraf-Oskouei & Najafi, 2013). Honey is a unique natural product that can be consumed as such or added to products. It does not require major processing or preservation (Bratu, 1998). Quality control is considered as a mark of the food industry. This is a prerequisite for the product competitiveness. Regarding the taste, colour, flavour, or consistency, the smallest exception will lower the standard (Trienekens & Zuurbier, 2008).

The content of hydroxymethylfurfural (HMF) represents its ability to indicate the lack of freshness of the honey or its adulteration (Shapla et al., 2018). Legislative standards state that commercial honey intended for human consumption must not have more than 40 mg of HMF per kilogram of honey (CAC, 2001; CD, 2001).

In managing the risks associated with the consumption of honey, all stages of production must be taken into account. The HACCP (Hazard Analysis Critical Control Point) system, although not mandatory, comes for the purpose of quality and safety control in the honey industry as well, being a preferred tool to ensure that consumers are offered safe food (Formato et al., 2011).

The HACCP methodology outlines seven principles, which include conducting hazard analysis, evaluating Critical Control Points (CCPs), establishing critical limits, implementing a monitoring system, defining corrective actions, creating verification procedures, and maintaining comprehensive documentation

(CAC, 1999). In HACCP programs, "control points" (CP's) will also be identified. Control points play a vital role in a food processor's extensive product control system and can complement HACCP by helping in the delivery of safe food products with consistently high quality to consumers (Humber, 1992).

The article describes a systematic approach based on qualitative characterization of polyfloral honey and the influence of thermal processing on hydroxymethylfurfural content, including through risk assessment within the HACCP system. Analysis of 11 samples of honey from the main areas of Alba County, Romania, provided basic data for HMF content of honey. A sample from Alba Iulia area containing 2.1 mg/kg HMF was the subject of a thermal treatment simulation at different temperatures, in time.

MATERIALS AND METHODS

Polyflower honey samples from different areas of Alba County, Romania (Table 1), have been tested to study their qualitative properties.

Table 1. The origin and geographical area of the polyflower honey samples

Sample	The origin area of honey	Areal
S1	Alba Iulia	Plain
S2	Abrud	Mountainous
S3	Aiud	Plain
S4	Baia de Arieș	Mountainous
S5	Blaj	Hilly
S6	Câmpeni	Mountainous
S7	Cugir	Hilly
S8	Ocna Mureș	Hilly
S9	Sebeș	Plain
S10	Teiuș	Hilly
S11	Zlatna	Sub-mountainous

The samples were obtained from different beekeepers. Each sample was collected in a sterile container and kept in a dark place at 2-8°C until it was analysed.

The following physico-chemical parameters were analyzed: pH water activity, moisture (water content), HMF, glucose and fructose content (SR 784-3, 2009; AOAC, 1995).

pH value: an aqueous solution of 10% honey was prepared. pH was measured with a PH-meter PHT 810 (1340-5810) (EBRO, Germany) (SR 784-3, 2009; AOAC, 1995).

The *water activity (a_w)* was determined with the Aquaspector apparatus AQS-2-TC (Nagy Messsysteme GmbH, Gäufelden, Germany) (SR 784-3, 2009; AOAC, 1995).

The *water content (moisture)* of honey was determined by refractive index (RI) at 20°C using an ABBE AR 2008 refractometer (Kruss Scientific GMBH, Hamburg, Germany). The water content (%) was set with the help of standard tables according to the refractive indices.

For the determination of *HMF in honey*, 10 g of honey was dissolved in approximately 25 mL of distillate water and transferred to a 50 mL volumetric flask; 2 mL of honey solution and 5 mL of p-toluidine (Sigma-Aldrich Production GmbH, Switzerland) were placed in two test tubes. In one tube 1 mL of distilled water was added (reference solution) and in the other 1 mL of barbituric acid (Kanto Chemical Co. Inc.) solution 0.5% (sample solution). The absorbance was read in 1 cm cuvettes at 550 nm with a Lambda 20 UV VIS Spectrophotometer (Perkin Elmer, Waltham, MA, USA). The HMF content was determined by the external standard method (p 99%, Sigma-Aldrich, Milan, Italy) and by using the proposed formula for the method.

Glucose: for a quantity of 20 ml of solution (obtained from 1 g of honey in 100 ml of distilled water), 50 ml of sodium bicarbonate solution (Sigma-Aldrich Production GmbH), 50 ml of sodium carbonate solution (Sigma-Aldrich Production GmbH) and 20 ml of iodine solution (Sigma-Aldrich Production GmbH) were added. The mixture was kept closed in the dark for 2 hours, after which 12 ml of sulfuric acid solution (Sigma-Aldrich Production GmbH) was added and titrated with thiosulfate solution (Sigma-Aldrich Production GmbH). In parallel, a sample was prepared using the same volume of water instead of the 20 mL honey solution.

$$\text{Glucose (\%)} = [9.005 \cdot (V-V1) \cdot 5]/10$$

where: V - the volume of the iodine solution that was consumed in the honey sample, V1 - the volume of the iodine solution that was consumed in the control sample (Marghitaș, 2008; Popescu & Meica, 1997).

Fructose: in 20 ml of solution (obtained from 3 g of honey in 200 ml of distilled water) were added 2.5 ml of NaOH 4N and 8 ml of iodine solution (Sigma-Aldrich Production GmbH). Then 6 ml of sulfuric acid (Sigma-Aldrich

Production GmbH) and sodium sulfite (Sigma-Aldrich Production GmbH) were added until a straw-yellow colour was obtained. 0.5 ml of starch solution (Sigma-Aldrich Production GmbH) was added and continued with sodium sulfite 2% (Sigma-Aldrich Production GmbH) until the solution became colourless. The solution was neutralized with 0.1N NaOH in the presence of phenolphthalein (Sigma-Aldrich Production GmbH) (Marghitaș, 2008; Popescu & Meica, 1997).

RESULTS AND DISCUSSIONS

The quality of a bee's honey can also be identified by the association of various physico-chemical properties. From this point of view, parameters such as: pH, water activity, moisture, fructose, glucose and HMF content and others are evaluated and monitored to appreciate the quality of honey. Table 2 shows the physico-chemical results of the honey samples collected from the towns of Alba County.

Table 2. The values of the physico-chemical parameters for the polyfloral bee honey samples from Alba County

Parameter / Source/Area	pH	a_w	Fructose, %	Glucoze, %	Moisture, %	HMF, mg/kg
Alba Iulia	3.70±01	0.541±0.018	38.4±1.0	28.7±0.6	16.7±0.7	2.1±0.3
Abrud	3.66±02	0.602±0.033	40.8±1.2	29.2±0.8	15.9±0.9	7.2±1.0
Aiud	3.92±04	0.574±0.012	41.2±0.9	27.0±1.2	16.4±0.7	11.0±1.1
Baia de Arieș	3.68±03	0.559±0.027	39.9±1.5	29.8±1.6	16.5±1.1	4.7±0.4
Blaj	3.74±01	0.580±0.039	38.3±1.8	30.4±1.3	16.2±1.2	6.5±0.9
Câmpeni	3.76±02	0.567±0.034	40.5±1.3	27.9±1.1	15.3±0.7	5.2±0.6
Cugir	3.63±03	0.522±0.046	44.4±1.7	28.6±0.9	15.2±0.6	8.0±0.5
Ocna Mureș	4.08±04	0.503±0.025	41.3±2.1	29.9±2.0	17.0±1.5	12.1±1.3
Sebeș	3.81±01	0.567±0.029	38.2±0.4	27.8±1.4	15.8±0.6	7.4±0.17
Teiuș	3.79±02	0.548±0.010	39.5±0.9	27.6±1.0	15.7±0.8	4.9±0.4
Zlatna	3.95±05	0.545±0.017	39.0±0.6	29.1±0.7	16.1±1.2	6.6±0.8

The physico-chemical parameters meet the standards of the current regulations - EU Honey Directive (CD, 2001). The pH values align with the well-established characteristic of honey was naturally acidic. The typical pH range of honey is 3.2-4.5 (Dilnawaz et al., 1995; Hamid & Saeed, 1991; Stenson et al., 1960; White et al., 1963). In the samples collected from Alba County, the lowest value recorded was from the Cugir area with a value of 3.63, and the highest, of 4.08, in the Ocna Mures area.

Typically, raw honey contains around 16-18% moisture. For Alba County, the range obtained for the water content of honey was 15.2-17%. However, it's internationally recognized that high-quality honey should ideally have a water content of less than 20% (Singh & Singh, 2018). The water activity (a_w) of honey typically falls within the range of 0.5 to 0.65 (Chen, 2019; Abramovic et al., 2008; Cavia et al., 2004; Chirife et al., 2006).

Honey is predominantly composed of carbohydrates, making up approximately 80% of its content, with the majority being glucose and fructose (Pauliuc et al., 2022). The fructose content in honey typically ranges from 21% to 43%, and the fructose-to-glucose ratio varies from 0.4 to 1.6 or even higher (Bobiș et al., 2018). The values recorded for the glucose and fructose content for Alba County, Romania fall within those stipulated in specialized literature.

Typically, freshly extracted honey maintains hydroxymethylfurfural (HMF) levels below 10 mg/kg. Elevated levels beyond this threshold may suggest overheating during the extraction process. Additionally, the HMF content in honey following processing and/or blending should not exceed 40 mg/kg (CAC, 2001).

HACCP serves as a method for identifying hazards and implementing control measures that prioritize prevention over primarily relying on testing end-products (Dahiya et al., 2009). In order to achieve the HACCP plan related to the bee honey production process, it is absolutely necessary to approach the detailed technological flow diagram first, for the honey under study and which will have a specific character, customized for each company. The flow diagram allows better identification of contamination points or paths throughout the production chain, which helps to more easily establish measures to

prevent physical, chemical and microbiological contamination (Bevilacqua et al., 2023).
Figure 1 shows the technological stages of bee honey conditioning, received from beekeepers in collection-packaging centers.

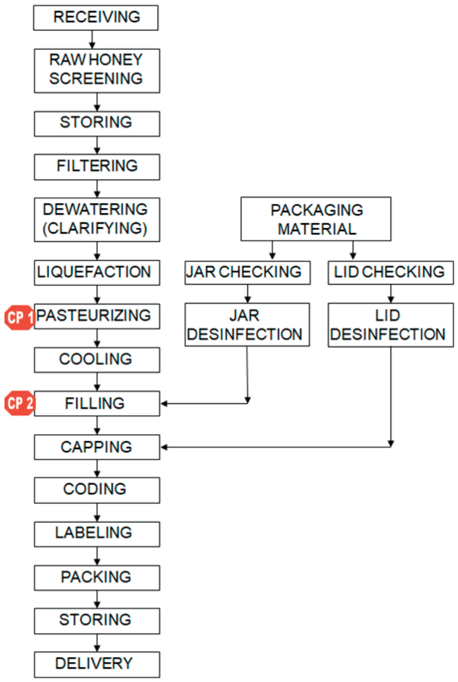

Figure 1. Control points that can lead to the increase of HMF content in process flow diagram of honey processing

HACCP is a management system where food safety is addressed throughout the technological flow through the analysis and control of biological, chemical and physical hazards. The HACCP plan is implemented to identify critical control points and control points (Formato et al., 2011).
The HACCP plan is implemented to identify critical control points (CCP's) and control points (CP's), the last one being used for quality specifications (CQP). A CCP indicates a high food safety risk (likely to occur) and a CP indicates a low food safety risk (not likely to occur). CQP implies identifying the quality of products, tools, and other aspects used in food processing. In the case of bee honey processing, the increase in the HMF value is considered a quality problem (Vica et al., 2009).
The problem, with the exception of the identification of potential fraudulent tendencies of falsification of honey, can be identified by analyzing certain degradation products (metabolites), such as hydroxymethylfurfuralor 5-hydroxymethyl-2-furaldehyde ($C_6H_6O_3$) (HMF) (Gregorc et al., 2020). The stages identified as points of attention (control) from the point of view of HMF content are presented in Table 3.
Following the analysis and evaluation of the dangers that may appear in all the technological stages of bee honey conditioning, received from the beekeepers, it was identified the need to include in the HACCP plan pasteurization and filling of jars, as points of attention (control) from the point of view of growth of the HMF content of honey.
In order to determine the variation of the HMF content of polyflora bee honey for 300 min, the evolution of the parameter was followed at 5 different temperatures between 30°C and 100°C.

Table 3. Analysis and assessment of the dangers related to the increase of HMF corresponding to the identified CP

Stage	Hazard type	Hazard description	Stage role	Control	Limit	CP
Pasteurization	Chemical	Excessive heating may cause increased HMF content and reduced enzyme (diastase) activity. It negatively influences the taste, aroma and colour of honey (becoming more "browning").	The heat treatment reduces the moisture content of the honey, destroying the yeast cells and liquefying the crystals, also increasing its shelf life.	Monitoring the time and duration of honey pasteurization. Ensuring that the working process and storage environment do not exceed 60°C.	HMF <40 mg/kg	CP1
Jar filling	Chemical	The use of hot sterilized jars increases the temperature of the honey and the risk of increasing the HMF content and reduced enzyme (diastase) activity.	Keeping the product in sterile (risk-free) packaging to ensure its preservation and to be easy to transport.	Cool the jars to room temperature (about 15 min) before adding the honey.	HMF <40 mg/kg	CP2

In Table 4, the HMF values resulting from the treatment of polyfloral bee honey at different temperatures over time are presented comparatively.

Table 4. The values of HMF content obtained at different temperatures over time

Time [min] \ Temp. [°C]	30	50	70	80	100
0	2.1±0.7				
30	5.3±0.8	10.2±0.3	11.5±0.2	11.7±0.5	15.8±0.7
60	5.4±0.5	10.8±0.8	12.2±0.7	19.6±0.4	46.3±2.8
90	5.7±0.3	11.7±0.5	14.5±0.3	20.4±0.2	50.7±4.1
120	6.6±1.1	12.3±0.9	17.8±0.15	27.3±0.9	60.2±3.7
150	9.0±1.4	15.5±1.6	19.4±2.2	36.1±1.4	74.7±2.2
180	11.4±2.3	17.2±1.0	20.3±1.8	42.9±3.1	82.3±3.8
210	15.2±4.0	22.0±3.3	44.2±4.9	50.7±3.8	84.1±4.5
240	17.1±2.8	29.6±2.4	52.1±6.7	63.9±2.5	86.5±3.9
270	19.5±3.9	32.8±1.8	60.6±2.0	74.8±4.7	88.0±2.3
300	20.1±2.7	37.5±4.1	65.0±3.3	86.6±2.9	90.4±3.7

Table 4 presents the variation of HMF with the temperature at which the polyfloral honey was treated. The HMF value shows an increasing trend over time for all the temperatures to which the honey was subjected, starting from 2.1 mg/kg, respectively registering a value of 90.4 mg/kg for the honey subjected to 100°C for 300 min.

In chemistry, it's uncommon for a process to be solely affected by a single variable parameter. Instead, it's intriguing to observe how other parameters, through their fluctuations, notably impact the variations observed in the outcome (Gluck, 1971). Using MATLAB, experimental data regarding the time variation of the HMF content for poly-floral honey samples at different temperatures were processed and analyzed. Figure 2 presents both the experimental data and the surfaces generated by statistical Mathematical model. A correlation was established between the HMF content of honey in time (t) and the applied temperature (T) using a two-degree polynomial relation with two independent variables.

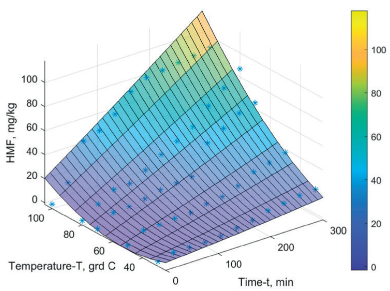

Figure 2. The variation of HMF content of polyfloral bee honey depending on the applied temperature and time

The second order regression equation for honey samples that correlates the variation of the HMF content with the time and the temperature has the following form:

$$\text{HMF (mg/kg)} = 21.2496 - 0.0585 \cdot t - 0.8637 \cdot T + 0.0035 \cdot t \cdot T + 3.6856 \cdot 10^{-5} \cdot t^2 + 0.0081 \cdot T^2$$

where: t - time, min; T - temperature, °C.

The accuracy of the correlation is confirmed by the adequacy indicators: the model accuracy indicator ($r^2 = 0.944$), the correlation coefficient ($r = 0.972$) and the standard deviation ($\sigma = 6.467$). The calculated correlation parameters validate a very good prediction capacity of the statistical mathematical model.

CONCLUSIONS

The physical properties and nutritional chemical composition of honey from Alba County, Romania were investigated to highlight the quality of the polyfloral honey from this region. The equation of the statistic model can approximate the variation in time of the HMF content at different temperatures. These represent a control and prediction tool for the appreciation of the product quality in time.

The system for food safety - HACCP acts through the forms of control, monitoring and verification, documents and pays more attention to prevention. In addition to the recording and monitoring systems of the critical control points, the attention points indicate a low food safety risk, but which will still generate honey quality problems. Thus, 2 stages were identified, the pasteurization and filling of the jars, which can lead to an increase in the HMF content, the control and the established limits.

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NETTLE BREAD, A POTENTIAL FUNCTIONAL PRODUCT

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Abstract

Stinging nettle (Urtica dioica) plants are considered plant superfood with medicinal properties. Stinging nettle plant can be found worldwide and were used to combat a wide range of diseases like: seasonal allergies, respiratory problems, arthritis, pain, anemia and lethargy, inflammations etc.), as textile or food since thousands of years. There are no informations regarding since when nettle started to be introduced in bread, but there are written food receipts since Roman times. The aim of this study is to present the possibility of using nettle powder (1, 5, 10%) of the total flour quantity, to improve bread taste and quality. Young nettle leaves were collected from a nonpolluted area (Bazos, Timis), dried under controlled environment, then powdered and stored at room temperature in stainless steel container. The bread with and without nettle powder (Control) was analyzed regarding moisture content, mineral composition using XRF analyzer, total antioxidant capacity and total polyphenol content. The results were statistically evaluated. By comparing plain white bread and bread enriched with nettle powder, it's observed the increase of mineral content, total phenolics and antioxidant activity.

Key words: *antioxidants, mathematical models, minerals, Urtica dioica.*

INTRODUCTION

The concern of consumers and producers of food products is to offer not only a safe, healthy food, but also to promote health. Due to this new trend the number of functional foods on the market is constantly growing (Maietti et al, 2021) (a growth rate of about 8–16% per year).

As bread and bakery products are amongst the most consumed food in the world (Gül et al., 2017), there is a high request of innovative functional products in this area.

Plant-derived bioactive compounds as functional food ingredients, capable to increase the mineral and antioxidants content, are some of the components that could be included in bread formulation (Maietti et al., 2021).

Stinging nettle (*Urtica dioica*) is an edible plant, highly valued for its nutritional and nutraceutical properties from the spontaneous flora of the world (Bhusal et al., 2022).

The nettle plant is rich in flavonoids, phenolic compounds, vitamins, minerals, organic acids, volatile compounds, fatty acids, carbohydrates isolectins, sterols, terpenes, and proteins (Said et al., 2015, cited by Bhusal et al., 2022).

The most representative minerals of the nettle plant are: calcium, potassium, magnesium, phosphorus, iron, sulphur, zinc, manganese, copper, and nickel (Pradhan et al., 2015).

Forêt, in 2021, mentioned that nettle bread is very rich in antioxidants and minerals and due to the fact that nettle powder is considered a low glycemic index food (Perez, 2022), its recommended to be added to the whole grains for obtaining a nutritious functional bread.

Nettle plants are very rich in minerals and compared "to wheat flour, nettle powder has much higher total ash content (0.57% for wheat flour and 17.67% for nettle powder), being probably, one of the richest sources of minerals among plant foods" (Man et al., 2019).

The addition of phenolic compounds affects dough's physical-chemical properties and qualities because of several interactions with flour ingredients and helps "to prevent the formation of carcinogens such as acrylamide during baking, thus functioning as an anti-carcinogenic agent in food systems" (Xu et al., 2019).

The aim of this study was to create a functional type of bread with nettle powder (1, 5, 10%) of

the total flour quantity, and to verify if the bread quality is improved.
In order to appreciate the quality of the new proposed bread assortments, samples of each bread assortment were analyzed based on total antioxidant capacity, total polyphenols content and mineral content.

MATERIALS AND METHODS

Collection of nettle plants
The nettle plants were collected from Bazos Forest, a region from Timis County, Romania. The leafy parts of the plants were washed, cut, shade dried at room temperature. The dried leaves were pulverized, packed in airtight sterile bags, labelled and stored for further analyses and uses.
Bread making
Ingredients for the plain bread (Control) were: 0.5 kg of flour type "000"; 300 mL of water; 40 ml of extra-virgin olive oil; 10 g of yeast; 10 g of NaCl.
Nettle-enriched bread was obtained by adding 1%, 5% and 10% nettle powder and brings in all three cases to 0.5 kg with flour type "000".
All samples, plain and nettle enriched doughs (kneaded and fermented) were partially baked at 100 °C for 60 min. Final products were oven-baked at 180°C for 60 min.
From each bread assortment was cut one slice of bread, oven dried and fine grinded for future analysis. All samples were homogenized and analyzed in triplicate.
XRF mineral analysis
For XRF (X-ray fluorescence) analyses of samples powder were used Hitachi X-MET8000 portable spectrometer. Each mineral detected in the analyzed material produces a set of characteristic fluorescent X-rays ("a fingerprint") that is unique for that specific element (Thermofisher, 2020).
All analyses were performed in triplicate and the results were reported in ppm.
Total antioxidant capacity (TAC) and total polyphenols content (TPC)
TAC was determinate using CUPRAC method and TPC using Folin-Ciocalteu method as described by Bordean in 2016.
All measurements were performed in triplicate and the antioxidant capacity of each extract was expressed in (μM Trolox/100 g FW).
The total polyphenol content was expressed in mg Gallic Acid/100 g FW.
Statistical analysis
The data were statistically evaluated using the software PAST Version 2.17c (Hammer et al., 2001) and MVSP.
For modelling the data and to have a view regarding the mineral distribution in the bread samples we have used spatial interpolation technique, named Kriging (Veer, 2013), which leverages the spatial autocorrelation structure captured by the semivariogram (Badia-Melis, & Ruiz-Garcia, 2016) to make predictions at unmeasured locations (Castrignanò & Buttafuoco, 2020).

RESULTS AND DISCUSSIONS

Fortification is the process of adding certain nutrients to foods to increase their nutritional value.
Fortified bread is recommended because of the addition of certain nutrients that can be essential for overall health.
The influence of nettle powder addition to bread is presented in Table 1 and Figure 1.

Table 1. Presentation of Nettle - enriched bread assortments characteristics

Bread characteristics	Control (PB1)	PB2	PB3	PB4
Color	Yellow	Yellow green	Brown green	Dark brown greenish
Taste	Plain	Plain	Slight taste of nettle	Strong taste of plant
Acceptability	7	7	6	3
	(1 = dislike very much, 4 = neither like nor a dislike, 7 = like very much)			

Legend: PB1 = plain bread (Control); PB2= Nettle enriched bread (1% nettle powder); PB3 = Nettle enriched bread (5% nettle powder); PB4 = Nettle enriched bread (10% nettle powder).

The obtained bread types can be described as follows:
PB1 = Plain bread (Control) is a product which looks well developed, rectangular in shape with shiny surface, golden yellow color, well-flushed core, pleasant aroma, pleasant taste, characteristic of well-baked white bread.
PB2= Nettle enriched bread with 1% nettle powder appears like a well-developed product, rectangular in shape, glossy surface, yellow-

green color, soft core, pleasant aroma, pleasant taste, presenting the characteristic of well-baked bread.

PB3 = Nettle enriched bread (5% nettle powder) is a well-developed product, rectangular in shape, matte surface, brown-green color, soft core, pleasant specific nettle aroma, pleasant taste, characteristic of baked bread.

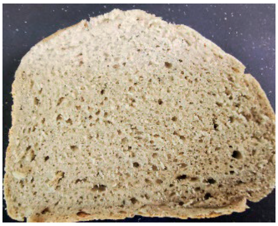
	**PB1**
	**PB2**
	**PB3**
	**PB4**

Figure 1. Bread assortments with nettle powder
Legend: PB1 = plain bread (Control); PB2= Nettle enriched bread (1% nettle powder); PB3 = Nettle enriched bread (5% nettle powder); PB4 = Nettle enriched bread (10% nettle powder)

PB4 = Nettle enriched bread (10% nettle powder) appears like a well-developed product, rectangular shape, matte surface, dark green color, dense core, pungent nettle aroma, specific taste of nettle bread.

In plant-based foods, especially in bread, the interactions between minerals, polyphenols, antioxidants, carbohydrates and fibers are very important because the functionality of each of the components might be affected (Schefer et al, 2021).

The mineral content and the variations between bread samples are presented in Figure 2.

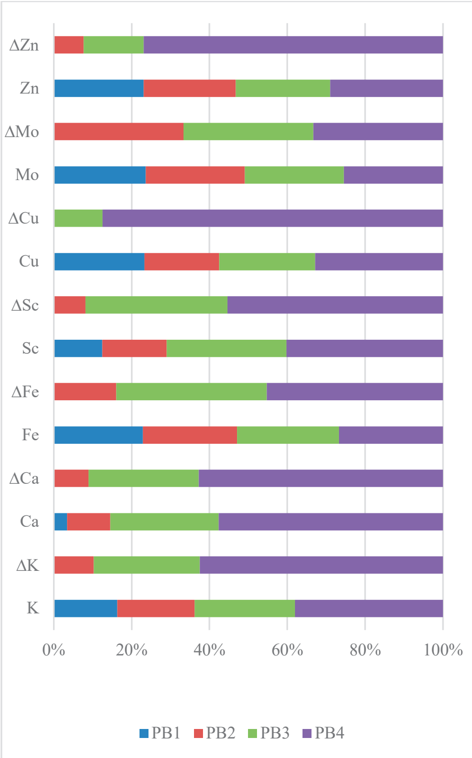


Figure 2. Mineral fingerprint of the investigated bread samples
Legend: PB1 = plain bread (Control); PB2= Nettle enriched bread (1% nettle powder); PB3 = Nettle enriched bread (5% nettle powder); PB4 = Nettle enriched bread (10% nettle powder), Δ = percentage change

As we can observe from Figure 2, most of the minerals show an increase of content (the percent is mentioned on the diagram).

The percentage of change ΔCa > ΔK > ΔSc > ΔZn > ΔCu > ΔFe > ΔMo. The mineral content is increasing proportional with the increase of nettle powder content.

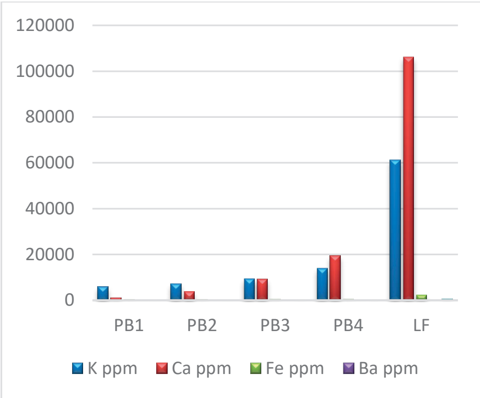


Figure 3. Mineral content of bread samples and nettle powder
Legend: PB1 = plain bread (Control); PB2= Nettle enriched bread (1% nettle powder); PB3 = Nettle enriched bread (5% nettle powder); PB4 = Nettle enriched bread (10% nettle powder), LF= nettle powder

As we can observe the minerals present in the nettle powder (LF) contribute substantially to the mineral content of the proposed bread types (PB2, PB3 and PB4) (Figure 3).
The spatial interpolation of data corresponding to the mineral composition of fortified bread samples is presented in Figure 4.

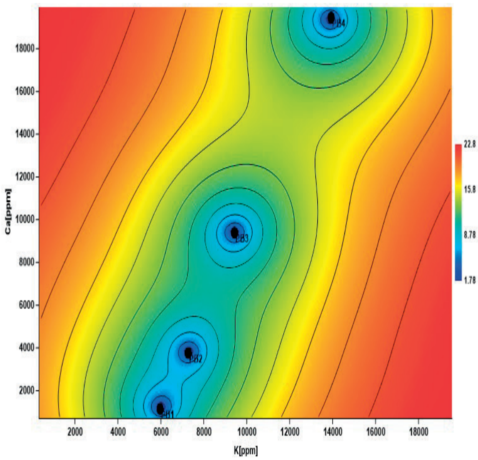


Figure 4. Spatial interpolation of mineral data of bread types
Legend: PB1 = plain bread (Control); PB2= Nettle enriched bread (1% nettle powder); PB3 = Nettle enriched bread (5% nettle powder); PB4 = Nettle enriched bread (10% nettle powder)

The figure shows the level of mineral composition optimization based on Kriging Algorithm and exponential optimized semivariogram.
The exponential semivariogram has the role to calculate the kriging weights for neighbouring sample points, in order to provide an estimate for the unmeasured areas of the bread samples.
The TAC and TPC values of functional bread are presented in Figure 5 and 6. The values are expressed as average values.

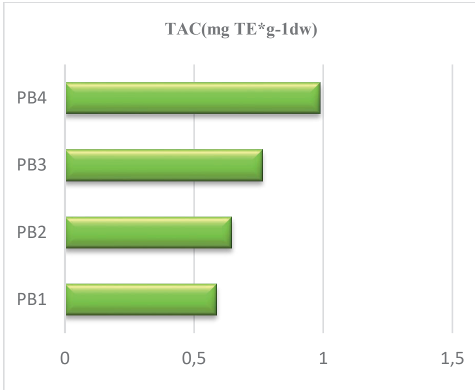


Figure 5. Representation of TAC of the investigated bread samples
Legend: TAC= total antioxidant capacity mg of Trolox equivalents (TE) per g of dry weight (dw).

As we can observe the total antioxidant capacity is increasing with the increase of nettle powder content. The bread with the highest content of nettle powder showing.

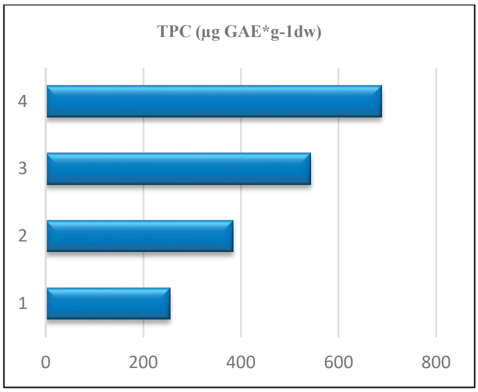


Figure 6. Representation of TPC of the investigated bread samples
Legend: TPC = total polyphenol content - µg of gallic acid equivalents (GAE) per g of dry weight (d.w.)

CONCLUSIONS

The increase of calcium, potassium and iron of the proposed bread assortments and the pleasant taste of the bread assortments (in special PB2 and PB3) recommends the use of nettles powder for optimization of bread mineral content.

The addition of nettle powder to the bread dough gives a special taste and texture to the bread and provides a large amount of easily assimilable nutrients, but will not provide the same amount of nutrients as conventional fortification.

The percentage of change $\Delta Ca > \Delta K > \Delta Sc > \Delta Zn > \Delta Cu > \Delta Fe > \Delta Mo$.

Although nettle fortification is not a common fortification, the high content of nettle powder recommends it for improving the iron, calcium and potassium content of bread.

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ARID CLIMATE IMPROVEMENT IN THE LOW PLAIN ARANCA WITH FRUIT TREE AND FOREST SHELTER - BELTS

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Abstract

The village territory Beba Veche is placed within the Low Plain Aranca in the western part of Romania of 9300 ha. The aridity of climate has been intensified because of wood cutting, and of an ample hydrotechnics and land improvement works. In present time there are 5-6 droughty years from ten years. The dominant soil types are Chernozems (44%) and Vertisols (37%). The shelter - belt must be composed of forest species but also from shrubs and fruit trees, like: Quercus, Fraxinus e., Robinia p., Alnus g., Cydonia o., Cerasus v., Malus d., Pirus s., Prunnus d.; Sorbus n., Corylus a., Hippophae r. The seedlings will be plant on 4 rows, with 2 m between rows and 1.5 m on the rows. The necessary of seedlings is about 375,000 seedlings and the costs can be recuperated in about 6-8 years.

***Key words**: climate, shelter-belt, vertisols wind.*

INTRODUCTION

The Banat Plain is graduated in the high plain (Vinga, Gătaia, Socol, Buziaș), terrace plain (Recaș, Sudriaș, Biniș, Mureș) and low plain (Bega - Timiș, Moravița, Jimbolia and Aranca).
As part of Banat Plain, Aranca Plain is characterized by the most dry climate from the Banat region (Rogobete et al., 2008; Rogobete et al., 2021).
Because until not long ago (19th century) the Low Plain Aranca were covered with water, ample hydrotechnics and land improvement have been effectuated (Man, 2015).
Beside the positive effects have existed some negative effects for example an overly coming – down for ground water table, up to three maters depth. Alongside with the precipitation deficit, it was been accentuated the necessity for shelter - belt.
The aridity of climate has been intensified also because the forest massif existing in the Banat Plain especially those around the settlements has been exhausted (Chisalita, 2015; Coste et al., 1997).
Similar phenomena has been noticed for Bărăgan Plain and Oltenia Plain by Lupu (Lupu, 1952) and Nesu (1999), which have proposed setting up forest belts for agricultural land.
It is appreciated that the forest belts will be diminish the evapo-transpiration, will maintain the snow layer a prolonged time, reduce the wind speed and in the same time contribute to increase the level of agricultural production.
It is necessary to mentioned the improvement the local climate, with positive effects of biodiversity. It can be added also the fruits production, wooden mass harvest and the presence multiplied of birds and other wild animals.

MATERIALS AND METHODS

In order to realize this thesis relative to shelter - belts, we have used the climatic data from weather station Sânnicolau Mare (30 km distance), the soil survey at scale 1:10.000 Beba Veche (OSPA - Timis - guide Rogobete), and a research theme from ICAS Timișoara (Adam and Rogobete, 2002) as principality bibliography sources. The materials used for shelter - belt in the Beba Veche territory were cadastral map 1:20000, drainage - system map 1:100000, soils map 1: 10000 (1994 year), climatic data with the frequence - speed and wind directions, and a list with adapted forest and fruits species for this area. The placement of the shelter belt in the perimeter of Beba Veche started to trace the network of the cadastral map. It has been realized an ideal network and after that was made a land recognition, and we have remarked that is practically impossible to realize a complete network of shelter – belts, because there is not an agreement with the landowners, which accuse of loose the land. Also, the Romanian Natioanl Society of Land Improvement has deemed that the shelter belt must be placement only on one site of the channels for access of a mechanics maintenance.

RESULTS AND DISCUSIONS

The territory Beba Veche is situated in the western part of Aranca Plain, at the frontier zone with Serbia and Hungary and river Mureș and Galatca.

Figure 1. Map of Aranca Low Plain

Aranca Plain is a low plain (Figure 1), formed bu subsidence and has only 77-83 m altitude with numerous old and abandoned riverbeds, the plain being until the 18th century a huge swamp (Tarau et al., 2019).

After the ample improvement works effectuated in the 19th and 20th centuries, these lands become agricultural and land in crop use (Figure 2).

Aranca subsystem drainage, with an area of 55,582 ha is part of Sânnicolau Mare system which has 9 pumping station, with 63.47 mc/s water discharge installed.

Beside the positive effects there are some negative effects, for example an overly coming - down for ground water table up to three meters depth. Consequently, the radicular system of the plants has lost an important source of water - capillary fringe.

Figure 2. Map with land improvement works

At the present time, the ground water level fluctuates between 0.5-5.0 m depth and the chemical composition of the ground water is variable from one to other drilling, for example in the clayey deposits the total soluble salts content arrives at 3.0-5.0 g/l (Rogobete et al., 1997).

The total area of Beba Veche is 9300 ha. Which is composed of 7200 ha agricultural land, 600 ha built up, roads, 1500 ha drainage canals, and waste land. Aranca river is connected with Tisa river and by drainage system with Mures river (OSPA, 1994).

Shelter - belts are necessary for:

- village pasture, with a total length of 8.6 km and an area of 12.3 ha;
- channels in the agricultural land with a total length of 62.9 km and a area of 63.8 ha;
- village and exploitation roads and canals, with total length of 45.1 km, on 37.5 ha.

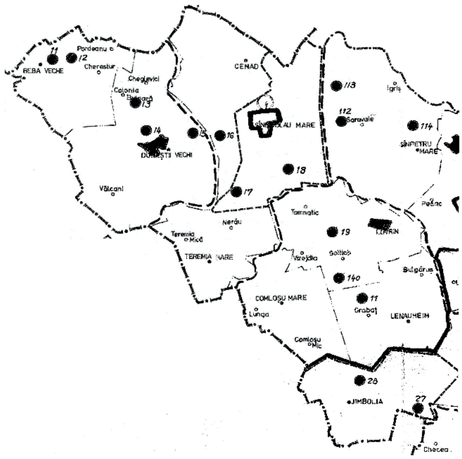

Figure 3. Hidrogeological control points

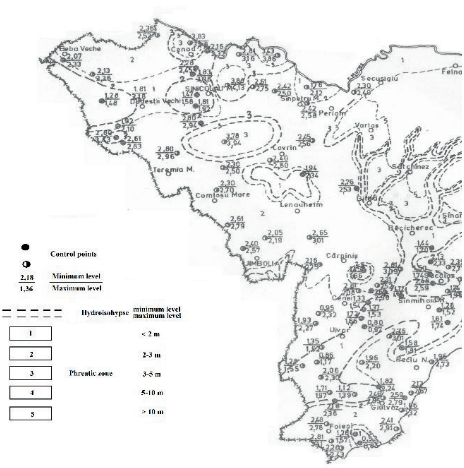

Figure 4. Hydrogeological map

From the climatic point of view, Aranca Plain is characterized by the temperate continental climate, with a topoclimate similar with steppe. The annual average temperature is 10.8ºC and 40 days tropical: July = 21.4ºC and August = 21.1ºC.

The multiannual average of precipitations is 536.3 mm, with the following values: July = 53.1 mm, August = 48.6 mm, September = 41.4 mm. It is necessary to specify it may be possible as 4-5 years from 10 years to be droughty, the precipitation deficit being accentuated by the wind with 30-40% (Mircov, 2015).

The initial natural vegetation of sylvosteppe, with *Quercus* sp. was replaced by agricultural crops and grassy vegetation, rich in halophite species, like Puccinelia, Statice etc.

The soil survey for Beba Veche (1994) and the map (Rogobete, 1997; Tarau et al., 2019) indicate for the agricultural Beba Veche territory (8817 ha) the soil cover (Table 1).

Table 1. Soil cover (8817 ha)

Soil type	AS	CZ	VS	GS	SN	Association
Surface, ha	528	3880	3263	160	104	882
%	5.99	44.01	37.01	1.81	1.18	10.0

Table 2. Quality class

Class	I	II	III	IV	V
%	10.31	26.85	27.21	34.39	1.24

From the Table 1, we can see that the dominant soil types are Chernozems (44%) and Vertisols (37%), which have the quality class I + II, respectively class III (approximately), but the soil cover from Beba Veche has important degradation processes, which affected all the soil types:

- water logging (surface water - 45% from total area, ground water = 67% from total area);
- salinization and sodication (78% from the total area);
- compaction (≈80 % from the total area).

The physical and chemical properties for the main soil types will be presented in the next tables, corresponding to the WRB system.

Table 3. Haplic Chernozems (phreatic moist - variety)

Horizons	Ap	Atp	Am	AmBv	Bv	C_{Ca}
Depth, cm	0 -15	-22	-39	-50	-83	121
Clay	31.0	30.0	31.8	31.6	30.2	27.2
Silt	25.9	22.9	22.5	24.7	20.0	22.0
OC	1.60	1.48	1.23	0.99		
CEC	25.35	24.18	25.90	25.25	22.79	18.90
BSP	86.6	87.4	87.8	90.2	91.5	100
pH_{H2O}	6.65	6.80	6.80	7.05	7.25	8.55
BD	1.30	1.40	1.33	1.33	1.33	1.40

The soil type Chernozems has the best physical, chemical and mechanical properties and represents 44% but some of them are gleyic or sodic, or with compaction phenomena in the plowpan horizon, Atp.

Tabel 4. Pellic - stagnic - gleyic Vertisols

Horizons	Azy	ABzyW$_3$	BzyGox	BzyCz
Depth, cm	14 - 26	42 - 69	99 - 130	130 - 163
Clay	74.6	77.0	78.5	77.6
Silt	13.3	11.2	10.2	10.4
pH_{H2O}	6.85	7.20	8.10	7.95
OC	2.41	1.82		
CEC	64.38	71.20	69.50	73.00
BSP	95.5	98.0	100	100
Bs	1.27	1.27	1.32	1.32
K	20	<10	<10	<10
FC	32.6	33.9	33.8	33.5
AW	8.4	10.7	10.0	12.0

The content of clay, wich is the biggest from all soil type in Romania. The soil retains a great quantity of water but the available water is very small, only 10% from the value of field capacity (> 33 %).

Since the mineralogical content of clay is dominated by smectite, which is expandable, the wetting and drying cycles cause these clays to expand and contract.

Deep craching occurs from the surface causes slickensides on the structure faces and oblique shear planes. These soils are increasingly being cultivated mechanically for a great diversity of crop. Although they have a relatively high water holding capacity, shallow rooting crops may suffer from drought stress Vertisols are low hydraulic conductivity and become very hard when dry. Agricultural use ranges from grazing, wood production to crop production (wheat, sorghum, rice).

Table 5. Calcaric Fluvisols (phreatic moist variety)

Horizons	A_{oK}	C_K	C_{IIK}	C_{vK}
Depth, cm	0 – 24	36 – 55	55 – 85	170 – 200
Clay	27.4	17.8	20.6	30.9
Silt	23.0	15.5	27.2	21.9
pH_{H2O}	8.15	8.35	8.40	8.75
OC	1.33	1.19	0.49	
CEC	28.30	25.00	23.30	26.00
BSP	100	100	100	100
BD	1.30	1.42	1.40	1.50
K	810	1010	400	110
FC	21.4	15.5	17.3	22.2
AW	130	10.7	10.0	12.0

The third as wide - spread, Fluvisols (6%) are in generally stratified, moderate fertility, the available capacity is better than of Vertisols, and the water movement are very good. Fluvisols can be very compact and many have a high groundwater table and exhibit gleyic properties. In many cases Fluvisols flooding can limits the use for arable cropping but they are widely used for grazing.

The climatic data, the frequency and intensity of the wind and the relief conditions, impose shelter - belt installation in order to improve environmental conditions.

The composition of the shelter - belt must be in accord with the soil cover, each one of vegetable species having typically requirements. We consider that it is necessary to introduce in the shelter - belt composition shrubs and fruit trees in order to increase the fruits resources in the Beba Veche settlement (Rubtov, 1974; Traci, 1985).

The proposed species list for shelter - belt is:

- Trees: *Quercus robur*, *Fraxinus excelsior*, *Robinia pseudoacacia*, *Tilia tomentosa*, *Alnus glutinosa*, *Cydonia oblonga*, *Juglans nigra*, *Cerasus avium*, *Cerasus vulgaris*, *Sorbus aucuparia*, *Malus domestica*, *Pirus sativa*;
- Shrubs: *Corylus avelana*, *Sambucus nigra*, *Hippophae rhumnoides*, *Prunnus domestica*, *Prunnus cerasifera*, *Prunnus vulgaris*, *Prunnus spinosa*, *Rosa canina*, *Ribes nigrum*, *Ribes rubrum*, *Crataegus monogyna*, *Syringa vulgaris*, *Rubus ideaeus*.

The sites which are predicted for shelter - belts planting and alignments presents very various aspects, such as:

- channels with different depths;
- with exploitation roads on the both sides;
- with only one road;
- with lateral road.

The shelter - belts planting can be realized on the side of channels which can have > 1.5 m depth or < 1.5 m depth:

- in the first case, plantation will be realized only of one side, with 10 m in width. The forest or fruit trees will be planting in the middle of the shelter - belt;
- in the second case will be planting 1-2 rows on the side of channel with *Salix alba* or *Cerasus avium*.

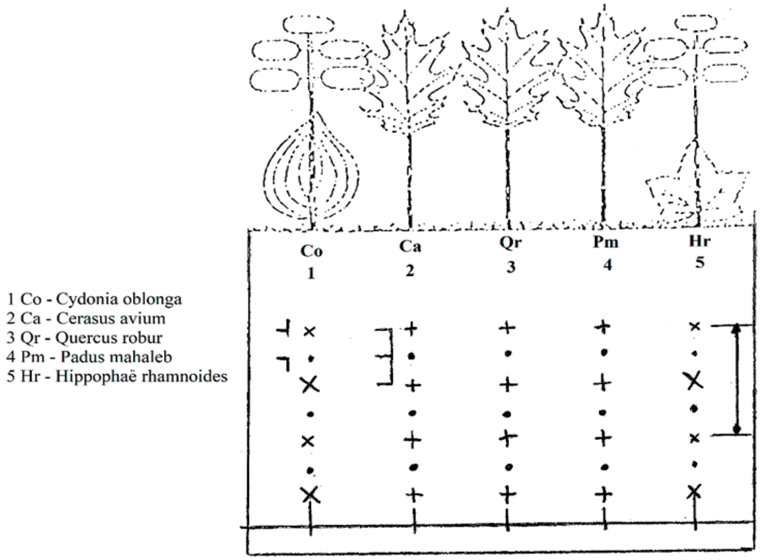

Figure 5. Mixed shelter - belt with Q. r. in the middle of row and fruit trees

The seedlings will be plant on 4 rows, with 2.0 m between rows and 1.5 m on the rows between seedlings.

The seedlings can be obtained in local nursery, which have 100-150 m^2, after a while of 2-4 years.

Table 6. Necessary Seedlings for shelter belts and alignments

- for pasture	31600 pieces
- in the perimeter of agricultural land	249380 pieces
- for alignments (secondary roads and channels)	43600 pieces
Total seedlings	324580 pieces

Table 7. All the costs for setting and maintenance shelter - belts (computation base on maize production and price)

year I	- tillage, planting, maintenance (mechanized and manual)	≈ 430 million lei
year II	- maintenance	≈ 269,414 million lei
Total year I + II ≈ 700 million lei		

A rough estimation of the total necessary seedlings for the Beba Veche village shows about 375.000 (with 20% insurance). Current expenditure with the establishment and maintenance in the first 4 years are estimated to be of about 250.000E.

After 10-12 years, the shelter - belts reach maturity and create a microclimate with a favorable effect on agricultural production, effect estimated through an increasing of about 8-10% for the level of the production.

At this must be added the plus production of wooden mass and fruits.

The gains obtained annually are estimated of about 80 mill E so that it can be recouped the initial investment in four years.

CONCLUSIONS

The shelter - belt realization presumes to use the cadastral maps of 1: 10.000 scale with the soil cover, with the drainage system, which has the stations pumping and the main and secondary drainage channels, the village roads and the exploitation roads, the hydrological and hydrographic maps and of course the climatic data.

These materials allow to realize the shelter - belt corresponding with the environmental factors and with the requirements of the used receipt, respectively the type of species. The economic computations prognosticate a retrieval for the cost after 10 years.

Shelter - belt, with forest and fruit trees improve the quality of life and the biodiversity.

Due to the great capital expenditures, we propose to realize only a network shelter - belts in a closed circuit which can delimits an area of about 200-300 ha and this area will be protected against winds from all directions.

The Hungarian researches have proposed to located shelter - belts on different angles in comparison with the principal wind direction.

Such an integrated system of shelter - belt is enough if covers 2-3% of agricultural land.

The placement of shelter - belt must to take account of the property owner their acceptance.

In variety of trees and fruit trees which be planted are determined by the soil types, climatic conditions and some plats characteristics, like drought resistance, depth of root and needs for firewood and fruits.

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STUDIES ON THE DETERMINATION OF IC50 VALUES OF ETHANOLIC AND METHANOLIC EXTRACTS FROM THE SPECIES *Amaranthus retroflexus* L (Amarantacae) - SPONTANEOUS FLORA

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Abstract

Antioxidant activity is a vital parameter in assessing the potential health benefits and practical applications of natural products. This study investigates the antioxidant potential of Amaranthus retroflexus L. extracts using the 2,2-diphenyl-1picrylhydrazyl (DPPH) method. The DPPH method, widely employed for its simplicity and reliability, relies on the ability of antioxidants to neutralize the purple-colored DPPH radical, leading to a color change in the solution. By establishing a concentration-response relationship through testing various concentrations of Amaranthus retroflexus L. extracts, a dose-response curve was constructed, pivotal for determining the IC50 value, which represents the concentration capable of scavenging 50% of free radicals. Lower IC50 values indicate stronger antioxidant activity. The findings reveal variations in the inhibitory potential of Amaranthus retroflexus L. extracts, suggesting their potential utility in pharmaceuticals, agriculture, and other industries. This study underscores the significance of exploring natural sources for antioxidant compounds and provides valuable insights into harnessing the antioxidant properties of Amaranthus retroflexus L. for various applications.

***Key words**: Amaranthus retroflexus L., half-maximal inhibitory concentration, concentration-response.*

INTRODUCTION

Amaranthus retroflexus L., popularly called the Red News, due to its valuable biological properties, rich phytochemical composition and extensive pharmacological activity, has become an area of widespread scientific and industrial interest.

In recent years, an increasing interest has been observed in plant raw materials, whose properties allow them to be used, both in food and for therapeutic purposes (Karamac et al., 2019). *Amaranthus retroflexus* L. is one of the most studied plants, as a natural source of a wide spectrum of biologically active compounds, relevant for reducing the risk of chronic diseases. This species has been well known since the time of the Aztecs, Mayans and Incas, spreading from the 16th-17th centuries to various other countries, as pseudo-cereals, vegetables, weeds or crops. *Amaranthus retroflexus* is a weed, with a cosmopolitan worldwide distribution (Baraniak and Kania-Dobrowolska, 2020). This species successfully invades and is adapted to a wide variety of climates (Alebrahim et al., 2021). The plant is native to North America and has become a naturalized weed in many countries, both in the northern and southern hemispheres (Weller et al., 2021). In Romania, it can be found throughout the country, in steppe areas, in the beech layer, especially on productive soils, fertilized with nitrogen. It is included in the list of weed species, causing losses to agricultural production (Dinu Mihaela et al., 2017).

This annual weed is occasional on cultivated land and in waste places such as garbage. It grows best at high temperatures and light intensities (Alebrahim et al., 2021), tolerating a wide pH range (4.2 to 9.1), although it is less common in acidic soils (Weller et al., 2021). The plant is widely used in the pharmaceutical industry, to produce drugs against atherosclerosis, gastric ulcers, tuberculosis, as well as as an antiseptic, antifungal and anti-inflammatory preparation. Amaranth seed oil exhibits hypolipemic, anti-atherosclerotic, hypotensive and antioxidant activity (Park et al., 2020). Therefore, its consumption can inhibit the development of food-related diseases

(Szwejkowska and Bielski, 2012). Since a significant content of squalene was found in Amaranth oil, this liver protective activity is due to this fact (Obiedzińska and Waszkiewicz-Robak, 2012). The oil can be used in the care of all skin types. It hydrates, soothes irritations, accelerates wound healing and has antimicrobial properties. It contributes to the regeneration, nourishment and strengthening of the epidermis, acting as an antioxidant (Lăcătusu et al., 2018). Some biochemical components - proteins, lipids, mineral substances, vitamins, are present in larger quantities in the spontaneous flora, compared to other cultivated species (Toader and Roman, 2007).

MATERIALS AND METHODS

The collection of samples was carried out from the seeds of the plants, harvested from the spontaneous flora, specific to the researched area (surroundings of Craiova). The material was identified in the Pharmacognosy laboratory of UMF Craiova, dried at ambient temperature. The collected samples are processed to extract the active compounds, which may be responsible for the observed inhibitory effects.
Obtaining the alcoholic extracts: the dry product was ground in a grinder with walls made of stainless material, in order to obtain fine powders. 0.200 ± 0.001 g of each dry, finely ground plant material was weighed on an analytical balance, with three decimal places, and 5 mL of alcohol (methanol 70%, respectively ethanol 70%), brought in previously at temperatures of 70°C.
The extracts were either shaken (200 rpm orbital shaker Panasonic MIR-S100) or ultrasonicated (ASonic PRO50 bath), then centrifuged at 3500 rpm for 10 minutes, the supernatant was collected and brought to a total volume of 10 mL with MeOH 70% or EtOH 70%
The following samples were obtained:
• *Amaranthus retroflexus* seeds ethanolic extract - shaking (Amaranthus SEA)
• *Amaranthus retroflexus* seeds ethanolic extract - ultrasonication (Amaranthus SEU)
Ultrasound-assisted extraction is an extraction technique that uses high-frequency sound waves (ultrasound) to disrupt cell walls and enhance the release of phenolic compounds from plant materials (Michalaki et al., 2023).

Determination of antioxidant activity by the DPPH method
The method started from the preparation of a $0.25x10^{-3}$ Mol/L DPPH solution in methanol and a $0.25x10^{-3}$ M trolox solution in ethanol. From each alcoholic extract, 5 different concentrations were made: 10, 25, 50, 75, 100 and 200 µl/ml. 10 µl of extract from each extract concentration and 150 µl of DPPH were pipetted into 96-well microplates. The absorbance was determined at 515 nm (Tecan Infinite M1000 Pro), at 5, 10, 15 and 30 minutes after starting the experiment (Figure 1).
The percent inhibition of DPPH was calculated as follows:
PI (%) decolorization = [1 – (Abs Sample/ Abs Blank] 100 (1).

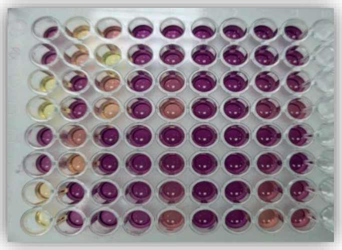


Figure 1. DPPH discoloration under the action of plant extracts of various concentrations

RESULTS AND DISCUSSIONS

Being in close correlation with antioxidant activity, the IC50 value (maximum inhibitory concentration 1/2) is the concentration of the sample capable of eliminating 50% of free radicals, in the DPPH (2,2-diphenyl-1picrylhydrazyl) method, for their evaluation. The study may reveal any variations in the inhibitory potential of *Amaranthus retroflexus* L., which may have implications for their potential use in different applications such as pharmaceuticals or agriculture. The concentration-response relationship is established by testing different concentrations of *Amaranthus retroflexus* L. extracts. These data are used to plot a dose-response curve, which is crucial for determining the IC50 value.
Antioxidant activity by the DPPH method
The DPPH method is a very widespread and simple method used to evaluate the antioxidant

activity of plant extracts or other types of samples (chemical compounds, food industry samples (La J, Kim MJ, Lee J, 2021). This method is based on the ability of antioxidant compounds to reduce the DPPH· radical, a purple-colored organic free radical (Figure 2), to an inactive, colorless form. The stronger the antioxidant activity of the sample, the faster reduction of the DPPH radical, implicitly a discoloration of the solution with plant extract (Iordănescu et al., 2021)

Antioxidant activity can be expressed as percent DPPH inhibition or as inhibitory concentration (IC50), which is the sample concentration required to halve DPPH· uptake. The lower the IC50 value, the stronger the antioxidant activity of the extract.

The strongest antioxidant activity was the methanolic extract obtained by ultrasound and shaking from Amaranthus seeds (Amaranthus SMU 37.81 µL extract/mL, Amaranthus SMA 57.16 µL extract/mL) (Table 1, Figure 3).

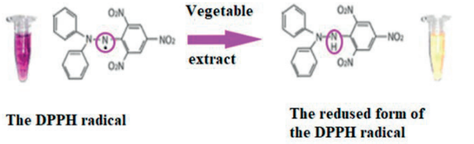


Figure 2. Reduction of the DPPH·radical to an inactive form

Table 1. Inhibitory capacity of extracts, expressed as IC 50 (µl extract/ml)

No.crt	Sample	IC 50 (µl) extract/ml	Standard deviation n=3 determinations
1	*Amaranthus* SEA	86.43	0.42
2	Amaranthus SEU	76.23	0.25

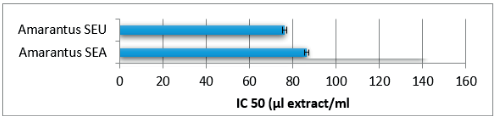


Figure 3. Inhibitory concentration of different types of extracts from *Amaranthus retroflexus* seeds

Table 2. 12/3-9 *Amaranthus retroflexus* seeds methanolic extract - stirring

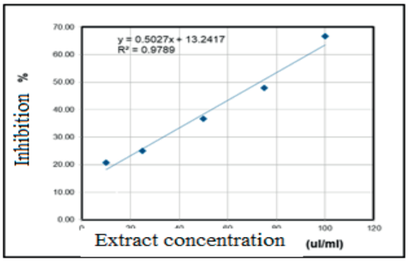
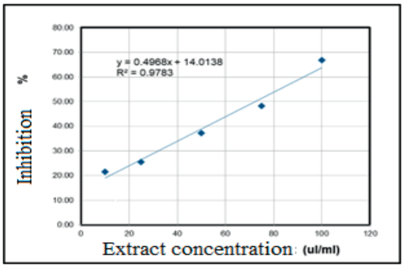
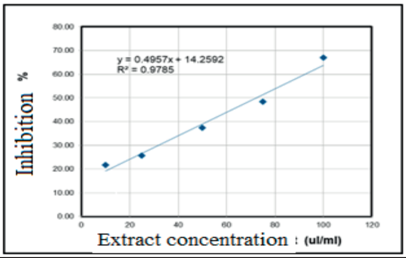
	IC 50 =(50-b)/a	a	b	r2	
12_3-9 rehearsal 1	73.12+	0.5027	13.2417	0.9789	
12_3-9 rehearsal 2	72.44	0.4968	14.0138	0.9783	
12_3-9 rehearsal 3	72.10	0.4957	14.2592	0.9875	
	Average	72.55	SD 0.52		

Table 3. 12/3-10 *Amaranthus retroflexus* seeds methanolic extract - ultrasonication

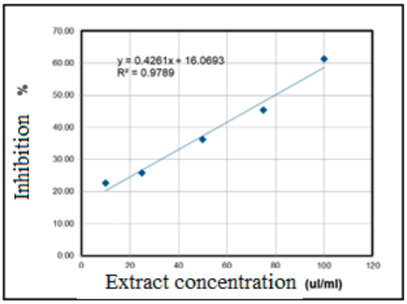
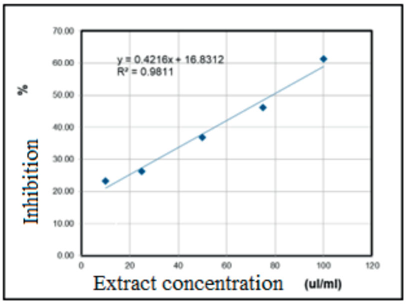
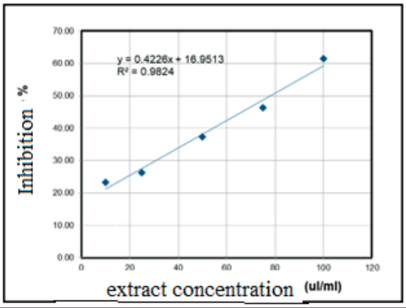
	IC 50 =(50-b)/a	a	b	r^2	
12_3-10 rehearsal 1	79.63	0.4261	16.0693	0.9789	
12_3-10 rehearsal 2	78.67	0.4216	16.8312	0.9811	
12_3-10 rehearsal 3	78.20	0.4226	16.9513	0.9824	
	Average 78.84	SD 0.73			

Table 4. 12/3-19 *Amaranthus retroflexus* seeds ethanolic extract - stirring

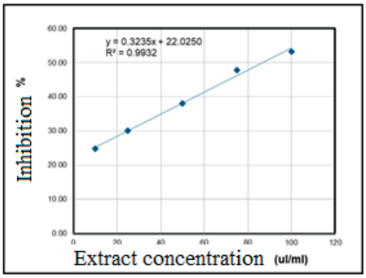
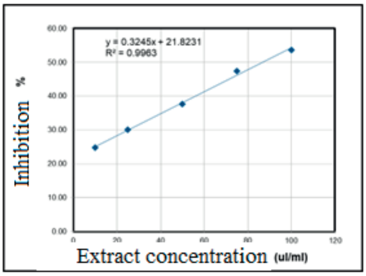
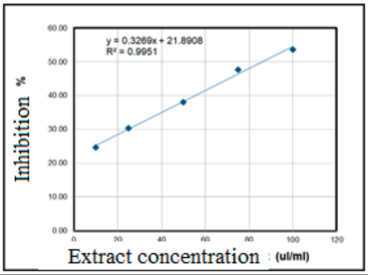
	IC 50 =(50-b)/a	a	b	r2	
12_3-19 rehearsal 1	86.48	0.3235	22.0250	0.9932	
12_3-19 rehearsal 2	86.83	0.3245	21.8231	0.9963	
12_3-19 rehearsal 3	85.99	0.3269	21.8908	0.9951	
	Average 86.43	SD 0.42			

Table 5. 12/3-20 *Amaranthus retroflexus* seeds ethanolic extract – ultrasonication

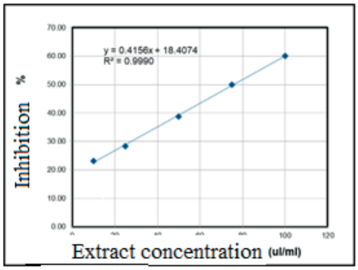
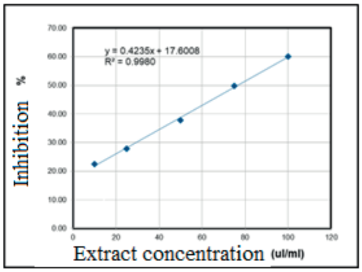
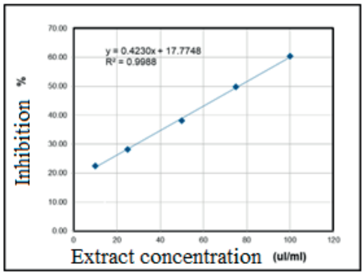
	IC 50 =(50-b)/a	a	b	r2	
12_3-20 rehearsal 1	76.42	0.4156	18.4074	0.9990	
12_3-20 rehearsal 2	76.50	0.4235	17.6008	0.9980	
12_3-20 rehearsal 3	76.18	0.4230	17.7748	0.9988	
Average	76.23	SD 0.25			

Table 6 shows a statistic of the inhibition capacity of the extracts at different time intervals. Figure 4 shows the inhibition of different types of extracts with the same concentration (100 µl/ml) at 5, 10, 15 and 30 minutes, respectively.

The seeds show increased inhibitory activity. In the vast majority of extracts, an increase in the inhibitory potency is observed through ultrasound, similar to the results obtained for total polyphenols.

Table 6. Determination of the inhibition capacity of the extracts, at various concentrations, over time

Concentration µl/ml	Average 5 min	SD	Average 10 min	SD	Average 15 min	SD	Average 30 min	SD
***Amaranthus retroflexus* seeds methanolic extract - stirring**								
10	**16.887**	**0.261**	**18.208**	**0.364**	**19.344**	**0.412**	**21.337**	**0.526**
25	**21.106**	**0.148**	**22.179**	**0.651**	**23.547**	**0.308**	**25.414**	**0.377**
50	**28.473**	**0.648**	**32.096**	**0.336**	**34.028**	**0.338**	**37.095**	**0.357**
75	**36.722**	**0.483**	**41.541**	**0.422**	**44.064**	**0.296**	**48.144**	**0.285**
100	**50.912**	**0.433**	**55.863**	**0.270**	**59.558**	**0.270**	**66.785**	**0.136**

			Amaranthus retroflexus **seeds methanolic extract - ultrasonication**					
10	**19.807**	**0.251**	**20.455**	**0.410**	**21.614**	**0.246**	**23.017**	**0.407**
25	**20.903**	**0.392**	**22.454**	**0.513**	**23.889**	**0.313**	**26.101**	**0.342**
50	**28.033**	**0.669**	**31.456**	**0.373**	**33.290**	**0.236**	**36.766**	**0.514**
75	**34.798**	**0.487**	**39.261**	**0.467**	**41.784**	**0.319**	**45.936**	**0.480**
100	**49.188**	**0.622**	**53.115**	**0.241**	**55.791**	**0.214**	**61.363**	**0.105**
			Amaranthus retroflexus **seeds ethanolic extract - stirring**					
10	**20.879**	**0.181**	**21.519**	**0.433**	**22.657**	**0.392**	**24.788**	**0.121**
25	**25.114**	**0.173**	**26.024**	**0.439**	**27.372**	**0.163**	**30.183**	**0.175**
50	**30.754**	**0.192**	**32.391**	**0.417**	**34.068**	**0.150**	**37.945**	**0.233**
75	**38.055**	**0.254**	**40.492**	**0.464**	**43.016**	**0.282**	**47.626**	**0.258**
100	**40.905**	**0.449**	**44.707**	**0.334**	**47.433**	**0.212**	**53.511**	**0.211**
			Amaranthus retroflexus **seeds ethanolic extract - ultrasonication**					
10	**19.600**	**0.124**	**19.946**	**0.334**	**20.971**	**0.118**	**22.730**	**0.321**
25	**23.346**	**0.052**	**24.405**	**0.372**	**25.357**	**0.193**	**28.134**	**0.260**
50	**31.095**	**0.094**	**32.563**	**0.305**	**34.675**	**0.180**	**38.169**	**0.460**
75	**37.860**	**1.003**	**41.939**	**0.363**	**44.682**	**0.141**	**49.855**	**0.123**
100	**45.545**	**0.297**	**50.256**	**0.392**	**53.329**	**0.327**	**60.129**	**0.157**

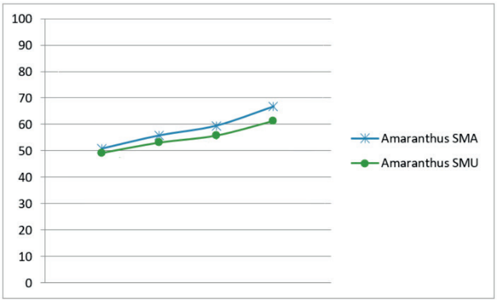


Figure 4. Time evolution of the inhibitory capacity at the concentration of 100 µl/ml

CONCLUSIONS

Comparing the two extractive methods (stirring and ultrasound) an increase in the extracted phenolic compounds using ultrasound is found.

In this way, ultrasound can speed up the extraction process, reducing the extraction time compared to agitation. The yield of extractives using ultrasound is improved, while also ensuring the preservation of thermo-sensitive compounds.

However, we must take into account that there are also certain factors that can affect this type of extraction, namely the frequency or the type of sample, but also the fact that, the prolonged use of high-intensity ultrasound, can lead to the heating of the sample, which can affect the stability some phenolic compounds.

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RESEARCH ON THE BIODIVERSITY OF CARABIDS (ORDER *Coleoptera*, FAMILY *Carabidae*), PREDATORY INSECTS IN SOME AGRICULTURAL ECOSYSTEMS ACCORDING TO THE APPLIED TECHNOLOGY AND IN THE CONTEXT OF NEW CLIMATE CHANGES

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Abstract

Research was done during 2023 on three field crops: wheat, corn and sunflower. The collection of the material was done with the soil traps Barber type, and the collection of biological material was made every two weeks, starting from May to September depending on the culture. We were organized two variants in the experimental stationary, depending on the technologies (treatment scheme applied) to each culture as follows: V1, the field where no chemical treatment was done (ecological variant); V2, the field to which chemical treatments were applied, if necessary to the seeds and during the vegetation period (conventional variant). From the material collected from the Barber soil traps during to the five months, were selected only the carabid species that were then identified. Regarding the analysis and interpretation of the results, two indices of the diversity of the species were also calculated, namely: the Sorensen index and the Spearman index whose values show how similar the cultures compared to each other are, two by two.

Key words: *predatory insects, agricultural ecosystems, climate changes, Barber traps.*

INTRODUCTION

Carabids comprise more than 20,000 species, spread all over the globe. In temperate regions it walks more on the ground and in the ground, under stones, leaves, etc. They are of varied size, they have their own habitus that makes them easily distinguishable. (Neculiseanu, 2000). Both larvae and adults are predators and useful to humans.

However, there are also harmful species, such as, for example, the species *Zabrus tenebrioides* (the scaly beetle) which both as a larva and as an adult is harmful to cereal crops, then *Harpalus pubescens* harmful to cereal crops (Antonescu, 2011).

In agricultural crops and even in forest areas, many carabid species are important ecological indicators responding immediately to human interventions, such as pesticides that cause paralysis or even death of adult insects or larvae shortly after the application of treatments.

For this reason and for other reasons (climate changes, etc.) many carabid species have decreased, are on the verge of extinction or have even disappeared.

A determining factor is also the anthropization of nature, in general and general excessive pollution (Ciochia et al., 1997).

MATERIALS AND METHODS

The collection of the material from this work was done with the help of Barber type soil traps (Mocanu, 2016), in the year 2022.

A number of three crops were studied: wheat, corn and sunflower, each with 2 variants:

- conventional variant, with seed treatments and during the vegetation period, with insecticide products;
- the ecological variant, without insecticide treatments.

Samples were collected at intervals of about two weeks, in total 10 harvests for corn and 6 harvests each for wheat and sunflower.

The harvest dates were:

- Harvest I on 15.05;
- Harvest II on 29.05;
- Harvest III on 12.06;

- Harvest IV on 26.06;
- Harvest V on 10.07;
- Harvest VI on 24.07;
- Harvest VII on 07.08;
- Harvest VII on 21.08;
- Harvest IX on 04.09;
- Harvest X on 18.09.

A salt solution (NaCl) 25 g/l was used to capture the material. At each collection, the solution inside the trap was replaced, and the material collected after being cleaned of plant debris or other impurities, soil debris, snails, mice, etc. was brought to the laboratory (Talmaciu, 2005) Only carabids species that were then determined were selected in the laboratory.

The determination of the biological material was made with the help of determinants books that belong Panin, 1951, Reitter, 1908-1916.

RESULTS AND DISCUSSIONS

In the wheat crop, 269 carabid specimens belonging to 27 species were collected in the untreated variant, while 142 carabid specimens belonging to 4 species were collected in the chemically treated variant (Table 1).

Table 1. The situation regarding the dynamic structure and abundance of carabid species in the wheat crop

No	Name of species	First vatiant (no treatments)						Total I	Second variant (with treatments)						Total II
		1	2	3	4	5	6		1	2	3	4	5	6	
1.	*Carabus coriaceus*	2	1	0	1	0	0	4	0	0	0	0	0	0	0
2.	*Carabus calceatus*	1	1	0	0	0	0	2	0	0	0	0	0	0	0
3.	*Pterostichus cylindricus*	17	16	23	15	5	8	84	26	18	19	17	10	7	97
4.	*Pseudophonus pubescens*	24	15	7	6	10	6	68	13	7	9	1	-	-	30
5.	*Harpalus distinguendus*	6	5	2	7	3	1	24	0	0	0	0	0	0	0
6.	*Calathus fuscipes*	1	3	2	4	2	0	12	0	0	0	0	0	0	0
7.	*Pseudophonus griseus*	5	3	3	3	7	1	22	0	0	0	0	0	0	0
8.	*Platynus assimilis*	1	2	1	0	0	0	4	0	0	0	0	0	0	0
9.	*Pterostichus niger*	3	5	4	4	1	1	19	1	1	3	1	1	3	10
10.	*Pterostichus vulgaris*	4	0	1	0	0	0	5	0	0	1	1	3	0	5
11.	*Amara aenea*	1	0	0	0	0	0	1	0	0	0	0	0	0	0
12.	*Amara familiaris*	1	0	0	0	0	0	1	0	0	0	0	0	0	0
13.	*Carabus intricatus*	1	0	0	1	0	0	2	0	0	0	0	0	0	0
14.	*Harpalus smaragdinus*	1	0	0	0	0	0	1	0	0	0	0	0	0	0
15.	*Amara similata*	1	0	0	0	0	0	1	0	0	0	0	0	0	0
16.	*Harpalus aeneus*	0	2	0	1	0	0	3	0	0	0	0	0	0	0
17.	*Carabus scabriusculus*	0	1	0	0	0	0	1	0	0	0	0	0	0	0
18.	*Carabus auronitens*	0	1	0	0	0	0	1	0	0	0	0	0	0	0
19.	*Nebria brevicallis*	0	3	0	2	1	0	6	0	0	0	0	0	0	0
20.	*Brachynus crepitans*	0	1	0	0	0	0	1	0	0	0	0	0	0	0
21.	*Calathus melanocephalus*	0	0	1	0	0	0	1	0	0	0	0	0	0	0
22.	*Pterostichus lepidus*	0	0	1	0	0	0	1	0	0	0	0	0	0	0
23.	*Pterostichus cupreus*	0	0	0	1	0	0	1	0	0	0	0	0	0	0
24.	*Carabus auratus*	0	0	0	1	0	0	1	0	0	0	0	0	0	0
25.	*Harpalus tardus*	0	0	0	1	0	0	1	0	0	0	0	0	0	0
26.	*Pterostechus nigrita*	0	0	0	0	1	0	1	0	0	0	0	0	0	0
27.	*Carabus cancellatus*	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Total		70	60	45	47	30	17	269	40	26	32	20	14	10	142

In the corn culture, a number of 520 specimens of Carabidae belonging to a number of 23 species were collected in the untreated variant, and a number of 421 specimens of Carabidae belonging to a number of 7 species were collected in the treated variant (Table 2).

Table 2. The situation regarding the dynamic structure and abundance of carabid species in the corn crop

No	Name of species	First vatiant (no treatments)										Total I	Second variant (with treatments)										Total II
		1	2	3	4	5	6	7	8	9	10		1	2	3	4	5	6	7	8	9	10	
1.	*Pseudophonus pubescens*	34	57	40	39	26	21	4	6	1	7	235	66	44	35	29	24	44	6	5	6	7	266
2.	*Pterostichus niger*	1	0	0	0	1	2	4	1	4	-	13	0	0	0	0	0	1	1	1	0	0	3
3.	*Pseudophonus griseus*	20	22	9	12	6	12	16	9	7	1	114	0	15	25	14	36	1	19	14	7	0	131
4.	*Harpalus distinguendus*	4	1	2	3	5	7	3	2	2	1	30	0	1	0	0	0	0	0	0	0	0	1
5.	*Nebria brevicallis*	2	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
6.	*Pterostichus cylindricus*	10	11	4	7	11	13	9	10	3	4	82	0	5	0	0	2	3	2	1	3	0	16
7.	*Harpalus aeneus*	1	1	0	0	1	0	2	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
8.	*Ophonus azureus*	1	3	1	3	0	1	0	0	0	0	9	0	0	0	0	0	1	0	0	0	0	1
9.	*Pterostichus koyi.*	1	0	0	1	0	1	0	0	0	0	3	0	0	0	0	0	1	0	0	0	0	1
10.	*Harpalus rufipes*	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
11.	*Ophonus puncticallis*	1	0	0	2	1	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
12.	*Carabus coriaceus*	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
13.	*Pterostichus melas*	0	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
14.	*Abax carinatus*	0	0	1	1	0	2	0	0	0	0	4	-	0	0	0	0	0	0	0	0	0	0
15.	*Calathus fuscipes*	0	0	1	1	1	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
16.	*Amara aenea*	0	0	0	1	1	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
17.	*Carabus canlceatus*	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
18.	*Harpalus tardus*	0	0	0	1	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
19.	*Amara apricaria*	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
20.	*Anisodactylus nemovivagus*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
21.	*Bembidion properans*	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
22.	*Pterostichus vulgaris*	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
23.	*Brachynus explodens*	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Total		77	95	59	71	55	63	38	28	17	14	520	66	65	60	43	62	51	28	21	16	7	421

In the sunflower culture, 507 specimens belonging to a number of 19 species were collected in the untreated variant, and 413 specimens of carabids belonging to a number of 8 species were collected in the treated variant (Table 3).

Table 3. The situation regarding the dynamic structure and abundance of carabid species in the sunflower crop

No	Name of species	First vatiant (no treatments)										Total I	Second variant (with treatments)										Total II
		1	2	3	4	5	6	7	8	9	10		1	2	3	4	5	6	7	8	9	10	
1.	*Pterostichus cylindricus*	17	16	4	20	9	15	8	11	7	3	110	22	29	15	17	16	8	9	13	5	4	138
2.	*Pseudophonus pubescens*	24	26	88	57	46	12	3	5	3	3	267	14	23	6	4	1	11	0	1	1	0	61
3.	*Pseudophonus griseus*	13	32	3	6	19	3	2	2	5	2	87	5	6	0	4	0	1	0	1	1	0	18
4.	*Harpalus distinguendus*	4	2	1	0	2	2	0	0	0	0	11	1	1	2	0	0	0	0	0	0	0	4
5.	*Amara similata*	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
6.	*Pterostichus niger*	2	0	0	1	0	1	2	1	1	1	9	1	2	2	6	0	0	0	0	4	2	17
7.	*Calathus fuscipes*	3	1	1	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
8.	*Harpalus tardus*	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
9.	*Platinus assimilis*	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
10.	*Harpalus aeneus*	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
11.	*Leistus ferrugineus*	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
12.	*Amara familiaris*	0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
13.	*Ophonus puncticollis*	0	1	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
14.	*Bembidion ruficorne*	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
15.	*Carabus coriaceus*	0	1	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
16.	*Pterostichus lepidus*	0	0	0	1	2	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
17.	*Nebria brevicollis*	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
18.	*Zabrus tenebrioides*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
19.	*Abax carinata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Total		69	83	99	85	79	33	15	19	16	9	507	43	62	25	31	18	20	9	16	11	6	241

Regarding the predatory carabids collected in the three crops, the situation is as follows:
- in the wheat crop, a number of 146 specimens belonging to 17 species were collected in the untreated variant, and 112 specimens belonging to a number of 3 species were collected in the chemically treated variant (Table 4).

Table 4. Structure and abundance of predatory carabids species collected from the wheat crop in the two variants

No.	Name of species	Variant	
		No treatments	With treatments
1.	*Carabus coriaceus*	4	0
2.	*Carabus cancellatus*	2	0
3.	*Pterostichus cylindricus*	84	97
4.	*Calathus fuscipes*	12	0
5.	*Platynus assimilis*	4	0
6.	*Pterostichus niger*	19	10
7.	*Pterostichus vulgaris*	5	5
8.	*Carabus intricatus*	2	0
9.	*Carabus scabriusculus*	1	0
10.	*Carabus auronitens*	1	0
11.	*Nebria brevicallis*	6	0
12.	*Brachynus crepitans*	1	0
13.	*Calathus melanocephalus*	1	0
14.	*Pterostichus lepidus*	1	0
15.	*Carabus auratus*	1	0
16.	*Pterostechus nigrita*	1	0
17.	*Carabus calceatus*	1	0
Total		146	112

A number of 116 specimens belonging to 13 species were collected in the untreated version of corn, and 20 specimens belonging to a number of three species were collected in the chemically treated version (Table 5).

Table 5. Structure and abundance of predatory carabids species collected from the maize crop in the two variants

No.	Name of species	Variant	
		No treatments	With treatments
1.	*Pterostichus niger*	13	3
2.	*Nebria brevicallis*	3	-
3.	*Pterostichus cylindricus*	82	16
4.	*Pterostichus koyi*	3	1
5.	*Carabus coriaceus*	1	-
6.	*Pterostichus melas*	2	-
7.	*Abax carinatus*	4	-
8.	*Calathus fuscipes*	1	-
9.	*Carabus cancellatus*	1	-
10.	*Bembidion properans*	1	-
11.	*Anisodactylus nemovivagus*	1	-
12.	*Pterostichus vulgaris*	1	-
13.	*Brachynus explodens*	1	-
Total		116	20

In the sunflower culture, 133 carabid specimens belonging to a number of 8 species were collected in the untreated variant, and 178 carabid specimens belonging to a number of 4 species were collected in the chemically treated variant (Table 6).

Table 6. The structure and abundance of predatory carabidae species collected from the sunflower crop in the two variants

No.	Name of species	Variant	
		No treatments	No treatments
1.	*Pterostichus cylindricus*	110	138
2.	*Pterostichus niger*	9	38
3.	*Calathus fuscipes*	5	0
4.	*Platynus assimilis*	1	0
5.	*Leistus forregineus*	1	0
6.	*Bembidion ruficorne*	1	0
7.	*Carabus coriaceus*	3	0
8.	*Pterostichus lepidus*	3	0
9.	*Nebria brevicallis*	0	1
10.	*Abax carinatus*	0	1
Total		133	178

Regarding the Sorensen Index that shows us whether the fauna of different ecosystems are similar or different and was calculated according to: Is=$\frac{2c}{a+b}$, in which:
Is - the Sorensen index;
a - the total number of existing species in the first compared fauna;
b - the total number of existing species in the second compared fauna;
c - the number of species common to the two compared fauna;
- from a qualitative point of view, the most appreciated carabid communities resulted from the comparison of the carabids from the treated wheat crop with the treated corn one (IS = 72.73), which leads to an appreciation of the two carabid entomofaunas, followed by the variant untreated wheat-untreated sunflower (IS = 65.12) and treated wheat-treated sunflower variant (Is = 50.00); at the opposite pole with low values of the Sorensen index were the variants: untreated wheat - treated wheat (Is = 12.99), untreated corn - untreated sunflower (Is = 17.95) untreated corn - treated sunflower (Is = 19.36) and the variant treated corn - untreated sunflower (Is = 21.74).
The Spearman index (Table 8) had the highest value for the untreated sunflower variant (0.90), followed by the treated wheat-untreated wheat variant (0.60) and then the treated maize-untreated maize variant (0.50).
The weakest similarities are found in the variants treated corn-untreated corn and treated wheat-untreated corn, which fall into value class 3 (0.41-0.60).

The greatest similarity of the carabid fauna was recorded in the treated sunflower-untreated sunflower variant, which falls into class 5 values (0.81-1.00).

Table 7. Presentation of the results regarding the Sorensen index

No.	Variant	Index value	Reference class
1.	Untreated wheat - Treated wheat	12.99	I - 0-20
2.	Untreated wheat - Untreated corn	29.42	II - 21-40
3.	Untreated wheat - Treated corn	41.81	III – 41-60
4.	Untreated wheat - Untreated sunflower	65.12	IV - 61-80
5.	Untreated wheat - Treated sunflower	34.29	II - 21-40
6.	Treated wheat – Untreated corn	22.23	II - 21-40
7.	Treated wheat- Treated corn	73.73	IV - 61-80
8.	Treated wheat – Untreated sunflower	15.00	I - 0-20
9.	Treated wheat – Treated sunflower	50.00	III - 41-60
10.	Treated corn - Untreated corn	46.67	III - 41-60
11.	Treated corn - Untreated sunflower	21.74	I - 0-20
12.	Treated corn - Treated sunflower	40.00	II - 21-40
13.	Untreated corn - Untreated sunflower	17.95	I - 0-20
14.	Untreated corn - Treated sunflower	19.36	I - 0-20
15.	Untreated sunflower - Treated sunflower	41.67	III - 41-60

Table 8. Presentation of the results regarding the Spearman index

Wheat		Corn		Sunflower	
No treatments	With treatments	No treatments	With treatments	No treatments	With treatments
0.60		0.50		0.90	

CONCLUSIONS

In all three cultures in the untreated variant, the number of specimens and carabid species collected is higher, the situation being as follows for the cultures:

- in the wheat crop, in the untreated variant, carabids belonging to a number of 27 species were collected, totaling a number of 209 specimens compared to the treated variant, where specimens belonging to only 4 species were collected, totaling a number of 142 specimens;
- in the wheat crop, in the untreated variant, carabid specimens belonging to 23 species were collected, totaling 520 specimens, while in the treated variant, specimens belonging to 7 species were collected, totaling 421 specimens;
- in the sunflower culture, 507 carabid specimens belonging to 16 species were collected in the untreated variant, and 413 carabid specimens belonging to only 8 species were collected in the treated variant.

Regarding the species of predatory carabid collected, for the 3 crops it is found that the most predatory species were collected in the untreated variant as well as the number of specimens, with only one exception, in the sunflower crop.

By cultures and variants the situation is as follows:

- in the wheat crop, 146 specimens of predatory carabids belonging to a number of 17 species were collected, while in the treated variant, 112 specimens were collected belonging to only 3 species;
- 116 specimens belonging to a number of 13 species were collected in the untreated version of corn, while 20 specimens belonging to only 3 species were collected in the treated version;
- in the sunflower culture, the untreated variant, 133 specimens belonging to 8 species were collected, and in the treated variant, 178 specimens belonging to 4 species were collected.

Regarding the values of the Sorensen and Spearman indices, which reflect the degree of similarity between the fauna of two or more communities, the situation is as follows:

- the highest value of the Sorensen index of 72.73, which shows a high degree of similarity, was for the variant treated wheat-treated corn, and the lowest value 12.99 was recorded for the variant untreated wheat-treated wheat;
- the Spearman index had the highest value of 0.90 for the variant treated sunflower - untreated sunflower, followed by the variant treated wheat - untreated wheat with a value of 0.60 and then the variant treated corn - untreated corn, with a value of 0.50.

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USING RAPD MARKERS TO ESTABLISH DNA FINGERPRINT AND TO STUDY THE GENETIC VARIABILITY DISCRIMINATION BETWEEN TWO ROMANIAN POTATO VARIETIES

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Abstract

In order to optimize the potato breeding program in Romania, molecular research was developed so that we can have the way of expressing the genetic potential of the new breeding creations. This study aimed to establish a unique DNA fingerprint for two new Romanian potato cultivars from NIRDPSB Brasov, using RAPD molecular markers for genetic variability discrimination between them. Amplification was carried out with 17 arbitrary primers, but only 14 produced amplified sequences. OPC 08 produced the most banding patterns. The level of similarity between these two varieties of potato has approximately the same value, suggesting a stronger degree of relationship between varieties. Although the examined varieties have different genetic origins, their shared geographical origin and belonging to the same species, this can explain nearly identical similarity coefficient between them and to use of only two varieties for discrimination of genetic diversity can do a high degree of similarity. Discrimination between varieties was limited by the RAPD technique, we recommend using a large number of varieties or a more precise technique for improved accuracy.

***Key words**: breeding, potato, PCR, RAPD, genetic diversity, discrimination.*

INTRODUCTION

The intensification of agriculture as a whole has led to significant genetic erosion, as well as soil depletion and environmental damage.
Currently, globally but also in Romania, remarkable results in potato breeding domain were obtained focusing on counteraction tendency of negative effects caused by the hyper-intensification of agriculture, by raising the novelty degree of techniques, proposed to be used in breeding programs (Stefan, 2012). Potato varieties must fulfill the requirements of the market and consumer preferences, as well as to show good agronomic performance in several environments and wide adaptation to productive systems, all thaw, potato needs to deal with some complicated issues that make potato breeding a special case in genetic improvement of crops (Munoz et al., 2018).
The variety represents the most important link in obtaining high and constant productions, without a continuous and progressive increasing of material and energy costs. In 2014, the 6th edition of The World Catalogue of Potato Varieties it appeared, which includes the description of more than 4500 potato varieties and about 1900 wild species, which are cultivated in more than 100 countries around the world (Hermeziu et al., 2020).
The large-scale cultivation of the potato is not only a matter of theoretical interest but is an integral part of diet of a large population worldwide (Caliskan et al., 2022).
The introduction and development of new potato cultivars (*Solanum tuberosum* L.) has been an important strategy to increase crop productivity of this important staple food, fourth after rice, wheat, and corn (Rocha et al., 2010). Breeding is the key factor in a complex equation that determines both agricultural production and the standard of civilization (Hermeziu et al., 2015).
Identification of crop plants, studies in their genetic diversity and relationships is crucial for the development of breeding programs (Onamu et al., 2016). The increasing number of potato cultivars and the importance of their choice make necessary to strengthen users guarantees concerning purity and identity (Yasmin et al., 2006), because due to Moisan-Thiéry et al.,

2001, the identification mainly based on phenotypic characters implies crop inspection at different stages and is often doubtful.
Molecular markers based on the deoxyribonucleic acid (DNA) sequence are more reliable in this regard (Raghunathachari et al., 2000). DNA fingerprinting analysis is defined by applying marking techniques at the molecular level, to identify cultivars. This technique has entered the spotlight in recent years, because of two multilateral agreements: the Intellectual Property Rights related to varieties' marketing (TRIPs) and the Convention on Biological Diversity (CBD). To implement the dispositions of the two conventions, there is a need to dispose by identity and ownership of the obtained genotypes (Sunil, 2000).
The developments of molecular genetics resulted in several procedures based on DNA for detecting genetic polymorphism (Collares et al., 2004). Randomly amplified polymorphic DNA (RAPD) is a technique based on the amplification of discrete regions of the genome by polymerase chain reaction (PCR), with short oligonucleotide primers of arbitrary sequence (Williams et al., 1990). Using DNA fingerprinting it was possible to distinguish between true mutations and epimutations, such as those caused by changes in DNA methylation (Vagnarelli et al., 1995).
This technique is being used successfully to identify, characterize, and estimate genetic divergence of potato cultivars (Rocha et al., 2002), being a simple and inexpensive method for accessing the variability at DNA level compared to other methods (Singh et al., 2021).
The aim of the study was to establish a unique DNA fingerprint for two new Romanian potato cultivars developed at the National Institute of Research and Development for Potato and Sugar Beet Brasov (NIRDPSB Brasov), employing RAPD molecular markers for molecular discrimination between them.
The main limitation in potato clonal selection is the time taken to obtain elite genotypes (Melo, 2011). Thus, the results will be useful for potato breeding programs, by increasing accuracy and safety, within the applied selection schemes. At the same time, the genetic base used in applied genetics research is diversified.

MATERIALS AND METHODS

Within the activities of NIRDPSB Brasov, pota.to breeding works are constantly oriented towards the creation of new potato varieties. Thus, the molecularly characterized biological material in this paper, it is represented by two new mid early potato varieties, respectively Cezarina (Figure 1) and Ervant (Figure 2), patented and registered in The Romanian Official Catalogue of cultivated plant varieties, in the year 2019.
The method of obtaining these varieties is sexual hybridization, followed by clonal selection. During breeding process, varieties were subjected to progressive selection (in vegetation and at harvest), in accordance with breeding objectives (morphological aspect, resistance to diseases and pests, tolerance to thermo-hydric stress and production capacity).
The molecular analysis was developed in the Genetics Laboratory of the Agriculture Faculty from University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

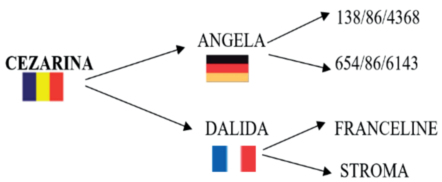

Figure 1. Genealogy of Cezarina potato variety

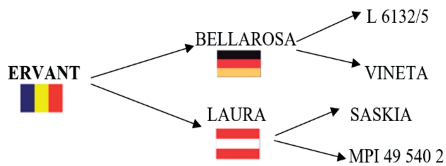

Figure 2. Genealogy of Ervant potato variety

DNA extraction was performed with the protocol described by Lodhi et al. (1994) modified and improved by adding ascorbic acid and diethyldithiocarbamic acid (DIECA), by Pop et al. (2003). The appreciation of purity and quantity of DNA was carried out with NanoDrop100 spectrophotometer. The molecular markers used were RAPD type. The DNA fingerprint obtained through the RAPD technique being able to become one of the most useful methods for variability discrimination between varieties.

A number of 17 decanucleotide primers were used for amplification, produced by Operon Technologies, Inc. USA (Table 1). The primers used to amplify the DNA sequences had a G-C nucleotide content between 50 and 80%.

Table 1. Primers used in DNA amplification

Crt. no.	Primer name	The nucleotide sequence of the primers (5'- 3')
1	OPAB 11	GTGCGCAATG
2	OPAB 10	CTGCTGGGAC
3	OPB 11	GTAGACCCGT
4	OPC 08	TGGACCGGTG
5	OPC 10	TGTCTGGGTG
6	OPA 20	GTTGCGATCC
7	OPE 14	TGCGGCTGAG
8	OPB 08	GTCCACACGG
9	OPB 09	TGGGGGACTC
10	AB 11	CAATCGCCGT
11	OPA 04	AATCGGGCTG
12	OPA 18	AGGTGACCGT
13	OPB 10	CTGCTGGGAC
14	OPB 17	AGGGAACGAG
15	OPA 01	CAGGCCCTTC
16	OPAB 12	GTGCGCAATG
17	OPC 09	TGGACCGGTG

Electrophoresis of amplified DNA products in agarose gel. Amplified DNA fragments were separated on 1% agarose gel with TAE buffer 1% and stained with safe-green (5 µl/100 ml gel) (Bioline). Throughout the whole process, keeping the voltage and current intensity constant is essential for a correct electrophoresis. Gels were run for 1.5 h at 80 V and visualized under UV light (UVP BioImaging Systems). Each product was visualized on gel with 100 bp DNA Step Ladder molecular marker (Fermentas) to calculate the bands sizes.

Images capture of electrophoresis gels and data analysis. The visible bands, with a certain predetermined intensity, were included in the analyses. These were automatically detected using the TotalLab 100 program. This program determines the size of DNA fragments by comparing them with a DNA standard (100 bp DNA Step Ladder). After comparison, it was possible to determine the size of each amplified fragment in base pairs (bp).

For the analysis of genetic diversity using dominant markers, a method is employed that involves calculating genetic distances between the analyzed taxa. Based on these distances, phenotypic trees are subsequently constructed. These trees can provide insights into how taxa are grouped, depending on their similarities or differences. The analysis and interpretation of the data was carried out using the PAST (Paleontological Statistics) program (Hammer and Harper, 2001) and Jaccard similarity coefficient. Dendrograms are typically constructed using outcomes derived from dominant markers, along with diverse distance or similarity coefficients among the analyzed taxa. The Jaccard similarity coefficient is a coefficient used in the analysis of genetic diversity and the construction of phylogenetic trees (Jaccard, 1901).

RESULTS AND DISCUSSIONS

DNA sample isolation results

DNA purity is one of the most important factors in the reproducibility of the RAPD method. Only using DNA template with high purity ensures reproducible results by the RAPD method. If the DNA is of adequate quality, the RAPD fingerprints will be identical in repetitions. The producer (www.promega.ro) recommends a good-quality of DNA when the report A260/A280 is between 1.7-2.0. Values lower than 1.7 indicate impurity with proteins, while value exceeding 2.0 indicate impurity with other contaminants, such as RNA. In his study, Piskata et al. (2019) has the same recommendation. According to Kasem et al., 2008, high-quality DNA is defined by predominantly featuring long, high molecular weight fragments, displaying an A260/280 ratio ranging between 1.8 and 2.0 and exhibiting an absence of contaminants such as polysaccharides and phenols.

The results obtained for DNA quality are discussed in association with the usefulness for downstream applications and availability of the DNA source in the target study (Lucena-Aguilar et al., 2016).

In our study the purity and quantity of DNA, gave as the following values, shown in Table 2.

Table 2. Purity and amount of DNA resulting from the application of the isolation protocol

Variety name	DNA purity 260/280	Amount of DNA isolated (ng/µl)
Cezarina	1.92	49.50
Ervant	1.88	48.01

As can be seen, the purity of the isolated DNA falls between the limits recommended by the literature, so it could be safely used for the next step, PCR amplification. Also, the amount of isolated DNA is considered to be optimal to continue molecular analyses.

DNA fingerprinting results using RAPD primers

DNA fingerprinting, referred to as DNA typing, DNA profiling or genotyping is a technique employed to distinguish individuals based on unique patterns present in their DNA samples (Adnan et al., 2023). These methods have been widely employed and continue to be utilized extensively for various studies from humans to animals, plants and fungus.

Also, was used to infer familial relationships and conducting phylogenetic studies between humans (Katki et al., 2010; Madboly et al., 2021), to determine the sex of individuals, as well as paternity/maternity and close kinship in divers animals (Chambers et al., 2014) or between different variety of crop plants (Adnan et al., 2023), genotypic distributions in natural populations of blackberries and raspberries (Nybom and Schaal, 1990), in sports of 'Red Delicious' apples (Nybom, 1990) or in establishment to paternity analysis in apples "*Malus* x *domestica*" (Nybom and Schaal, 1990), in rice, where this methods use oligonucleotide probes specific for simple repetitive DNA sequences (Ramakishana et al., 1994) or in plant and fungi (Weising et al., 1995). Application of oligonucleotide probes for DNA fingerprinting is a sensitive tool for genome diagnosis in cultivated beet (Schmidt et al., 1993). Vosman et al. (1992) used DNA fingerprinting for identification of highly polymorphic DNA regions in tomato, also the list can continue with Nidudur and Sanjeet (2022) which showed the relevance of DNA fingerprinting in crop improvement. To select genotypes in terms of sexual type, color and size of the fruit, Vázquez and his team (2016) used different PCR molecular markers.

In our study the RAPD molecular markers used were able to identify and amplify DNA fragments, specific to each variety studied. Thus, from 17 RAPD primers, the following amplified unique DNA sequences: OPAB 11, OPAB 10, OPB 11, OPC 08, OPC 10, OPA 20, OPB 09, AB 11, OPA 04, OPA 18, OPB 10, OPB 17, OPA 01 and OPAB 12. The primers OPE 14 and OPC 09 did not amplify any fragment and OPB 08, amplified only in Ervant cultivar. Primer AB 11 was not considered for analysis because the program did not identify the amplified bands due to their low intensity (Figure 3 and Figure 4). Morales et al. (2011), used 40 RAPD markers, of which only 11 were polymorphic, the rest of primers did not amplify or presented low amplification quality and were not considered in the final data analysis.

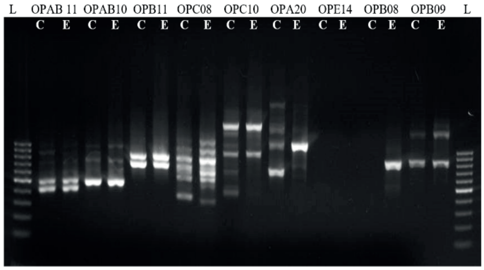

Figure 3. Amplification of DNA fragments using 9 RAPD primers for varieties Cezarina (C) and Ervant (E)

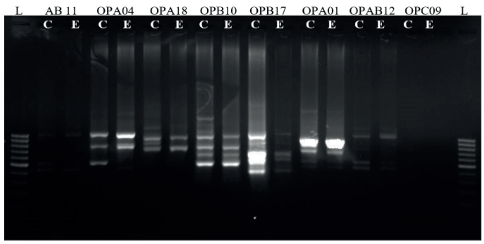

Figure 4. Amplification of DNA fragments using 8 RAPD primers for varieties Cezarina (C) and Ervant (E)

The other RAPD primers are considered polymorphic, succeeding in differentiating the two varieties, due to different values between the amplified fragments.

Seven RAPD primers, including OPB 10, were utilized by Sandhya et al. (2018), to assess the diversity both within and among twelve populations of three mushroom species: *Ganoderma lucidum*, *Leucoagaricus* sp. and *Lentinus* sp., where the size band was between 320 and 2400 pb, while in our study, it ranged from 543 to 1200 bp in the Cezarina variety and 543 to 1085 bp in the Ervant variety with four identical bands size in varieties (see Table 3). Also, this primer generated an appropriate amplified fragment in size, between 718 and

1259 pb to identified different varieties in *Mentha* sp. (Berindean et al., 2021).

Table 3. DNA fingerprints obtained using RAPD molecular markers

Primer RAPD	Amplified band size (bp)	
	CEZARINA	ERVANT
OPAB 11*	441	441
	404	404
OPAB 10*	928	928
	712	712
	441	441
OPB 11*	801	801
	690	690
OPC 08	928	1088
	766	862
	690	766
	566	626
	333	566
	-	348
OPC 10	1354	1354
	837	862
	606	-
	404	-
OPA 20	1607	-
	1088	1088
	606	-
OPB 08	-	800
OPB 09	-	1234
	837	837
OPA 04	1085	1085
	822	823
	543	-
OPA 18	956	1042
	927	-
	822	822
OPB 10	1200	-
	1085	1085
	822	822
	738	738
	543	543
OPB 17	1085	-
	738	738
	619	650
	483	-
OPA 01*	956	956
	822	822
OPAB 12	1114	1114
	619	-
	543	-
Total bands	**38**	**30**
Total bands used in analysis	**31**	**23**

*Monomorphic primers

The OPC 10 primer in our study generated four polymorphic bands in Cezarina variety (range from 404 to 1354 pb) and only two polymorphic bands in Ervant cultivar (range from 862 and 1354). As we can see the last band size amplified is the same in both varieties. The same primer did not generate banding patterns for estimate genetic diversity present in eight varieties of soybean, even if when it was in pair with OPD 4 or OPD 14 (Ramavtar et al., 2019).

The RAPD primer OPC 08 produced the most banding patterns, with five for the Cezarina variety and six for the Ervant variety, and the size of the bands ranged between 333 and 928 bp, respective 348 and 1088 bp. This primer shows two specific bands for both varieties, 566 pb and 766 pb respectively. The average number of polymorphic bands/germplasms for the OPC 08 was 3.05 and has shown a particular fragment of 750 bp to Safedvelchi and Elavazhai cultivars (commercially grown banana), within the research led by Hinge and his team (2022).

The following RAPD primers that generate only one amplified pattern are OPA 20 amplified only one band with a size of 1088 bp in the Ervant variety, but the same band it was amplified in Cezarina variety too (one of the three), while OPB 09 amplified a band of 837 bp in size in the Cezarina variety, which appear in Ervant variety, too. Additionally, the OPAB 12 primer amplified only one band with a size of 1114 bp in the Ervant variety, but the same band also appeared in the Cezarina variety.

Also, as seen in Table 3, four of the RAPD primers used (OPAB 11, OPAB 10, OPB 11, OPA 01) are considered monomorphic, because they failed in differentiating the two potato varieties by amplifying fragments, so they were not considered for analysis.

Only clear and reproducible bands were counted, so from a total of 38 fragments amplified in the Cezarina variety, 31 were considered relevant for analysis. Similarly, from the 30 fragments amplified in the Ervant, only 23 were considered for analysis.

Table 3 shows the fragments (genetic fingerprints) in base pairs (bp) obtained after PCR amplification and using the RAPD molecular marker method for discrimination of potato genetic diversity. Each band amplified with RAPD primers was manually scored as present (1) or absence (0).

The level of similarity between these two varieties of potato was analyzed with Jaccard coefficient.

The genetic distance obtained among the varieties has approximately the same value (0.578 and 0.580) suggesting a stronger degree of relationship between varieties (Table 4).

Table 4. Jaccard`s correlation between the genetic distance of the varieties

Coefficient	Cezarina	Ervant
Cezarina	1	0.580
Ervant	0.578	1

Based on genetic distance, the dendrogram shows us a high degree of similarity between the varieties, even though varieties are different from a genetic origin perspective (Figure 5).
The current potato breeding process generally starts with planting tens of thousands of genetically unique individuals, with only ~1% retained because they meet expectations for market class based on visual criteria.

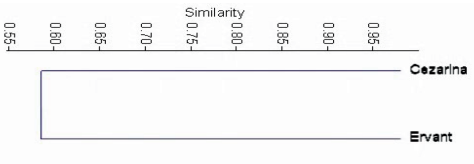

Figure 5. Phylogenetic tree generated by computing genetic distances among RAPD primers, utilizing the Jaccard similarity coefficient and PAST software

In contrast to other raw material crops, there has been no improvement in genetic potato yield over the last 100 years, primarily due to the highly heterozygous autotetraploid genome of commercial potato (Agha et al., 2023).
Given the complex genetic structure of the cultivated potato (2n=4x=48) and the strong segregation of characters in the descendants obtained by sexual hybridization, but also due to the linkage phenomenon, often unfavorable, it can reach a high degree of similarity between cultivars within a collection.
The random amplified polymorphic DNA technology is a powerful tool for searching for species-specific markers and can detect slight intraspecific differences with great accuracy, it is confirmed by the larger number of studies carried out at the molecular level on many species.
The use of RAPD primers was thought to be a useful method for distinguishing between newly bred cultivars, breeding lines and potato genetic resources (Seo et al., 2001).
However, the RAPD technique can generate multiple polymorphic bands between varieties. But this polymorphism does not reflect the difference between specific sequences for each cultivar, even if they belong to the same species. Kim et al. (2021) in his study showed genetic divergence between the China elite local lines and foreign sources of *Ricinus communis* L. and the overall genetic variation was not extremely large despite the geographical distance.
The potato varieties we examined (Ervant and Cezarina) have different genetic origin, but they have the same geographical origin and also, they are from the same species, so this can explain why the similarity coefficient is nearly identical between them. And the limited number of varieties used (only two varieties), to generate discrimination of genetic diversity leads to obtaining a high degree of similarity. RAPD analysis stands out as the most cost-effective and straightforward method for DNA genotyping, making it well-suited for routine analysis with multiple samples (Dhurva et al., 2012). In our case, RAPD markers has not been able to discriminate at the genomic level. For more and exactly results is needed to use a higher number of varieties and molecular techniques more accurate, like SSR. In Hussein et al.'s study (2023), the comparison of SSR and RAPD markers for genetic diversity in some mango cultivars led to the opinion that applying SSR markers yielded significantly higher values for other genetic diversity parameters when compared to RAPD markers.

CONCLUSIONS

Using DNA analysis methods allowed for obtaining a genetic fingerprint characteristic of each genotype.
Out of the 17 RAPD primers used, 13 successfully amplified a distinct DNA sequence in the examined varieties. Two primers, OPE 14 and OPC 09, did not produce any fragments. Another primer, OPB 08, exclusively amplified in the Ervant cultivar, while the AB 11 primer was not considered for analysis due to the low intensity of its amplified bands. Fragments obtained after amplification vary in size and number from one primer to another. The primer that generated the most bands, both for Cezarina and Ervant was OPC 08.
The Jaccard coefficient yielded a genetic similarity of approximately equal values (0.578 and 0.580) between Ervant and Cezarina, signifying a robust connection between the varieties. This observation is further supported by the dendrogram obtained, reinforcing the indication of a strong relationship. Even though the genetic fingerprinting technique employed produced DNA polymorphic fragments, it is unable to discriminate between the varieties at

the genomic level. For more and exactly results is needed to use a higher number of varieties or a more precise technique for improved accuracy.

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THE BIODIVERSITY OF COLEOPTERO-FAUNA FROM WHEAT CROPS IN THE CONDITIONS OF THE NEW CLIMATE CHANGES AND THE PREMERGING PLANT

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Abstract

The observations were made in a wheat crop located in NE Moldova, Romania. In characterizing the climate in the NE of Moldova, we rely on long-term meteorological observations from the Plant Protection Center. In order to achieve the research objectives, were used three experimental variants:1st variant, wheat after wheat; 2nd variant, wheat after sun flower; 3rd variant, wheat after corn. In wheat crops, 43 harmful species were reported, grouped into 3 categories: species that caused damage with an attack rate of 3-20%, even if chemical treatments were performed, species that produced a sporadic attack, under 1% and species reported only in cereal crops without causing damage. A new concept has emerged, namely integrated control, which can be defined as a form of applied ecology, dividing pest populations, predator and parasite populations on the other in agrobiocenosis.

***Key words**: biodiversity, wheat, Barber traps.*

INTRODUCTION

Beetles (order Coleoptera) are the group with the largest number of insects in terms of species abundance, with about 400,000 species described (Jäch M.A. and Balke M. 2008) and divided into 211 families (Cesar J. Benetti et al., 2018).
The vast majority of species are terrestrial and only a small percentage can be considered aquatic. Due to this, the Coleoptera is one of the largest orders of aquatic invertebrates (Cesar J. Benetti et al., 2018).
No element satisfies human requirements as economically nutritious and active as wheat bread. Wheat is rich in the protein (7-22%) which is represented by protamine (35-45%); glutamine (35-40%); globulins (15-20%) and albumin (2.5%). All of these ensure the growth and development of the body and play a very important biocatalytic and energetic role (Gîdei and Popescu, 2009).
The large number of the pests that attack wheat crops make the organization of their control occupy an important volume of the concerns of farmers.
In recent years, technologies to control wheat pests have registered methodological recommendations related to the impact of climate change on the structure and attack of pests (Malschi et al., 2018), (Gîdei and Popescu, 2009) as well as the practice of the system of minimum soil works, without plowing, adequate for soil protection and conservation of soil water supply, in drought conditions (Carlier L. et al., 2006; Herbert et al., 2007).
Pest control should be applied in conjunction with integrated pest management, which consists of a pest management set and uses a balanced approach to all control methods (agro-phytotechnical, physical-mechanical, biological and chemical) to maintain the ratio. of pests or their attack at a level where no significant crop losses occur.
This integrated control system is a conglomeration of methods, means and products that are applied according to different classification principles in crop technology to reduce damage.
In order to control it is necessary to take into account of the taxonomic studies (determination of host and parasite species), biology studies (food source, mode of attack and feeding, stages of development, duration of each stage, mode of multiplication, number of generations, etc.),

ecology studies (influence of climatic factors, establishing the relationship between the attacked species and the pest, establishing the relationship between pests and parasitic species, the importance of parasites in limiting the pest population) (Neculiseanu, 2000).
These studies refer to the possibility of controlled human intervention, in order to reduce damage and restore biocenotic balances in ecosystems and which will form the basis for the development of biological and integrated pest control schemes.

MATERIALS AND METHODS

The informations from this paper were obtained from observations made in the period 2020-2021 in a wheat crop located in the NE part of Moldova.
In order to evaluate the potential of the available agro-climatic resources, were taken into account of the agrometeorological data registered with the Agroexpert system. By constantly monitoring and supervising the phenomen of risk / thermal and water stress, the most effective measures can be applied to prevent and reduce the negative effects on the wheat crop.
Collection of entomological material using soil traps type Barber. Soil traps type Barber consisted of inserting 500 ml plastic boxes with of 10 cm in diameter and 8 cm a height of buried in the ground into the soil, in which a solution of formalin (40%) diluted with water was placed up to a concentration of 5%. The pits were made with a spade, and the boxes were buried very carefully so that the edge of the trap was at ground level so that the insects would not be hindered by any obstacle in its vicinity (Varvara et al., 1991) (Figure 1).
The traps were installed at a distance of about 6 m between them. The number of Barber soil traps used to obtain the most accurate data was determined by the location of the collection, ranging from 15 to 50 (Diaconica, 2019).

Figure 1. Installing traps in experimental lots

The experience was organized in 3 variants:
- V1 wheat after wheat;
- V2 wheat after sunflower;
- V3 wheat for corn.

The location of the traps was made in a row with 6 traps per variant, at a distance of 6 m between them. The samples were collected in each of the two years of observation (2020 and 2021) during April-August, at intervals of about 7-10 days.
At each harvest, the contents of the box were wrapped in gauze, and each sample was labeled with the date of collection, the trap number and the variant (Figure 2).

Figure 2. The gathering and determination of the collected material (original)

In the laboratory, the material was cleaned of plant debris and then washed under running water. The material was selected by order or species, and the insects were determined using the determinant (Gîdei 2009; 2012).
Following the identification of harmful species in the crops studied, a series of indicators such as frequency, intensity and degree of attack were followed.
The *attack frequency* (F %), which represents the ratio between the number of attacked plants or plant organs analyzed and is calculated according to the formula: $F\,\% = \frac{n}{N} \times 100$
The *intensity of the attack* (I %), actually represents the percentage of plants, or of attacked organs of the plant destroyed by the pest.
Depending on this scale, the *attack intensity* (I %) is calculated according to the formula:

$$I\,\% = \frac{\sum (i \times f)}{n}$$

The degree of attack (GA) is equal to the product of these two indicators.

$$GA = \frac{F\% \times I\%}{100}$$

RESULTS AND DISCUSSIONS

The climate of the area is part of the temperate continental climate with regions of steppe.

It is an essential ecosystem component in the appearance and development of this region, being of temperate continental type, with moderate-continental (Central European) nuance at the level of the high hills and excessively continental (Eastern European).

In our research, the climate component has a very important role, both in the biology of insects in the agricultural ecosystem and in the phenology of crop plants.

The average temperature (Figure 3) of the agricultural year 2020-2021 is comparable to the multiannual one, but during the vegetation period the values of the average temperatures recorded in the air were lower than the normal values, due to the high amplitude between the temperature during the day and night.

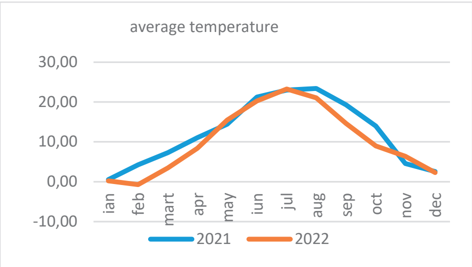

Figure 3. The average temperature of the agricultural year 2020-2021

The annual water regime (Figure 4) is surplus compared to the normal one, but during the vegetation period the sum of the registered precipitations satisfies the necessity of the wheat crop.

The trend of rainfall characteristics is the presence of torrential rains, the increase of the number of rains greater than 0.1 mm and less than 5.0 mm and the decrease of the number of recoverable rains (> 5 mm). Knowledge of agro-meteorological characteristics is necessary in establishing the trend of thermal and water risk on wheat crops.

In 2021, the Phytosanitary Office issued two warnings on 29.04. and 18.05.2021, regarding the control of foliar diseases and pests of wheat crops.

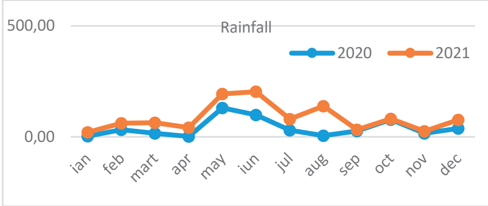

Figure 4. The annual water regime in year 2020-2021

In 2020, two warnings were issued on 06.04. and 21.05.2020, by the Phytosanitary Office, regarding the control of foliar diseases and pests of wheat crops.

For the control of foliar diseases: *Erysiphe graminis, Puccinia* spp., *Septoria tritici, Fusarium* spp., It is recommended to use Priaxor EC, Topsin 500 SC, Nativo PRO 325 SC, Evolus, Mystik PRO, Protector Super 250 EC Karate Zeon, Fastac Active, Priaxor EC, Biscaya 240 OD and Orius 25 EW.

Figure 5. Aspects of the presence and attack of harmful species in wheat culture

For the control of foliar diseases: *Erysiphe graminis, Puccinia* spp., *Septoria tritici, Fusarium* spp., it is recommended to use the products Priaxor EC, Nativo PRO 325, Mystik PRO and to control the pests of cereals Alfametrin 10 EC, Mavrik 2 F, Karate Zeon, Evolus, Priaxor EC, Orius 25 EW.

The species that caused some damage with an attack rate of between 3-30%, even if some treatments were performed. *Eurygaster* spp., *Aelia* spp., *Opatrum sabulosum, Otiorrhynchus pinastri, Epicometis hirta, Phyllotreta nemorum, Phyllotreta vittula, Zabrus tenebrioides and Haplotrips tritici* had the highest attack rate.

- species that have produced a sporadic attack, less than 1%, such as: *Pseudophonus rufipes, Selatosomus latus, Dorcadion fulvum, Otiorrhynchus lingustici, Selatosomus bipustulatus, Atomaria linearis, Harpalus tardus, Phyllotreta atra, Otiorrhynchus raucus, Harpalus distinguendus*.
- species reported only in cereal crops without causing damage, among which we mention:

Pedinus femoralis, Pseudophonus griseus, Aphthona euphorbiae, Pentodom idiota, Apion tenue, Apion urticarium, Apion virens, Ceutorrynchus scapularis, Dorcadion pedestre, Longitarsus pratensisetc.

Tabel 1. Pests reported during the wheat crop research period

Scientific name	Order / Family	Observations
Eurygaster spp.	Heteroptera /Scutelleridae	7-10 % attack
Aelia spp.	Heteroptera /Pentatominae	3-5 % attack
Opatrum sabulosum	Coleoptera /Tenebrionidae	15-20% attack
Otiorrhynchus pinastri	Coleoptera /Curculionidae	10-15% attack
Epicometis hirta	Coleoptera /Cetoniidae	7-10 % attack
Phyllotreta nemorum	Coleoptera /Chrysomelidae	3-5 % attack
Tanymecus dilaticollis	Coleoptera /Curculionidae	10-30% attack
Lema melanopa	Coleoptera/Chrysomelidae	7-10% attack
Anisoplia segetum	Coleoptera/Rutelidae	8-15% attack
Zabrus tenebrioides	Coleoptera /Carabidae	5-8% attack
Phyllotreta vittula	Coleoptera /Chrysomelidae	2-3% attack
Haplotrips tritici	Thysanoptera /Phlaeothripinae	15-20% attack
Agriotes lineatus	Coleoptera /Elateridae	9-11% attack
Pseudophonus rufipes	Coleoptera/Carabidae	Signaled
Selatosomus latus	Coleoptera/Elateridae	Signaled
Dorcadion fulvum	Coleoptera/Cerambycidae	Signaled
Otiorrhynchus lingustici	Coleoptera/Curculionidae	Signaled
Harpalus distinguendus	Coleoptera /Carabidae	Signaled
Atomaria linearis	Coleopter/Cryptophagidae	Signaled
Harpalus tardus	Coleoptera/Carabidae	Signaled
Phyllotreta atra	Coleoptera /Chrysomelidae	Signaled
Otiorrhynchus raucus	Coleoptera/Curculionidae	Signaled
Selatosomus bipustulatus	Coleoptera/Elateridae	Signaled
Pedinus femoralis	Coleoptera/Tenebrionidae	Sporadic
Pseudophonus griseus	Coleoptera/Carabidae	Sporadic
Aphthona euphorbiae	Coleoptera/Chrysomelidae	Sporadic
Pentodom idiota	Coleoptera /Dynastidae	Sporadic
Apion tenue	Coleoptera/Curculionidae	Sporadic
Apion urticarium	Coleoptera/Curculionidae	Sporadic
Apion virens	Coleoptera/Curculionidae	Sporadic
Ceutorrynchus scapularis	Coleoptera/Curculionidae	Sporadic
Dorcadion pedestre	Coleoptera/Cerambycidae	Sporadic
Longitarsus pratensis	Coleoptera /Chrysomelidae	Sporadic
Malachius marginellus	Coleoptera/Melyridae	Sporadic
Otiorrhynchus fuscipes	Coleoptera/Curculionidae	Sporadic
Otiorrhynchus laevigatus	Coleoptera/Curculionidae	Sporadic
Bruchus affinis	Coleoptera /Chrysomelidae	Sporadic
Oscinella frit	Diptera /Chloropidae	Sporadic
Chlorops pumilionis	Diptera /Chloropidae	Sporadic
Phorbia securis	Diptera/Anthomyiidae	Sporadic
Mayetiola destructor	Diptera/Cecidomyiidae	Sporadic
Schizaphis graminum	Hemiptera /Aphididae	Sporadic
Delia coarctata	Diptera/Anthomyiidae	Sporadic

From the analysis of the entomofauna dynamics of beetles collected in 2020 in the three experimental variants, a large variation of the number of species and individuals is found from one collection to another (Tabel 2).

Table 2. The abundance of beetle species collected in 2020 in wheat culture

Variant	Rec. I 29.04	Rec. II 17.05	Rec. III 29.05	Rec. IV 13.06	Rec. V 27.06	TOTAL
V1	252	194	94	60	49	649
V2	360	158	56	40	33	647
V3	253	315	67	39	96	770
	865	667	217	139	178	2066

The largest number of individuals was collected in April, at the first harvest of variant V2, wheat after sunflower, when were gathered 360 beetles. The fewest specimens, 33 in number, were collected at the aVa harvest, also at V2 (Figure 6).

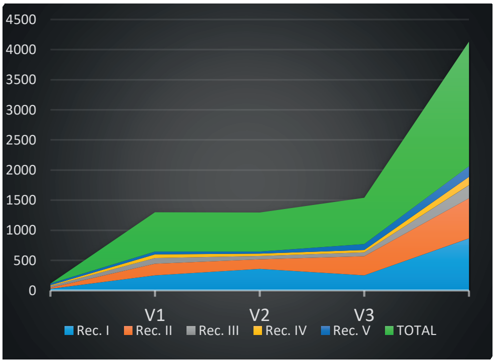

Figure 6. Analysis of the entomofauna dynamics of beetles collected in 2020

From the analysis of the entomofauna dynamics of beetles collected in 2021 in the three experimental variants, we find a large variation in the number of species and individuals, from one collection to another (Table 3).

Tabel 3. The abundance of beetle species collected in 2021 in wheat culture

Variant	Rec. I 24.05	Rec. II 30.05	Rec. III 07.06	Rec. IV 18.06	Rec. V 22.06	Rec. VI 06.07	Rec. VII 02.08	TOTAL
V1	232	165	165	129	77	102	50	920
V2	120	132	186	70	44	52	31	635
V3	166	132	161	89	126	78	23	775
	518	429	512	288	247	232	104	2330

The largest number of individuals was collected in May, at the first harvest of variant V1, wheat after wheat, when was gathered 232 of beetles. The fewest specimens, 23 in number, were collected at the 7th harvest on 02.08, in the variant wheat after corn (Figure 7).

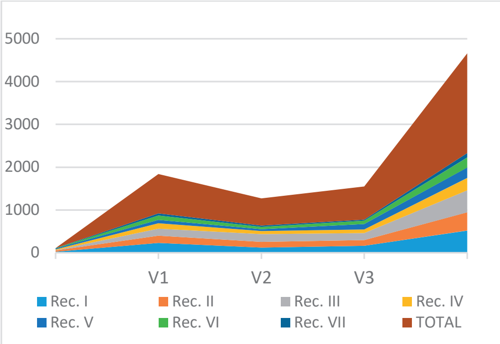

Figure 7. Dynamics of the beetle species collected in 2021

The beetle species were divided into 3 categories taking into account the food they consume, as follows:
- useful species - predators, which feed on insects or other invertebrates;
- species - harmful to some cultivated plants;
- species of beetles that do not cause damage, but have a phytophagous food regime.

Following the analysis of the collected material, in connection with the mode of feeding, the situation in the research year 2020 is as follows (Tables 4, 5, 6):
- 29 species, totaling 826 specimens of beetles are cited in the literature as harmful, representing 40%.

The most commonly encountered were: *Epicometis hirta* (187 specimens), *Opatrum sabulosum* (179 specimens), *Phyllotreta nemorum* (135 specimens), *Pentodon idiota* (91 specimens), *Tanymecus dilaticollis* (44 specimens) (Tabel 4).
- the useful species of beetles collected (28) totaled 626 specimens, representing 30.3% of the total. The most commonly encountered were: *Conosoma bipunctata* (279 specimens), *Pterostichus marginalis* (265 specimens), *Colodera nigrita* (53 specimens), *Coccinella 7 punctata* (18 specimens) (Tabel 5).

Coccinella 7 punctata is a shock predatory carabid found in cereal crops, causing medium, equal to or less than degrees of aphids, such as those caused by chemical treatments.
Harpalus aeneus feeds on eggs and larvae of larvae, eggs and larvae of firing beetles and other insects.
Pterosthichus nigrita has adults and larvae predators of the larvae and pupae of the flower mosquito of wheat flowers.
Anthicus floralis has omnivorous adults, consuming small arthropods, pollen, fungi and anything else they can find. Some species are cited in the literature as biological control agents because they may eat eggs or larvae of pests.
- 38 species (617 specimens) of beetles are designated as non-harmful, representing 29.7%. Among them, the most numerous were: *Drasterius bimaculatus* (146 specimens), *Dermestes laniarius* (107 specimens), *Formicomus pedestris* (100 specimens), *Anthicus antherinus* (83 specimens) and *Pteryngium crenatum* (79 specimens) (Tabel 6).

Tabel 4. Harmful beetle species in wheat crops in 2020

Name	Number of specimens/Variant		
	1	2	3
Epicometis hirta	58	111	18
Opatrum sabulosum	73	70	36
Phyllotreta nemorum	21	108	6
Pentodon idiota	33	40	18
Tanymecus dilaticollis	20	15	9
Harpalus distinguendus	16	10	7
Agriotes lineatus	11	9	12
Pedinus femoralis	23	6	-
Phyllotreta atra	-	17	5
Phyllotreta nodicornis	-	17	5
Aphthona euphorbiae	10	-	2
Otiorrhynchus laevigatus	6	2	1
Orchestes fagi	7	-	-
Pseudophonus rufipes	6	-	-
Harpalus tardus	4	2	-
Pseudocleonus cinereus	2	3	-
Anobium punctatum	-	5	-
Tanymecus palliatus	-	-	5
Blaps mortisaga	2	1	1
Stomodes gyrosicollis	-	3	-
Ceutorhynchus punctiger	2	-	-
Cassida nobilis	2	-	-
Otiorrhynchus singularis	2	-	-
Zabrus blapoides	1	1	-
Paradons quadrisignatus	-	-	2
Anisoplia segetum	1	-	-
Oulema melanopa	-	1	-
Selatosomus latus F	-	-	1
Zabrus tenebrioides	-	-	1
TOTAL 29 species and 823 samples		823	

Tabel 5. Useful beetle species identified in wheat crops in 2020

Name	Variant 1	Variant 2	Variant 3
Conosoma bipunctata	11	4	264
Pterostichus marginalis	3	3	259
Colodera nigrita.	10	28	15
Coccinella 7 punctata	12	5	1
Ityocara rubens	1	-	12
Microletes maurus	-	7	5
Metabletus truncatelus	5	5	-
Idiochroma dorsalis	1	-	8
Brachynus explodens	-	-	6
Pterostichus lepidus	3	-	-
Anisodactylus binotatus			3
Calathus fuscipes	-	1	2
Pterostichus aterrimus var. niger	1	1	-
Coccinulla quatuordecimpustulata sinensis Wse	2	-	-
Pterostichus cupreus	-	1	1
Sipalia circularis	-	1	1
Calosoma inquisitor	1	-	-
Necrophorus antennatus	1	-	-
Callistus lunatus	1	-	-
Cypticus quisquilius	1	-	-
Broscus cephalotes	1	-	-
Coccinella 5 punctata	1	-	-
Staphylinus caesareus	-	1	-
Astenus filiformis	-	1	-
Hister quadrimaculatus	-	-	1
Tachyusa constricta	-	-	1
Paederus limnophilus	-	-	1
Metabletus foveatus	-	-	1
TOTAL 28 species and 626 samples		626	

Tabel 6. The beetle species collected in 2020 that do not cause damage to the crop plants

Name	Variant 1	Variant 2	Variant 3
Drasterius bimaculatus	115	31	-
Dermestes laniarius	28	19	60
Formicomus pedestris	41	22	37
Anthicus antherinus	13	57	13
Pteryngium crenatum	8	9	62
Anthicus floralis	5	5	31
Pleurophorus caesus	4	8	14
Cryptophagus dentatus	21	-	-
Tachyporus ruficolis	3	-	15
Anthicus humeralis	13	-	4
Silpha obscura	-	1	12
Anthicus gracilis	11	-	-
Hypnoidus pulchellus	4	5	1
Oxyporus rufus	9	-	-
Amara aenea	1	4	2
Cercyon lateralis	-	-	7
Anthicus humilis	6	-	-
Corticaria longicornis	3	-	3
Cartodere ruficollis	-	-	6
Ophonus sabulicola	-	-	4
Cetonia aurata	2	-	1
Harpalus smaragninus	-	-	3
Paramecosoma melanocephalum	-	-	3
Amara eurynota	-	-	3
Aphodius fimetarius	-	2	-
Cantharis fusca	-	-	2
Anthicus quadriguttatus	-	-	2
Onthophagus taurus	-	1	-
Mycetophagus populii	-	1	-
Psammobius porcicollis	-	1	-
Ophonus azureus	-	-	1
Atomaria fuscicollis	-	-	1
Bidessus geminus	-	-	1
Harpalus cupreus	-	-	2
Scirtes hemisphaericus	-	-	1
Cryptophagus dorsalis	-	-	1
Cerylon ferrungineum	-	-	1
Melanotus brunnipes	-	-	1
TOTAL 38 species and 617 samples		617	

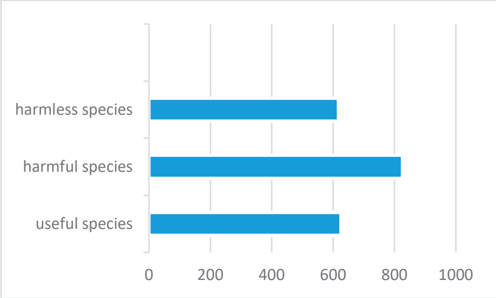

Figure 8. The number of copies of beetles collected in 2020 according to their food spectrum

In the research year 2021, from the investigation of the collected material, depending on the mode of feeding, the situation is as follows (Tables 7, 8, 9):

- a number of 32 species totaling 970 specimens of beetles are cited in the literature as harmful, representing 41.6%.

The most abundant species were: *Opatrum sabulosum* (493 specimens), *Otiorrhynchus pinastri* (305 specimens), *Pentodom idiota* (30 specimens), *Otiorrhynchus orbicularis* (26 specimens), *Harpalus distinguendus* (24 specimens), *Agriotes ustulatus* (17 specimens). (Tabel 7).

- there were 28 useful beetle species collected, with a total of 338 specimens, representing 14.5% of the total. The most abundant species were: *Idiochroma dorsalis* (87 specimens), *Coccinella 7 punctata* (55 specimens), *Conosoma bipunctatum* (48 specimens), *Pterostichus cupreus* (21 specimens), *Metabletus truncatelus* (12 specimens), *Micraspis 12 punctata* (12 specimens) (Tabel 8);

- a number of 34 species totaling 1022 specimens of beetles are cited in the literature as being non-harmful, representing 43.8%. Among these, the most abundant were: *Dermestes laniarius* (512 specimens), *Formicomus pedestris* (208 specimens), *Anthicus humilis* (82 specimens), *Anthicus floralis* (72 specimens) and *Pteryngium crenatum* (65 specimens) (Tabel 9).

According to agricultural science, the choice of a good precursor plant can increase the production by 50 percent more than monoculture (Figure 10).

Tabel 7. Harmful beetle species identified in wheat crops in 2021

Name	Variant		
	1	2	3
Opatrum sabulosum Linnaeus	237	104	152
Otiorrhynchus pinastri Herbst.	93	87	125
Pentodom idiota Hbst.	7	13	10
Otiorrhynchus orbicularis Herbst	5	7	14
Harpalus distinguendus Duftscmid	16	5	3
Agriotes ustulatus Schaller	3	4	10
Epicometis hirta Poda	7	3	1
Tanymecus dilaticollis Gyll	-	2	9
Harpalus aeneus Fabricius	6	1	2
Agriotes lineatus Linnaeus	2	3	1
Aelia spp Linnaeus	4	-	-
Dorcadion fulvum Scopoli	-	1	2
Otiorrhynchus lingustici Linnaeus	-	1	2
Otiorrhynchus raucus Fabricius	1	-	2
Pseudophonus griseus Panzer	2	-	1
Atomaria linearis Stephens	-	-	2
Harpalus tardus Panzer	1	-	1
Phyllotreta atra Fabricius	2	-	-
Phyllotreta vittula Redtenbacher	2	-	-
Zabrus tenebrioides Goeze	-	2	-
Aphthona euphorbiae Schrank.	1	-	-
Apion tenue Herbst.	1	-	-
Apion urticarium Herbst.	-	-	1
Apion virens Herbst	-	-	1
Ceutorrynchus scapularis Germar	-	-	1
Dorcadion pedestre Poda	-	1	-
Longitarsus pratensis Panzer	1	-	-
Malachius marginellus Fabricius	-	-	1
Otiorrhynchus fuscipes Stierlin & W.G.	1	-	-
Otiorrhynchus laevigatus Latreille	-	-	1
Pseudophonus rufipes De Geer	1	-	-
Selatosomus bipustulatus Linnaeus	-	-	1
TOTAL 32 species and 970 samples		970	

Tabel 8. Useful beetle species identified in wheat crops in 2021

Name	Variant		
	1	2	3
Idiochroma dorsalis Pontoppidan	11	13	63
Coccinella 7 punctata Linnaeus	20	16	19
Conosoma bipunctatum Grav.	10	16	22
Pterostichus cupreus Linnaeus	6	3	12
Metabletus truncatelus Linnaeus	4	2	6
Micraspis 12 punctata Linnaeus	5	4	3
Crypticus quisquillius Linnaeus	3	8	-
Hister stercorarius Hoff.	1	2	3
Carabus coriaceus Linnaeus	4	-	1
Anisodactilus binotatus Fabricius	1	-	3
Calathus fuscipes Goeze	2	-	2
Carabus cupreus Linnaeus	-	2	1
Pterosthichus nigrita Paykull	2	-	1
Anisodactilus signatus Dejean	2	-	-
Cymindis axillaris Fabricius	-	-	2
Exosoma lusitanicum Linnaeus	-	2	-
Abax ovalis Duftschmid	1	-	-
Carabus crenatus Duftschmid	-	1	-
Cicindela campestris Linnaeus	-	1	-
Cynegetis punctata Linnaeus	-	1	-
Harpalus calceatus Duftschmid	-	-	1
Hister bimaculatus Gyll	-	1	-
Metabletus obscuroguttatus Duftschmid	-	1	-
Platynaspis luteorubra Goeze	-	-	1
Scopaeus laevigatus Gyllenhal	1	-	-
Stilicus angustatus Gravenhorst	-	1	-
Tachinus subterraneus Linnaeus	1	-	-
Zyras collaris Markel.	-	-	1
TOTAL 28 species and 338 samples		338	

Tabel 9. The beetle species collected in 2021that do not cause damage to the crop plants

Name	Variant		
	1	2	3
Dermestes laniarius Illiger	213	187	112
Formicomus pedestris Rossi	88	57	63
Anthicus humilis Germar	45	20	17
Anthicus floralis Linnaeus	34	18	20
Pteryngium crenatum Fabricius	24	11	30
Letrus apterus Laxmann	3	2	3
Harpalus azureus Duft.	1	3	3
Pleurophorus caesus Panzer	-	3	4
Zabrus blapoides Creutzer	1	3	3
Amara eurynota Panzer Panzer	-	2	3
Ophonus azureus Fabricius	3	1	1
Podonta nigrita Fabricius	-	1	4
Amara aenea De geer	4	-	-
Anthicus quadriguttatus Rossi	-	4	-
Cetonia aurata Linnaeus	1	1	2
Tachysa constricta Linnaeus	1	2	-
Bidessus unistriatus Schrank	-	1	1
Cantharis fusca Linnaeus	1	-	1
Cartodere ruficollis C. G. Thomson	-	1	1
Otho spondiloides	1	-	1
Philontus speldens Steph.	-	1	1
Silpha obscura Linnaeus	1	-	1
Atomara turgida Reitter	-	1	-
Combocerus glaber Schaller	-	-	1
Cryptophagus subdepressus Gyll.	-	1	-
Dolichosoma lineare Rossi	-	-	1
Hypnoidus pulchellus Linnaeus	-	-	1
Medon obsoletus Nordmann	1	-	-
Ophonus obscurus Fabricius	1	-	-
Oxypora annularis	1	-	-
Oxypora vittata	-	-	1
Poecilus dimidiatus G.A.Olivier	1	-	-
Quedius cruentus Olivier	-	-	1
Quedius molochinus Grav.		1	
TOTAL 34 species and 1022 samples		1022	

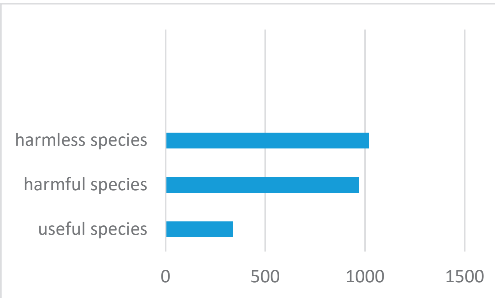

Figure 9. The number of copies of beetles collected in 2021 according to their food spectrum

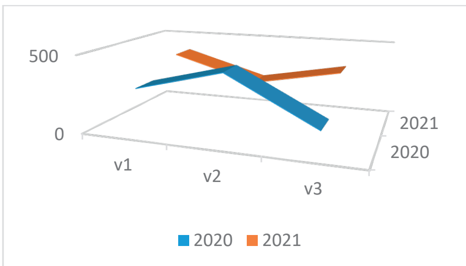

Figure 10. Species of harmful beetles identified in the wheat crop during the research period, depending on the precursor plant

CONCLUSIONS

Wheat and corn also have common diseases that we must take into account. Wheat after wheat crop can only be applied for one year, so we risk multiplying the diseases and pests of wheat, which then creates the major problems of declining production, or to combat chemicals, repeated work and chemical pollution. The advantage of wheat in a single year is that, when harvested in the summer, it allows soil work to be carried out, chemical fertilizers can be applied and weeds can be controlled.

Wheat growing in rotation with corn is also required for economic reasons. Thus, the total production of wheat and corn grown in rotation cannot be matched by any other pair of wheat with another plant.

However, wheat cannot be sown after maize if a large amount of Atrazine-based herbicide has been used in maize cultivation (more than 3 kg/ha; Atrazine has a residual negative effect on wheat).

Sunflower is a poor precursor to wheat, due to the fact that it impoverishes the soil in nutrients and water, which makes the preparation of the land difficult and of inferior quality.

If it is harvested on time and as soon as possible, the crop has been well maintained by the plowshares, plowed deep and fertilizers are used, sunflower can become a good precursor for wheat.

Because during the research period, in variant V2, wheat after sunflower, the number of harmful species is the lowest (16 species), compared to V1 and V3 when a higher number of harmful species were collected (20 and 18 species respectively), we recommend the use in practice of the wheat variant after sunflower.

As the greatest diversity in terms of the abundance of species collected was also recorded in the V2 wheat variant after sunflower, we recommend this variant in agricultural practice.

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THE EVALUATION OF THE BIOMASS QUALITY OF TALL OATGRASS, *Arrhenatherum elatius* (L.) Beauv, AND PROSPECTS OF ITS USE IN MOLDOVA

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Abstract

The main objective of this research was to evaluate the quality indices of green mass, hay, silage and straw from the local ecotype of tall oatgrass, Arrhenatherum elatius, grown in monoculture in the experimental plot of NBGI, Chisinau, Republic of Moldova. The results revealed that the harvested green mass contained 25.0-29.8% dry matter. The dry matter of the whole plant contained 80 g/kg CP6yy, 382 g/kg CF, 83 g/kg ash, 411 g/kg ADF, 710 g/kg NDF, 38 g/kg ADL,118 g/kg TSS, 373 g/kg Cel, 299 g/kg HC, with nutritive and energy value 58.2% DMD, 51.3% DOM, 9.29 MJ/kg ME and 5.31 MJ/kg NEl. The biochemical composition, nutritive and energy value of tall oatgrass hay: 77 g/kg CP, 414 g/kg CF, 80 g/kg ash, 436 g/kg ADF, 740 g/kg NDF, 40 g/kg ADL, 98 g/kg TSS, 396 g/kg Cel and 304 g/kg HC, 9.00 MJ/kg ME and 5.02 MJ/kg NEl. The tall oatgrass fermented fodder- silage is characterized by pH = 4.26, 5.9 g/kg acetic acid, 18.3 g/kg lactic acid and free of butyric acid, 88 g/kg CP, 405 g/kg CF, 100 g/kg ash, 420 g/kg ADF, 724 g/kg NDF, 26 g/kg ADL, 74 g/kg TSS, 394 g/kg Cel, 304 g/kg HC, with nutritive and energy value 56.5% DMD, 9.19 MJ/kg ME, 5.21 MJ/kg NEl. The tall oatgrass straw contained 50 g/kg CP, 51 g/kg ADL, 85 g/kg TSS, 405 g/kg Cel, 286 g/kg HC. The tall oatgrass substrates used for anaerobic digestion have optimal amount of lignin and hemicellulose, the estimated biochemical methane potential varied from 341 to 361 l/kg VS. The tall oatgrass straw and hay may be used as feedstock for the production by cellulosic ethanol, the estimated theoretical ethanol yield from cell wall carbohydrates averaged 507-509 L/t.

Key words: *Arrhenatherum elatius, biomass quality indices, forages, substrates for renewable energy production.*

INTRODUCTION

The family Poaceae containing 777 plant genera and 11461 accepted species, constitutes one of the world's most economically important plant groups. The *Plant List* includes 96 scientific plant names of species rank for the genus *Arrhenatherum*, 9 of these are accepted species names with Eurasian and North African distribution. In the spontaneous flora of the Republic of Moldova, there is one species, *Arrhenatherum elatius* (L.) Beauv is commonly known as tall oat-grass, bulbous oat grass or false oat-grass.

Arrhenatherum elatius is a perennial plant, loosely cespitose, sometimes rhizomatous, rhizomes to 3 mm thick. The culms grow 50-140(180) cm tall, with 4-5 nodes, glabrous, unbranched, the basal internodes may be swollen or not swollen; the nodes – glabrous or occasionally puberulent to densely hairy. Young leaves convolute; sheaths split, with overlapping margins; ligules membranous, 1- 3 mm long, obtuse to truncate, usually ciliate; blades without auricles, long (up to 40 cm), their width increasing from the base to about two-thirds of their length, then decreasing to give an acuminate tip; upper surface, smooth, ribless; lower with marked keel. Flag leaves shorter, widest at their base and the keel not well developed. The inflorescences are lax panicles, 20-30 cm long and 2-7 cm wide green, shiny, becoming stramineous, sometimes purple-tinged; branches 15-20 mm long, ascending to divergent, verticillate, usually spikelet-bearing at the base; pedicels 1-10 mm long. Spikelets usually 2-flowered, the lower male only, the upper hermaphrodite; sometimes a third, fourth or even fifth floret may be present, hermaphrodite or rudimentary; the lowest floret also sometimes hermaphrodite. Glumes membranous, unequal, the lower 1-nerved and shorter, the upper 3 -nerved and longer. Lower floret with a long, twisted, geniculate awn, inserted one-third from base of the lemma; the upper floret generally awnless, but when present usually straight and inserted near the tip of the lemma. Flowering time - May-June, cross-pollinated and wind-pollinated. Caryopses 4-5 mm long, about 1.2 mm wide, ellipsoid, densely hairy, yellowish. The weight of 1000 seeds 2.6-3.1 g. $2n = 14, 28, 42$. It is propagated by sowing. The fibrous roots are stout. The lowest temperature for seed germination of tall oat-grass is 3-4°C, seedlings can tolerate low temperatures from -2°C to -4°C, adult plants can still grow slowly at low temperatures from -3°C to -4°C, but suffer at -6°C. In winter, the plants tolerate temperatures down to -23°C. *Arrhenatherum elatius* occurs on different types of soils, with a pH between 5 and 8 and medium salinity, prefer full sun or half-shady places. It is a mesophytic to xerophytic species of open habitats: dry grassland, edges of woods, disturbed ground, successional fields, hayfields, pastures, thickets, and roadsides, sometimes becoming a dominant grass in fields. It can colonize and stabilize limestone scree, bare calcareous cliffs, maritime shingle and coastal dunes. *Arrhenatherum elatius* is grown as a forage grass; it recovers quickly after grazing, but it does not withstand overgrazing. It is sown in mixtures with other species for grassland restoration (Medvedev & Smetannikova, 1981; Maczey, 2015).

Currently, *Arrhenatherion elatioris* is being assessed for its potential suitability for energy biomass production (Moudrý et al., 2010; Raclavská et al., 2011; Ebeling et al., 2013; Boob et al., 2019; Danielewicz et al., 2019; Jezerska et al.,2019;Waliszewska et al., 2021).

The main objective of this research was to evaluate the quality indices of green mass, hay, silage and straw from the local ecotype of tall oatgrass, *Arrhenatherum elatius*, as feed for livestock, also as substrates for renewable energy production.

MATERIALS AND METHODS

The local ecotype of tall oatgrass, *Arrhenatherum elatius*, grown in monoculture in the experimental sector of the National Botanical Garden (Institute) of Moldova, Chişinău, N 46°58′25.7″ and E 28°52′57.8″, served as subject of research and the fodder plants: meadow fescue, *Festuca pratensis,* common oat *Avena sativa* 'Sorin' and winter barley *Hordeum vulgare* 'Excelent', were used as controls. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. The samples were collected in the second growing season and the first cut was done in the pre-flowering stage. The harvested plants were chopped into 1.5-2.0 cm small pieces, with a laboratory forage chopper; the dry matter content was detected by drying the samples to a constant weight, at 105°C. The prepared hay was dried directly in the field. For ensiling, the green mass was chopped by using a forage chopping unit, shredded and compressed in well-sealed glass containers, stored at ambient temperature (18-20°C). After 45 days, the containers were opened, and the sensorial and chemical characteristics of the prepared silages were determined in accordance with standard laboratory procedures and the Moldavian standard SM 108 for forage quality analysis. Straws were collected after the grains have been removed. For chemical analyses, plant samples were dried in a forced air oven at 60 °C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm and some of the main biochemical parameters were assessed: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM) and digestible organic matter (DOM) were determined by the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 at the Research and Development Institute for Grasslands, Braşov, Romania. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures.

The carbon content of the substrates was determined using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations of Dandikas et al. (2015). The theoretical ethanol potential (TEP) was calculated according to the equations of Goff et al. (2010) based on conversion of hexose (H) and pentose (P) sugars.

RESULTS AND DISCUSSIONS

Analysing the results of the fresh mass quality of the local ecotype of tall oatgrass, *Arrhenatherum elatius* (Table 1) we would like to mention that the dry matter contained 80 g/kg CP, 382 g/kg CF, 83 g/kg ash, 411 g/kg ADF, 710 g/kg NDF, 38 g/kg ADL, 118 g/kg TSS, 373 g/kg Cel, 299 g/kg HC. The tall oatgrass green fodder is characterized by a higher content of minerals and structural carbohydrates and lower concentration of total soluble sugars than the fodders from control variants. The *Arrhenatherum elatius* green fodder, as compared with the *Festuca pratensis* green mass has a higher content of crude protein, but as compared with the *Avena sativa* green mass, it contains a lower amount of acid detergent lignin. The high amount of neutral detergent fibre in oatgrass green fodder contributed to the reduction of digestibility, relative feed value and energy concentration.

Table 1. The biochemical composition and the nutritive value of the harvested green mass from *Arrhenatherum elatius*

Indices	*Arrhenatherum Elatius*	*Avena sativa*	*Festuca pratensis*	*Hordeum vulgare*
Crude protein, g/kg DM	80.00	95.00	71.00	119.00
Minerals, g/kg DM	83.00	65.00	80.00	75.00
Crude fibre, g/kg DM	382.00	356.00	359.00	349.00
Acid detergent fibre, g/kg DM ,	411.00	374.00	388.00	367.00
Neutral detergent fibre, g/kg DM	710.00	629.00	686.00	628.00
Acid detergent lignin, g/kg DM	38.00	46.00	33.00	29.00
Total soluble sugars, g/kg DM	118.00	167.00	174.00	163.00
Cellulose, g/kg DM	373.00	328.00	335.00	338.00
Hemicellulose, g/kg DM	299.00	258.00	298.00	261.00
Digestible dry matter, g/kg DM	561.00	598.00	587.00	603.00
Relative feed value	74.00	89.00	80.00	89.00
Digestible energy, MJ/ kg	11.18	11.84	11.64	11.92
Metabolizable energy, MJ/ kg	9.18	9.72	9.56	9.79
Net energy for lactation, MJ/ kg	5.31	5.73	5.57	5.81

In the specialized literature, there are data on the green mass quality of *Arrhenatherum elatius* plants. According to Skládanka et al. (2008) the forage dry matter from *Arrhenatherum elatius* plants contained 30.2% CF, 60.5% NDF, 35.9% ADF, 5.46 MJ/kg NEl; *Dactylis glomerata* plants -28.9% CF, 57.1% NDF, 35.1% ADF, 5.54 MJ/kg NEl; *Festulolium* plants contained 26.9% CF, 58.9% NDF, 32.3% ADF, 5.84 MJ/kg NEl. Tomić et al. (2005) reported that the grass quality of *Arrhenatherum elatius* grown the pasture associations was 6.28% CP, 30.07% CF, 8.11% ash. Reiné et al. (2020) studied the nutritional quality of meadow plant species and remarked that *Arrhenatherum elatius* contained 421 g/kg DM with 7.6% CP, 4.5% ash, 1.6% EE, 66.5% NDF, 35.2% ADF, 3.0% ADL, 61.5% DDM, 0.13% P, 0.50% Ca, but *Festuca arundinacea* contained 455 g/kg DM with 7.2% CP, 4.4% ash, 2.0% EE, 73.3% NDF, 41.1% ADF, 4.0% ADL, 56.8% DDM, 0.12% P, 0.35% Ca. Skládanka et al. (2010), compared the forage quality of grass green mass and found that the chemical composition of *Arrhenatherum elatius* plants was 7.92-9.49% CP, 29.34-30.25% CF, 55.48-61.20% NDF with 71.80-78.0% OMD; *Dactylis glomerata* plants - 9.00-9.17% CP, 27.52 -30.33% CF, 56.59-57.79% NDF with 70.4-76.9% OMD; *Festuca arundinacea* × *Lolium multiflorum* plants – 7.11-7.54% CP, 25.36-29.79% CF, 56.10-61.25% NDF with 71.8-78.0% OMD.

Hay represents a low-cost roughage source of nutrients and is vital to keep livestock healthy and productive, particularly in the autumn - middle spring period. We would like to mention that the hay prepared from tall oatgrass plants (Tables 2) contained 77 g/kg CP, 414 g/kg CF, 80 g/kg ash, 436 g/kg ADF, 740 g/kg NDF, 40 g/kg ADL, 98 g/kg TSS, 396 g/kg Cel and 304 g/kg HC. The digestibility and the energy concentration of the hay from tall oatgrass plants was 54.1% DDM, 10.82 MJ/kg DE, 8.88 MJ/kg ME and 5.02 MJ/kg NEl.

Table 2. The biochemical composition and the nutritive value of the hay from *Arrhenatherum elatius*

Indices	*Arrhenatherum elatius*	*Avena sativa*	*Festuca pratensis*	*Hordeum vulgare*
Crude protein, g/kg DM				
Minerals, g/kg DM	77.00	105.00	79.00	90.00
Crude fibre, g/kg DM	80.00	74.00	85.00	69.00
Acid detergent fibre, g/kg DM	414.00	381.00	364.00	435.00
Neutral detergent fibre, g/kg	436.00	404.00	391.00	456.00
DM	740.00	660.00	681.00	736.00
Acid detergent lignin, g/kg DM	40.00	50.00	32.00	45.00
Total soluble sugars, g/kg DM	98.00	111.00	153.00	51.00
Cellulose, g/kg DM	396.00	354.00	359.00	401.00
Hemicellulose, g/kg DM	304.00	256.00	290.00	280.00
Digestible dry matter, g/kg DM	541.00	574.00	584.00	534.00
Relative feed value	68.00	81.00	80.00	67.00
Digestible energy, MJ/ kg	10.82	11.40	11.59	10.69
Metabolizable energy, MJ/ kg	8.88	9.36	9.51	8.78
Net energy for lactation, MJ/ kg	5.02	5.39	5.54	4.79

During the process of preparing tall oatgrass hay, we observed an increase in the concentration of structural carbohydrates, lignin, and a decrease in the crude protein, minerals and total soluble sugar content, which has a negative effect on dry matter digestibility and energy concentration as compared to harvested green mass. The tall oatgrass hay is characterized by lower amount of crude protein, acid detergent lignin, total soluble sugars and minerals than common oat hay. As compared with the meadow fescue hay, the tall oatgrass hay did not differ significantly in the concentration of crude protein, but had high concentration of cell wall fractions (NDF, ADF, ADL), minerals and reduced content of total soluble sugars, dry matter digestibility and energy supply. The prepared tall oatgrass hay had lower content of crude protein, acid detergent fibre, acid detergent lignin and high content of total soluble sugars and minerals than hay prepared from the winter barley. According to Medvedev & Smetannikova (1981), tall oatgrass hay contained 7.6-12.7% CP, 1.6-3.4% EE, 23.2-32.0% CF, 36.0-50.0% NFE, 7.0-10.0% ash.

Silage fodder is a key element for good farm animal feeding. The investigated silage from *Arrhenatherum elatius* plants was distinguished by homogeneous light olive colour, pleasant smell specific of pickled watermelon, the consistency was preserved, in comparison with the initial green mass, without mould and mucus. The quality indices of the silage prepared from *Arrhenatherum elatius* are shown in Table 3.

The fermentation profile of the prepared tall oatgrass silage was pH = 4.26, 4.5 g/kg free lactic acid, 2.8 g/kg free acetic acid, 13.8 g/kg fixed lactic acid, 3.1 g/kg fixed acetic acid, butyric acid was not detected. It was determined that the pH of the tall oatgrass silage was higher, but the concentration of organic acids is lower than in the meadow fescue silage. The concentrations of nutrients in the tall oatgrass silage dry matter were: 88 g/kg CP, 405 g/kg CF, 100 g/kg ash, 420 g/kg ADF, 724 g/kg NDF, 26 g/kg ADL, 74 g/kg TSS, 394 g/kg Cel, 304 g/kg HC, with nutritive and energy value 56.5% DMD, 11.25 MJ/kg DE, 9.19 MJ/kg ME, 5.21 MJ/kg NEl.

As compared with the whole plant fodder in the *Arrhenatherum elatius* silage, there was detected an increase in the content of crude protein, minerals, cellulose and hemicellulose, and a reduction in the acid detergent lignin, the dry matter digestibility and energy concentration do not differ essentially. We would like to mention that *Arrhenatherum elatius* silage is characterized by a high content of cell wall fractions (NDF, ADF, ADL) and low content of crude protein, total soluble sugars, minerals, dry matter digestibility and energy concentration as compared with the control - winter barley silage. As compared with the meadow fescue silage, the tall oatgrass silage did not particularly differ in the concentration of structural carbohydrates, dry matter digestibility and energy supply, but had high level of crude protein and acid detergent lignin and reduced content of total soluble sugars. Dinić et al. (2008) studied the biochemical composition of silages made of various crops, grown in Germany, and remarked that tall oat grass silage from plant mowing in earring stage had pH 4.38 and contained 308.3 g/kg DM with 5.28% lactic acid, 1.89% acetic acid, 0.76% butyric acid, 15.60% CP, 5.01% EE, 69.01% NDF, 33.87% ADF, 18.75%TSS, 35.13%HC, 10.44% ash, 0.54% Ca and 0.35% P, but the silage from plant mowing in the blooming stage respectively - pH 4.13, 322.5 g/kg DM 5.66% lactic acid, 2.14% acetic acid, 0% butyric acid, 13.82% CP, 4.82% EE, 70.44% NDF, 34.03% ADF, 15.93% TSS, 36.41% HC, 9.76% ash, 0.49% Ca and 0.31% P. Crop residues are important feed resources for livestock.

Table 3. The quality indices of the silage from *Arrhenatherum elatius*

Indices	*Arrhenatherum elatius*	*Festuca pratensis*	*Hordeum vulgare*
pH index	4.26	4.08	4.04
Organic acids, g/kg DM	24.20	42.10	38.40
Free acetic acid, g/kg DM	2.80	3.50	3.40
Free butyric acid, g/kg DM	0.00	0.00	0.00
Free lactic acid, g/kg DM	4.50	6.50	10.50
Fixed acetic acid, g/kg DM	3.10	4.30	3.60
Fixed butyric acid, g/kg DM	0.00	0.00	0.00
Fixed lactic acid, g/kg DM	13.80	27.80	20.90
Total acetic acid, g/kg DM	5.90	7.8.00	7.00
Total butyric acid, g/kg DM	0.00	0.00	0.00
Total lactic acid, g/kg DM	18.30	34.30	31.40
Acetic acid, % of organic acids	24.38	18.52	18.20
Butyric acid, % of organic acids	0.00	0.00	0.00
Lactic acid, % of organic acids	75.62	81.48	81.80
Crude protein, g/kg DM	88.00	80.00	129.00
Crude fibre, g/kg DM	405.00	394.00	338.00
Ash, g/kg DM	100.00	96.00	114.00
Acid detergent fibre, g/kg DM	420.00	411.00	347.00
Neutral detergent fibre, g/kg DM	724.00	718.00	571.00
Acid detergent lignin, g/kg DM	26.00	22.00	18.00
Total soluble sugars, g/kg DM	74.00	132.00	129.00
Cellulose, g/kg DM	394.00	389.00	329.00
Hemicellulose, g/kg DM	304.00	307.00	224.00
Digestible dry matter, g/kg DM	565.00	569.00	619.00
Digestible energy, MJ/kg DM	11.25	11.32	12.21
Metabolizable energy, MJ/kg DM	9.19	9.29	10.02
Net energy for lactation, MJ/kg DM	5.21	5.31	6.10
Relative feed value	73.00	74.00	100

The optimal use of straw in animal diets depends on the availability of detailed information on their chemical composition, biological properties and nutritional value, which may vary between type of agroecosystem, plant species, cultivars. The results regarding the nutritive quality of the *Arrhenatherum elatius* seed straw are shown in Table 4. It has been found that tall oat grass straw is characterized by high content of protein (50 g/kg), minerals (77 g/kg), total soluble sugars (85 g/kg) and low cell wall concentration (742 g/kg) compared with the control variants – winter barley and meadow fescue seed straws, which has a positive effect on nutritive value and energy concentration. Renewable energy sources coming from agricultural crops could play an important role in terms of energy supply and positive environmental effects. Bioethanol, a renewable energy source, is one of the alternatives to petroleum. The use of ethanol as a fuel for internal combustion engines has certain advantages as compared with gasoline: the octane number is higher, which leads to greater detonation resistance; the freezing point of ethanol is lower, CO_2 emissions are also lower. The production of cellulosic ethanol via biological conversion consists of three critical steps: pretreatment of biomass, hydrolysis of sugar polymers (cellulose, hemicellulose etc.) to sugar monomers (hexose, pentose) and fermentation of sugar monomers to ethanol (Kumar & Murthy, 2011; Cerempei, 2016).

Table 4. The biochemical composition and the nutritive value of the straw from *Arrhenatherum elatius*

Indices	*Arrhenatherum elatius*	*Festuca pratensis*	*Hordeum vulgare*
Crude protein, g/kg DM	50.00	39.00	45.00
Crude fibre, g/kg DM	443.00	463.00	496.00
Ash, g/kg DM	77.00	75.00	59.00
Acid detergent fibre, g/kg DM	456.00	478.00	550.00
Neutral detergent fibre, g/kg DM	742.00	771.00	792.00
Acid detergent lignin, g/kg DM	51.00	54.00	68.00
Total soluble sugars, g/kg DM	85.00	72.00	79.00
Cellulose, g/kg DM	405.00	424.00	482.00
Hemicellulose, g/kg DM	286.00	293.00	242.00
Dry matter digestibility, %	534.00	517.00	461.00
Digestible energy, MJ/kg DM	10.69	10.38	9.38
Metabolizable energy, MJ/kg DM	8.78	8.52	7.71
Net energy for lactation, MJ/kg DM	4.79	4.54	3.72
Relative feed value	67.00	62.00	54.00

The composition of cell walls and the theoretical ethanol potential of investigated hay and straw substrates are presented in Table 5. We would like to mention that the investigated substrates of tall oat grass varied in terms of cellulose content (396-405 g/kg) and hemicellulose (286-304 g/kg), a fact that affected the concentration of pentose and hexose carbohydrates, and the theoretical bioethanol potential reached values of 507-509 l/t organic matter. In comparison with substrates from *Festuca pratensis* and *Hordeum vulgare* the hay substrate from *Arrhenatherum elatius* had higher bioethanol potential, but straw substrate - lower bioethanol potential values.

Table 5. The composition of cell walls and the theoretical ethanol potential of investigated substrates from *Arrhenatherum elatius*

Indices	*Arrhenatherum elatius*		*Festuca pratensis*		*Hordeum vulgare*	
	Hay	straw	hay	straw	hay	straw
Acid detergent fibre, g/kg	436.00	456.00	391.00	478.00	456.00	550.00
Neutral detergent fibre, g/kg	740.00	742.00	681.00	771.00	736.00	792.00
Acid detergent lignin, g/kg	40.00	51.00	32.00	54.00	45.00	68.00
Cellulose, g/kg	396.00	405.00	359.00	424.00	401.00	482.00
Hemicellulose, g/kg	304.00	286.00	290.00	293.00	280.00	242.00
Hexose sugars, g/kg	72.11	74.59	65.55	76.82	72.69	97.87
Pentose sugars, g/kg	50.00	47.04	47.70	48.20	46.05	39.81
Theoretical ethanol potential, L/t	509.00	507.00	472.00	521.00	495.00	574.00

Several literature sources describe the composition of cell walls in grass straw and its cellulosic ethanol potential. Goff et al. (2010) found that, for sorghum biomass, the theoretical ethanol potential ranged from 560 to 610 L/t of dry biomass. Kumar & Murthy (2011) reported that straw of tall fescue contained 94.6% solids, 10.6% ash, 6.7% CP, 19.08 % water extractives, 0.32% ethanol extractives, 32.4% glucan, 14.6% xylan, 2.9% galactan, 3.0% arabinan, 15.6% acid-insoluble lignin, 1.3% acid-soluble lignin and estimated ethanol potential to be 360.57 L/ton biomass. Danielewicz et al. (2019) mentioned that tall oatgrass biomass contained 34.7% cellulose, 15.4% lignin and 3.8% extractives; tall wheatgrass – 42.3% cellulose, 18.3% lignin and 2.3% extractives, tall fescue 34.6% cellulose, 15.9% lignin and 2.1% extractives and *Miscanthus* whole plant 40.5% cellulose, 20.6% lignin and 3.9% extractives. Doroftei et al. (2021) found that the studied straw of perennial grass seed crop contained 371-

518 g/kg cellulose and 223-314g/kg hemicellulose, the estimated theoretical ethanol yield varied from 432 to 605 L/t. Waliszewska et al. (2021) compared the chemical composition of grass species and remarked that *Arrhenatherum elatius* biomass contained 15.00% extractives, 35.46% cellulose, 17.54% lignin, 68.63% holocellulose, 33.17% hemicellulose, *Dacylis glomerata* respectively 10.59% extractives, 37.71% cellulose, 19.33% lignin, 69.19% holocellulose, 31.48% hemicellulose and *Phalaris arundinacea* - 12.77% extractives, 38.68% cellulose,15.42% lignin, 70.39% holocellulose, 31.71% hemicellulose.

The use of phytomass as substrate for biogas production directly harvested fresh mass or ensiled mass has recently become of major interest in Europe. The results regarding the quality of the *Arrhenatherum elatius* substrates and the potential for obtaining biomethane are shown in Table 6. We would like to mention that the nitrogen content in *Arrhenatherum elatius* substrates varied from 12.8 g/kg to 14.1 g/kg, the estimated content of carbon ranged from 500.0 g/kg to 509.0 g/kg, the C/N = 35.5-39.8, but the substrates from the control plant species contained 11.4-20.6 g/kg nitrogen, 492.2-519.0 g/kg carbon and C/N = 24-45. Essential differences were observed between the concentrations of acid detergent lignin and hemicellulose. As we have mentioned above, the process of ensiling decreased the lignin content and increased hemicellulose content, which had a positive effect on the activity of methanogenic bacteria. The biochemical methane potential *Arrhenatherum elatius* substrates varied from 341 l/kg VS in green mass substrates to 361 l/kg VS in ensiled mass substrates, being much higher than in *Avena sativa* substrate. The best biomethane potential were achieved in *Hordeum vulgare* silage substrates.

Table 6. The biochemical biomethane production potential of the investigated substrates from *Arrhenatherum elatius*

Indices	*Arrhenatherum elatius*		*Festuca pratensis*		*Hordeum vulgare*		*Avena sativa*
	green mass	silage	green mass	silage	green mass	silage	green mass
Crude protein, g/kg DM	80.00	88.00	71.0	80.00	119.00	129.00	95.00
Nitrogen, g/kg DM	12.80	14.10	11.4	12.80	19.00	20.60	15.20
Ash, g/kg DM	83.00	100.00	80.00	96.00	75.00	114.00	65.00
Carbon, g/kg DM	509.00	500.00	511.1	502.20	513.90	492.20	519.00
Ratio carbon/nitrogen	39.80	35.50	45.0	39.20	27.00	24.00	34.00
Acid detergent lignin, g/kg DM	38.00	26.00	33.0	22.00	29.00	18.00	46.00
Hemicellulose, g/kg DM	299.00	304.00	298.0	307.00	261.00	224.00	258.00
Biomethane potential, L/kg VS	341.00	361.00	347	367.00	361.00	379.00	329.00

There are different results reported in research studies conducted by other authors.

Ebeling et al. (2013) found that dependent of harvest dates and levels of fertilizer application the specific methane yield of *Arrhenatherum elatius* biomass substrates ranged between 311 and 347 l/kg VS; *Dactylis glomerata* substrates 313-334 l/kg; *Lolium perenne* substrates 320-335 l/kg VS and *Festuca rubra* substrates 312-331 l/kg VS.

Goliński & Goliński (2013) reported that the harvested biomass from the semi-natural grasslands that were mainly represented by *Arrhenatherion* alliance contained 308 g/kg dry matter, 10.35% CP, 6.36% ash, 50.98% NDF, 31.61% ADF and and methane yield was 338 l/kg VS. Boob et al. (2019) remarked that the methane yield of the biomass from *Arrhenatherion* grasslands was 300 l/kg VS. Von Cossel et al. (2019) found that the first-cut biomass substrate from *Arrhenatherion* grasslands were characterized by 240-297 g/kg DM, 7.0-8.1% ash, 4.7-5.7% lignin, 29.3-31.9% Cel, 20.7-25.2 % HC, 1.4-1.7% N and the methane yield ranged from 289 to 297 l/kg VS. Meserszmit et al. (2021) mentioned that the herbage from *Arrhenatherum elatius* and *Dactylis glomerata* plant community contained 8.00% CP, 3.17% EE, 57.30% NDF, 16.56% HC, 29.47% Cel, 11.24% lignin, 7.73% ash, and methane yield was 249 l/kg VS, but the herbage from *Agrostis capillaris* and *Festuca rubra* plant community - 9.29% CP, 2.71% EE, 55.73% NDF, 19.46% HC, 24.06% Cel, 12.21% lignin, 6.51% ash, and the methane yield was 244 l/kg VS, respectively.

CONCLUSIONS

The local ecotype of tall oatgrass, *Arrhenatherum elatius*, is suitable for grassland restoration and also may be used as fodder for livestock, also as substrate for bioethanol and biomethane production as a source of renewable energy.

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A NEW RAPESEED TREATMENT COMPOSITION FOR IMPROVED GERMINATION

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Abstract

Seed coating practice is used to improve plant cultivation by adding fungicides and insecticides in the form of an artificial outer layer. This technique of treatment and coating of seeds can help to improve germination and early establishment of plants. The combined application of rapeseeds treatment products with bioactive compounds and binder formulation leads to better protection of plants against pests, drought, and better ease of handling. In this context, the synthesis of a new coating composition for rapeseeds treatment was investigated. Both the raw material and the final product were analyzed using specific methods. The influence of the new coating composition on germination was studied. Experimental data indicated an increase in germination after 5 days at a temperature of 20°C for rapeseeds treated with new coating formulation which contains 18.76% hydrolyzed collagen and 3.7% thyme essential oils microencapsulates.

***Key words**: coating, formulation, germination, thyme essential oil, collagen hydrolysate.*

INTRODUCTION

Seed coating is the practice of covering seeds with external materials to improve handling, protection, and, to a lesser extent, germination enhancement and plant establishment. With an annual value exceeding US$1billion dollars, this technology is mostly the preserve of the private research sector, with few links to the scientific community (Pedrini et al., 2017).
Seed coating was developed as a common practice to improve plant cultivation by adding fungicides and insecticides in the form of an artificial outer layer. This practice can contribute to a certain extend to germination enhancement and early plant establishment. In addition to delivering active ingredients, artificial coatings modify the physical properties of the seeds by improving seed handling through standardization of seed weight and size. Thicker coatings are usually applied to small or morphologically uneven seeds (Chena et al., 2020).
The coating includes three techniques routinely used for seeds: film coating, encrusting and pelleting. Coating plant seeds before planting is a common practice in modern agriculture. The term "coated seed" has been applied to a seed, which was either pelleted, coated, or covered with an adhesive film. Coated seed may, in some circumstances, be produced by a dry powder process, which can have several disadvantages, such as poor adherence, non-uniform application, generation of significant amounts of dust, etc. Coating with wet powder method such as a polymer is a successful technology which can be used either singly or in a combination of other pesticides as a formulation to protect the seeds from biotic stresses like insect-pests and also reduce losses and protect from environmental concerns (Chandrika et al., 2017).
Seed coating is a mechanism involving the application of necessary materials in such a way that they affect the seed or soil at the seed–soil interface. The coating can protect the seed from many kinds of diseases and pests (Lawinska, 2019). The physical properties and thickness of the seed treatment/coating are critical factors that influence seed germination and seedling vigor. A thick hard seed coating can reduce, delay, or cause abnormal

germination or may even be toxic, while a minimal, fragile seed coating can break or disintegrate before planting or not have a high enough dosage of an active ingredient to be effective (Qiu et al., 2020).
Several problems have been encountered in the development of seed treatments. For example, binder formulations that have been developed have one or more of the following disadvantages: low formulation stability, low seed flowability, high levels of dust- off of the pesticide, and other coating ingredients from the seed before planting, and poor plantability characteristics. In addition to being unpleasant for personnel who handle the treated seeds, dustiness increases potential exposure to inherent hazards of the active ingredient(s) present in the coating. Dusting also has the possible effect of reducing the efficacy of the coating due to the loss of a portion of the active ingredient(s) from the seed. Another major problem encountered in the development of binder formulations is that various ingredients that have been included in binder formulations in an effort to impart beneficial properties have an overriding negative effect on the flowability of the coated seeds (Wagner, 2013). Therefore, specialized seed coating formulations must be developed and evaluated to be utilized effectively for any given plant species and agronomic purposes (Qiu et al., 2020).
Hydrolyzed collagen (protein hydrolysate) applications were reported to enhance plant growth and yield in field tomato (Parrado et al., 2008), greenhouse tomato, papaya, maize seedlings, broccoli, and tomato seedlings. Protein hydrolysates were also reported to have ameliorating effects on abiotic stress in plants (Ertani et al., 2013). An alfalfa plant-derived biostimulant increased maize plant biomass under salinity stress, and enhanced K^+ accumulation and reduced Na^+ accumulation inroots and leaves (Ertani et al., 2013). Combined application of plant-derived protein hydrolysate and beneficial microorganisms improved lettuce root system architecture, chlorophyll synthesis, and proline accumulation and enhanced lettuce tolerance to salinity and alkalinity (Wilson et al., 2018). An alternative to primary protein resources is secondary resources such as rawhide waste from the leather industry, which is not used, although they may lead to similar performance to that of major resources and exclude contamination risks because they have already undergone severe enough chemical processing to destroy bacteria and viruses. Also, for the extraction of proteins used in agriculture, secondary collagen resources, such as by-products from the leather processing industry, represent a viable alternative (Niculescu, 2017). A collagen-based product used to coat the surface was first produced and used in the EU and Russia. Importantly, processing collagen-based materials into valuable materials for agriculture will help solve the problem of solid waste generated by the leather industry. Processing collagen-based materials into valuable materials for agriculture will help solve the problem of solid waste generated by the leather industry (Lawinska et al., 2019). Rapeseed (*Brassica napus* spp. *napus* L.) is an important oil crop ranked on third place as oil plant production, used for producing biodiesel as well as in human alimentation and in chemical industry. (Haraga et al., 2023). Rapeseed crop is a very sensitive crop to germinative seedbed preparation due to small seed dimension and a need of moist for germination. Lack of water in soil could have as effect a bad germination and low rate of cover of soil, late emergence of plants and increase susccebility of plants to dissease and pest attack (Haraga et al., 2023). To overcome some abiotic stress factors plant needs the application of product to stimulate their grow and development, increase the yield and quality (Mihailov, 2024). The biostimulants affect the rates of physiological activity to stimulate natural processes (Nickell et al, 1982; Raza et al., 2021). The main problem in the technology of winter rapessed crop comes down to overcoming abiotic and biotic stress in the early stage of development of plants wich coul leads to a failure of sown area (Mihailov, 2024).
Thyme is an aromatic plant belonging to the Lamiaceae botanical family, shows a polymorphic variation in monoterpene production, the presence of intraspecific chemotype variation being common in the genus *Thymus* (Thompson et al., 2003). Advan et all, 2006 have reveal that beneficial effect through the additive or synergistic action of several chemical. Essential oils are known to

possess antimicrobial activity (Sebesan et al., 2008). Compound isolated from thyme plants (*Thymus vulgaris* L.) eliminate pathogenic microorganisms (Sebesan et al., 2008) and could be used in organic farming. This paper reports the synthesis of a new coating composition used for the rapeseeds treatment based on collagen hydrolysates, microcapsules with thyme essential oils, chelated micronutrients, and surfactants. The influence of the different type of compositions on germination of rapeseeds have been studied.

MATERIALS AND METHODS

Seeds

Rapeseeds *(Brassica napus* spp. *napus* L.) from variety Sammy with large grain (4.5-4.8 g TGW) with high oil content (40-41%) and low glucosinats content have been used in experiments.

Chemicals

Thyme essential oil (*Thymus vulgaris* L.) was purchased from Naturela, collagen hydrolysates were provided by the National Research and Development Institute for Textile and Leather - INCDTP-ICPI, the other reactive used have been procured from Scharlab SL Spain.

Synthesis of thyme essential oil microcapsules

Microencapsulation of thyme essential oil (*Thymus vulgaris* L.) was performed according to Enascuta et al., 2018, by a complex coacervation method in the first phase followed by the crosslinking phase in the presence of ultrasound (Enascuta, 2013).

Synthesis of the new coating composition for rapeseeds treatment

The new coating composition used for rape seeds treatment includes collagen hydrolysates, containing peptides with low molecular weights, having the role of stimulating the germination process; microcapsules with essential oils, with the role of biopesticide and preservation with controlled release; chelated micronutrients for plant growth and surfactants, to ensure the quality of the suspension.

The aqueous solution containing collagen hydrolysate was obtained by stirring at 500 rpm and a temperature of 30°C. In a reaction vessel containing the previously obtained hydrolyzed collagen solution (18.76%) was gradually added for 30 minutes under stirring and at room temperature 3.7% microcapsules with thyme essential oil, urea, and micronutrient chelates. For a better homogenization, the obtained bioactive composition was introduced in a blender at 3000 rpm.

Binder formulation

In a reaction vessel, the binder formulation is obtained by gradually adding under magnetic stirring polymers such as carboxymethylcellulose, hydroxymethylcellulose, starch, and polyvinyl alcohol. For a better adherence to the seeds is inserted a compound coating in the form of a powder of bentonite and dolomite.

The laboratory plant used to cover the seeds with the treatment composition consists of a mixing tank that can rotate around the axis. The speed of the mixing tank is variable, with values between 20 and 150 rotations/min.

Experiments regarding the germination effect of the rape seeds treated using the new coating composition (F1) were performed in the laboratory and the obtained values being compared with untreated rape seeds (Mt) and rape seeds treated with a coating composition with a lower concentration (F2) of collagen hydrolysates (12.3%) and microcapsules of thyme essential oils (1.2%).

After coating, the seeds were placed on filter paper dishes, 100 seeds/dish in 4 repetion, and then placed in the thermos-cabinet germinator at 20°C and 80% relative humidity in light condition. Sammy hybrid rapeseeds with TGW (Thousand Grain Weight) 4.68 g were used for testing.

Chemical characterization

The chemical composition of thyme essential oils was acquired using GC-MS/MS TRIPLE QUAD (Agilent 7890 A) with DB-WAX capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) and helium as carrier gas at 1 mL/min (Enascuta, 2018).

The morphology of the microcapsules was done using a scanning electron microscope (SEM) with a scanning electron microscope FEI QUANTA 200 (FEI Company, USA).

The new coating composition used for rape seeds treatment was analyzed in terms of total organic nitrogen (SR EN 15478:2009), ash (AOAC 920.153), density (SR ISO 758:1995), pH (SR EN 10523:2012), total sulfur (SR ISO 10084:1995) by gravimetric methods and Mn, Zn, Cu, Mg by ICP-OES method.

RESULTS AND DISCUSSIONS

Chemical composition of Thyme essential oil

The major components identified in the essential oil of Thyme essential oils were Thymol (39.29%), Isothymol (31.53%), Limonene (13.67%) and p-Cymene (8.13%), as shown in the Figure 1.

It has been observed that thyme essential oil is an important biocide; the large amount of important compounds gives the new coating composition for rapeseeds treatment both biocidal properties and preservatives properties.

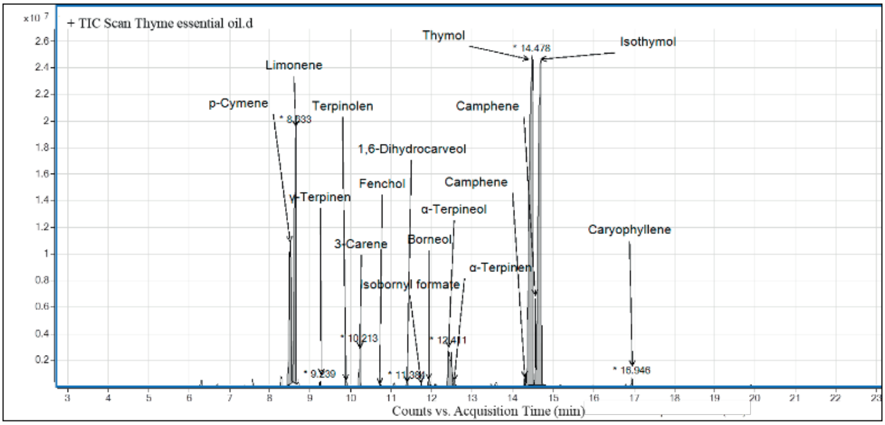

Figure 1. Chemical composition of Thyme essential oil

Chemical composition of collagen hydrolysate

The chemical composition of collagen hydrolysate was found to be the following: dry substance: 34.66%, total nitrogen: 14.8% and ash: 4.24%, protein substance: 83.18 and pH: 7.78.

Scanning electron microscope (SEM) analysis of microcapsules Thyme essential oil

Images obtained by electron microscopy looks like the rough surface of microcapsules with a few visible holes, cracks, and pores (Figure 2).

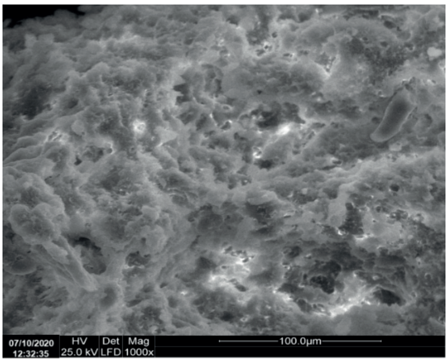

Figure 2. SEM imagines of Thyme oil microcapsules surface

Sponge-shaped structure with a large number of pores of very small size is also related to the loss of water trapped by the collagen hydrolysate network during the preparation of the sample for SEM.

Chemical characterization of the new coating composition

The values obtained from the chemical characterization of the new coating composition (F1) for the treatment of rapeseeds (Table 1) are compared with the values composition (F2) with a lower content of hydrolyzed collagen and a lower concentration of thyme oil microcapsules.

Table 1. Chemical characterization of the new coating composition

Characteristics	Values	
	F1	F2
pH	8.52	7.85
Density, g/cm^3	1.0734	1.0623
Total organic nitrogen (TKN), % (w/w)	4.76	2.76
Total sulfur, % (w/w)	0.15	0.12
Ash, % (w/w)	0.83	0.41
Mn (λ=257,610 nm), mg/kg	225	132
Cu (λ=327,393 nm), mg/kg	130	112
Zn (λ=213,857 nm), mg/kg	100	102
Mg (λ=280,271 nm), mg/kg	141	128

Influence of the new coating composition on germination

The experiences were of bifactorial type, A Factor represents the rapeseeds tested with two accessions: untreated seeds - a1, seeds coated with the new coating formulation - a2 and seeds treated with different coating formulation - a3.

B Factor represents the temperature from the growth chamber after applying the treatments of the seeds.

Because the germination values showed very large variations depending on the germination temperature, for a uniform evaluation of the impact of the treatment with the new coating composition on the germination, the determinations were performed at 5 days.

Table 2 shows the influence of treatment applied to rapeseeds with the new coating composition on germination, for each test temperature (A x B).

It was found that for the low temperatures 5°C the application of seed treatments composition determined statistically assured increases of germination, while at temperatures of 15°C and 20°C respectively although increases are recorded in all cases, germination increases are statistically ensured only in the case of the variant treated with F1.

Regarding the influence of test temperature on germination, there are significant increases in germination with increasing temperature for seeds treated with new coating composition (93.5 at 20°C).

Table 2. Influence of treatment applied to rapeseeds with the new coating composition on germination

		a_1 Mt	a_2 F1	a_3 F2
b_1 5ºC	%	66	71	68.5
	dif	Mt	5	2.5
b_2 10ºC	%	77.5	81.75	79.5
	dif	Mt	4.25	2
b_3 15ºC	%	88	91.5	89.75
	dif	Mt	3.5	1.75
b_4 20ºC	%	90.25	93.5	91.5
	dif	Mt	3.25	1.25
DL 5 %	3.125			
DL 1 %	4.238			
DL 0.1 %	6.514			

In the case of variants in which the test temperature was 5°C and 10°C, respectively, it is found that the products had the most intense impact; the tested products increase the resistance of the seeds to thermal stress.

Due to the optimal concentration of hydrolyzed collagen, the highest germination value 93.5 was observed at a temperature of 20°C for F1 while the untreated sample showed a value of germination of only 90.25. Coating of seeds with the new coating product based on thyme essential oil coarcevated and collagen hydrolysate in high concentration (F1), present a significant increase of germinative rates at all temperature levels and also an increase of germination rate comparative with coating product based on collagen with lower concentration (F2)

CONCLUSIONS

The synthesis of a new coating composition for rapeseeds treatment have been investigated. Thyme essential oil with antibacterial, antifungal and preservative properties have been analyzed using GC-MS/MS TRIPLE QUAD, the morphology of the essential oil microcapsules have been made using a scanning electron microscope (SEM) analysis and the composition of the new product have been determined using standard methods of analysis. The influence of the new coating composition on germination have been studied through laboratory experiments. Experimental data indicated a significant increase in germination after 5 days at a temperature of 20°C for rapeseeds treated with new coating formulation which contains 18.76% collagen hydrolysate and 3.7% thyme essential oils microencapsulates. For a lower qualitative germination condition (lower temperatures 5, 10 or 15°C the influence of the coating formulation (F1) have a higher influence on g process the highest influence of coating product have been reported at lowest germination temperature.

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THE ROLE OF TECHNOLOGY IN AGRICULTURAL FARM MANAGEMENT

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Abstract

Agriculture is a fundamental pillar of the global economy, contributing to food security, job creation and economic development. Effective farm management becomes crucial to maximize productivity and sustainability in this context. Agricultural farms can be classified according to their size: small, medium and large. Each category presents specific challenges and opportunities, and optimal management must be tailored to these characteristics. This article aims to explore the role of technology in agricultural farm management, analyzing the various solutions and applications available and providing conclusions and proposals for the future. This article looks at the use of drones and monitoring systems, smart irrigation systems, precision agriculture, the use of blockchain technology in the agricultural supply chain, and the integration of IoT in agriculture.

Key words: *farm management, innovative technologies, precision agriculture.*

INTRODUCTION

Agriculture, one of the oldest human activities, has evolved significantly over time, becoming increasingly sophisticated and efficient. In the digital age we live in, technology plays an increasingly important role in all aspects of life, including agriculture.

Today, farmers face many challenges, including increasing demand for food, climate change, dwindling natural resources and the need to maintain environmental sustainability. In this context, technology becomes an essential tool for optimizing the management of agricultural farms and improving efficiency, productivity and sustainability.

The optimal management of agricultural farms requires an adaptive and strategic approach, taking into account the specifics of each category of farm. By implementing modern technologies, using data (Kamilaris et al., 2017), diversifying production (Schöning et al., 2023), and collaborating and adopting sustainable practices (Jeroen et al., 2012), farmers can significantly improve the performance and sustainability of their holdings (Prisacaru & Caradja, 2019).

The implementation of modern technologies in agriculture (Alotaibi & Nadeem, 2021; Bhatia & Bhat, 2020) allows farmers to better manage their resources, increase productivity and improve the sustainability of their operations (Vesa, 2008). From the use of drones and monitoring systems to precision agriculture and IoT integration (Jirapond et al., 2019; Piancharoenwong & Badir, 2024; Ravesa & Shabir, 2022), technology brings numerous benefits that can radically transform the way agricultural activities are conducted.

MATERIALS AND METHODS

To achieve the main goal of the paper, research reports and specialist articles were studied to identify current trends, challenges and proposed solutions to emphasize the role of technology in the management of medium-sized farms. This will allow a deep understanding of the management dynamics of medium-sized farms and provide a solid basis for formulating relevant conclusions and proposals.

RESULTS AND DISCUSSIONS

1. Use of drones and monitoring systems

Drones and monitoring systems are a major innovation in agriculture, giving farmers the ability to collect accurate and detailed data about field conditions. These technologies

enable real-time crop monitoring, problem detection and prompt intervention to minimize losses and maximize yields.
Drones equipped with multispectral cameras can capture detailed images of fields, identifying areas affected by disease, pests or nutritional deficiencies. This data is then analyzed to provide accurate recommendations on the necessary treatments. For example, drones can detect water stress in crops, allowing farmers to adjust irrigation to save water and maintain plant health.
Monitoring systems based on sensors placed in the field can measure soil moisture, temperature, nutrient levels and other important parameters. This information is transmitted in real-time to farmers, who can make informed decisions about resource management and application of agricultural inputs. Continuous monitoring also allows for early identification of problems and their prevention, thus reducing the need for costly interventions and significant damage.

2. Smart irrigation systems

Climate change and increased competition for water resources have made efficient water management essential in agriculture. Smart irrigation systems use sensors and monitoring technologies to assess soil moisture, water needs and weather conditions, providing accurate and efficient irrigation.
These systems allow the automatic adjustment of applied water quantities according to the specific needs of crops and environmental conditions. For example, a smart irrigation system can reduce or stop irrigation on rainy days, thus saving water and reducing costs. At the same time, it can increase the amount of water applied during periods of drought, ensuring that plants receive enough water to grow healthily.
In addition to water savings, smart irrigation systems help reduce soil erosion and improve soil quality. By precisely applying water, excessive soil saturation and nutrient runoff are avoided, thus maintaining long-term soil fertility.

3. Precision agriculture

Precision agriculture is a modern approach that involves the use of advanced technologies to precisely monitor and manage agricultural operations. This is based on the collection and analysis of data to optimize the use of resources and increase productivity.
One of the pillars of precision agriculture is the use of GPS systems for accurate land mapping and monitoring of agricultural operations. Farmers can create detailed field maps, identifying specific problem areas such as nutrient-poor soil or areas of low crop density. This information allows the precise application of agricultural inputs, such as fertilizers and pesticides, according to the needs of each area.
Sensors and drones are also essential in precision agriculture, providing detailed and real-time data on the condition of crops. This information is analyzed to provide accurate recommendations on required treatments, irrigation optimization and resource management. For example, soil sensors can measure nutrient and moisture levels, allowing farmers to adjust fertilization and irrigation to maximize yields.
Agricultural management software plays a crucial role in integrating and analyzing the collected data. These applications allow farmers to monitor crop performance, plan farm operations and make informed decisions based on hard data. The software can also provide weather forecasts, pest warnings and recommendations to optimize production.

4. Using blockchain technology in the agricultural supply chain

Blockchain is a data storage and transmission technology that ensures transparency, safety and efficiency in the agricultural supply chain. By using blockchain, information about the origin, processing and transport of agricultural products can be securely and transparently recorded, thus ensuring their quality and authenticity.
In the agricultural supply chain, blockchain can be used to track products from farm to consumer. Every stage of the process, from harvesting and processing, to transport and sale, can be recorded on a blockchain, providing complete traceability. This allows any problems, such as food contamination or fraud, to be quickly identified and the necessary corrective action taken.

Blockchain can also improve the efficiency and transparency of business transactions. By using smart contracts, farmers and traders can enter into automated agreements based on predefined conditions. For example, a smart contract can stipulate that payment for agricultural products will be made automatically upon their delivery in good condition. This reduces the risk of misunderstandings and delays in payments, ensuring a more efficient and secure supply chain.

5. Integration of IoT in agriculture

IoT is another technology that is transforming the way farmers manage farm operations. IoT devices such as soil sensors, weather sensors, and Internet-connected agricultural equipment enable real-time monitoring of environmental conditions and crop performance.

IoT soil sensors can measure moisture, temperature and nutrient levels, providing farmers with detailed information about soil health. This data is transmitted in real-time to farmers, who can make quick and informed decisions about irrigation, fertilization and other necessary interventions. Continuous soil monitoring also allows for the early identification of problems and their prevention, thus reducing the need for costly interventions and significant damage.

IoT weather sensors can provide accurate forecasts and warnings of extreme weather conditions, allowing farmers to plan their operations based on weather conditions. For example, sensors can warn farmers of impending frost, allowing them to take measures to protect crops. These warnings can also help farmers optimize the timing of planting and harvesting, thereby maximizing yields and reducing the risks associated with adverse weather conditions.

Internet-connected agricultural equipment such as smart tractors and combines enable remote monitoring and control of farming operations. This equipment can be programmed to perform specific operations such as planting, irrigating and harvesting based on data collected by IoT sensors. This enables the automation and optimization of agricultural processes, reducing the need for manual intervention and improving efficiency.

SWOT analysis of the role of technology application in agricultural farm management

Strengths

1. Increasing operational efficiency:
- The use of technology, such as precision agriculture, automation and digitization, allows for more efficient management of resources and daily activities.
- Real-time monitoring of crops and soil reduces losses and optimizes the use of agricultural inputs (water, fertilizers, pesticides).

2. Cost reduction:
- Automation and robotization can significantly reduce labour costs.
- Using data and predictive analytics helps prevent problems and reduce risk, thereby saving financial resources.

3. Improving product quality:
- Precise monitoring of growing conditions and optimal application of inputs lead to obtaining higher quality agricultural products.
- Post-harvest technologies such as humidity and temperature sensors help maintain product quality during storage and transport.

4. Access to information and data:
- Modern technologies enable the collection and analysis of a large amount of farm data, which can be used to make informed and strategic decisions.
- Digital farm management platforms provide an overview and planning tools that improve overall management.

Weaknesses

1. High initial costs:
- Implementation of advanced technologies requires significant financial investment, which can be an obstacle for small and medium farms.
- Equipment and software maintenance and upgrade costs can also be high.

2. The need for technical skills:
- Effective use of modern technologies requires advanced technical skills and continuous training.
- The lack of qualified personnel in the use and maintenance of technological equipment can represent a disadvantage.

3. Dependence on infrastructure:
- Advanced technologies depend on basic infrastructure such as internet connection and electricity, which can be deficient in rural areas.

- Technical problems or equipment breakdowns can cause interruptions in farm activity.
4. Data security:
- Using digital platforms involves cyber security risks such as data loss or theft.
- Protecting sensitive information and ensuring compliance with data protection regulations requires additional measures and costs.

Opportunities

1. Continuous innovations:
- The rapid development of agricultural technologies provides continuous opportunities to improve farm management.
- Early adoption of new technologies can provide a competitive advantage in the market.
2. Access to new markets:
- Modern technologies allow product traceability and compliance with quality standards, facilitating access to national and international markets.
- Digital marketing and e-commerce platforms open up new sales and promotion channels.
3. Sustainability and ecological responsibility:
- Efficient use of resources and reduction of negative impact on the environment contribute to agricultural sustainability.
- Green technologies and sustainable practices can attract environmentally concerned consumers and potential investors.
4. Funding and subsidies:
- There are numerous funding programs and grants available to farmers who want to adopt advanced technologies.
- Government and international organization support for the digitization of agriculture can facilitate access to funds and resources.

Threats

1. Market and price volatility:
- Fluctuations in the prices of agricultural inputs and final products can affect the profitability of technology investments.
- Economic and commercial uncertainties can negatively influence profitability and long-term planning.
2. Climate change:
- Extreme and unpredictable climatic conditions can negatively affect the efficiency of agricultural technologies.
- Adaptation to climate change requires additional investments and the development of new technological solutions.
3. Regulations and compliance:
- Legislation on the use of technology and data protection may impose additional constraints and costs on farmers.
- Changes in agricultural regulations and policies can create uncertainties and risks for technology investments.
4. Resistance to change:
- Conservatism and reluctance to change can delay the adoption of advanced technologies in agriculture.
- Cultural and educational barriers can limit the acceptance and effective use of new technologies by farmers.

CONCLUSIONS

- Improved efficiency and productivity: the use of technology in the management of agricultural farms leads to a significant increase in efficiency and productivity, by optimizing the use of resources and agricultural processes.
- Improved sustainability: modern technologies contribute to reducing the impact on the environment, by reducing the consumption of water, energy and chemical inputs. They also enable more efficient management of natural resources and maintenance of soil fertility in the long term.
- Food quality and safety: the integration of technology in the agricultural supply chain ensures the traceability and authenticity of food products, helping to guarantee their quality and safety. Blockchain and other data storage technologies enable the rapid identification of problems throughout the agri-food chain and the prevention of fraud.
- Adaptability to climate change: IoT technologies and monitoring systems allow farmers to quickly adapt to climate change and effectively manage the risks associated with adverse weather conditions. This contributes to reducing vulnerability and maintaining the stability of agricultural production.
- Automation and cost reduction: Smart farm equipment and automated irrigation systems enable the reduction of manual intervention and operational costs. Automating agricultural processes improves efficiency and allows farmers to

focus on other important aspects of farm management.

Proposals regarding the implementation of technology in agricultural holdings

- Investment in technology and innovation: farmers should be supported in adopting modern and innovative technologies through subsidy and financing programs. Investment in research and development is also essential to continue innovation in agriculture.
- Education and training: farmers need to be educated and trained on the use and benefits of modern technologies. Training programs, information and knowledge transfer, and workshops can help farmers understand how to effectively integrate modern technology into their daily operations.
- Digital infrastructure: the development of digital infrastructure, high-speed internet networks and IoT infrastructure, is essential for the effective implementation and use of modern technologies in agriculture. Companies in the technology sector should work together to ensure access to the necessary infrastructure.
- Collaboration and partnerships: farmers, researchers, technology companies and government institutions should collaborate to develop and implement innovative technology solutions to provide to farmers. Partnerships between different actors in the agricultural sector can facilitate the exchange of knowledge and resources, helping to optimize the management of agricultural farms.
- Sustainability policies: promoting policies and regulations that encourage the use of sustainable technologies in agriculture that include environmental protection measures, efficient management of natural resources and reduction of greenhouse gas emissions.

In conclusion, technology and innovation play an essential role in transforming and optimizing agricultural farm management. Through the use of drones, smart irrigation systems, precision agriculture, blockchain technology and IoT, farmers can improve the efficiency, productivity and sustainability of their operations. Investments in technology, education and continuous training of farmers, but also the development of digital infrastructure and the promotion of sustainability policies are essential to fully exploit the potential of technology in agriculture. This will enable all farmers to meet future challenges and ensure sustainability and global food security.

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