



UNIVERSITY OF AGRONOMIC SCIENCES  
AND VETERINARY MEDICINE OF BUCHAREST  
FACULTY OF AGRICULTURE



# SCIENTIFIC PAPERS

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# SOIL SCIENCES



## THE USE OF SATELLITE IMAGES FOR THE IDENTIFICATION OF SALINIZED SOILS IN BRĂILA COUNTY

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### Abstract

*The high salt content in the soil raises special problems regarding the use of soils affected by salinity and alkalinity. In Romania, there is an area of approximately 614,000 ha of land where salinization-alkalization processes occur, causing a low fertility of these soils and at high concentrations, these soils cannot be used for agricultural production. In order to identify the areas occupied by these soils, research was carried out based on satellite images in three localities in Brăila county: Măxineni, Racoviță and Tudor, where laboratory determinations such as: soil reaction, content in chlorides, sulfates, magnesium, calcium, sodium and potassium. With the help of remote sensing and satellite images, maps were made regarding the salinity index (SI), Bare Soil index (BSI), Soil Adjusted Vegetation Index (SAVI), Tasseled Cap Transformation Wetness (TCW). Our study demonstrated the ability of remote sensing techniques and geographic information systems to survey, monitor, identify, analyze and classify land degradation manifestations and to detect spatial and temporal variables occurring in the study area. The digital indicators (INDEX) contributed to highlighting the terrain characteristics very effectively helping in clarifying the spatial distribution picture and the quantitative and qualitative evaluation of the types of soil degradation manifestations in the studied area.*

**Key words:** salinity, soil, remote sensing, GIS, deterioration index.

### INTRODUCTION

In all countries, there are concerns regarding the improvement of soils affected by salinization and of those at risk of salinization, for the purpose of increasing agricultural production, reducing the content of soluble salts in the soil and therefore, increasing the range of crops suitable for these lands, obtaining the techniques of the elements required for improving reproduction technologies, environmental protection and the economic efficiency of improvement technologies, as well as for increasing the standard of living in areas with saline and alkaline soils (Coteț Valentina, 2010). Soil salinity adversely affects seed germination, crop productivity and soil and water quality, especially in semi-arid and arid regions of the world, leading to loss of arable land and soil degradation. It is continuously increasing at an alarming rate and is accepted as a widespread environmental problem that endangers agricultural practices, as detailed by scientists in different parts of the world, at different intervals of time (Zhu, 2001; Metternicht and Zinck, 2003; Zheng et al., 2009). In general, there are

two main reasons for soil salinity. One relates to human-induced activities and the other is due to natural factors. Extensive use of poor quality irrigation water, due to the occurrence of extreme drought events, combined with intensive fertilizer application are the main human-induced activities resulting in soil salinization (Perez-Sirvent et al., 2003; Barbouchi et al., 2014).

Soil salinization is a dynamically unfolding process with significant social and economic aspects. Thus, for the sustainable management of arable regions and natural ecosystems, adequate and accurate information on the spatial magnitude and variability of salinity distribution is essential in order to monitor it in a timely manner and to prevent further growth of salt-affected areas (Allbed and Kumar, 2013). Periodic monitoring of soil salinity is a must for proper management of soil and water resources, however it is quite difficult to detect the required soil information in regions affected by salinity as referred by Taha Gorji et al. (2015). The Food and Agriculture Organization of the United Nations (FAO) has estimated that nearly 397 million hectares of the world's surface are

covered by salinized and alkalinized soils including almost all continents. Research carried out worldwide has established that soil salinity can be assessed using salinity indicators such as the Difference Vegetation Index (NDVI) with which soil salinity can be assessed (Albed and Kumar, 2013).

To overcome this limitation, several techniques have been developed to assess soil salinity. One such technique is based on remote sensing (RS), which has demonstrated considerable success in mapping and assessing soil salinity (Wu W, Mhaimeed A.S., 2014; Asfaw E., 2018; Garcia L., 2005).

Metternicht (1998), Metternicht and Zinck (2003), Eldeiry and Garcia (2010) and Furby et al. (2010) noted that significant results could be obtained by studying the spectral properties and radar backscatter of saline soils. Some researchers (e.g., ref. Brunner P., Li H., 2007) have studied soil salinity based on moisture content using the normalized difference infrared index. Other researchers have evaluated the relationships between soil salinity and vegetation indices (Ibrahim M., Koch B., Data P., 2020). Other studies analyzed soil salinity using thermal and short infrared wavelength bands (Ibrahim M., Abu-Mallouh H., 2018) to examine the relationship between soil salinity and land surface temperature (LST). These studies used satellite images containing thermal bands, such as Moderate Resolution Imaging Spectroradiometer (MODIS), which provide useful information on soil properties (Zhang T.-T., Zeng S.-L., Gao Y., Ouyang Z.-T., 2011). Recently, multispectral data derived from sources such as System Pour I, Observation de la Terre (SPOT), IKONOS, Quick Bird, Indian Remote Sensing and Landsat satellites have been used to explore soil salinity maps. Several other indices, such as the salinity index and soil-adjusted vegetation index (Brunner P., Li H., Kinzelbach W., Li W., 2007), are also commonly used to monitor soil salinity. However, Eldeiry and Garcia and Hu et al. recommended the combined use of the spectral response index and the best band (Ibrahim M., Ghanem F., Al-Salameen A., Al-Fawwaz A., 2019).

RS instruments and data must be integrated with field measurements of salinity to perform soil

salinity assessment and monitoring. RS is an effective tool for spatial analysis of soil salinity in arid and semi-arid areas. We, therefore, aim to estimate soil salinity in Abu Dhabi using specific spectral indices combined with field measurements. The soil salinity mapping model developed in this study is based on soil electrical conductivity (EC) and shows a promising correlation, which can be further improved by considering the soil salinity-LST relationship. This model is useful to develop effective soil salinity forecasting strategies for sustainable development and land management.

## MATERIALS AND METHODS

The methods used were:

Determination of pH in aqueous suspension 1:2.5; SR 7184-13:2001; PTL 04.

Determination of soluble salts from aqueous extract 1:5 (carbonates, bicarbonates, chlorides, sulfates, calcium, magnesium) STAS 7184/7-87; PTL 18.

Determination of soluble forms: potassium (K), sodium (Na); PTL 18.

Determination of electrical conductivity and estimation of the total content of soluble salts; STAS 7184/7-87 ch. 3.2; PTL 0.

Research was carried out in 2021 in three localities in Brăila County, respectively Măxineni, Racovița and Tudor, in order to determine the distribution area of soils affected by salinization and alkalization processes. Soil samples were collected at a depth of 0-20 cm and 20-40 cm and the following chemical analyzes were performed: soil reaction in aqueous suspension 1:2.5, determination of soluble salts from aqueous extract 1:5 (carbonates, bicarbonates, chlorides, sulphates, calcium and magnesium), determination of soluble forms of potassium and sodium and determination of electrical conductivity and estimation of total soluble salt content. With the help of Landsat satellite images, maps were processed and made regarding the salinity index (SI), Bare Soil index (BSI), Soil Adjusted Vegetation Index (SAVI), Tasseled Cap Transformation Wetness (TCW). The INDEX digital indicators contributed to highlighting the characteristics of the land in the researched area and the manifestation of degradation phenomena.

## RESULTS AND DISCUSSIONS

Regarding the reaction of the analyzed soils, the highest values on the 0-20 cm depth were recorded at profile 1 and 2 in the town of

Racovița, where the reaction shows values of 7.96 and 8.04 (slightly alkaline reaction). All the points analyzed show a slightly alkaline reaction at the depth of 0-20 cm, and the lowest value was of 7.42 at profile 2 from the town of Tudor.

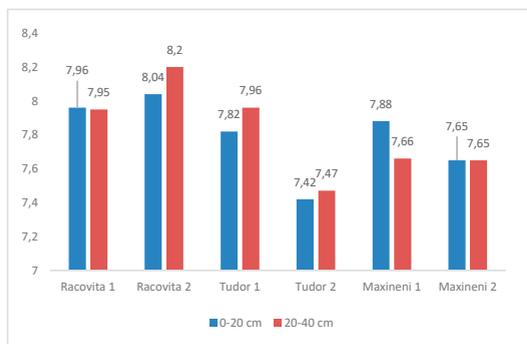


Figure 1. Soil reaction and its distribution in the investigated area

As for the depth of 20-40 cm, the highest values of the reaction were recorded at the profiles from the Racovița locality, respectively of 7.95 and 8.2 - slightly alkaline reaction, while the lowest value of the soil's reaction at this depth was recorded at profile 2 from the land of Tudor.

Regarding the content of  $\text{SO}_4^{2-}$  at the soil surface and the depth of 0-20 cm, the highest value of 347 me/100 g was recorded at profile 2 from Măxineni, indicating a moderately salinized soil, while the lowest values were recorded at the profiles from Racovița.

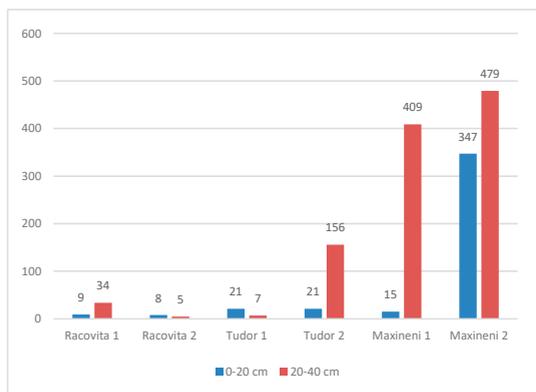
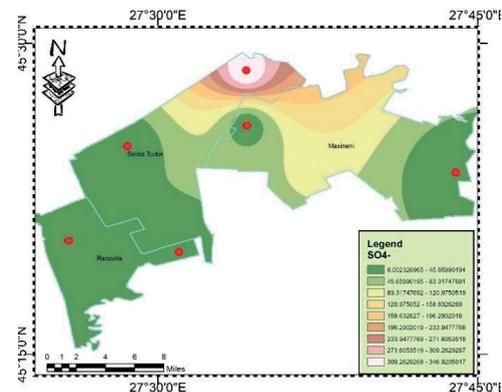
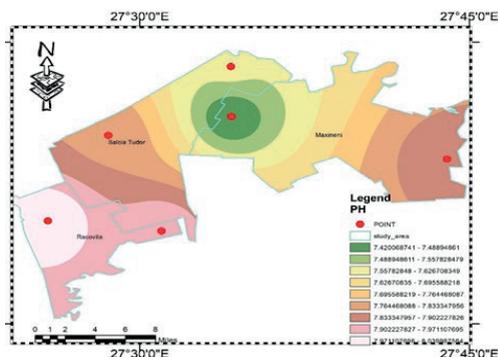


Figure 2.  $\text{SO}_4^{2-}$  content and distribution

At the depth of 20-40 cm we observed high values in the Măxineni profiles and in the Tudor profile 2, with values that vary between 156 and 479 me/100 g of soil, indicating a highly salinized soil. The highest  $\text{Cl}^-$  content at a depth of 0-20 cm was recorded at profile 2 from

Măxineni, with a value of 215 me/100 g soil and in the case of profile 2 from Tudor, where the soil is weakly salinized and moderately salinized at profile 1 from Măxineni. The lowest values were recorded at the two profiles from Racovița.



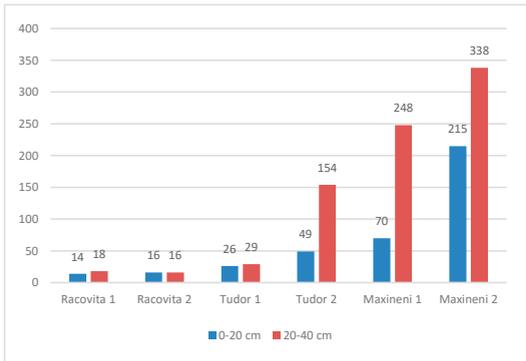
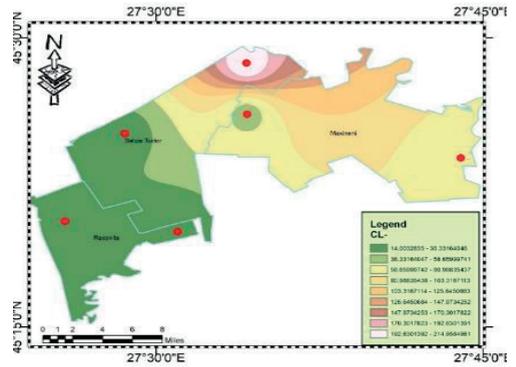


Figure 3. Cl<sup>-</sup> ion content and distribution



In the case of the 20-40 cm depth, values between 154 me/100 g of soil were recorded at profile 2 from Tudor and 248, respectively 338 me/100 g of soil at the two profiles from Măxineni, which means that the soils are slightly to strongly salinized.

It can be seen that both SO<sub>4</sub><sup>2-</sup> and Cl<sup>-</sup> contents in the analyzed soils increase with depth, therefore, at a depth of more than 40 cm, the soils become moderately to strongly salinized.

The Ca content is high in all samples analyzed at the depth of 0-20 cm with the highest values of 86 mg/100 g soil at profile 2 from Măxineni. A very low value of 7 mg/kg was recorded in profile 1 from Măxineni.

At the depth of 20-40 cm, higher calcium content values of 41 mg/kg were recorded at profile 2 from Tudor and at Măxineni with 81 mg/kg in profile 1 and 98 mg/kg in profile 2.

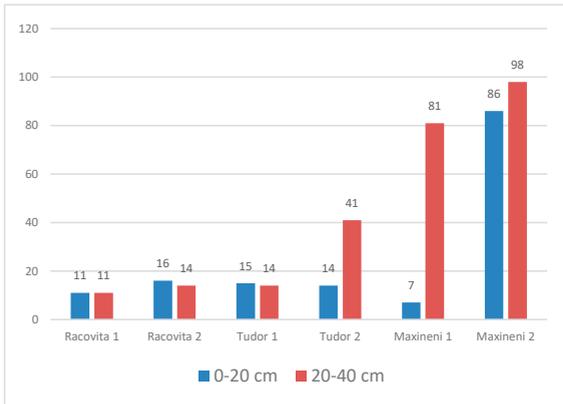
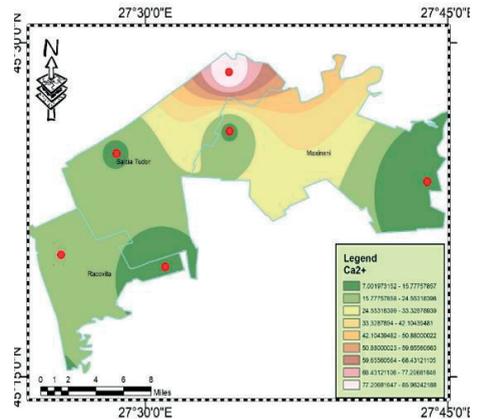


Figure 4. Calcium ion content and distribution



The magnesium content at the depth of 0-20 cm varies between 0.24 and 75 mg/kg, which represents a low to very low content.

And at the depth of 20-40 cm magnesium has a low to very low content with maximum values reaching 108 mg/kg in profile 1 from Măxineni.

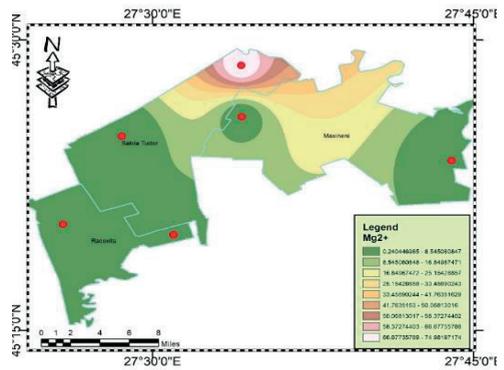
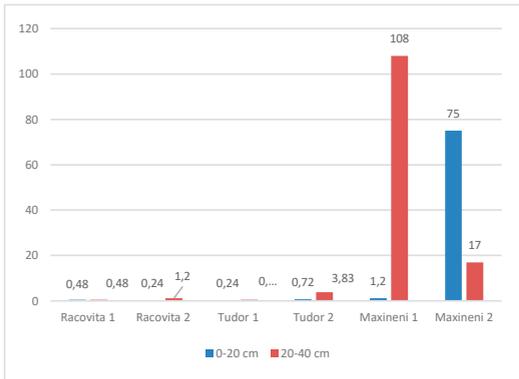


Figure 5. Magnesium content and distribution

The highest values of 58 and 89 mg/kg of the sodium content are at the profiles from Măxineni and the lowest at the profiles from Racovița, with a maximum sodium content of 2 mg/kg.

Along with the depth, the sodium content in the soil also increases, even if the values are low in the Racovița profiles, at the other point's very high sodium values were recorded at a depth of 20-40 cm.

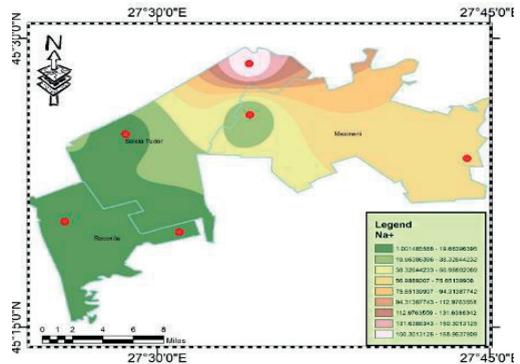
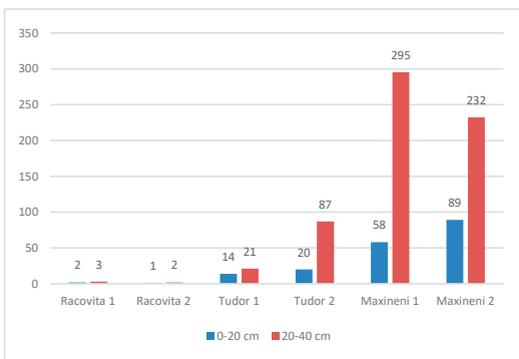


Figure 6. Sodium ion content and distribution

The potassium content varies between 8 and 15 mg/kg at the depth of 0-20 cm, which represents a low and very low content. The state of potassium supply at the depth of 20-40 cm is

medium only in profile 1 from Măxineni, with a value of 26 mg/kg and in the other profiles the potassium content in depth is low.

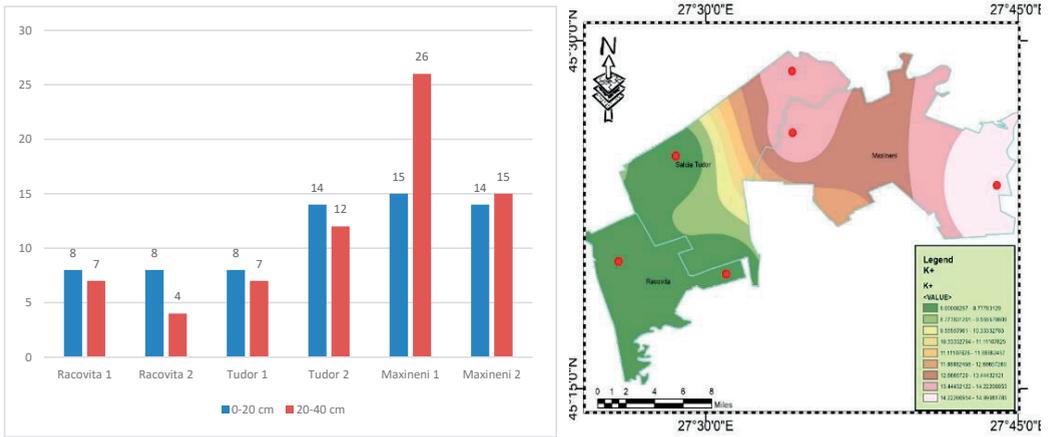


Figure 7. Potassium ion content and distribution

It can be seen from the samples analyzed in the three locations that the salinization and alkalinization of the soils increases with the depth due to the influence of groundwater loaded with

soluble salts especially of the sodium ion. Following the research carried out, 6 types of salinity index values were identified in the study area.

Table 1. Distribution of surfaces according to the salinity index

Interpretation	The salinized surface, km	Percent
No salinity	524	18.07%
Very low salinity	916	31.58%
Moderate salinity	768	26.46%
High salinity	483	17%
Excessive salinity	210	7%
<b>Total</b>	<b>2901</b>	<b>100%</b>

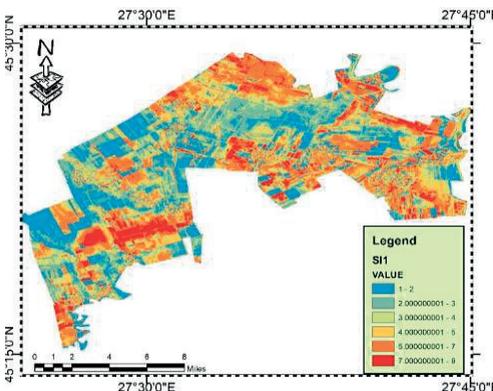


Figure 8. Salinity index (SI)

The Bare Soil index (BSI) was calculated to identify arid land, which includes non-cultivated areas (houses, roads, urban areas, rural urban areas, eroded areas and non-agricultural areas).

The results shown in Table 2 and Figure 9 indicate that there are five types of barren land guidance areas.

The reason may be due to the high percentage of salinity and the impact of difficult climatic conditions and high temperatures, which affect plant growth and thus lead to a decrease in the area covered by vegetation, especially in summer.

Table 2. Distribution of surfaces according to The Bare Soil index (BSI)

Interpretation of the aridity index	The salinized surface, km	Percent
Very dense vegetation	223	7.69%
Dense vegetation	633	21.81%
Harvested vegetation	1007	34.71%
No vegetation	768	26%
Degraded	271	9%
<b>Total</b>	<b>2901</b>	<b>100%</b>

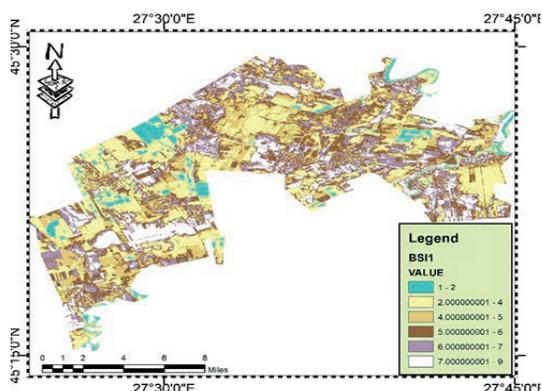


Figure 9. Bare Soil index (BSI)

### Soil Adjusted Vegetation Index (SAVI):

Soil Adjusted Vegetation Index (SAVI) is calculated as a ratio of R to NIR values with a ground brightness correction factor (L) defined as 0.5 to accommodate most land cover types. In Landsat 8,  $SAVI = ((Band\ 5 - Band\ 4) / (Band\ 5 + Band\ 4 + L)) * (1 + L)$ .

The soil brightness correction factor (L) was varied from 0 to 1 and from 1 to 100 to analyze the effect and sensitivity of NIR-red data space translation on vegetation index improvement and to determine whether a single optimal L value can be applied to a wide range of vegetation densities. Each shaded area illustrates the variations in vegetation index from a constant amount of vegetation on the two soil backgrounds. At  $L = 0$ , the NDVI behavior is shown with the width of the shaded area representing smooth variations from light to dark. As the adjustment factor (or change of

origin) is increased, there was a continuous decrease in soil-induced variations for small amounts of vegetation ( $LAI = 0-0.5$ ), and, at  $L = 1$ , soil influences nearly disappeared into these canopies. With higher vegetation density ( $LAI = 1$ ), the optimal fit was found at  $L = 0.75$  because soil influences reappeared at higher "L" values. With even higher vegetation densities, the optimum "L" values decreased to lower values. It was found that the optimal adjustment factor is linearly correlated with LAI ( $r = -0.990$ ).

The results presented in Table 3 and Figure 10 indicate that in the study area there are five ranges of SAVI index values, in different degrees, distributed between agricultural land, natural vegetation and growing vegetation. The reason is due to the impact of soil salinity and its critical degradation, as well as the decrease in the percentage of cultivated land.

Table 3. Soil Adjusted Vegetation Index (SAVI)

SAVI Interpretation	Area, km	Percent
Water	18	0.62%
Dense vegetation	1266	43.63%
Moderate vegetation	977	33.68%
No vegetation	641	22%
<b>Total</b>	<b>2901</b>	<b>100%</b>

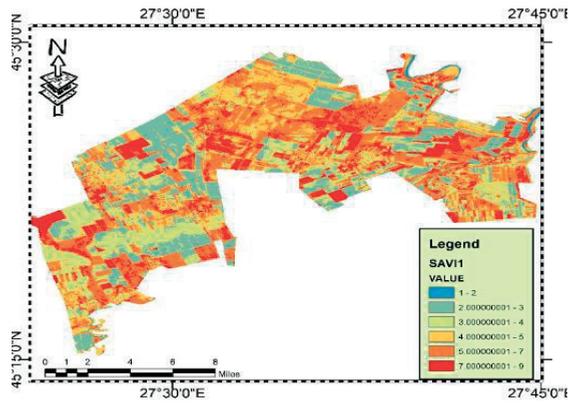


Figure 10. Soil Adjusted Vegetation Index (SAVI)

### Tasseled Cap Transformation Wetness (TCW)

Tasseled Cap Transformation Wetness (TCW) was updated by Crist (1985) for use with Landsat TM data. It has been used to determine the amount of moisture retained by vegetation or soil, thus called humidity, as well as other

indicators that indicate vegetation and soil radiance. TCW images (representing a measure of humidity determined by comparing the visible and near-infrared spectral response with the short-infrared spectral response) were derived from the ETM\_ images of the study area using the ER transform algorithm.

Table 4. Tasseled Cap Transformation Wetness (TCW)

Interpretation, Tcw	Area, km	Percent
<0	996	34.34%
>0	1905	65.67%

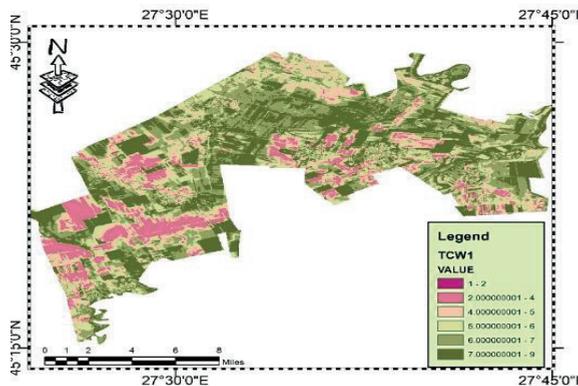


Figure 11. Tasseled Cap Transformation Wetness (TCW)

### Topsoil Grain Size Index (GSI)

Topsoil Grain Size Index (GSI) was developed based on the field survey of soil surface spectral reflectance and laboratory analyses of soil grain composition. GSI found has close correlation to the fine sand or clay-silt-sized grain content of the topsoil in sparsely vegetated arid land of Inner Mongolia, China. A high GSI value corresponds to the area with high content of fine

sand in topsoil or low content of clay-silt grains.

The GSI can be simply calculated by:

$$GSI = (R - B) * (R + B + G) \\ = (B4 - B2) * (B4 + B2 + B3)$$

Where: R, B, and G are the red, blue, and green bands of the remote sensing data, respectively. GSI value is close to 0 in the vegetated area, and for water bodies it is a negative value.

Table 5. Topsoil Grain Size Index (GSI)

Interpretations	Area, km	Percent
Water	227	7.82%
Degraded	1282	44.18%
Moderately degraded	903	31.13%
Severe degradation	490	17%
Very severe degradation	0	0%
<b>Total</b>	<b>2901</b>	<b>100%</b>

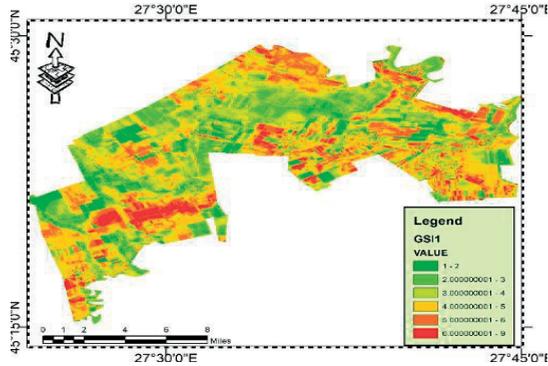


Figure 12. Topsoil Grain Size Index (GSI)

All the results obtained in the laboratory and the processing of satellite images indicated that there is a correlation between the results, especially in the area of Măxineni, where there is a high percentage of salts in the soil. The high content of salts in the soil led to the lack of land cover with vegetation.

## CONCLUSIONS

- Natural factors have a direct impact on land degradation problems in our study area. The main factors to mention are climate change, soil and water surface degradation, lack of vegetation cover and groundwater, which have led to the spread and exacerbation of deterioration manifestations in the study area, where climatic action played a major role, with effects such as high temperatures, lack of rain and wind speed which led to the drying of the soil surface, making it vulnerable to erosion and salinization processes and leading to an increase in degraded land.
- Through our study, it became clear to us that the problem of land degradation is prevalent in most of the study area in its various manifestations, but it clarified the occurrence of soil salinization is the most prominent.

- Our study demonstrated the ability of remote sensing techniques and geographic information systems to survey, monitor, identify, analyze and classify land degradation manifestations and to detect spatial and temporal variables occurring in the study area.
- The digital indicators (INDEX) contributed to highlighting the characteristics of the land very effectively by clarifying the spatial distribution picture and the quantitative and qualitative assessment of the types of soil degradation manifestations in the study area, such as the images resulting from the natural difference index of the vegetation cover, Salinity Guide, Arid Soil, Soil Degradation Guide.
- There are clear spatial changes in the intensity of salinization processes in the study area, but by changing water management, through appropriate irrigation practices, can often lead to increased crop yields in saline soil conditions.
- Landsat satellite visual images are used for regional studies that are carried out over a relatively large area, due to the relatively low spatial resolution, which varies between 6-15 meters, the accuracy of its results is increased by spectral, spatial enhancements

and matching operations with reference information, whether it's maps or images.

- Studies have confirmed that geospatial data allows us to obtain graphical and non-graphical information, considering the estimation of land degradation risks through geographic mapping. Land data is extremely important for modeling the erosion of degraded plots and presenting this data in a digital format has significant benefits in terms of fast processing and quality of information obtained.
- Studies have shown that the use of remote sensing techniques and geographic information systems has enabled researchers to obtain accurate and sufficient information and data to understand the causes of land use changes and also to lessen the risk of land degradation as computerized processing, review and additionally exposures to the information acquired in various forms (maps, graphs, tables, text, etc.) are provided. For that reason, they offer some special benefits, including obtaining thematic maps, the ability to process large and multiple heterogeneous databases with spatial reference and high flexibility in terms of IT system configuration, allowing modification of a wide variety of applications and users.
- Previous research has shown us the possibility of combining data related to different things and analyzing the information obtained by computer processing of primary data, as compared to classical procedures (where the calculation areas are larger and of variable size). In this case, land degradation is determined at the primary surface level (pixels/cell). Another great advantage of GIS technology is that it is possible to integrate all factors (natural and human) at the cellular level. Computer processing of data that characterizes the factors that determine the initiation and maintenance of a land degradation process generates multiple possibilities for agricultural land simulation.
- Greater accuracy of research on land degradation due to erosion can also be achieved with GIS technologies by diversifying data acquisition methods (including photogrammetry and remote

sensing) and by continuously developing and updating databases.

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## ASSESSMENT OF THE GENETIC CONDITION OF THE ORDINARY CHERNOZEM IN THE AREA OF THE STEP OF THE SOUTH PLAIN, IN NATURAL AND AGRICULTURAL REGIME

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### *Abstract*

*Chernozems have been and will continue to be the main base of agricultural production. The statements of the founder of soil science, Dokuchaev V.V., that chernozems are and will be the breadwinner and the main breadbasket of mankind, remain truly modern. Fertile chernozem is the main base for agricultural and crop production. Soil scientists consider it the main granary of mankind, the king of soils). The main purpose of the research is the comparative analysis of the genetic peculiarities of chernozems evolved in natural and arable regime, the appreciation of the indices modified by the agropedogenetic process in the Steppe Area of the Southern Plain of the Republic of Moldova. In the laboratory it was determined: the hygroscopic water content, the density of the solid phase of the soil; particle size and microaggregate composition; hygroscopicity coefficient; humus content; carbonate content; soluble salt content; current reaction (pH); the content of exchangeable cations, etc. A detailed study of morphogenetic properties showed that chernozems under natural vegetation have: a powerful, well-developed soil profile; dark, almost black color, gradually weakening with depth; a well-defined granular structure in most of the humus strata; weakly compacted composition, gradually increasing in the lower horizons; the absence of noticeable signs of eluvial-illuvial differentiation of the soil profile; uneven, lingual boundary of the transition of the humus horizon into the parent rock; the presence of a carbonate horizon, confined, as a rule, to the lower boundary of the humus stratum and characterized by various forms of carbonate neoformations. The thickness of the natural soil profiles (profile 13) and arable soil (profile 14) up to the parent rock varies from 160 cm to 151 cm accordingly and is certified as strong deep. The soil corresponds to the non-carbonate class, the carbonates being absent in horizon A. The maximum content of carbonates is registered in the BC horizon and constitutes 11.9% in the natural one and 10.7% in the arable one.*

*Key words:* ordinary chernozem, genetic condition, natural and agricultural regime, Steppe Area of the Southern Plain.

### INTRODUCTION

It would be a huge mistake to think that soil fertility is constant over time, that it is a certain statistical value. Generalizations made in different countries confirm that the condition of the soil cover has deteriorated in the last decade. Every educated person understands that to obtain today's crops, nutrients were used that accumulated in the soil after the mineralization of plants growing on them and from other sources over several millennia. We are pleased with the growth in yields, but along with the growth in yields, soil depletion also increases, which is associated with an increase in the removal of nutrients from it. In the minds of many people, including experts in the field of agriculture, there is an opinion about the extremely high fertility of chernozem soils. This

idea has evolved over the centuries. But chernozem soils contain in their composition a relatively small amount of mobile forms of nutrients (Виноградов, 1969).

The significance of chernozem is far from being exhausted solely by its agricultural value. Chernozem soil is an excellent habitat for a huge variety of animals, plants, microorganisms. Therefore, the degradation, and even more so the destruction of chernozems, leads to a reduction in the biological diversity of the living world of the Earth, to the irretrievable loss of part of its genetic fund, created by the evolution of life on our planet over many millennia (Афанасьева, 1966; Докучаев, 1952; Савич, 2014).

Compared to other types of soils, chernozems are characterized by an ideal balance of all factors of soil formation. However, despite the

natural perfection, chernozems inevitably evolve under the influence of natural and, especially, anthropogenic factors. It is assumed that the increasing anthropogenic load on soils will lead to qualitative and quantitative changes in the state of chernozems. In this regard, knowledge of the direction of changes in the modern soil-forming process is undoubtedly relevant. Without this, it is difficult to imagine the future state of not only soils, but nature as a whole (Лыков, 1985; Шевченко, 1984).

In arable chernozems, a significant transformation of the morphological and morphogenetic properties of soils is noted. Changes here acquire a directed, irreversible character. At the first stages of development, these transformations are localized in the upper part of the soil stratum; subsequently, they spread in depth, covering, depending on the duration of soil use, the entire profile (Докучаев, 1952; Шишов et al., 2004).

Morphological features of soils reflect the intensity of processes and regimes occurring in soils. However, at present they do not have an exact quantitative assessment, the relationship between them is not described by certain mathematical equations. This reduces the accuracy of assessing the fertility and degradation of soils by their morphological properties, reduces the objectivity of assigning soils to certain types and taxonomic units of a lower hierarchical level (Байбеков, 2007; Орлов, 2001; Савич, 2006).

In modern agriculture, the most important task is to preserve and expand the reproduction of soil fertility. It is due to the fact that the intensification of agriculture is accompanied by an increase in crop yields, which dramatically increases the removal of nutrients from the soil, increases the decomposition of humus, and reduces its content and soil fertility (Гришина, 1973; Лыков, 1985; Шевченко, 1984). Humus is the most important indicator of soil fertility, a source of nutrients for plants.

In the practice of soil science, the humus content and its constituent components are generally expressed as a percentage of the soil mass. However, this indicator does not reflect the peculiarities of the agricultural use of soils, primarily because of the unequal, often significantly different, values of their bulk density. As a result, for a correct and reliable

comparison of genetically similar soil varieties in the virgin-arable land system, it is necessary to operate with data on humus reserves, the calculation of which takes into account not only changes in the humus content, but also the density of soil composition. With long-term plowing of chernozems, the humus content decreases, but their bulk density increases markedly. Therefore, real (in terms of reserves, t/ha) and apparent (in % of mass) humus losses in soils during their long-term plowing can differ significantly. However, at present, there is not enough data in the scientific literature on a comparative assessment of the long-term dynamics of the humus of agrochernozems, expressed as a percentage of the soil mass and reserves in t/ha (Сапожников, 1994; Шишов et al., 2004).

The plowing of virgin chernozems on upland and their long-term agricultural use is accompanied by a significant decrease in the humus content within a 1.5-meter soil layer. At the same time, the most noticeable losses of humus occur in the upper part of the humus horizon of soils, and further down the profile, the differences gradually smooth out. Thus, in the 0-10 cm layer of arable chernozems, compared to virgin areas, the humus content decreased by 2.15-2.35%, or in relative units, by 25-26%. Thus, plowing and long-term agricultural use of ordinary chernozems in agricultural production leads to dehumification.

## MATERIALS AND METHODS

The ordinary chernozem research was located in Cahul district, Ursoaia village under steppe vegetation. These soils occupy an area of about 650 thousand ha. The pedological study in the field was carried out by placing two main soil profiles (profile 13 - natural and profile 14 - agricultural). From the soil profiles, 12 samples identified on genetic horizons were taken for laboratory analysis. 2 geodetic pickets were fixed for the spatial positioning of the soil profiles. The works were carried out in the WGS-84 coordinate system, later transferred to the national coordinate system MoldRef-99. The actual material was obtained by averaging large arrays of stock, literature and own experimental data for each taxon. Only sections laid down in landscape and ecological conditions typical for

chernozem formation were taken into consideration, namely: upland areas of watersheds; automorphic conditions of soil formation; well-preserved meadow-steppe vegetation, rich in species composition; carbonate loess-like rocks, clayey and heavy loamy granulometric composition; absence of visible signs of manifestation of erosion processes.

According to the pedagogical district, the researched territory falls within the Southern Plain Steppe Zone (III), the Bessarabian Southern Plain Steppe district (13) with carbonate and common chernozems. Through field pedological research, laboratory and office work, it was found that the soil cover on the researched sector is represented by the subtype of clay-clayey carbonate chernozem on clay, natural (profile 13) and clay-clay carbonate chernozem on clay, agricultural (profile 14). Archival materials available from the Institute of Pedology were used as information “Nicolae Dimo” Agrochemistry and Soil Protection (IPAPS “N. Dimo”), its Institute of Land Use Planning and Organization (IPOT). Morphological and analytical data on genetic horizons were introduced in the geoinformation system of the Soil Quality Database within the “Data of the Pedological Center” of IPAPS “N. Dimo” ([http://gis.soil.msu.ru/soil\\_db/moldova/](http://gis.soil.msu.ru/soil_db/moldova/) and the SoilDB CPanel web application, [http://gis.soil.msu.ru/soil\\_db/assessment/](http://gis.soil.msu.ru/soil_db/assessment/)) of the Euro-Asian Soil Partnership under DO IT office, field and laboratory research methods were used.

## RESULTS AND DISCUSSIONS

Thus, as a result of modern agricultural use in chernozems, the following phenomena are observed:

1. Transformation of the humus profile, which manifests itself in a change in color, thickness, content and quality of humus;
2. Transformation of the carbonate profile, fixed in a change in its thickness, depth of occurrence, forms of newly formed carbonates, and the nature of migration processes;
3. Formation of neohorizons of agrogenic nature: compacted (“plow sole”) - in the lower part of the arable layer, textured clayey - in the sub-humus part, iron segregation zone - in the lower part of the profile;

4. Changes in the structural organization of the humus strata of the profile, manifested in the deformation of the shapes, sizes, faceting of peds, their packaging, etc.;

5. Transformation of the composition of the soil mass, expressed in a change in the density of the composition, the density of the solid phase of the soil, porosity, etc.;

6. The appearance (especially in old-arable chernozems) of silty-clay-humus film formations - cutan on the faces of peds in horizons AB and B.

Profile no. 13 were placed under shrub vegetation. The object is located on a quasi-horizontal terrain with a degree of inclination of up to 1 ° (Photo 1) at an altitude of 170.4 m on a south-westerly exposure. The effervescence is recorded from 47 cm. The morphological description of the profile is presented below.



Photo 1. The profile of the ordinary chernozem (natural)

Morphological characteristic of Profile no. 13. Ordinary chernozem:

**A<sub>t</sub> (0-8 cm)** - gray, dry, loose, glomerular-grainy structure, finely porous with very frequent pores, abundant shrub and grassy roots, clay-loamy texture, gradual passage.

**A (8-47 cm)** - gray color with a brown tinge, dry, compact, glomerular structure, fine porous with very frequent pores, grass roots and trees, clay-loamy texture, clear passage.

**Bl<sub>k</sub> (47-64 cm)** - brown, dry, compact, glomerular-lumpy structure, porous with frequent pores, pseudomycelias, shrub roots, clay-loamy texture, clear passage.

**B2k (64-96 cm)** - dark yellow, dry, compact, massive lumpy structure, fine and frizzy pores, carbonate pseudomycelias, crotovina, roots, clay-loamy texture, clear passage.

**B2kCk (96-160 cm)** - yellow with white, dry, compact, unstructured, finely porous, pseudomycelias and carbonate concretions, beloglasca, roots, clay-loamy texture, clear passage.

**Ck (160-175 cm)** - yellow, dry, compact, unstructured, roots, clayey texture.

Profile no. 14 was placed on a quasi-horizontal ground with a degree of inclination of up to 1 ° (Photo 2) at an altitude of 170.7 m with a south-western exposure. The category of land use is agricultural. The effervescence on the profile appears from 48 cm.



Photo 2. The profile of the ordinary chernozem (agricultural)

The morphological description of Profile no. 14 ordinary chernozem (agricultural) is presented below.

**Ap (0-31 cm)** - gray color, dry, loose, glomerular structure, fine porous with very frequent pores, roots, clay-loamy texture, clear passage.

**A (31-48 cm)** - gray color with a brown tinge, dry, slightly compact, glomerular-Bulgarian structure, fine porous with frequent pores, roots, clay-loamy texture, clear passage.

**B1k (48-66 cm)** - brown, dry, compact, glomerular-lumpy structure, porous with frequent pores, pseudomycelium, roots, clay-loamy texture, clear passage.

**B2k (66-93 cm)** - dark yellow, dry, compact, lumpy structure, fine pores and freckles,

carbonate pseudomycetes, crotovina, roots, clay-loamy texture, clear passage.

**B2kCk (93-151 cm)** - yellow with white, dry, compact, unstructured, finely porous, pseudomycelias and carbonate concretions, beloglasca, roots, clay-loamy texture, clear passage.

**Ck (151-170 cm)** - yellow, dry, compact, unstructured, roots, clayey texture.

The thickness of the natural soil profiles (profile 13) and arable soil (profile 14) up to the parent rock varies from 160 cm to 151 cm accordingly and is certified as strong deep. The soil corresponds to the non-carbonate class, the carbonates being absent in horizon A. The maximum content of carbonates is registered in the BC horizon and constitutes 11.9% in the natural one and 10.7% in the arable one.

After morphogenetic characterization, the state of natural soil settlement is loose at the surface and compact in depth, with glomerular-grainy and grainy structure in horizons A, followed by glomerular-lumpy and lumpy structure in horizon B, and in the horizon and transitional horizon solification (C) is unstructured. In the profile of agricultural soil, the state of settlement is loose on the surface, compact in depth. The arable profile has a glomerular structure in the first horizon, glomerular-lumpy to the B1k horizon, lumpy in B2k and unstructured in depth.

The thickness of the humus layer places soils in the class with a strong deep humus profile (96-93 cm). The humus content in the natural surface layer is 6.26%, which corresponds to the humiferous class, moderately humiferous (3.25%) for horizon A.

At a depth of 96 cm it decreases to 1.35% and at a depth of 0.88-0.36%. The humus content in the Ap horizons of the agricultural profile has a moderately humiferous class containing 3.12%. For horizons A and B1k it varies between 2.87% and 2.31%. At a depth of 93 cm the content decreases to 1.33% and at a depth of 0.66-0.57%.

The apparent density in the natural soil varies from 1.11 g/cm<sup>3</sup> in the superficial horizon the value increasing in depth up to 1.46 g/cm<sup>3</sup>, and in the agricultural one 1.14 g/cm<sup>3</sup> in the arable horizon increasing in depth up to 1.44 g/cm<sup>3</sup>. The natural soil in the first two horizons has a neutral current reaction at (pH (H<sub>2</sub>O) = 7.10),

and at a slightly alkaline depth at (pH (H<sub>2</sub>O) = 8.05-8.35). The agricultural soil in profile has a low alkaline current reaction (pH (H<sub>2</sub>O) = 7.67-8.30).

The physical, physico-chemical and chemical parameters of the researched soils are shown in Figures 1-3.

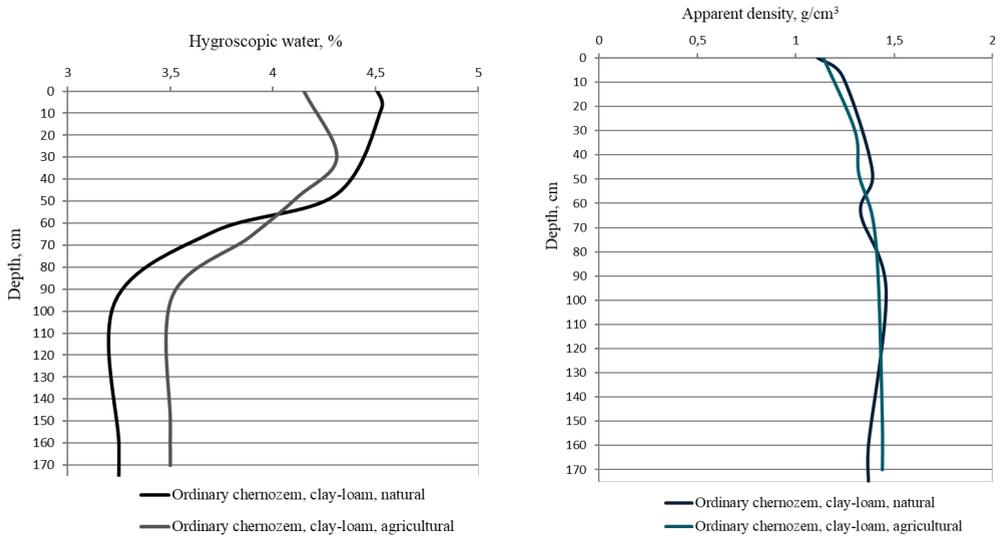


Figure 1. The values of the hygroscopic water, (%) and bulk density (g/cm<sup>3</sup>)

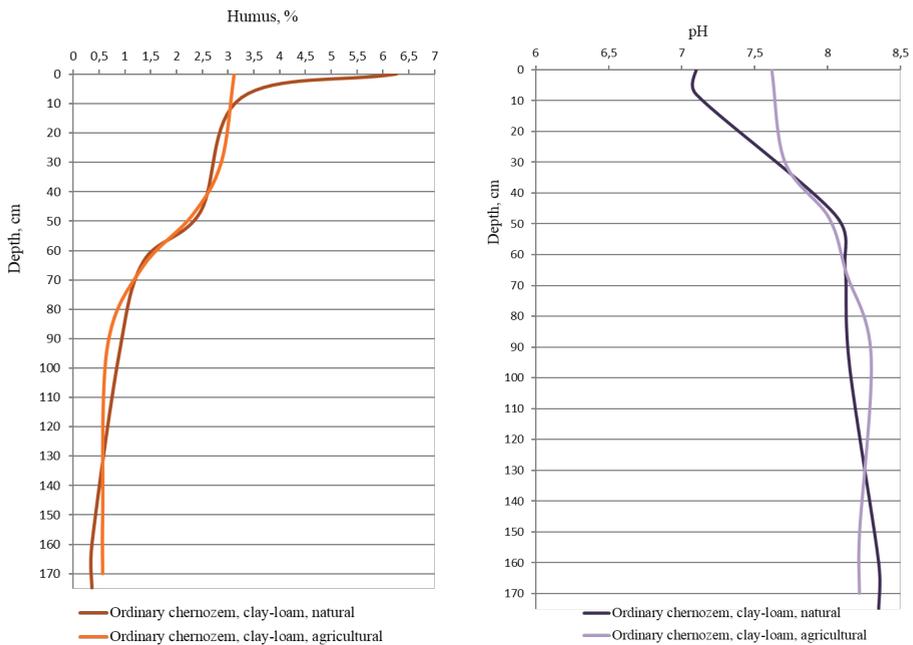


Figure 2. The values of the humus (%) and pH

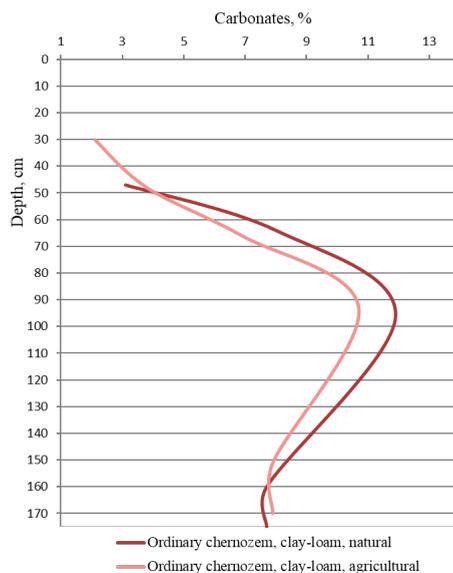


Figure 3. The values of the carbonates (%)

The sum of exchangeable cations varies on the natural profile from the large class ( $\Sigma$ cat. 32.32-30.26 me/100 g soil) in the A<sub>1</sub>, A and middle horizons ( $\Sigma$ cat. 16.64-22.34 me/100 g soil) in depth. Arable soil corresponds to the large class ( $\Sigma$ cat. 28.94-26.93 me/100 g soil) in the first two horizons Ap and A and middle ( $\Sigma$ cat. 15.87-20.62 me/100 g soil) in depth. The adsorbent

complex of the soil has the degree of calcium saturation 88-72% in profile 13 and 87-77% profile 14, the relative magnesium content is 12-25% and 12-21% respectively, and the exchangeable sodium makes 1-2% for both profiles. The soils are unaltered, and the ratio of calcium to magnesium is 7:1 in the first two surface horizons for both profiles (Table 1).

Table 1. Exchangeable cation content of the researched soils

No. profile	Name of the soil	Index	Depth, cm	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	Summ	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>
				me/100 g soil				% of summ		
P13	P13 Ordinary chernozem (natural)	A <sub>1</sub>	0-8	28.21	3.94	0.17	32.32	87	12	1
		A	8-47	26.53	3.56	0.17	30.26	88	12	1
		B1k	47-64	18.76	3.41	0.17	22.34	84	15	1
		B2k	64-96	12.90	3.34	0.40	16.64	78	20	2
		BCk	96-160	12.62	4.51	0.39	17.52	72	26	2
		Ck	160-175	12.36	4.15	0.39	16.9	73	25	2
P14	P14 Ordinary chernozem (agricultural)	Ap	0-31	25.29	3.48	0.17	28.94	87	12	1
		A	31-48	23.42	3.34	0.17	26.93	87	12	1
		B1k	48-66	17.09	3.36	0.17	20.62	83	16	1
		B2k	66-93	13.30	3.42	0.41	17.13	78	20	2
		BCk	93-151	13.04	3.44	0.42	16.90	77	20	2
		Ck	151-170	12.17	3.28	0.42	15.87	77	21	3

The dry residue from natural profile 13 includes values of 0.039-0.052%, and in profile 14 agricultural it increases varying between 0.066

and 0.073%. All the highlighted horizons are free of soluble salts. The anionic part is clearly predominated by HCO<sub>3</sub><sup>-</sup> with a content of 0.22-

0.90 me/100 g and  $\text{SO}_4^{2-}$  with 0.07-0.34 me/100 g, and in the cationic part of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with a content of 0.38-0.60 and 0.07- 0.30 me/100 g accordingly. The chemistry of the surface soil layer is sulfate-hydrocarbonate/ carbonate in the

surface layers and hydrocarbonate/carbonate in depth (natural profile) and for the arable one hydrocarbonate-chloride-sulfatic/carbonate (see Table 2).

Table 2. The chemical composition of the aqueous extract of the researched soils

No. profile	Name of the soil	Index	Depth, cm	Dry residue, %	$\text{HCO}_3^-$	$\text{Cl}^-$	$\text{SO}_4^{2-}$	$\text{Ca}^{2+}$	$\text{Mg}^{2+}$	$\text{Na}^+$
					me/100 g soil					
P13	P13 Ordinary chernozem (natural)	A $\ddot{t}$	0-8	0.048	0.30	0.13	0.34	0.60	0.12	0.05
		A	8-47	0.039	0.22	0.08	0.29	0.48	0.07	0.04
		B1k	47-64	0.04	0.48	0.10	0.11	0.38	0.27	0.04
		B2k	64-96	0.042	0.52	0.09	0.07	0.58	0.04	0.06
		BCK	96-160	0.048	0.50	0.10	0.18	0.55	0.14	0.09
		Ck	160-175	0.52	0.55	0.08	0.24	0.45	0.30	0.12
P14	P14 Ordinary chernozem (agricultural)	Ap	0-31	0.067	0.42	0.23	0.18	0.68	0.11	0.04
		A	31-48	0.066	0.48	0.26	0.15	0.72	0.11	0.06
		B1k	48-66	0.071	0.52	0.21	0.30	0.79	0.19	0.05
		B2k	66-93	0.073	0.53	0.23	0.35	0.92	0.13	0.06
		BCK	93-151	0.067	0.48	0.24	0.23	0.70	0.20	0.05
		Ck	151-170	0.072	0.48	0.26	0.42	0.90	0.19	0.07

The texture of the natural soil in the A $\ddot{t}$  horizon is loamy (physical clay content 39.24%), followed by clay-loamy (physical clay content 47.14-51.51%), and in the parent rock loamy (physical clay content 42.83%). In the arable

one up to the Bck passage horizon, the textural class is clay-clay with a physical clay content varying from 47.8% to 50.70%. In the parent rock it is clayey, the physical clay content is 44.00% (Tables 3 and 4).

Table 3. Soil texture

Horizon	Depth, cm	Content of fractions (%) with diameter (mm)							Kd
		1-0.25	0.25-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	$\Sigma < 0.01$	
P13. Ordinary chernozem (natural)									
A $\ddot{t}$	0-8	8.09	15.03	37.64	6.61	4.76	27.87	39.24	4
A	8-47	0.48	7.57	44.52	8.31	11.00	28.12	47.43	4
B1k	47-64	0.17	8.00	44.69	6.53	12.51	28.10	47.14	4
B2k	64-96	0.12	5.37	43.00	7.72	18.97	24.82	51.51	9
BCK	96-160	1.31	7.52	41.10	9.42	14.69	25.96	50.07	10
Ck	160-175	0.29	8.70	48.10	7.53	10.66	24.64	42.83	9
P14. Ordinary chernozem (agricultural)									
Ap	0-31	0.6	7.6	45.9	10.2	8.2	27.5	45.9	6
A	31-48	0.3	9.2	44.7	8.1	9.7	28.0	45.8	7
B1k	48-66	0.2	4.3	44.7	8.3	14.2	28.2	50.7	7
B2k	66-93	0.3	8.3	42.8	8.4	11.4	27.5	48.7	7
BCK	93-151	0.2	7.4	45.2	9.0	12.1	26.8	47.2	7
Ck	151-170	0.3	8.2	47.4	7.5	9.1	27.4	44.0	6

Table 4. Microaggregate composition of the soil

Horizon	Depth, cm	Content of fractions (%) with diameter (mm)					
		1-0.25	0.25-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001
P13. Ordinary chernozem (natural)							
A <sub>1</sub>	0-8	8.58	41.71	37.76	5.29	3.51	1.15
A	8-47	0.59	32.53	46.94	7.72	9.02	1.16
B1k	47-64	0.26	38.89	43.46	6.40	7.97	1.02
B2k	64-96	0.31	36.45	40.40	7.56	13.01	2.27
BCk	96-160	1.39	28.07	45.56	8.59	10.68	2.71
Ck	160-175	0.52	29.63	51.30	5.70	7.55	2.30
P14. Ordinary chernozem (agricultural)							
Ap	0-8	0.7	30.2	51.9	9.1	6.5	1.6
A	8-47	0.2	28.4	52.4	8.9	8.1	2.0
B1k	47-64	0.1	25.7	51.1	10.9	10.1	2.1
B2k	64-96	0.2	26.9	51.3	8.6	11.1	2.0
BCk	96-160	0.2	30.6	55.0	7.6	5.7	1.9
Ck	160-175	0.2	31.1	54.4	7.2	5.8	1.7

As is known, particles of silt and coarse dust predominate in the composition of the granulometric fractions of these varieties. Then, in descending order, follow: fine, medium dust and fine sand fractions. The absolute minimum falls on coarse and medium sand. In the genetically conjugated series from podzolized to ordinary chernozems, a heavier particle size distribution is clearly seen, accompanied by an increase in the content of silt, fine dust and a decrease in the amount of coarse silt particles. As a result, in this series there is a change in the ratio of the two dominant fractions: silt and coarse dust. In ordinary chernozems, silty and then coarse silt particles predominate.

Inside the profile distribution of these fractions is characterized by an increase in the proportion of silt and a relative decrease in the silt fraction from top to bottom. The noted quantitative changes in the content of fractions in the series under consideration are due, in our opinion, to differences in the intensity of the soil-forming process and the migration of silt along the profile. This is confirmed by the data on the granulometric composition of arable chernozems, where the balance of silt in relation to the parent rock is even more shifted to the negative side compared to virgin analogues.

The use of chernozems in agricultural production leads to an increase in the proportion of aggregates over 10 mm, a decrease in granular and dusty fractions, and a decrease in the water

resistance of structural elements. In general, the analysis of the physical and water-physical properties of virgin and arable chernozems showed that there is a correlative relationship between the humidization of the water regime and the deterioration of the physical properties of chernozems.

Studies of the hydrological regime have established that in virgin chernozems, the greatest amount of moisture, as a rule, is observed in the spring after snowmelt. During the growing season, moisture consumption by virgin vegetation is carried out mainly from the upper meter layer, where the greatest seasonal changes in humidity are observed. Summer precipitation mainly moistens the uppermost soil layer to a depth of 20–30 cm.

In arable chernozems, the water regime develops differently. In the first half of the growing season, the change in humidity in arable chernozems is quite close to that in chernozems under natural vegetation.

Differences are observed in the second half of summer. During this period, on the virgin lands, the vegetation continues to vegetate and, consequently, consume moisture, while on the arable land, the desuctive consumption of moisture stops after harvesting and it is lost from the soil only as a result of physical evaporation. "Underutilization" of moisture at the end of summer, as well as a lower consumption by cultivated vegetation during the growing season,

determine here the annual increment of moisture in comparison with the steppe by an average of 20–40 mm with a fluctuation range of 10–140 mm.

The annual underutilization of moisture leads to an increase in the depth of spring moisture and more frequent through wetting of the soil profile than on virgin lands. i.e., the water regime of arable chernozems, although it remains periodically leaching, is shifting to a more humid side in terms of quantitative indicators. In the steppe chernozems - ordinary chernozems - shifts towards the humidification of the water regime during plowing are even more pronounced, due to the fact that the place of xerophytic steppe vegetation is occupied by mesophytic cultivated plants.

## CONCLUSIONS

Under the influence of long-term cultivation of ordinary chernozems, processes of anthropogenic degradation develop in them. The properties of old-arable ordinary chernozems are currently deteriorating significantly.

Degradation processes are clearly diagnosed by morphological changes in the structure, overconsolidation, deficient balance of macro- and microelements, erosion and other characteristics.

Given that at present the use of organic and mineral fertilizers is carried out in minimal volumes, it is not necessary to expect a significant improvement in the agrophysical and agrochemical properties of soils under modern conditions.

To stop the further development of the soil dehumification process, it is necessary to use crop residues and straw as organic fertilizers as widely as possible, as well as introduce green manure fallow and post-stubble green manure into production.

The texture of the natural soil in the A<sub>1</sub> horizon is loamy (physical clay content 39.24%), followed by clay-loamy (physical clay content 47.14–51.51%), and in the parent rock loamy (physical clay content 42.83%). In the arable one up to the B<sub>ck</sub> passage horizon, the textural class is clay-clay with a physical clay content varying from 47.8% to 50.70%. In the parent rock it is clayey, the physical clay content is 44.00%.

The dry residue from natural profile 13 includes values of 0.039–0.052%, and in profile 14 agricultural it increases varying between 0.066 and 0.073%. All the highlighted horizons are free of soluble salts. The anionic part is clearly predominated by HCO<sub>3</sub><sup>-</sup> with a content of 0.22–0.90 me/100 g and SO<sub>4</sub><sup>2-</sup> with 0.07–0.34 me/100 g, and in the cationic part of Ca<sup>2+</sup> and Mg<sup>2+</sup> with a content of 0.38–0.60 and 0.07–0.30 me/100 g accordingly. The chemistry of the surface soil layer is sulfite-hydrocarbonate/carbonate in the surface layers and hydrocarbonate/carbonate in depth (natural profile) and for the arable one hydrocarbonate-chloride-sulfatic/carbonate.

It has been established that the structure of the soil cover of the studied area is composed of ordinary chernozem, medium-thick, medium-humus, ordinary chernozem, medium-thick, low-humus, ordinary calcareous, medium-thick, low-humus.

## ACKNOWLEDGEMENTS

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## SOME ASPECTS OF THE FOREST SOILS GENESIS FROM THE FOREST-STEPPE OF THE REPUBLIC OF MOLDOVA

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### *Abstract*

*The genesis of contemporary soils, formed under the vegetation of deciduous forests, grey and brown soils, as well as their evolution over time as a result of long-lasting climatic oscillations and anthropogenic impact, are treated differently; some of their peculiarities in different climatic subzones of the Republic of Moldova are not always taken into account when developing their classification. Taking into account the recommendations of the World Reference Base of Soils to name high-ranking soil taxonomic units (at the type level) in one word, in the proposed classification system, forest soils have now been renamed as: xero forestry chernozems, greyzems, brownzems. Moldova is located at the intersection of three biogeographical areas whose natural conditions have a decisive impact on the evolution of all components of the environment, including soils. The anthropic use of forest soils in arable lands causes unprecedented changes in landscapes, ecosystems and environment, and the current climatic situation corresponds to the formation of chernozems throughout Moldova.*

**Key words:** xero forestry chernozems, climate change, pedogenesis, forest steppe.

### INTRODUCTION

Moldova is located at the intersection of three biogeographical areas whose natural conditions have a decisive impact on the evolution of all components of the environment, including soils. At the same time, the anthropogenic use of soils in forest lands has led to unprecedented changes in the landscapes of ecosystems and the environment.

The beginning of the soil cover formation on the Republic of Moldova territory refers to the border between the Pleistocene and Holocene periods (Адаменко и др., 1996). After this time, as a result of climate change and geographical areas, Moldova's forest-steppe soils have undergone a series of development stages, that were most prominently reflected in their profiles during the transition from the cold steppe to forest, or from the forest to the semi-humid or semi-arid steppe, which led to the formation of the polygenetic soil cover (Александровский, 1988, 2006; Florea, 2005; Leah & Cerbari, 2021).

The genesis of contemporary soils formed under deciduous forest vegetation, recently called

greyzems, brownzems, xero forestry chernozems and those progressed in arable chernozems from deciduous forest soils, as well as their evolution over time as a result of long-term climatic oscillations, anthropogenic impact, population migration (Xenopol, 2006) is treated differently. Some of their genetic peculiarities in different climatic subzones of the Republic of Moldova are not always taken into account at the soil classification (Cerbari, 2008; Cerbari & Lungu, 2011; Florea et al., 1987; Florea & Muntean, 2003; Ursu, 2011; Граги, 1977).

Contemporary climatic conditions throughout the Republic of Moldova correspond to the formation of chernozems (Крупеников, 1967). This is also confirmed by the spread in the north part of Moldova of the cambic and luvic arable chernozems progradated from the postarable greyzems, initially formed under the deciduous forests.

Taking into account the recommendations of the World Reference Base of soils (2014) to name top-level soil taxonomic units (at the type level) in one word, in the actual soil classification system proposed, that forest soils have been named now as follows: greyzems, brownzems,

xeroforestry chernozems (Cerbari, 2008; Ursu, 2001; 2011).

## MATERIALS AND METHODS

The study objects were greyzems (grey soils), brownzems (brown soils) and xeroforestry chernozems, natural (virgin) and anthropogenic modified from the forest-steppe of Northern, Central and Southern Moldova. The properties of these soils were obtained based on the pedological research materials, carried out in recent years.

In the field research, laboratory and office, the methods and the soils evaluating criteria and properties were made according to sources (Florea et al., 1987; Monitoringul..., 2010; Ghid de autoevaluare..., 2018; Cerbari, 2008; Теория и методы..., 2007; WRB, 2014).

The characteristic of greyzems properties (grey soils) was performed based on the data obtained for the soil profiles researched for soil monitoring in the 2007-2020 years on the Northern Moldavian Plateau and the Dniester Plateau. In the 2020, the virgin and arable greyzems from the northwestern part of the Northern Moldavian Plateau (Briceni) and from the Dniester Plateau (Rezina) formed in conditions of practically humid temperate climate were researched.

The north-western part (Briceni) is characterized by absolute heights within 242-284 m and a wetter climate than in the other administrative districts, located on the plateau in its eastern, central and southern part. The Dniester Plateau, being in direct contact with the Eastern European plain, is characterized by a colder climate and absolute heights within the limits of 271-351 m. In both cases, the textural differentiation of virgin greyzems is pronounced (Table 1).

## RESULTS AND DISCUSSIONS

The area of soils, developed under the deciduous forest, from North to South of Moldova was formed under the influence of the following factors:

- the latitude climate zone in the northern part of the Republic Moldova with a semi-humid temperate climate (Greyzems - the sum of active air temperatures higher than  $10^{\circ} =$

$2700-2900^{\circ}$ , the humidity coefficient Ivanov-Văsoțchii,  $K = 0.7-1.0$ );

- the latitude climate zone in the central part of the Republic of Moldova with contrasts warm climate semi-humid to semi-arid (Brownzems - the sum of active air temperatures higher than  $10^{\circ} = 2900-3100^{\circ}$ , humidity coefficient Ivanov-Văsoțchii,  $K = 0.6-0.7$ );

- the latitude climate zone in the southern part of the Republic of Moldova with a contrast semi-arid climate (chernozems xeroforestry - the sum of active air temperatures higher than  $10^{\circ} = 3100-3200^{\circ}$ , humidity coefficient Ivanov-Vashoschii,  $K = 0.5-0.6$ ).

The profiles of virgin soils from the forest-steppe of the Republic of Moldova shows in Figures 1-4. The values of the main physical and chemical indices of the soils formed under the deciduous forests of the forest-steppe of the Republic Moldova presents in the Table 1. Below are present a brief description of the listed types of soils formed under deciduous forests in the forest-steppe area of Moldova.

### *Greyzem typical on the Northern Moldova Plateau.*

The genesis of greyzems (grey soils) were first studied by Докучаев (1949) and Виленский (1958), Вильямс (1949) and others. The greyzem was determined as a zonal type of soil formed in the vegetation and climatic conditions characteristic of forest-steppe forests. The greyzems formation is manifested by the gradual decrease of the influence of pedogenesis podzolic process from north to south, from the area of coniferous forests with podzolic soils to the area of deciduous forests (Докучаев, 1949). The hypothesis put forward by Виленский (1957) predicts the secondary origin of grey soils (greyzems) as a result of the oscillation of the southern and northern boundary of the forest-steppe and the manifestation of the processes of degradation and progradation of greyzems. At present, these oscillations took place, but they were not large and have influenced the progression or regradation of soils on relatively small areas (Lupașcu et al., 1998; Хотинский, 1986; Чендев, 2003).

Regarding the genesis of greyzems (grey soils) in the forest-steppe area, several hypotheses have been presented into three groups:

1. Primary formation as a special type of soil, developed under deciduous forests (Докучаев, 1949).

2. Secondary formation of greyzems following the degradation of chernozem soils and the planting of woody vegetation on these surfaces (Виленский, 1957).

3. Greyzems formation from virgin (natural) podzolic soils following the development of the process of substituting the woody vegetation with the steppe and meadow grassy vegetation (Вильямс, 1949).

The authors are of the opinion that following processes participate in different proportions in the formation of the greyzems and brownzems profile: podzolic; *in situ* alteration (cambic) and lessivage - clay migration.

In conditions of percolative water regime or periodically percolative a soil profile with an eluvial horizon is formed at the top and illuvial in the middle and lower part of these soils.

The different correlation of the podzolic process, lessivage, alteration *in situ* and the humus accumulation process leads to the formation of greyzems (grey soils) and brownzems with different properties. In colder or wetter climates (humidity coefficient Ivanov-Vashoschii,  $K = 0.8-1.0$ ) under the deciduous forest, the podzolic process becomes more intensive and typical greyzem is formed, rarely - greyzems albic.

In contrast thermal conditions, characteristic for loamy-sandy soils on the Codrii Plateau (altitude 200-400 m) and the clayey-loamy soils of the hilly periphery of the Codrii (altitude 170-200 m) the brownzems are formed, textural differentiated under the action of the *in situ* alteration process and lessivage. The contemporary soils of the forest-steppe of Moldova are mostly polygenetic, their formation evolving through different phases of pedogenesis.

The typical virgin greyzem from the northwestern part of the Northern Moldova Plateau (Figure 1, profile 11) has a type profile:  $AEh_f (0-8\text{ cm}) \rightarrow AEh (8-29\text{ cm}) \rightarrow EBhtw (29-47\text{ cm}) \rightarrow Btw (47-68\text{ cm}) \rightarrow BCtw1 (68-96\text{ cm}) \rightarrow BCtw2 (96-110\text{ cm}) \rightarrow Ck (110-130\text{ cm})$ . The soil characterized by a differentiated texture by the eluvial-illuvial process of pedogenesis. The index of textural differentiation (Idt) of the profile of these soils for the illuvial horizon,

compared to the eluvial horizon, reaches values 1.7-1.9. Soils with such Idt values are evaluated as moderately texturally differentiated soils.

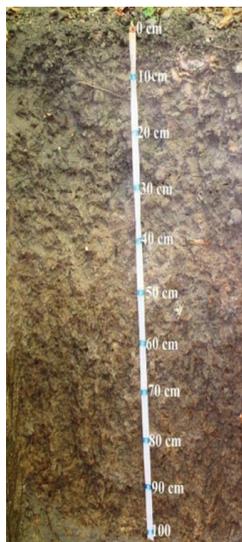


Figure 1. Profile 11. Greyzem typical, moderately humic with semi-deep humiferous profile, semi-deep clayey-loamy, moderate differentiated textural, virgin (altitude 258 m, oak and carpen forest, Briceni)



Figure 2. Profile 12. Greyzem albic, stagnic slitized, gleyed from the surface, clayey-loamy 0-37 cm, loamy-clayey 37-80 cm, strongly differentiated textural (altitude 249 m, hornbeam forest, Rezina)

Textural division of the typical greyzem (profile 11), located in the northwestern part of the Northern Moldavian Plateau, occurred under the full action of the three processes: *in situ* alteration; lessivage of the alteration colloidal material; podzolic process - migration of water-soluble organo-mineral compounds of Fe and Al on the profile.

In this concrete case, the main role in the textural differentiation of the researched soil profile, returns to the podzolic process (Rode, 1984). Indirect, this is also confirmed by the value of  $pH_{KCl} = 4.1-4.3$  which indicates for the eluvial and illuvial horizons in the depth range 8-96 cm a strong and very strong aggressive acidity (Table 1).

At the same time, the values of hydrolytic acidity in the depth range of 8-96 cm are medium and vary within the limits of 4.1-5.9 me/100 g soil.

The color of the eluvial horizons of the soil is grey and the illuvial horizons - dark brown (Figure 1) as a result of hemosorption and formation on the particles surface of film from the organo-mineral compounds of Fe and Al, dissolved in water (chelates). Illuviation of iron and aluminum chelates is the main process involved in the pedogenetic process of podzolization of these soils (Cerbari, 2001).

According to the data presented in Table 1, the thickness of the humiferous profile is 47 cm, the humus content in the genetic horizons can be appreciated as follows: AEh<sub>t</sub> 0-8 cm - high content; AEh 8-27 cm - submoderate content; EBhtw 27-47 cm - small content.

**Greyzem albic on the Dniester Plateau.** The virgin stagnic albic greyzem (Figure 2, Profile 12) was located on the horizontal surface of the Dniester Plateau in a hornbeam forest. Absolute altitude - 249 m. The soil is characterized by profile type: AEh<sub>t</sub> (0-5 cm) → AEhg (5-20 cm) → BEhtg (20-37) → Btwg1 (37-60 cm) → Btw2 (60-80 cm) → BGtw (80-100 cm) → Cwg (100-120 cm).

The values of the physical and chemical properties of albic greyzem are presented in Table 1. The greyzem albic is characterized by a strongly differentiated textural profile. The values of the textural differentiation index for illuvial horizons of soil are high - 19-21. The soil is characterized by high aggressive acidity which has led to the textural differentiation of its profile. The pH<sub>KCl</sub> values are equal to 3.2-3.6, characteristic for manifestation of the podzolic process. At the same time, the hydrolytic acidity is very high - 12-13 me. The sum of the exchangeable cations (Ca<sup>2+</sup> + Mg<sup>2+</sup>) is small in the eluvial horizons and medium in the illuvial horizons.

The greyzem albic stagnic is characterized by a superficial humic profile. The humus content is very high in the organic surface layer 0-5 cm (8.00%). This layer suddenly passes into the low humic albic eluvial horizon (1.99%). The soil is leached by carbonates on the entire profile. The soil reaction is strongly acidic. Gleyzation and acidity in the local conditions of horizontal surface without leaks, temperate continental climate with humidity coefficient - 1.0, are the factors that ensured the gleyic process manifestation of Fe and Al migration and formation of the albic eluvial horizon.

**Brownzem typical virgin** (Figure 3, Profile 60), located on the quasi-horizontal surface, above the village of Ivancea (Orhei) is characterized by profile type: AEh<sub>t</sub> (0-8 cm) → AEh (8-20 cm) → BEhtw (20-31 cm) → Bhtw (31-52 cm) → BCw (52-70 cm) → BCwk (70-90 cm) → Bck (90-120 cm) → Ck (120-150 cm).

Due to the intensive brown color of the mineral part of the soil and the weakly manifestation of lesvage, the genetic horizons of the brownzem profile are poorly differentiated.

The results for Profile 60 presented in Table 1 confirms that typical virgin brown soils are poorly differentiated texturally. The texture of these soils is clayey-loamy to loamy in the surface horizons AE and clayey-loamy to loamy-clayey in the illuvial-cambic horizons Bhtw, for which the clay content is 8-13% higher than in the eluvial horizon AE.

The decrease in the clay content in the illuvial horizon AE is due to its leaching in the horizons in the central part of the soil profile. Clay surplus from the illuvial-cambic horizon is formed both as a result of its leaching from the AE horizon, as well as as a result of the *in situ* alteration process (for the most part). However, the moderate *in situ* alteration of the soil material of the middle part of the profile and the poor leaching of the colloids from the top of the soil led to poor textural differentiation of the profile and the coating of soil particles with iron hydroxide films.

Lack of iron and aluminum in lysimeter water (Граци, 1977) confirms the very weakly manifestation in these soils of the podzolic process. According to the data, typical virgin brownzems is characterized by favorable physical properties in the surface layer 0-30 cm and unfavorable in the cambic illuvial horizon. Apparent density in this horizon, usually varies in the range of 1.60-1.65 g/cm<sup>3</sup>, which leads to a worsening of its quality and often at the manifestation of the stagnant gleyzation.

The soil reaction is slightly acidic in the AEh<sub>t</sub> horizon (pH = 6.6); strongly acidic in the AEh horizon; slightly acidic in the BEhtw, Bhtw, BCw horizons; weakly alkaline in the Bck and Ck horizons. The carbonates in the form of bioglasca and vines are contained in the limits of 12.5-24.4% in the horizons Bck and Ck, starting with a depth of 70 cm.

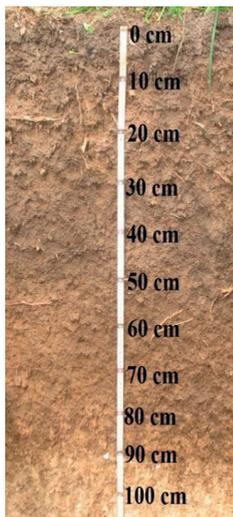


Figure 3. Profile 60. Brownzem typical, submoderate humic with semi-deep humiferous profile, clayey-loamy, poorly differentiated textural, virgin (altitude 197 m, oak forest, Orhei)

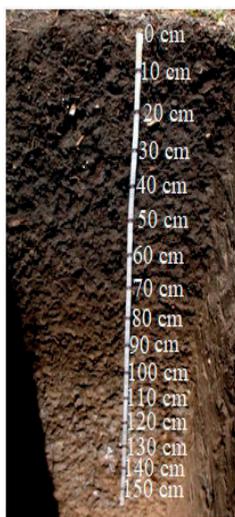


Figure 4. Profile 17. Chernozem typical, humic with deep strongly humiferous profile, clayey-loamy, virgin (altitude 160 m, fluffy oak forest, Anenii Noi, Calfa and Hirbovăt)

The soil is characterized by a semi-deep humiferous profile (thickness about 50 cm). The humus content on the soil profile varies from 8.99% in the horizon 0-8 cm up to 1.35% in the iluvial horizon at a depth of 30-52 cm.

***Chernozems typical virgin from the xerophytic deciduous forests of southern Moldova.*** These soils widespread in the southern part of Moldova with a recently semi-arid climate were first described by Крупенников (1967) and called "chernozems xeroforestry" (Ursu, 2011) described these soils under fluffy oak forests with specific grass cover.

Considering the morphological composition of these soils, according to recently characteristic for typical chernozem, was called typical moderately humiferous chernozems (Ursu, 2011). The word "xeroforestry" is not coerspund to the soil subtype level. However, the humus content within the range of a soil subtype is an index with very large spatial variation.

The typical chernozems from the southern semi-arid area of Moldova are spread over several large areas (the periphery of the Tigheci height with an altitude of 130-190 m), which gives the possibility to appreciate this territory as the

beginning of the forest-steppe zone in Republic of Moldova.

Note that chernozems cannot evolve in any forest. Thus, we assume that the invasion of the semi-arid area of southern Moldova of the fluffy oak forest took place in the Little Ice Age between 1250-1850s, when there was a severe cooling of the climate in Europe. In the Little Ice Age the winters were very cold with a lot of snow, and the summers were wetter than now. This led to the invasion of forests on the already existing areas of ordinary chernozems. The microclimate formed by the forest with grassy vegetation contributed to the progradation of common chernozems into typical chernozems.

The characteristic of chernozems formed under xerophytic forests in south-eastern Moldova (Figure 4) were based on data obtained for soil profile 17, located in the forest near Calfa commune, south-eastern Moldova, which is a specific ecological niche.

The primary denudation surface of this alluvial territory was formed in the late Pliocene. Recently, this surface has altitudes of 150-200 m and represents a slightly undulating plain and at the same time strongly fragmented towards the Dniester valley and its tributary valleys.

Surface rocks are composed of Quaternary loessoid deposits with a thickness of 2-5 m to 20 m, followed by Pliocene alluvial deposits. The loessoid clayey-loamy deposits are characterized by a high content of the coarse dust fraction that ensures a positive impact on the soil physical and mechanical properties.

The deep valleys of small rivers running from the northwest to the southeast serve as passageways for raindrops formed over the Black Sea. The heights surrounding these valleys stop the movement of moist air masses. As a result, the humidity regime on these heights is quite favorable and large fragments of xerophytic forests (Hârbovăt forest) have still been preserved here. In the southern area warm semi-humid - dry. Solar period - 310-320 days. Duration of insolation - 2200-2300 hours. Average annual temperature - 9.5°-10°C. The sum of t°C>10° - 3100-3300°. Annual amount of precipitation - 500-550 mm. Potential evaporability - 850-900 mm.

Table 1. Physical and chemical properties of soils formed under deciduous forests in the forest-steppe of the Republic of Moldova

Depth, cm	Fractions particle size, mm; content, %		Index of textural differentiation	Apparent density, g/cm <sup>3</sup>	Humus, %	CaCO <sub>3</sub> , %	pH	pH <sub>KCl</sub>	Hydrolytic acidity, me/100 g soil	
	<0.001 mm	< 0.01 mm								
Profile 11. Grizem typical, moderately humiferous with semi-deep humiferous profile, semi-deep clayey-loamy 0-47 cm, loamy-clay in depth. moderately texturally differentiated, virgin										
AEht 0-8	20.6	46.4	<b>1.0</b>	1.09	5.61	0	5.9	5.6	5.7	
EAh 8-27	21.5	48.1	<b>1.0</b>	1.17	2.24	0	5.6	5.0	5.9	
Ebhtw 27-47	28.7	50.7	<b>1.4</b>	1.40	1.10	0	5.6	4.3	5.0	
Btw 47-68	36.3	63.0	<b>1.7</b>	1.61	0.83	0	5.8	4.1	4.8	
BCtw 68-96	37.7	61.6	<b>1.8</b>	1.64	0.47	0	5.9	4.3	4.1	
Ctw 96-110	39.7	61.6	<b>1.9</b>	1.63	0.42	0	7.0	6.0	1.1	
Cwk 10-130	37.6	6.4	<b>1.8</b>	1.59	0.41	5.9	7.0	-	-	
Profile 12. Grizem albic, slitized stagnic, gleyzed from the surface, clayey-loamy 0-37 cm and loamy-clay 37-80 cm, strongly differentiated textural, virgin										
AEhtg 0-5	17.9	41.6	<b>1.0</b>	0.85	8.00	0	5.7	5.2	11.4	
AHhg 5-20	24.2	45.3	<b>1.0</b>	1.35	1.99	0	4.4	4.5	13.5	
Ebtwg 20-37	34.0	56.2	<b>1.4</b>	1.61	0.57	0	4.8	4.4	12.9	
Btwg 37-60	46.0	62.0	<b>1.9</b>	1.64	0.58	0	4.9	3.4	13.8	
Btwg 60-80	50.6	63.6	<b>2.1</b>	1.66	0.59	0	5.0	3.3	12.5	
Gtw 80-100	44.2	57.4	<b>1.8</b>	1.63	0.29	0	4.6	3.5	12.4	
Gtw 100-120	40.6	54.4	<b>1.7</b>	1.61	0.24	0	4.7	3.5	12.0	
Profile 60. Brunezem typical submoderate humerous with semi-deep humiferous profile, clayey-loamy, weakly differentiated texturally, virgin										
AEht 0-8	26.3	48.3	<b>1.0</b>	0.81	8.99	0	6.6	-	1.9	
AH 8-20	27.1	49.7	<b>1.0</b>	1.16	2.77	0	4.9	-	11.7	
BEht 20-30	34.6	54.7	<b>1.3</b>	1.38	1.88	0	6.5	-	4.1	
Bhtw 30-52	39.8	58.4	<b>1.5</b>	1.58	1.35	0	6.2	-	3.2	
Bw 52-70	35.5	58.5	<b>1.3</b>	1.60	0.88	0	6.3	-	2.9	
BCwk 70-90	32.6	56.4	<b>1.2</b>	1.58	0.84	12.5	7.5	-	0	
BCk 90-120	26.2	49.6	<b>1.0</b>	1.55	0.78	24.4	8.0	-	0	
Ck 120-150	20.0	38.1	<b>1.0</b>	1.48	0.27	24.0	8.0	-	0	
Profile 17. Chernozem typical clayey-loamy with a strongly deep humiferous profile texturally undifferentiated, virgin (xerophytic deciduous forest in southern Moldova)										
Aht 1 0-12	29.8	50.5	<b>1.0</b>	1.06	6.30	0	7.0	-	1.5	
Aht2 12-27	31.6	52.5	<b>1.1</b>	1.20	4.97	0	6.9	-	1.8	
Ah 27-47	32.9	53.3	<b>1.1</b>	1.32	3.60	0	6.9	-	2.2	
ABh 47-67	33.9	53.3	<b>1.1</b>	1.45	2.63	0	6.8	-	2.0	
Bhk1 67-90	34.4	53.2	<b>1.2</b>	1.45	1.80	2.8	7.4	-	0	
Bhk2 90-109	34.5	53.9	<b>1.2</b>	1.45	1.46	9.0	8.0	-	0	
BCk 109-130	34.4	54.9	<b>1.2</b>	1.44	1.00	12.5	8.2	-	0	
BCk2 130-150	34.7	55.3	<b>1.2</b>	1.44	0.82	13.9	8.1	-	0	
Ck 150-200	34.4	55.4	<b>1.2</b>	1.40	0.65	11.5	8.0	-	0	

Hydrothermal coefficient after Ivanov-Vashotskii,  $K = 0.55-0.65$ . Duration of the vegetation period - 179-187 days. Frequency of droughts - 2 times in 10 years.

The typical chernozem (virgin) from Hârbovăț forest is characterized by a type profile: *Aht1* (0-12 cm) - *Aht2* (12-30 cm) - *Ah* (30-47 cm) -

*ABh* (47-67 cm) - *Bh1* (67-90 cm) - *Bhk2* - *BCk1* - *BCk2* - *Ck* (Figure 4).

Effervescence - from 90 cm. Carbonates in the form of pseudomycelias - from 90 cm, rare accumulations of bioglobulin - from 180 cm The physical and chemical properties of the chernozem are presented in Table 1.

The content of physical clay on the soil profile is quite homogeneous and varies in the limits of 50.5-54.9%, and of the clay itself varies in the limits of 29.8-34.7%.

According to the values of the apparent density, the typical chernozem is very loose in the horizon A<sub>h</sub>1 (0-12 cm), loose in the horizon A<sub>h</sub>2 (12-27 cm), non slitized in the horizon Ah (27-47 cm), weakly compacted in the horizons AB<sub>h</sub>, B<sub>hk</sub>1 and B<sub>hk</sub>2 in depth range 47-100 cm.

This soil is characterized by excellent physical quality. According to the humus content, the researched soil is deeply humic, leached by carbonates to a depth of 67 cm, pH<sub>H<sub>2</sub>O</sub> value = 6.8-7.0. This soil, according to the values of the listed properties, has a very high potential fertility (Table 1).

## CONCLUSIONS

Silvosteppe on the Republic Moldova territory practically extends over most, from North to South under deciduous forests the following types of soils have been highlighted: greyzems; brownzems; typical chernozems.

Typical virgin greyzems, brownzems and chernozems, formed under deciduous forest vegetation on the Republic of Moldova territory are polygenetic soils and have undergone various phases of pedogenesis as a result of climate change in the Holocene, anthropogenic impact (deforestation and arable land use; nomads, the disappearance of agriculture and the restoration of secondary steppe grass vegetation on former arable land, the invasion of fluffy oak forests on chernozems formed under the steppe vegetation of southern Moldova and their progression to typical chernozems with deep humiferous profile was short glacier).

The textural differentiation of the typical greyzem profile from the northwestern part of the Northern Moldavian Plateau occurred under the integral action of three pedogenesis processes: alteration *in situ* of the soil material; leaching of the colloidal material of the alteration.

The formation of brownzems on the Codrii Plateau is due to the synergistic action of the contrasts semi-arid climate in the atmosphere during the warm period of the year for the local latitude and the specific climate in the loamy-

sandy soil. The high sand content sufficiently increases the aridity and contrast of the hydrothermal regime in this soil.

In the hydrothermal conditions of the Codrii Plateau on the parent clayey-loamy rocks, rarely found here, due to the fact that these rocks do not increase the aridity of soil, greyzems and not brownzems were formed.

Iron plays an important role in the genesis of soils in the forest-steppe zone: it is the third element in the soil by content, compared to other elements and, possessing the property of changing its valence and properties, is a diagnostic feature of the direction of pedogenesis. and soil subtype; determines the color of the soil, conditioned by the degree of hydration of the iron as a result of the modification of the hydrothermal regime, it has the property of changing its valence and properties, it represents a diagnostic character of the pedogenesis direction of the soil type and subtype; determines the color of the soil, conditioned by the degree of hydration of the iron as a result of the change of the hydrothermal regime.

In the field the greyzems and the brownzems are different: the typical virgin greyzems are characterized by the light color of the eluvial horizon and the dark brown of the illuvial horizon, as a result of the covering of the soil particles with organo-mineral films of the iron chelates of 100-120 cm; typical virgin brownzems are characterized by a light brown color of the eluvial horizon and a brown or reddish-brown color of the illuvial-cambic horizon, the carbonates appear deeper than 70-100 cm of profile.

In order to increase the flow of organic matter in arable grey soils and brown soils, it is necessary to organize legume seeds (autumn or spring vech and peas) for use as green manure in the agricultural sector of Moldova (Leah, Cerbari, 2020).

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## SOME ASPECTS AS A RESULT OF THE EVALUATION OF HUMUS CONTENT IN ERODED SOILS

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### *Abstract*

*Visible soil erosion processes began to develop in the territory of Moldova around the second half of the 19<sup>th</sup> century. For a long time, their quantitative indicators of its spatial spread were not due to the lack of such cartographic data. After the early 1950<sup>s</sup>, soil mapping in agricultural units and administrative districts. Fragmented relief is one of the indispensable conditions for the development of slope processes, including erosion processes. Anthropogenic impact plays an important role in increasing the areas affected by erosion. The purpose of this paper is to examine the content and distribution of humus in eroded soils in the Republic of Moldova to assess their fertility. As a result of the evaluation of the humus losses on the three degrees of erosion, it was found that, for chernozems, they correspond approximately to the row: 20, 40 and 60%. Some trans-boundary passages are observed in carbonate chernozems - being the least resistant to erosion (28, 48 and 69%) and an insignificant variation are characteristic of typical chernozems and to a lesser extent leached (17, 39, 60 and 16, 38, 64%), but in soils with a high erodibility these figures of humus losses, almost equal. Brown soils are related to erosion on average for chernozems approximately 20, 40, 65%. We can state that, exclusively, it presents the gray soils in which the degree of erosion, weak and moderate, is distinguished by lower humus losses. If we refer to the whole, then the series of 20, 40 and 60% humus losses in the eroded soils compared to the standard, we are quantitatively characterized by their real degradation effect, quite terrible, caused by erosion. But from a practical point of view it is essential, because these figures characterize the real humus degradation of the underlying soil layer (50-100 cm), which has a significance for assessing their fertility especially for perennial crops, which develops a deep root system.*

**Key words:** *humus content and losses, soil erosion process, soil type and subtype.*

### INTRODUCTION

Humus is an essential component of the soil on which its main qualities and peculiarities depend. The humus content also conditions the taxonomic level in the systematics of some soils. The results of the generalization of contemporary analytical data allow the introduction of a system of quantitative indices for assessing the degree of humification of taxonomic units and their division by gender (Ursu, 2014). In the process of pedogenesis in the soil profile, a chemical with a very complicated composition is formed and accumulates, which has been called humus. This substance is formed as a result of the interaction of soil biota with organic residues from the decomposition of organic matter. Humus is the main component and conditions the main properties of the soil. Over time, these indices gave way to the taxonomic level, currently we are still considered in contemporary

classifications (SWSR, 2015). However, humus has not lost its primary role in pedogenesis, a current problem of the condition of reclaimed soils is dehumidification.

In the Republic of Moldova, the role of humus in soil pedogenesis and taxonomy is aware (used in the classification of chernozems), but the humus content is not limited by quantitative indices (Ursu, 1999). The problem is that the humus content is dependent on many factors: phytocenosis (steppe, meadow, forest), particle size composition (clay, dust, sand), land use (plantation, pasture, protection strip), the degree of degradation and the method of technogenetic transformation of the exploited soils (erosion, plowing, clearing, etc.). On the territory of the Republic of Moldova, grass steppes (fescue-grass) on virgin chernozems have not been preserved. The total capitalization of chernozems and their work over hundreds of years have caused their degradation - destructuring and dehumidification with all the

respective consequences. The maintenance of chernozems with perennial grasses and the establishment of forest plantations (anti-erosion forest strips, etc.) within 4-5 decades can obviously restore the upper part of the chernozem profile (horizon A) - partly grainy structure and humus content (Ursu, 2005). Such "improvements" occur only in slightly degraded soils, which have kept their vertical profile. In heavily eroded soils these processes occur very slowly.

In the Republic of Moldova, due to inadequate and extensive human activities, other objective and subjective factors, the humus content in soils decreased from 5-6% to 2.5-3.0%, the surface of eroded land increasing to about 850 thousand ha, which constitutes over 35 percent of the agricultural land area. The surface of landslides affected by landslides has also increased, which currently constitutes over 55 thousand ha of active landslides and about 350 thousand ha of landslides in a state of stagnation (Ursu, 2013; Крупеников, 1967). The landslide led to the removal to the earth's surface of the underlying horizons of low-humid soils of the initially eroded soils. The surface humus horizons were buried to a depth of 30-50 cm, thus being preserved.

Microtraising and weeding between rows in fruit plantations has led to a decrease in erosion processes. As a result, the processes of accumulation of organic matter have intensified in the former underlying horizons moderately humiferous, returned to the surface by unclogging. In the fields where the vineyards and orchards were cleared about 20 years ago, and the lands were turned over to arable land, the erosion processes intensified, the fertile soil losses increased. Therefore, with the recent deforestation of old vineyards and orchards and the transfer of arable land, we can predict an intensification of erosion processes on the territory of the reception basin. According to the humus content in the recently arable-postarable layers (0-30 cm), the researched soils not eroded and weakly eroded are moderately humiferous (humus content 3.0-3.3%), those moderately eroded - submoderate humifers (2.7-2.9%), and the strongly eroded ones - weakly humic (1.9-2.3%). The problem of increasing the flow of organic matter in both eroded and not eroded

soils is the main one in maintaining their quality status and production capacity (Cojocaru, 2016).

## MATERIALS AND METHODS

Pedological research carried out for various purposes necessarily includes the analytical assessment of the humus content in different horizons. However, these results are not taken into account in the name of the soil. In the current classification (Ursu, 1999) the humus content conditions the division of the typical chernozem into moderately humiferous and weakly humiferous (usual). This division mainly refers to the reclaimed land, but the quantitative criterion is missing.

Recently, research conducted in downy oak forests has highlighted the taxonomic variability of chernozems at the subtype level (leachate, typical, carbonate) and humus content (Ursu 2012; 2013; Ursu et al., 2012). Thus, at present we have a considerable variability of chernozems with different humus content (and with different thickness of humified profiles), which is not taken into account in the current classification. Research on typical gray soils in different regions and under different types of forests, has established an obvious difference in humus content in the upper and underlying horizons (A1, A2 and B1). This quantitative index in the previous classifications was taken into account when assessing the degree of podzolization (podzolic ash, poorly podzolic ash). The humus content in brown soils and in the types of azonal soil classes (lithomorphic, hydromorphic, halomorphic, dynamomorphic) is not taken into account.

Given the role of humus in pedogenesis, I consider it appropriate to introduce into the taxonomic system of Moldovan soils the quantitative indices of humus content at the level of gender (content) and at different levels (profile thickness). The following is a system of quantitative indices for different soil types. The assessment of these indices was performed by analyzing the database, collected in recent decades, and adapting them to the taxonomic classification system of the higher soil units of Moldova (Ursu, 2011).

In brown soils, the humus content in the 0-10 cm layer varies between 4.5 and 7.7% in the profiles dug in the forest and 3.2-1.1% in the reclaimed

soils. In white and typical ash soils, the humus content is 6.8-2.9% under forests and 3.8-1.6% in plowing, in soft ash, respectively 10.9-8.9 and 4.1-2.6%. The humus content in chernozems is also quite variable. The most humidifying is the clay-iluvial chernozems, leached and typical under the forests of common oak (clay-iluvial), fluffy oak (leached and typical) and forest strips (4.5-11.3%). Weak humifiers are the typical chernozems and carbonate dehumidified as a result of recovery and work over the centuries. The humus content (Ursu, 2014) in lithomorphic, hydromorphic, halomorphic and dynamomorphic soils varies depending on different factors (granulometry, phytocenosis, recovery, clogging, improvement, etc.).

## RESULTS AND DISCUSSIONS

The content and distribution of humus per profile in eroded soils serves not only to specify their classification status (degree of erosion) but also to a lesser extent to assess their fertility (reclamation) and characterize most other

ecological functions of soils as their degree of erosion. manifestation is in a rather high dependence (close to the linear one) on the amount of humus (Крупеников, 1990; 1997; Добровольский, Никитин, 1990).

Under the influence of erosion, the general humus impoverishment of the soils on appreciable spaces takes place. Such a situation should not only be considered as a simple summation of the loss of humus by separate portions of land, but should be seen as something of a principle that turns the soil cover into another essence. Kovda B.A. (1981) put forward an idea of assigning a special status to the separate humus sphere in the biosphere by presenting the spatial junction of the humiferous horizons as such as the soils of the earth's crust. Humosphere - after the gradations of erosion in the erosional queens it loses successively its characteristic aspects it moves away from the initial humispheric level. This can be seen from the string of humus content in the chernozems shown and the gray soils as a measure of the increase in erodibility (Table 1).

Table 1. Average indices of humus content and losses (%) in the 0-50 cm layer in chernozem subtypes and gray soils by degree of erosion

Soil types and subtypes	Standard %	weak eroded		moderately eroded		strongly eroded	
		1	2	1	2	1	2
Carbonate chernozem	3.36	2.40	28	1.75	48	1.03	69
Ordinary chernozem	3.47	2.65	21	1.94	44	1.01	62
Typical chernozem	3.74	3.11	17	2.23	39	1.50	60
Levigated chernozem	3.40	2.84	16	2.01	38	1.22	64
Average - for chernozems	3.52	2.75	20,5	1.98	42	1.19	64
Dark gray soils	2.49	2.02	20	1.48	40	0.87	65
Gray soils	1.82	1.55	16	1.35	25	0.72	60

Note: 1 - average; 2 - % losses compared to the standard

There is some difference in some soil subtypes but the principle (trend) of humus loss on the three degrees of erosion for chernozems corresponds approximately to the range: 20, 40 and 60%. Some "overrun" is observed in carbonate chernozems - the least resistant to erosion (28, 48 and 69%) and a small "lag" are characteristic of typical chernozems and to a lesser extent leached (17, 39, 60 and 16, 38, 64%) but at high erodibility the loss figures almost equal. Dark gray soils are related to the average for chernozems (20, 40, 65%).

Exclusivity shows gray soils in which the degree of erosion is weak and moderate, distinguished

by lower losses. But overall, the range of 20, 40 and 60% humus losses from the standard quantitatively characterizes the really terrible degradation effect caused by erosion: there is a rapid and increasing extinction of the humus sphere. According to the principle in Table 1, the losses by the soils of the amount of humus in the layer 50-100 cm are also calculated. Of course, only in the case of heavily eroded soils do we have to deal with real losses, but in other cases this situation is related to the proximity to the surface due to the erosion of the less humic layers of the soil. But from a theoretical point of view it is important and practically essential that

these figures characterize the real humus degradation of the second half meter of the soil, which has a significance for assessing their

fertility especially for perennial crops, which develop a deep root system (Table 2).

Table 2. Average indicators of humus content (%) in the 50-100 cm (Hoyp, 2001) layer at subtypes of chernozems and gray soils by degree of erosion (conventional signs are the same as in Table 1)

Soil types and subtypes	Standard %	weak eroded		moderately eroded		strongly eroded	
		1	2	1	2	1	2
Carbonate chernozem	1.66	0.97	42	0.71	57	0.47	71
Ordinary chernozem	1.73	1.26	26	0.89	47	0.60	65
Typical chernozem	1.83	1.42	21	1.14	37	0.67	63
Levigated chernozem	1.95	1.36	24	0.97	50	0.46	77
Average - for chernozems	1.77	1.25	28,5	0.92	48	0.55	69
Dark gray soils	1.09	0.84	26	0.65	46	0.53	51
Gray soils	0.72	0.58	20	0.29	41	0.36	50

For the 50-100 cm layer the dispersion of the values and the values of losses themselves is somewhat higher than for the 0-50 cm layer; the average value for chernozems is a string: 28.6; 48 and 69% compared to 20.5; 42 and 64% in the 0-50 cm layer. At the quantitative level, it can be concluded that as the erosion increases,

there is an intensified degradation in terms of humus content and in the second half of the soil. The values obtained (Table 3) also have a significance for the correction with the help of laboratory analyzes of the data of the field diagnosis of the soils regarding the determination of the degree of their erodibility.

Table 3. Average depth, in cm of humus content of 1% in Moldovan soils with full profile and eroded (Hoyp, 2001)

Soil subtype	Full profile	Eroded soil		
		weak	moderately	strongly
<b>Chernozems</b>				
Carbonate	110	70	50	30
obişnuit	110	90	70	40
Typical	110	90	70	60
Levigated	110	90	70	40
Dark gray soils	80	70	50	20
Gray soils	60	60	40	20

An unanimously accepted index for diagnosis and soil appreciation contributes to the depth of the layer with humus content over 1% (Table 3). Deep chernozems, all their subtypes have on average such an amount of humus at a depth of 110 cm. In case of weak erosion at the carbonate chernozem this value is reduced up to 70 cm at the rest of the subtypes up to 90 cm at the degree of moderate erosion at the carbonate chernozem up to 50 cm at others - 70 cm. At strong erosion the variety of values is expressed more clearly, for example at carbonate chernozem only 30 cm; in the usual and leached - 40, in the typical - 60 cm. In both subtypes of gray soils all values are essentially lower and in strongly eroded varieties it decreases by up to 20 cm.

The value of these quantities (Table 3) is important for solving such problems as:

2) the possibility to perform unclogging; 3) alienation of land "at rest" through debauchery.

The varieties of strongly eroded carbonate chernozems of both subtypes of gray soils can in no way be used as arable land, vineyards and orchards but must be expropriated for cultivation and at the same time the hope for the relative restoration of the soil profile is not too great here. it is probably possible to create very shallow soils over time in conditions of their permanent and regular use for fodder uses.

It is necessary to make a remark regarding the standards: in the period of 15-20 years that have passed since the editing of the guidelines on the statistical parameters of the soils, the thickness of their genetic horizons has not changed but the humus content especially in the 0-30 cm layer, indisputably reduced, it must be assumed by 0.1-

0.3%. But mass figures on this issue do not exist. Therefore, we consider that it is possible to use as standards parameters of the humus content mathematically representative, established according to the large numerical totality obtained before. In this case it is about full profile soils (standards). Regarding the eroded soils of three degrees of erosion, here things are presented in a simpler way: their surfaces have increased but the characteristics of the humus content of all gradations have remained unchanged. We will examine the content and composition of clay minerals in Moldovan soils as this parameter as well as the particle size composition also to some extent influences the anti-erosion resistance of soils.

Of these minerals, which fall into the fraction of clay only soil particles with a size of less than 0.001 mm play the most essential role in this respect is played by the illit-smectitic group and that of the illite (as a whole in the past, and sometimes even now mineral is called montmorellonite). In the subtypes of chernozems with full profile in their 0-30 cm layer, the amount of smectite and illit-smectitic oscillates within the limits of 13.4% (carbonate chernozem) up to 16.8% (podzolytic chernozem) in the corresponding horizon B 12.8-19.6%.

According to the illiterate, the image of its distribution on the profile is essentially completely different: analogous figures constitute 10.5-16.4% and 7.2-13.6%, which is with depth the content of this clay mineral decreases. Overall, it can be said that the chernozems of Moldova are characterized by a fairly high content of clay minerals. (Почвы Молдавии. Том I, 1984, p.111). You can also find the corresponding figures for all other chernozem subtypes to 4-5 depths of the profile, there are also data for brown and gray soils (Алехеев, 1999).

For eroded soils and only for chernozems, very little is given. After smectite, smectite-illite the differences in the degree of erosion are significant, and after illite they are quite essential: for example in the horizon Ap (0-40 cm) of the carbonate chernozem the amount of illite is 9.1-10% in the varieties eroded in depending on the gradations, 8.9-7.1% (Алехеев, Арапу, 1987) which is as the erosion increases, the soil resistance to erosion based on

this indices must decrease but it must be assumed that the significance of this parameter in general is less essential.

The most initial figures for the entire republic on the spread of eroded soils refer to 1967 when eroded soils (which is moderately and heavily eroded, ravaged and affected by landslides) occupied 641.7 thousand ha or 19% of the territory of Moldova; eroded ash soils cultivable by these degrees of erosion - 66.2 thousand ha or 2% (Крупеников, 1967, p.144). According to the pedological provinces (according to their separation at that time) the eroded chernozems were distributed as follows in % of the surface of the province: northern Moldovan forest-steppe - 19.5; Central Moldova forestry - 17.7; plain near the Danube (Predanubian); Ukrainian steppe plain - 15.9% (Крупеников, 1967, p. 367). Such data were reported on pedological districts and sub-districts (Крупеников, 1967, pp. 380-382). Here we have a relatively lower figure for the central province (17.7%) which, as it has been known since then, has suffered more than other provinces due to erosion, this is explained by the fact that the calculations were carried out for the whole area without excluding the areas occupied by forests, which here are the most (about 40%) and the soils under forest vegetation are practically not affected by erosion. In general, it can be said that by the end of the 1960<sup>s</sup>, the deterioration of the republic's soils caused by erosion was already very significant and alarming (21%, which is about one-fifth of the territory of Moldova). Next, a calculation was made of full-profile and eroded soils of three degrees of erosion - according to the soil map of Moldova. They were more precisely genetically differentiated and quite reasonably determined soil surfaces in terms of their subtypes, and among them full profile and eroded three degrees after the state in the early - mid '80<sup>s</sup>.

The complete data of these estimates are given in volume 2 "Почвы Молдавии" in special tables (pp. 20-21). We chose from these those materials that refer to eroded soils (Table 4). Regarding the accuracy of the ground map at a scale of 1: 50000 it has not been discussed and decided mono-semantically that it is accurate, as this notion is appropriate to the given scale map and was drawn up by generalizing the maps at a scale of 1: 10000 or more precisely. In general,

of course, some small areas merged, and at the same time, naturally, the degree of information on the map decreased. However, since the merger of accounts was often done in one direction or another, its final result had to be obtained more or less truthfully starting from the law of large numbers.

Turning to the analysis of Table 4, it should be noted that we do not have a better and more complete source of quantitative information about the soil cover of the republic. The share of all eroded soils changed slightly compared to 1967 (21%) according to new data, it accounted for 22.5% of the country's territory. If we

consider only soils that are subject to erosion and exclude those that are exempt from it (alluvial soils from meadows, alluvial soils, wetlands, etc.) then the total level of erosion of the soil cover will increase up to 31%. reaching almost a third of the total area, which is the excess is about 30%. But about 10-15 years have passed since the map was drawn up, and naturally the eroded soil surfaces have now grown. This is an indisputable fact, but we do not know its precise quantitative expression. It will be shown below that we may have some new opinion on the issue at hand (Table 4).

Table 4. Surfaces of Moldovan soils (Hoy, 2001) with full profile and eroded

Soil type and subtype	Full profile		Eroded soil							
			Weak		moderately		strongly		total	
	1	2	1	2	1	2	1	2	1	2
Brown	19332	92.5	956	4.6	603	2.9	0	0	1559	7.5
Light gray	3905	96.2	145	3.6	9	0.2	0	0	154	3.8
Gray	102283	77.2	17471	13.2	11167	8.4	1546	1.2	30184	22.8
Dark gray	119781	78.0	24583	16.0	8510	5.5	738	0.5	33831	22.0
Carbonated chernozems	359612	55.8	160122	24.2	101082	15.5	32864	5.0	294068	44.2
Ordinary chernozems	492148	77.6	101574	16.0	36355	5.8	4023	0.6	141952	22.4
Forest xerophytic	14579	80.9	2966	16.3	596	3.3	0	0	3562	19.6
Typical chernozems	217780	77.4	46838	16.6	14947	5.3	1972	0.7	63757	22.6
Levigated chernozems	270461	68.4	84844	21.4	34778	8.8	5452	1.4	125074	31.6
Podzolytic chernozems	97364	82.7	15203	12.9	4899	4.2	271	0.2	20373	17.3
Compactly	11596	84.5	1228	9.0	664	4.9	217	1.6	2109	16.5
Solonized and salted	10504	61.0	3005	17.4	3438	20.0	278	1.6	6721	29.0
Rendzine	13824	42.7	6457	19.9	9970	30.8	2146	6.6	18573	57.3
Total area	1733169		455392		227018		49507		741917	
% of the surface of Moldova (3 million 376 thousand ha)	-	52	-	14	-	7	-	1.5	-	22.5
% of the surface of soils subject to erosion (2 million 482 thousand ha)	-	69.5	-	18	-	9.5	-	2.0	-	31

Note: 1 - thousand ha; 2 -% of the subtype area

Carbonate chernozems (44.2%) are the most subject to erosion, followed by leached chernozems (31.6%). Two central subtypes of common and typical chernozems (22.4 and 22.6%) are close to each other and also to the value of the total damage (22.5%). Leaving aside the little-spread soils, we note that the ash and dark ash soils (22.8 and 22% as well as the

podzolytic chernozems (17.3%) on significant areas are occupied by forests and therefore the figures obtained for them are reduced according to after the total surface of the soils moderately and strongly eroded in the first place are the carbonate chernozems (20.5%) and levigated (10.2%), ordinary (6.4%) and typical (6%) are

close and in this case by each other and by the average index per republic as a whole (8.5%). As a clear reason for the reduced erosion resistance by carbonate chernozems, the peculiarities of their granulometric composition

serve (Table 5); among them almost 40% of the soils have a muddy texture and even lighter than the number of chernozem subtypes, in this respect they suddenly surpass all the others.

Table 5. Distribution of some Moldovan soils (Hoyp, 2001) according to particle size distribution

Soils	% of subtype area			
	clay-loamy and loam-clayey	Clay	clay-sandy	sandy and sandy-loamy
Gray	43.5	30.5	18.4	7.6
Dark gray	65.1	22.1	7.5	2.3
Chernozems				
Carbonated	58.9	36.7	3.2	1.0
Ordinary	79.0	17.8	2.2	1.0
Typical	89.8	8.4	0.9	0.9
Levigated	83.6	11.4	3.0	2.0
Podzolytic	76.8	10.2	2.4	0.6

The influence of the degree of clay on the predisposition of fragile soils and surface rocks to erosion has been discussed above. According to their granulometry, chernozems are related to forest soils, but in this case the general erosion effect is reduced due to the fact that they are probably half under the forest. Regarding the levigate chernozems, it is not possible to talk about the influence of the specificity of the granulometry on their low resistance against erosion, here they probably influence their acid reaction in the superficial horizons, unsaturated in the bases, the less hydrostable structure.

We must be interested not only in the eroded soil surfaces but also in their dynamics: according to the very logic of this process, the eroded soil surface can only increase from year to year: in previous capitals it has been shown that this increase goes fast and as if in two ways: 1) it increases the total surface of the eroded soils; 2) in their composition with exceeding rhythms increases the share of moderately and strongly eroded soils. It was not possible to compare this dynamic for the entire surface of Moldova and the "key method" was applied, which is widely used to describe different geographical, climatic and biological spatial objects. The ground plans of the households were selectively drawn up - 171 in number (this constituted about 20% of their total number) with the interval of their mapping separated from each other by approximately 18-25 years. The households were located to certain pedo-geographical (natural) regions, respectively, for reasons that

this method mirrored the situation more objectively than the sighting in the administrative districts (Захаров, 1978, Крупеников, 1992). Depending on the area of the districts and partly on the presence of materials, the number of control households in the districts varied from 10 to 16.

## CONCLUSIONS

The organic part of each soil is distinguished by the composition and content of humus in the upper horizons and the thickness of the humus layer.

The humus content is a very important integral criterion for the characteristic of the pedogenesis of the soil profiles, the avoidance of the degradation processes.

In the current classification of soils, which refers to the higher taxonomic units, the humus content is not taken into account, because this soil-specific index is at a lower taxonomic level than the subtype.

In order to characterize the soils, taking into account their current state, degradation trends, etc., a system of quantitative indicators of humus content at the taxonomic level of gender is proposed for all higher units.

It is recommended to use the full name of the soil, including at the level of genus: typical brown soil moderately humiferous; levigated humiferous chernozem in the deep, etc.

The humus content can also serve as a characteristic of azonal soils. Quantitative

indices of humus content in the soil characterize not only the properties, but also the pedogenetic conditions, especially biotic factors, biocenoses. The degrading effect of soil erosion is not limited to the removal of fertile layers and the deterioration of its physical, chemical, water and biological properties. The indirect impact of the erosion process on the components of the environment refers to the entrainment in the circuit of some organic and mineral compounds from outside the agricultural lands. The evaluation of these losses was made based on the value of the mass of soil evacuated by erosion and the content of organic matter and nutrients in the washed soil.

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## PECULIARITIES OF ALLUVIAL SOILS FORMATION FROM THE LOWER BOTNA RIVER MEADOW

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### Abstract

*The lower Botna meadow is located on the territory of Causeni district, 6 km south of the Botna river into the Dniester meadow, and characterized by the same absolute altitudes as the Dniester meadow. As a result of overflow waters, the Lower Botna meadow, a permanent swamp territory was formed with an area of about 2500 ha. Dams were built in the Dniester meadow in the 1950s, and the Botna meadow is no longer flooded by the floods of the Dniester. The swamp was drained, the groundwater level dropped to 1.5-2.5 m. The dry land has been used in agriculture for more than 60 years. At present, the soil covers of this territory, according to the WBR-14 classification, is formed by deep humic clayey alluvial soils with extremely deep humiferous profile. The humus content greater than 1.00% to a depth of 2 m and content of mobile phosphorus is 10-12 mg/100 g soil on the entire humifer profile. These endemic soils are described in Moldova for the first time and, due to the high content of humus (4.5-5.0%) and mobile phosphorus (12 mg/100 g), they are an extremely good object for the implementation in the organic farming.*

**Key words:** meadow, humus, Botna, mobile phosphorus, flood.

### INTRODUCTION

The genesis characteristics of the deeply humic alluvial soils were studied in the lower meadow of the Botna River. The Lower Botna meadow is located on the territory of Căușeni district, 6 km southwest of the Botna river overflowing the Dniester river, dreaming of the city of Tiraspol. The meadow is characterized by the same absolute altitudes as other meadows of the Dniester River. The absolute altitude of the surface of the lower Botna meadow varies: from 4 - 5 m at the outflow of the Botna river in the Dniester river on the territory of the meadow facing the village of Chircăiești; from 5-6 m - on the territory facing the village of Cărnățeni (16 km above the overflow site) and 6-7 m near the Căușeni (19-20 km above the overflow of the Botna in the Dniester river).

From the right side of the Botna river meadow, at its overflow in the Dniester river near the Chițcani commune, a horn-shaped hill advanced to the Dniester riverbed, which led to the major narrowing of the Dniester river meadow and the partial redirection of the annual floods to 18-20 km above the Botna river meadow. As a result of the annual floods that have been repeated regularly for millennia, a permanent swamp

territory with an area of about 1800-2000 ha has formed the Lower Botna meadow (Figure 1).



Figure 1. Lower Botna dried meadow on the territory of Chircăiești village, Căușeni district, R. Moldova

Dams were built in the Dniester river meadow in the 1950s, and the Lower Botna meadow was not flooded. In the 1950-1960 period, the natural swamp formed over the many years in the Lower Botna meadow was drained. The groundwater level in this territory has dropped to 1.5-2.5 m. The dry land 60 years ago was used in irrigated agriculture. Currently, the soil cover of this territory, according to the WBR-14

classification, consists of deeply humic alluvial soils (2.4-2.5% humus) with a carbon content in the layer 0-50 cm higher than 1.4%. These soils are post-marshy loamy, with extremely deep humus profile to a depth of 1.7-2.0 m (humus content greater than 1.00% and mobile phosphorus content 10-12 mg/100 g on the entire humiferous soil profile). These endemic soils in Moldova are described for the first time and, due to the high content of humus (4.5%-5.0%) and mobile phosphorus in the arable layer, it is an extremely favorable object for the implementation in the organic agriculture.

## MATERIALS AND METHODS

In the research process of the ground cover of the Lower Botna meadow, the methods approved in the Republic of Moldova were used to carry out pedological research in the field, laboratory and office. In the field were placed 2 soil profiles (7 and 8) with the depth up to the groundwater (Figure 2).

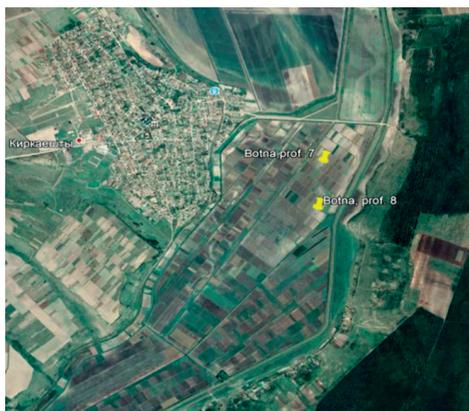


Figure 2. Location of the profiles 7 and 8 in the Lower Botna meadow on the territory of Chircăiești commune, Căușeni district, R. Moldova

The groundwater level in the recently dried meadow varies from a depth of 2.5 m in the middle of the meadow (profile 7) to 1.5 m - in the meadow next to the riverbed (profile 8).

The bulk density in the field was determined by the cylinder method and the resistance to soil penetration with the Golubeva penetrometer. For laboratory analysis, samples were taken from each genetic horizon of the soil profiles.

The analyzes were performed according to the standardized methods in use.

## RESULTS AND DISCUSSIONS

The researched soil profiles 7 was located in the middle of the meadow, and profile 8 - located near the Botna riverbed on the agricultural lands of Chircăiești village. The identification of soil taxonomic units and value classes for soil classification indicators at lower level was carried out on the basis of the Soil Classification of the Republic of Moldova (Cerbari, 2001; Крупеников, Подымов, 1987), Russian Classification System (Егоров, Фридланд, 1977), World Reference Base for Soil Resources WRB (2014); Methodology for elaborating pedological studies, Part III. Ecopedological indicators (Florea et al., 1987).

The soil profiles 7 and 8 is characterized by clayey granulometry (particle-size distribution) and analogous morphological indices, but the thickness of their genetic horizons differs due to the different depth of the groundwater level (Figures 3 and 4).

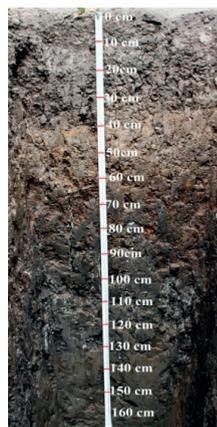


Figure 3. Profile 7. Deep humic alluvial soil after dried swamp (central meadow)



Figure 4. Profile 8. Deep humic alluvial soil after dried swamp (meadow near the river)

The researched soils has a profile type: *Ahp* - humiferous horizon, humus content 4.2-4.7%; *ABhr* - humiferous submoderate horizon with accumulations of iron oxides in capillary water; *Bh<sub>yz</sub>* - humic horizon with accumulations of the gypsum and soluble salts in the water of the

capillary fringe; *BGh* - humic gleyic horizon; *G* - mineral gleyic horizon deeper than 170 cm. The humus of the humiferous horizons of the studied soil is gray-black. All the genetic horizons described have gone through the organic-gleyic phase of pedogenesis at different times. Permanent annual deposition of solid material in turbid stagnant water, *in situ* alteration of sedimentary deposits, as a result of gleyization and enrichment of sediments with organic material in swamp conditions led to the formation of deep humic clayey soils with extremely deep humiferous profile (Figures 3 and 4).

As a result of arable land use after drying, it has been found that they have a high long-term fertility. However, the irrational exploitation of the soils in the last decades after the agrarian reform (the sudden replacement of the conventional tillage technology with the basic surface tillage according to the Mini-till technology) led to the strong compaction of the recently uncultivated soil section of the process of dehumidification and destructuring of this layer. The physical and chemical properties of the researched soils are presented in Table 1.

Table 1. Physical and chemical properties of humic alluvial soils in the Lower Botna meadow

Depth, cm	Soil particle content, %; diameter, mm		AH*	D*	BD*	RP*	Humus, %	CaCO <sub>3</sub> , %	pH
	< 0.001	< 0.01							
Profile 7. Dried post-marsh humic alluvial soil with extremely deep humiferous profil, fine clayey, weakly carbonated, gleyization in profile and deep gleyic, arable (central part of the meadow)									
Ahp1 0-20	48.7	86.9	6.0	2.57	1.29	18	4.71	1.4	7.2
Ahp2 20-40	49.4	85.8	5.9	2.62	1.49	24	3.77	3.3	7.2
ABhr 40-90	52.5	90.2	6.1	2.65	1.49	24	2.69	4.1	7.1
Bhzy 90-130	62.6	90.1	6.8	2.67	1.51	25	2.16	2.5	7.1
BGh 130-170	63.6	90.4	5.8	2.66	-	25	1.73	1.9	7.1
G 170-250	49.2	80.0	4.8	2.68	-	-	0.83	2.2	7.4
> 250 cm	Groundwater appeared at a depth of 260 cm, with a stabilized level at a depth of 250 cm from the earth's surface								
Profile 8. Dried post-marshy humic alluvial soil with extremely deep humiferous profile, fine clayey, weakly carbonated, gleyization on the profile and deep gleyic, arable (meadow nearby the river)									
Ahp1 0-20	47.5	85.3	5.4	2.60	1.27	15	4.26	2.9	7.4
Ahp2 20-35	48.1	83.9	5.4	2.63	1.41	25	4.17	3.2	7.3
ABhr 35-60	45.5	80.8	6.3	2.65	1.42	23	2.18	6.1	7.4
Bhzy 60-90	52.9	84.9	5.9	2.66	1.44	22	2.25	5.2	7.4
BGh 90-135	61.4	85.2	6.2	2.68	-	20	3.17	2.5	7.5
BGh 135-150	60.3	86.8	6.8	2.69	-	19	2.87	2.2	7.6
> 150 cm	Groundwater appeared at a depth of 160 cm, with a stabilized level at a depth of 150 cm from the earth's surface								
*AH - hygroscopic water, %; D - density of the solid part, g/cm <sup>3</sup> ; BD - bulk density, g/cm <sup>3</sup> ; RP - penetration resistance, kgf.									

Due to the clayey granulometry the compaction of the soil in general and especially in drying condition is strong. In the last 30 years, the organic fertilizers have not been applied into the soil. The flow of fresh organic matter in this layer has clearly decreased which has led to a decrease in soil resistance to compaction. In the 2000 year, the soil on the field where profile 7 was placed was worked with the subsoil to the depth of the former arable layer 35-40 cm and on the field where profile 8 was placed was ploughed to a depth of 20 cm. The 20-35 cm section of soil that has not been ploughed for many years has been strongly

compacted. The roots of agricultural plants hardly penetrate into this compacted layer or pass only through cracks.

Both fields in the 2020 - an extremely drought year were sown with corn. The corn harvest was formed only on the field by the river from the capillary fringe water account, located in the soil at the depth of 50-150 cm. The toxic salts content in this stratum of 50-100 cm, in which the roots of corn have penetrated, constitutes 0.054-0.088%, that corresponds to the salinization degree of soil: from salinized to weakly salinized.

The information on the salt content, reaction and ionic composition of the aqueous extract of the alluvial soils in the lower Botna meadow based on the data obtained for soil profile 7 (deep

humic alluvisoil in the central part of the meadow) and soil profile 8 (deep humic alluvisoil located near the riverbed) is presents in Tables 2-5.

Table 2. Salt content, reaction and ionic composition of the aqueous extract in the alluvial soil in the lower Botna meadow (profile 7)

Depth, cm	Dry residue, %	pH	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	$\frac{Na^+}{Ca^{2+}+Mg^{2+}}$
			mg/100 g soil %						
0-20	0.106	7.75	<u>0.48</u> 0.029	<u>0.07</u> 0.002	<u>1.10</u> 0.053	<u>0.77</u> 0.015	<u>0.81</u> 0.006	<u>0.07</u> 0.002	0.04
20-30	0.081	7.45	<u>0.52</u> 0.032	<u>0.10</u> 0.004	<u>0.67</u> 0.032	<u>0.63</u> 0.013	<u>0.53</u> 0.006	<u>0.13</u> 0.003	0.11
30-90	0.517	7.55	<u>0.35</u> 0.021	<u>0.23</u> 0.008	<u>6.62</u> 0.318	<u>4.71</u> 0.094	<u>1.53</u> 0.018	<u>0.96</u> 0.022	0.15
90-130	0.903	7.35	<u>0.25</u> 0.015	<u>1.70</u> 0.060	<u>10.69</u> 0.513	<u>6.25</u> 0.125	<u>2.47</u> 0.030	<u>3.92</u> 0.090	0.45
130-170	0.713	7.27	<u>0.20</u> 0.012	<u>2.50</u> 0.088	<u>7.40</u> 0.355	<u>4.94</u> 0.099	<u>1.68</u> 0.020	<u>3.48</u> 0.080	0.52
230-250	0.199	7.52	<u>0.34</u> 0.021	<u>0.53</u> 0.019	<u>2.05</u> 0.098	<u>1.04</u> 0.021	<u>1.01</u> 0.012	<u>0.87</u> 0.020	0.42

Table 3. Content of soluble salts (me/100 g) in the alluvial soil from the lower Botna meadow (profile 7)

Depth, cm	Ca(HCO <sub>3</sub> ) <sub>2</sub>	CaSO <sub>4</sub>	Na <sub>2</sub> SO <sub>4</sub>	MgSO <sub>4</sub>	MgCl <sub>2</sub>	NaCl	Toxic salts, %	
							total	from the residue
0-20	0.5	0.3	0.1	0.7	0.1	-	0.052	49
20-30	0.5	0.1	0.1	0.4	0.1	-	0.040	49
30-90	0.4	4.4	1.0	1.3	0.2	-	0.157	30
90-130	0.3	6.0	3.9	0.8	1.7	-	0.405	45
130-170	0.2	4.7	2.7	-	1.7	0.8	0.317	44
230-250	0.3	0.7	0.9	0.5	0.5	-	0.116	58

Table 4. Salt content, reaction and ionic composition of the aqueous extract in the alluvial soil in the lower Botna meadow (profile 8)

Depth, cm	Dry residue, %	pH	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	$\frac{Na^+}{Ca^{2+}+Mg^{2+}}$
			me/100 g soil %						
0-20	0.141	7.68	0.38	0.07	1.66	0.84	1.15	0.12	0.06
			0.023	0.002	0.080	0.017	0.014	0.003	
20-35	0.102	7.77	0.45	0.14	1.00	0.74	0.67	0.18	0.13
			0.027	0.005	0.048	0.015	0.008	0.004	
35-60	0.101	7.90	0.55	0.21	0.79	0.70	0.35	0.50	0.48
			0.034	0.007	0.038	0.014	0.004	0.012	
60-90	0.163	7.80	0.55	0.68	1.22	1.02	0.47	0.96	0.64
			0.034	0.024	0.059	0.020	0.006	0.022	
90-135	1.142	7.40	0.30	0.48	14.85	10.37	2.65	2.61	0.20
			0.018	0.017	0.713	0.207	0.032	0.060	
135-160	0.869	7.32	0.24	0.89	10.84	6.17	2.10	3.70	0.45
			0.015	0.030	0.520	0.123	0.025	0.085	

Table 5. Content of soluble salts (me/100 g) in the alluvial soil from the lower Botna meadow (profile 8)

Depth, cm	Ca(HCO <sub>3</sub> ) <sub>2</sub>	CaSO <sub>4</sub>	Na <sub>2</sub> SO <sub>4</sub>	MgSO <sub>4</sub>	MgCl <sub>2</sub>	NaCl	Toxic salts, %	
							total	from the residue
0-20	0.4	0.5	1.1	1.1	0.1	-	0.076	54
20-35	0.4	0.3	0.2	0.5	0.1	-	0.052	51
35-60	0.6	0.2	0.5	0.1	0.2	-	0.054	53
60-90	0.6	0.5	0.8	-	0.5	0.2	0.088	54
90-135	0.3	10.1	2.6	2.3	0.5	-	0.335	29
135-160	0.2	5.9	3.7	1.2	0.9	-	0.351	40

According to the data obtained for profile 7, regarding the content of toxic salts, the deeply humic alluvial soil is not salinized in the first 100 cm, but is moderately salinized in the depth of 100-200 cm. The soil salinization type is sulphatic. Gypsum (CaSO<sub>4</sub>) predominates in the composition of soluble salts, that is important in terms of the protection of alluvisols from solonization. The content of toxic salts varies in

the second meter of the profile within the limits of 0.335-0.351%. The soil cover of the Lower Botna meadow has historically formed under hydromorphic conditions under the influence of both surface runoff and groundwater after the wetland has dried up. Tables 6 and 7 presents data on the total mineralization, chemical composition and toxic salts content of the surface waters Botna and Dniester rivers.

Table 6. Chemical composition of surface water of the Botna and Dniester rivers and groundwater in the central part of the meadow (profile 7) and in the meadow near the river (profile 8)

Water source	Mineralization, mg/l	pH	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	Sum	SAR	PMg, %
			me/l mg/l									
River Dniester	432	7.17	-	<u>5.8</u> 354	<u>1.25</u> 44	<u>0.42</u> 20	<u>3.62</u> 72	<u>2.98</u> 60	<u>0.87</u> 20	<u>14.94</u> 570	0.5	4.5
River Botna	3190	8.76	<u>1.22</u> 37	<u>7.06</u> 431	<u>10.94</u> 383	<u>28.00</u> 1344	<u>2.70</u> 54	<u>8.52</u> 102	<u>34.78</u> 800	<u>93.28</u> 3151	14.7	76
Profile 7	3784	7.03	-	<u>20.50</u> 1250	<u>12.42</u> 435	<u>26.37</u> 1266	<u>36.00</u> 720	<u>12.86</u> 154	<u>10.43</u> 240	<u>118.58</u> 4065	2.1	26
Profile 8	4260	7.18	-	<u>12.54</u> 765	<u>13.11</u> 459	<u>37.50</u> 1800	<u>30.42</u> 608	<u>11.64</u> 140	<u>21.09</u> 485	<u>126.63</u> 4257	4.6	28

Table 7. Content of soluble salts in surface waters (Dniester) and groundwater, me/l

Water source	Ca(HCO <sub>3</sub> ) <sub>2</sub>	CaSO <sub>4</sub>	Mg(HCO <sub>3</sub> ) <sub>2</sub>	MgSO <sub>4</sub>	MgCl <sub>2</sub>	Na <sub>2</sub> CO <sub>3</sub>	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Sum	Toxic salts, % of total
River Dniester	3.62	-	2.18	-	0.80	-	0.42	0.45	7.47	51.5
River Botna	2.70	-	3.14	-	5.38	1.22	28.00	5.56	43.3	89.6
Profile 7	20.50	15.50	-	0.44	12.42	-	10.43	-	59.29	35.9
Profile 8	12.54	17.88	-	-	11.64	-	19.62	1.47	63.15	47.7

According to the degree of mineralization and chemical composition the water from the Dniester River is very good and can be used without restrictions to irrigate the soils of the Lower Botna meadow. Botna river water, according to SAR and PMg values, does not meet the quality requirements for irrigation water (Tables 6 and 7). At present, the territory carbonated and are characterized by favorable pH values in the range of 7.2-7.4.

of the Lower Botna meadow is used for arable land and is characterized by a favorable unstable groundwater regime. In the event of irrational soil and water management, the hydrological regime may become unfavorable (that happens as a result of incorrect irrigation). The arable post-marsh deep humic aluvial soils from the Lower Botna meadow are poorly. An extremely important index of these soils is the very high content of mobile phosphorus on

the entire humic profile, up to a depth of about 2 m (>10 mg/100 g soil). Such soils, with historical analogous solifaction conditions (gradual synergistic accumulation over thousands years of fine alluvial-proluvial deposits mixed with organic ones, rich in mobile phosphorus, formed in swamp pedogenesis codes) are very rare. Phosphorus is a strategic element that makes possible the use alluvial soils from the Lower Botna meadow in the organic farming.

## CONCLUSIONS

At present, the soil cover of the Lower Botna meadow is used in non-irrigated agriculture. In the post-sovietic period, about 30 years ago, after its dried in the 1960s, the territory with these endemic soils was irrigated. After the land reform of 1990s the irrigation system was completely damaged and soils are no longer irrigated.

The process of pedogenesis in synergistic conditions of swamp and periodic flooding of the Dniester meadow over a long period of time, followed by drained and use as arable land for about 60 years, led to the formation of the deep humic alluvial soils (humus in upper layer is 4.0-5.0%) with extremely humiferous profile to a depth of 1.7-2.0 m and very rich in mobile phosphorus.

The researched soils are characterized by a high level of natural fertility. Rich in humus and mobile phosphorus it is recommended to restore the unfavorable physical properties of the arable soil layer by increasing the flow of qualitative organic matter in this layer.

Reducing the negative consequences of climate change that directly affect the degradation of the post-arable layer 0-30 or 0-35 cm is possible only by moving to green farming that provides

for the systemic use of green mass of leguminous ameliorative plants (autumn and spring vetch or peas) as an organic fertilizer coupled with the gradual periodic implementation of the mini-till system.

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## ASPECTS REGARDING THE SHELTERBELTS ESTABLISHMENT IN BĂRĂGAN PLAIN

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### Abstract

*The Romanian Academy owns large areas of agricultural land located in the Bărăgan Plain, more precisely in the counties of Călărași and Ialomița, under the administration of the "Patrimoniu" Foundation (FP). In the context of current climate changes challenges, as global warming and its negative effects, the Romanian Academy acted both by organizing scientific debates, to raise awareness and establish a common action plan, but also by taking direct and effective measures, to be followed as good practice examples. One of these measures is the program for the establishment of a network of shelterbelts on the agricultural lands owned by the Academy. The program is running in the period 2014-2024 and starting with 2017, the planting action was carried out, managing to plant around 155 ha of shelterbelt until 2021, that were maintained accordingly to each location needs, with the appropriate plant protection measures. After five years of experience in carrying out the program of establishing the shelterbelts, viable solutions that can be applied in the future were defined: mechanization of works by using modern planting equipment, as it was the Forest seedlings planting equipment EPF 1, and the Hand Drilling Machine for seedlings replacement; the use of a high quality plant material, as oak seedlings grown in seedling trays for the replacement of the dead plants, achieving very good percentages of rooting), the application of pre-emergent herbicides, which delayed the weeds infestation in newly established plantations and which are reactivated at the first rain, eliminating in some cases the hoeing or mechanical weed control but also the use of foliar fertilizers based on macroelements, amino acids and microelements, which regulate the water stress during the summer period of the seedlings and grant significant annual growth.*

**Key words:** shelterbelts, planting technologies, soil management practices, economic efficiency.

### INTRODUCTION

The Bărăgan plain, known for its fertile lands, face major ecological issues due to insufficient precipitation and harsh climatic conditions, as the summer extreme temperatures, rainfall deficits coupled by high evapotranspiration values, and these phenomena have increased both in magnitude and frequency in the last 30 years, in the global warming context.

As the analyzed areas includes mainly steppe lands, and just a little of forest-steppe, the differences between the two are very important. Etymological, the terms "forest steppe" or "antesteppe" defines the region in between the forest zone and the steppe, while the "steppe" (word of Slavic origin) represents the "a very

large unforested land", regardless of the way the land is used. In Romanian language, the word "steppe" was used for the first time by the botanists. In the forestry area, the term seems to have been introduced by C. Huffel (1888) who speaks of "afforestation of steppes in Braila and Ialomița counties". Until then, only the terms "Bărăgan field" or "plains" were used (Pascovschi and Donita, 1967; Giurgiu, 1995). In the plain area, the oak-related species forests are the most stable ecosystems, able to withstand the actions of extreme climatic factors. Unfortunately, the areas with oak forests are in a continuous decrease, especially in the last 250 years (Giurgiu, 2010; Dolocan, 2012), their fragmentation, destruction and isolation causing profound changes to the surrounding

environmental conditions, which become more and more harsh.

In this context, the afforestation in the plain area, through mixed agricultural and forestry crops, also known as agroforestry, become more and more popular, supporting both productions and ecosystems. Within these agroforestry systems, the plantation of several rows of forest trees species, also known as shelterbelts, is the most successful way of bringing back nature on intensively anthropized agricultural lands (Bettles et al., 2021; Santiago-Freijanes et al., 2021). To mitigate the negative effects of climate change, forest ecosystems can be sustainably used, as they achieve a very good carbon fixation, substantially higher than that of agricultural crops (Dhyani et al., 2021; Nath et al., 2021; Siarudi et al., 2021).

The shelterbelts reduce climatic extremes – the effects of droughts, storms by reducing wind speed by up to 50% and stopping soil erosion, especially wind (Andreu et al., 2017), benefit snow storage, prevent evaporation and implicitly determine the conservation of water in the soil (Mize et al., 2008).

The shelterbelts have a beneficial action on the growth of biodiversity by creating optimal conditions for the perpetuation of animal species, birds (Beillouin et al., 2021; Mupepele et al., 2021), by supporting populations of insects that exercise biological control of pests of agricultural crops.

Shelterbelts have an important role in increasing crops production. In Ukraine, a 25-year study of barley crop showed that shelterbelts presence increased yields by 17-18% in the drought years, by 13-15% under normal conditions and with 6-9% in favorable years, having average rainfall. This was reflected in the increase of net income of farms by 27-57% in drought years and by 13-26% in rainy years (Miloserdov, 1989). Also, shelterbelts may represent a good income source, by its secondary, non-wood products that may be valorized, e.g., black locust honey was the most promising non-wood forest product for Ialomița County in an analysis in 2017, done by Enescu.

Knowing the beneficial effects of shelterbelts, there were several legislative attempts to support the increase of areas covered by shelterbelts: (1) the law no. 289/15.05.2002 on shelterbelts ("Legea privind perdelele forestiere de

protecție"), republished and updated; (2) the law no 46/19.03.2008 - The forestry Code (Codul silvic), republished and updated; (3) the state aid scheme "Support for the first afforestation and the creation of forested areas" related to Measure 8 "Investments in the development of forested areas and improving the viability of forests", Sub-measure 8.1 "Afforestation and the creation of forested areas" within the PNDR 2014-2020, implemented by the Payments and Intervention Agency for Agriculture (APIA). The Forestry Code, by the article 88, sets up a bold objective for 2035 - the afforestation of two million hectares of land beside the forestry foreseen area. Unfortunately, the state aid scheme had little effects in the first years and the APIA launched new sessions every year, including in 2022, also improving the application procedures (APIA, 2022).

The current government attempts to enforce the shelterbelts plantation, through the Emergency Ordinance no. 35/2022, in accordance with the provisions of the Romania's recovery and resilience plan (PNRR), the aims to implement forested areas on 25000 ha by the end of 2023 and has the obligation to reach another 31000 ha in the period 2024-2026. The bravery of these measures relies on the fact that at the level of 2020 less than 200 ha of new forests were established (Euronews, 2022). This objective aims to revive the establishment of new forests in the lowland area, the costs of design, establishment and maintenance of forest crops being settled, granting an annual payment of 456 euros/year/ha for the carbon stored in the biomass, for a period of 20 years.

The Romanian Academy, aware of the role of shelterbelts for the plain area, initiated with its own funds through the "Patrimoniul" Foundation, on its agricultural lands, a program aimed at establishing over 170 ha forest curtains, mainly in Călărași and Ialomița counties (Mușat et al., 2021). By disseminating the results obtained through its initiative, it is intended, in addition to the raising awareness of the shelterbelts necessity, proving their positive effects and the opportunity of implementation of an agroforestry system, to demonstrate to the landowners and farmers that it is possible to carry out such successful projects, with low expenses and maximum effects, showing an

example of good practices and an efficient management of agroforestry lands.

The current paper illustrates different aspects regarding the shelterbelt's establishment in Bărăgan plain, as a good practice example for those who intend to apply to APIA or PNRR measures.

## MATERIALS AND METHODS

### Characterization of physico-geographical conditions

The shelterbelts establishment in Bărăgan plain will be illustrated by presenting two locations in South-eastern part of Romania where shelterbelts were installed by the project initiated by the Romanian Academy.

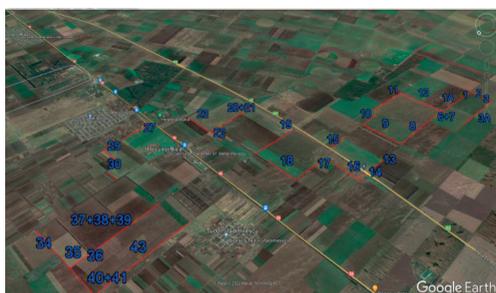


Figure 1. Shelterbelts for the protection of agricultural fields in Perișoru area

The two locations are both situated in Călărași County, in Perișoru area (Figure 1), where the soil is represented by a typical chernozem and in Grădiștea area, where the soil is a calcaric fluvisol, in a meadow area (Figure 2).



Figure 2. Shelterbelts for the protection of agricultural fields in Grădiștea area

The main physical and geographical conditions of the two areas and the differences between them are illustrated in Table 1. The main differences are due to the types of soil that formed in different geological conditions the soils from Perișoru being more fertile and appropriate for a large variety of crops.

The tree species were chosen according to the local climatic conditions (rainfall deficit, high summer temperatures, bright sunshine, etc.) and planted in two different plant compositions.

Table 1. The geographical, geological and climate conditions of Perișoru and Grădiștea

Location	Geology and lithology	Climatic factors	Groundwater	Soil types
Perișoru (35-40 m altitude)	-The Bărăgan plain, on the Moesian Platform, - sedimentary deposits, - fluvio-lacustrine deposits - loess and löessoid deposits overlap, some sands; - alluvial deposits, often covered by löess	<i>min. t</i> > -30°C <i>January av.</i> -2...-4°C <i>max. t</i> >40°C <i>July av.</i> 22-23°C <i>aagr</i> - 125-127 kcal/cm <sup>2</sup> ; <i>aaat</i> - 10.8-11.0°C <i>dwf</i> - 190-210 d, <i>aar</i> - 450-550 mm	0-5 m in river meadows, 2-5 m in ravine depressions, 5-15 m in most interfluves	chernisols, includes typical chernozem and vermic soils
Grădiștea (15-20 m altitude)	-The Danube meadow; - fluvial and swampy deposits, - clays (both sandy or löessoid), - fine and coarse sand, - sand s homogenized with gravel	<i>min. t</i> > -30°C <i>January av.</i> -2...-4°C <i>max. t</i> > 38°C <i>July av.</i> 22-23°C <i>aagr</i> - 125-127 kcal/cm <sup>2</sup> ; <i>aaat</i> - 10.8-11.0°C <i>dwf</i> - 190-210 d, <i>aar</i> - 400-500 mm	1-2 m in the spring 2-3 m during summer and autumn	limnosols, alluviosols, gleisols

*min.* - minimum temperature

*January av.* - January average temperature

*max. t* - maximum temperature

*July av.* - July average temperature

*aagr* - annual average global solar radiation

*aaat* - average annual air temperature

*dwf* - days without frost/year

*aar* - average annual rainfall.

According to the advice received from the National Institute for Research and Development in Forestry (INCDS) specialists, in Grădiștea were used *Quercus pedunculiflora*, *Prunus cerasifera* *Fraxinus ornus* *Acer tataricum*, *Pyrus pyraister* *Prunus mahaleb* while in Perișoru only a mix of *Ulmus pumila* and *Gleditsia triacanthos* was planted. The plant species were associated to grant a strong vertical layer arrangement in front of the prevailing wind and to comply with the ecological requirements of each species.

## RESULTS AND DISCUSSIONS

The program for the establishment of shelterbelts on the agricultural lands of the Romanian Academy was set up to be carried out in the period 2014-2024 and the planting have started in 2017.

The designer proposed a system of seven rows, with a planting scheme of 2 x 1 m, using 5000 saplings/ha of the two compositions mentioned before.

The technology used for the pilot project, at the shelterbelts establishment was the mechanized planting, using the equipment for planting forest saplings (EPF 1) produced by the National Institute of Research – Development for Machines and Installations Designed for Agriculture and Food Industry – INMA Bucharest (INMA) (Figure 3).



Figure 3. Mechanized planting with EPF 1, 2018, Grădiștea

The machine proved its usefulness and efficiency by managing the planting of ~1.2 ha in just one day, using reduced labor force,

consisting of teams of only two workers and a tractor driver, the establishment costs being reduced with about 30 %, compared to manual planting.

The use of EPF 1 machine requires small saplings, with a root of 25-30 cm and a stem of 30-35 cm, so plant grooming (pinching and pruning) is necessary before planting. In order to reduce the costs of this operation, from the second year of implementation of mechanized planting, the purchase of small-sized saplings was envisaged, and the Counties forestry nurseries of Călărași and Ialomița, but also private nurseries were visited in order to establish the plots from which the saplings will be used, and a representative of the project was present during the plants preparation for planting (sorting, pruning and transportation). Since 2017, when the project implementation started, to the current 2022 season, 155.4 ha of shelterbelts, with a length of over 107000 m and an average width of 15 m have been established (Table 2).

Table 2. The shelterbelts planted in the period 2017-2022

Locality	County	Area (ha)	Length (m)
Bucu	Ialomița	12.2	7400
Grădiștea	Călărași	74.4	52080
Perișoru	Călărași	44.9	31430
Borcea	Călărași	23.9	16730

Even the EPF 1 equipment was used at maximum capacity, some shelterbelts were also planted manually, due to the climate conditions. The distribution of manual and mechanized plantings in Grădiștea area is presented in Figure 4. Of the total area planted, 50.4 % (37.5 ha) was planted by mechanized means and 49.6% was planted manually.

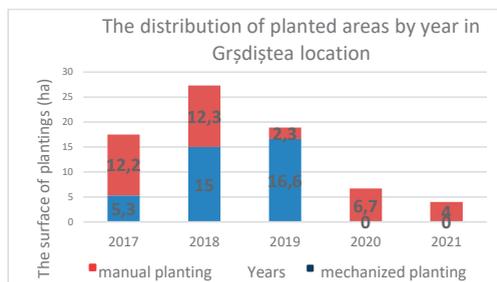


Figure 4. Distribution of shelterbelts planted areas, Grădiștea

In the years 2017-2019, when during the cold season heavy precipitations occurred, manual planting was the only option to plant the saplings, as the tractor and the seedlings planting equipment were impossible to be used. From the year 2020, the planting was done exclusively mechanized. The situation was the same for Grădișteța and Perișoru areas.

The works of filling the losses (dead plants) in the years following the shelterbelts establishment, as the planting equipment could not be used, were done by an auger, a team of two workers making an average of 2500 tree holes, much above the norm for manual digging (Figure 5). In this case too, the reduction of expenses was substantial.

One of the problems faced by the "Patrimoniul" Foundation was the lack of regulations for the payment of forestry workers, which required the execution of the planting and maintenance works to be done directly by the employees of the foundation, with the support of the forestry staff of the Penteleu Forest Service of the Romanian Academy.



Figure 5. Drilling with the auger, 2019, Perișoru

Another good practice solution for the losses replacement is the use of containerized oak saplings supplied by Ialomița Forestry Department (Figure 6), as their use led to very low percentage of seedlings loss and very fast start of the new growths.

In the first years after planting, maintaining the soil clean and prevent the competition between weeds and saplings is one of the intensive labor requiring works.



Figure 6. Containerized brown oak saplings used for planting

Taking into account the acute lack of labor force in forestry, and the very high expenses that manual workforce brings, the share of manual works was decreased by using a large range of herbicides and by increasing the number of mechanized interventions on the interval between seedlings. The forestry and plant protection specialists recommended the use of pre-emergence and early post-emergence herbicides to control dicotyledonous and monocotyledonous weeds (oxifluorfen in the first year and a mix of isoxaflutol and tiencarbazon-metil, with a ciprosumfamid as a safener in the second year), applied immediately after pruning the planted seedlings on the clean soil. The results were surprising, as these herbicides managed to delay by 30-80 days the first hoeing and allowed a better start of seedlings in the absence of strong competition from other plants. At the same time, these herbicides can reactivate after rains, so they significantly reduced the workload on the following manual interventions (Figure 7).



Figure 7. The effect of herbicides at 40 days after their application (mix of isoxaflutol, tiencarbazon-metil, ciprosumfamid)

Starting 2020, a dedicated tractor was purchased for mechanized hoeing, by successive passes on the interval between rows, with a tiller and vegetable mass chopper, eliminating the risk of growth competitions with the weeds (Figure 8).



Figure 8. Mechanized hoeing at Perișoru

In the second year, in the heavily infested areas, total herbicides were applied using electric pumps equipped with protective funnels for directing the jet and protecting the plants. Very good results were obtained by applying foliar fertilizers based on macro-elements, aminoacids, and microelements during the summer period. These allowed the plants to regulate the water stress of the seedlings and lead to annual shoots growths of over 120 cm (Figure 9), even when temperatures above 35°C and prolonged droughts were recorded in the summer.



Figure 9. Brown oak in Grădiștea, 4<sup>th</sup> year of vegetation

## CONCLUSIONS

After five years of implementation of the shelterbelts program for the protection of agricultural lands, some conclusions may be drawn:

- also in the forestry sector, in the context of acute labor force shortage and increasing prices, mechanization is a viable solution both for planting works, using modern equipment/machines (as EPF -1, for planting, augers for trees planting holes), and for maintenance works, as the application of a range of pre-and post-emergence herbicides to control dicotyledonous and monocotyledonous weeds that delay the first hoeing and reduce the volume of manual labor; or the use of hoeing machines for maintenance between rows;

- use of a high-quality plant material is the key for successful installment of the shelterbelts (the forestry departments should produce and sell more high quality seedlings easily, without restrictions);

- use of foliar fertilizers based on macro-elements, amino acids and microelements should become a widespread practice, due to the benefits on water stress reduction and plants annual growth.

Regarding the plant's composition, the mix of Turkestan elm and honey locust can perform the protection function starting the 3<sup>rd</sup>-4<sup>th</sup> year of vegetation, and is recommended for all perimeter shelterbelts, while the mixture based on oak-related species most likely requires 5-6 years until the maturity, being recommended for interior and secondary shelterbelts.

Considering the presented results, we recommend to landowners and farmers to switch to agroforestry systems on their lands by setting up shelterbelts to protect the agricultural fields.

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## EVOLUTION OF SOIL ECOSYSTEM SERVICES UNDER DIFFERENT CROPPING SYSTEMS: A REVIEW

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### Abstract

*Soil can provide essential ecosystem services that include the supply of food, feed, raw materials and biofuels. It also has an important regulatory role (carbon sequestration, water purification, reduction of contaminants, pest control, climate, nutrient cycle and biological habitat regulation etc.). The simultaneous provision of these multiple services is the result of complex interactions between different aboveground and belowground communities across ecosystems. When a system is not well managed, persistent losses in the ecosystem services can occur. E.g. land use changes affect the structure, function and efficiency of ecosystems, thereby impacting the value of the ecosystem services. Also, various agricultural management practices lead to increased food production, but at the same time affect the ecosystem functions. In this context, the main objectives of this study are to evaluate the evolution of the soil ecosystem services under different cropping systems such as certain crop species, monoculture or various types of crop rotation, organic, mineral or integrated fertilization, soil tillage etc., and to understand the interrelation between soil and ecosystem services.*

**Key words:** soil, ecosystem services, cropping systems, sustainability.

### INTRODUCTION

Soil represent a key component of the ecosystem due to their slow forming and recovery rate (Pulleman et al., 2012; Jónsson et al., 2016) and the foundation of most essential ecosystem services (Breure et al., 2012; Ferrarini et al., 2018). It sustains human life through the provision of food, feed, raw materials, etc., represents a reservoir of biodiversity, which is essential for a range of ecosystem processes such as decomposition, mineralisation, and nutrient cycling, serves as a repository for the carbon and nutrient elements that sustain life, retains the water for plants growth and soil organisms and limits the soil loss, contributes to the composition of the atmosphere and impact climate (FAO, 2015; Nielsen et al., 2015; Silver et al., 2021) (Figure 1). Also, soils can provide cultural ecosystem services (i.e. recreation, spirituality, knowledge, aesthetics etc.) (Power, 2010). Soil functions are strongly interrelated and they are used to assess soil ecosystem services (Prado et al., 2016; Pereira et al., 2018). Agricultural ecosystems cover approximately

40% of the Earth land area and are providers of ecosystem services such as pollination, pest control, genetic diversity for future agricultural use, soil retention, soil fertility maintenance and nutrient cycling that support the provisioning services (Power, 2010).

As economy developed and human production activities increased, the structure and functions of agricultural ecosystems changed, resulting in a series of ecological and environmental problems (e.g. cropland quality decline and its overall function) (Tao et al., 2022).

Intensive agriculture focusses on profit maximization achieved by high yields, the technologies applied being based on high consumption of industrial resources (Luty et al., 2021). The Green Revolution of the 1950s and 1960s led to tripled cereal crop production with just a 30% increase in land area cultivated due to crop genetic improvement, combined with enhanced inputs and irrigation. Intensification of the agricultural system did not end with the Green Revolution. From 1985 to 2005, global crop production increased by another 28% (Bennet et al., 2021).

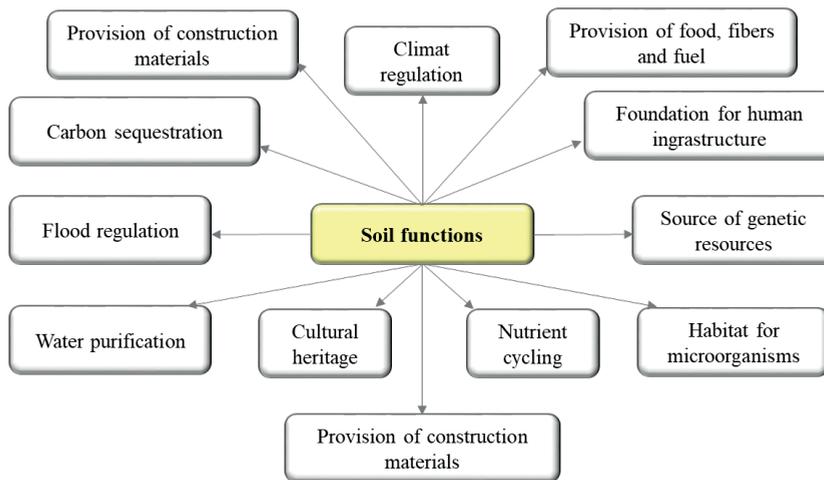


Figure 1. Soil functions diagram (modified after FAO, 2015)

In conventional agriculture, agroecosystems are “often maintained in a state of nutrient saturation and are inherently leaky as a result of chronic surplus additions of nitrogen and phosphorus” (Power, 2010). From 1965 to 2000, nitrogen fertilizers use increased 6.87 times and phosphorous fertilizers use increased 3.48 times. Thus, the increasing agricultural intensity generates pressure not only on land resources but also across the whole environment (Tilman, 1999; Kanianska, 2016).

Nitrogen chemical fertilizers raise a series of issues related to the potential effects on biodiversity and ecosystem functioning (Socolow, 2016). More than half of the nitrogen in agriculture is not properly used and is lost to the environment by nitrate leaching and emissions of ammonia (NH<sub>3</sub>), nitrous oxide (N<sub>2</sub>O) and nitrogen oxide (NO), out of which the volatilization loss of ammonia represents 10 to 60% of the total nitrogen intake (Sun et al., 2019). Also, different practices within intensive agriculture (monoculture or short crop rotation, conventional tillage - CT, use of pesticides, intensive grazing and livestock production) are known for their negative impact on soil ecosystem services in the agricultural areas (e.g. acidification, salinization, erosion, compaction, water use increase, soil fertility loss, greenhouse gas emissions and increased pollution) (Gomiero et al., 2011; Pereira et al., 2018).

A response to the contrast between sustaining human population growth through agricultural production and maintaining the ecosystem

functions within conventional agriculture could be the promotion of alternative agricultural production systems, which were brought to global attention in the mid 80’s and are closely linked to the idea of sustainability (O’Donoghue et al., 2022).

Conservation agriculture practices, which include reduced tillage or no-tillage, retention of crop residues on soil, and crop rotations, including cover crops, aim to increase crop yields by enhancing several regulating and supporting ecosystem services (Palm et al., 2013). They can reduce erosion due to residues retention on soil surface and increase water infiltration and decrease runoff with no-till. The benefits of conservation agriculture on ecosystem services (nutrient cycling, C sequestration, and pest and disease control) can vary depending on soil management, soil type, and climate (Palm et al., 2013). Conservation agriculture practices can limit the soil fertility and agroecosystem functioning by increasing water deficiency for crops in drought periods, pest infestation due to entire maintenance of crop residue on soil surface and the risk of off-site water source contamination because of organic nutrient management (Stavi et al., 2016).

Organic agriculture system is subordinated to the rhythm of natural processes (Luty et al., 2021). Its practices rely on and benefit from biological cycles (i.e. appropriate selection of crop rotations or cover crops, biological control etc.) (Boone et al., 2019), may lead to the

reduction of greenhouse gases emissions, and a better biodiversity, water use efficiency, soil, and air quality (Gomiero et al., 2011), but “require more land to produce the same amount of output and therefore, their better environmental results might be cancelled” (Boone et al., 2019).

The concept of integrated farming systems appeared in Western Europe in the 1980s and combines natural processes with agricultural activity. Integrated farming includes the application of phytosanitary products and organic and chemical fertilizers, but at minimum levels to prevent the spread of nutrients outside the agroecosystems. Also, it promotes crop rotation, especially with leguminous species and intercrops, which addresses the concept of ecosystem services (Luty et al., 2021).

The negative effects of agricultural production on ecosystem services can be avoided through the adoption of sustainable agricultural practices that include soil conservative tillage (conservation tillage - CstvT, reduced tillage - RT, minimum tillage - Mt and no-tillage - NT), crop diversity practices (e.g. intercropping or the use of multi-year crop rotations), returning of organic matter to soil, cultivation of carefully selected cover crops, mulching - which protects soil and increase its fertility, integrated pest management (Valieva et al., 2010). Also, a constant and balanced use of fertilizers and organic amendments (compost, biochar etc.) can contribute to the soil structural stability and quality restoring (Siedt et al., 2021). The sustainable practices we focused on in this study were the use of organic fertilisers, various crop rotations and conservation tillage.

Sustainable agricultural practices can improve the efficiency of resources in agriculture and the sustainability of agroecosystems, but more research is needed to identify ways to strengthen the resilience of systems as well as their sustainable performance, with high productivity, stable yields, maximum resistance to stressors and a positive response to favourable conditions, leading at the same time to long-term economic and social sustainability, multiple ecosystem services and a minimal impact on the environment (Peterson et al., 2018).

The main objective of the study is to assess the evolution of the soil ecosystem services under different cropping systems such as certain crop

species, monoculture, or various types of crop rotation, organic or mineral fertilization, soil tillage etc. and to understand the interrelation between soil and ecosystem services, focusing only on soil structure, soil organic matter and organic carbon contents under different cropping systems.

## MATERIALS AND METHODS

A number of 114 research papers published between 1990-2022 in different multinational indexed open access journals like *Elsevier*, *Springer*, *Taylor & Francis*, *MDPI* or research networking sites such as *Wiley Online Library*, *Web of Science*, *ResearchGate* were used for this review. A search query was applied for titles that included the following terms: “*soil ecosystem services*”, “*monoculture*”, “*crop rotation*”, “*organic amendments*”, “*chemical fertilisers*”, “*conventional tillage*”, “*conservation tillage*”, “*reduced tillage*”, “*no-tillage*”, “*soil structure*”, “*soil physical properties*”, “*organic matter*” or “*organic carbon*”.

There are many research papers that focus on the relationship between soil and ecosystem services, but this subject cannot be exhausted since, at the global level, there is a wide range of ecological conditions in which agriculture is performed and at the same time there is a wide range of agricultural practices whose impact on the environment is very variable.

This study focusses on the current knowledge on: i) cropping systems (monoculture, 2-3 years crop rotations, long-term crop rotation); ii) soil tillage systems (conventional, conservation, reduced or no-tillage); iii) fertilisation methods (chemical and organic fertilisers) and their effects on the ecosystem services.

## RESULTS AND DISCUSSIONS

### *Overview*

*Monoculture* or the practice of growing one crop species at a time year after year (Aman, 2020) emerged a couple of centuries ago as a cropping system that allow farmers for a more efficient planting and harvesting, use of fewer types of expensive equipment and labour. After 1945, monoculture evolved globally and currently supplies most of our food and a significant share

of non-food crops. Despite its advantages, monoculture is amongst the most controversial features of today's agriculture (Balogh, 2021). Over the years, in monoculture system, standard and persistent agronomic practices were employed (i.e. the use of similar pesticides, fertilizers, farm machinery, tillage depth, etc), which led to the evolution of certain weeds, pests, and diseases, depletion of nitrogen, phosphorus, and potassium from soil, the reduction of essential nutrients content in the agricultural soils, the alteration of soil physico-chemical and biological properties (Pervaiz et al., 2020).

*Crop rotation* is a practice that farmers have been using for centuries. It can be simple, where two crops are alternated every year or can follow a more complex pattern, several crops being rotated (Plourde et al., 2013). Simplified corn-soybean rotation is under expansion during the past several decades in the U.S., due to "increased food and industrial uses, economic and world trade benefits, and tremendous efforts devoted to genetic improvement and infrastructure development" (Wang et al., 2021). In the European Union (EU), crop rotations last 3 to 5 years in conventional agriculture and 5 to 10 years in organic agriculture and can include different species and strategies to achieve the desired outcome (Mudgal et al., 2010). For example, 3-year rotations were recently studied under the conditions of a stagnant argic faeoziom type soil (Moraru and Rusu, 2013), as well as under the specific climatic conditions of the sylvosteppe area in the south-est of Romania (Figure 2), on a red preluvosoil (Duşa M., 2022). Different socio-economic and technological factors influenced the abandonment and rediscovery of crop rotation over time. Since the Green Revolution, farmers have been choosing simplified rotation because of innovations in machinery, better performing varieties, chemical fertilisers, efficient pest and weed control as well as market opportunities. Breeding and the improvement of watering practices allowed the development of more diversified crop rotations (Mudgal et al., 2010). From 1950 to 1970, the choices of crop rotation were greatly influenced by the changes in the world economic situation. The economic factor that had an impact on the crop rotations was the good market prices for maize and wheat, with

dropping demands on grain leguminous crops which were taken out from crop rotations in Europe. The energetic crisis in 1970 increased fuel prices, the imported crops from other continents became more expensive and farmers had to diversify the production and to implement crop rotation in their management practices (Mudgal et al., 2010).

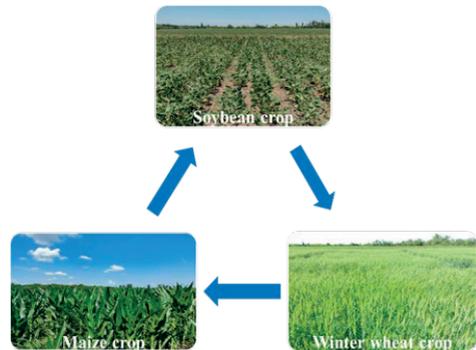


Figure 2. Winter wheat - maize - soybean crop rotation diagram in Moara Domneasca Experimental Field for 2021-2023 period

Diversified crop rotations have positive effects on ecosystem services: they suppress weeds and pests thus reducing the costs of chemical fertilisers and pesticides, reverse soil degradation by increasing the soil microbial biomass C and N pools, reduce soil erosion (Wang et al., 2021) by improving soil structure (Hoss et al., 2018). Long-term crop rotations also reduce the risks of eutrophication due to runoff and leaching and improve crop yield (Hunt et al., 2019). There are also some disadvantages of crop rotations but those are low compared to benefits: less profitability (when farmers are forced to reduce the area cultivated with the most profitable crop), sometimes decreased crop flexibility and some rotations involve high inputs (Selim M., 2019).

#### ***Crop rotation and soil physical properties***

The sustainability of an ecosystem can be estimated by assessing and monitoring soil properties, which are sensitive, over time, to various changes that occur and through which the quality of soil can be evaluated (Novak et al., 2019). Soil physical quality affects the conditions under which plants absorb nutrients and how chemical reactions or biochemical and microbiological transformations take place (e.g.

oxidation and reduction processes, transfer, or immobilisation of pollutants in soil, etc.) (Pranagal et al., 2021). Also, high quality soils should be resistant to degradation and resilient in their ability to recover from unfavourable conditions (Larkin et al., 2021).

There are not many studies on the evolution of soil physical properties under long-term *monoculture* and under the conditions of typical agricultural production. However, according to Pranagal et al. (2021), long-term wheat monoculture didn't have a significant negative impact on soil physical properties. For instance, the values for field air capacity of soil (which provides information on soil oxygenation, gas exchange, soil organisms activity, oxidation and reduction processes) under monoculture were higher than those for the soil under crop rotation (under wheat monoculture, the soil contained more air -  $0.144 \text{ m}^3 \text{ m}^{-3}$  - compared to the soil under crop rotation -  $0.117 \text{ m}^3 \text{ m}^{-3}$ ). It is important to say that the changes in the field air capacity caused by long-term wheat monoculture didn't led to significant deterioration of soil aeration. Also, under monoculture, soil bulk density was lower ( $1.69 \text{ Mg m}^{-3}$ ) than in crop rotation ( $1.75 \text{ Mg m}^{-3}$ ) (Pranagal et al., 2021).

A long-term experiment with winter wheat monoculture on a Spolic technosol from Poland showed that soil had visible large pores, developed mainly due to the presence of wheat stalks and roots which were mixed each year during ploughing, and the highest values of bulk density or total porosity (Kofodziej et al., 2016). An increase of soil bulk density and a decrease of porosity under monoculture can be explained by the deep plants root distribution in soil, the decrease of water content around root system and thus the intensification of soil compaction (Wu et al., 2021).

*Crop rotation* can be an eco-friendly measure, providing diversification in crop management systems and modifying the high pressure on the agricultural ecosystem (Saulic et al., 2022). There is a strong correlation between the species included in the crop rotation system and soil properties. So, "deep-rooted crops should ideally follow shallow-rooted ones to preserve subsoil structure" (Lampkin, 1990). This alternation of crops leads to uniform root distribution, good soil structure, increased soil

porosity and permeability, reduced soil bulk density, improved soil aggregate stability, increased water retention and stability, and increased resistance to soil erosion (Yu et al., 2022).

Crop rotation with grain leguminous and perennial forage species can have beneficial effects on soil physical properties by increasing microporosity, hydraulic conductivity and aggregate stability and decreasing bulk density and penetration resistance (Gotze et al., 2016). Under a crop rotation with lupin and canola, soil had a lower shear strength and a greater porosity than those with cultivated field pea and barley (Ball et al., 2005). Also, a crop rotation practiced in an organic farming system that included red clover had a strong influence on soil pore-size distribution. Here, the highest soil mesoporosity ( $0.2\text{-}30 \text{ }\mu\text{m}$ ) and the lowest microporosity ( $< 0.2 \text{ }\mu\text{m}$ ) were registered compared to other crop rotations (spring barley - buckwheat or buckwheat - white mustard - buckwheat - rye -spring wheat) (Feiziene et al., 2016).

However, some authors showed that short crop rotations, such as corn-soybean or wheat-soybean reduce macro-aggregation because of low residue input by soybean (Agomoh et al., 2021). Zuber et al. (2015) reported that the inclusion of soybean in rotation with corn led to reduced water aggregate stability as compared to corn monoculture. This can be explained by the low residue accumulation and soil organic matter depletion. Also, in corn-soybean rotation, the soil remains uncovered over-winter and this can increase the risk of erosion (Blanco-Canqui, 2018).

A 3-year wheat-soybean-maize crop rotation significantly reduced the bulk density in the 0-20 cm and 20-40 cm soil layers and increased soil porosity (Wu et al., 2021). In crop rotation, maize can be considered a suitable species due to its fibrous roots that produce high levels of macro-aggregation (Bronick and Lal, 2005). Diversification of crop rotations by including cereals, cover crops or overwinter crops can be a strategy to imitate the structure of natural ecosystems, and may improve soil ecosystem services (Taveira et al., 2020). Complex crop rotations which include alfalfa improve soil saturated water content and aggregation compared to simple rotations because perennial

leguminous have deep tap roots that lift the soil for better water holding capacity. As a result of improved soil aggregation, the abundance of larger soil pores increases and this contributes to a higher saturated water content and hydraulic conductivity (Kiani et al., 2015).

Making a comparison with an annual crop rotation without grassland, Van Eekeren et al. (2008) showed that a 3-year annual crop rotation preceded by 3-year of grassland lead to an improved soil structure (i.e. higher percentage of crumbs and sub-angular blocky elements), and a lower bulk density.

Within an experiment with two crop rotations which included corn-corn-oats-spring barley (with red clover under-seeded in oats and spring barley) and corn-corn-soybean-soybean and 4-year corn monoculture, the poorest soil structure was recorded in monoculture and a good soil structure in corn-corn-oats-spring barley rotation. Also, the topsoil structural quality was better preserved in the diverse rotation compared to monoculture or corn-corn-soybean-soybean rotation (Munkholm et al., 2013).

The influence of crop rotation on soil structure depends both on the chosen species for rotation as well as on soil management practices (Ball et al., 2005).

### ***Tillage systems and soil physical properties***

The need to minimize the impact of agricultural practices on soil structure is one of the main purposes of land management (Pagliai et al., 2004). But tillage systems can have a different effect on soil physical properties because of the variation of tillage intensities (Bhattacharyya et al., 2008).

According to Pagliai et al. (2004), *conventional tillage* modifies the most, soil physical properties having a negative impact on soil structure due to surface crust. Also, the elongated transmission pores decreased significantly, thus indicating that the soil structure became compact, and a plough pan was developed at the lower limit of cultivation.

*Conservation tillage* (which includes reduced tillage and no-tillage) is a sustainable management practice associated with improved water infiltration and conservation, reduced erosion and improved soil structure (Žurovec et al., 2017).

*Reduced tillage* can have a strong influence on the soil aggregates stability compared to conventional tillage (Pagliai et al., 2004; Daraghmeh et al., 2009; Obalum et al., 2019) but its positive effect on soil depends on climatic conditions and soil type (Daraghmeh et al., 2009).

Within an experiment with different tillage practices conducted in Germany over a period of 37-40 years, Jacobs et al. (2009) reported that reduced tillage improved the aggregate stability and increased the concentrations of soil organic carbon and nitrogen within the aggregates in the upper soil compared to conventional tillage. Also, compared to CT, RT decreased the soil bulk density and increased the proportion of larger aggregates, thus increasing soil structure (Daraghmeh et al., 2009).

However, Schluter et al. (2018) observed that under CT and RT, differences in soil structure were determined only in a shallow depth. Thus, ploughing led to soil loosening and increase of macroporosity and macropore connectivity. Instead, the absence of ploughing caused compaction and a decrease of hydraulic conductivity that wasn't recovered even if the earth-worms population from the soil increased (Figure 3).

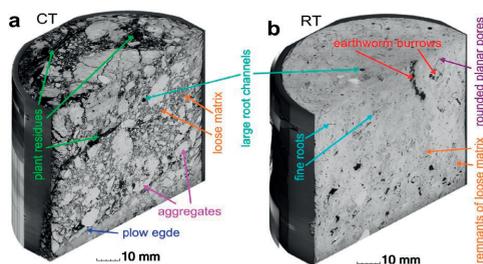


Figure 3. Microstructure of soil at a depth of 13-23 cm, in a) conventional tillage and b) reduced tillage (reprinted after Schluter et al., 2018)

Regarding the soil pore system, Pagliai et al. (2004) reported that under minimum tillage, there was an increase of the storage pores (0.5-50  $\mu\text{m}$ ) and of the amount of elongated transmission pores (50-500  $\mu\text{m}$ ) mainly due to the improvement of soil water content and to the increase of available water for plants.

Under certain environmental conditions, *no-tillage systems* (NT) may have some advantages over CT such as the improvement of soil

aggregate stability and reduction of soil loss, increasing soil water availability and the number of bio-pores that can facilitate root growth (Martinez et al., 2008). Thus, comparing the effects of CT and NT treatments on the soil physical properties it was observed that NT systems enhanced the soil aggregate stability and decreased soil water infiltration and coarse porosity (Martinez et al., 2008). Also, the values obtained by Moreira et al. (2016) for bulk density and pore size distribution under NT system indicated an improvement of soil physical properties and showed a physical balance condition, which can be modified only by short term events like weather conditions variability, intensive machinery traffic or changes in crops grown in the crop rotation system.

Even if NT system has some environmental advantages compared to the conventional one, there are also some important negative effects of this system on soil physical properties such as surface compaction through the reduction of macropores, mainly when heavy machineries are used in high soil moisture conditions (Sokolowski et al., 2020).

Conservation tillage can be applied depending on soil conditions. For instance, in soils with fine texture and poorly drained, minimum tillage (MT) is encouraged, while in light-medium texture and well drained soils, NT will be beneficial (Bussari et al., 2015).

### ***Soil organic matter under different cropping system***

#### ***Crop rotation and soil organic matter***

Soil organic matter (SOM) is a key element of soil properties and processes (bulk density, structure, temperature, water relations, nutrient availability, and biological activity) (Miles et al., 2008) that can be affected by agricultural practices (Liu et al., 2006). Therefore, improving the content of organic matter in the soil could be a strategy to make resilient agroecosystems (Allam et al., 2022). Dynamics and the quantity of SOM are affected by the land use changes and management and this can be measured in practice as soil total organic carbon (TOC) (Apezteguia et al., 2009). Plant diversity can influence the ecosystem processes including SOM dynamics (McDaniel et al., 2014). The crops included in rotation can have different

effects on the quantity and quality of SOM through their biomass, plant residues characteristics, root system and their influence on the soil microbial community (Raphael et al., 2016). The studies conducted over time showed that crop rotation can decrease, increase, or have no effect on SOM concentration (McDaniel et al., 2014).

Within an experiment which focused on the assessment of the SOM stratification under 3 crop rotations namely continuous corn, 2-year corn-soybean and 3-year corn-oat-alfalfa combined with three levels of tillage intensity, Deiss et al. (2021) showed that, under NT, crop rotation with oat and alfalfa maintained or increased SOM accumulation. The high root biomass produced by perennials can lead to increased SOM accumulation. Moreover, corn-soybean crop rotation decreased SOM accumulation compared to continuous-corn and corn-oat-alfalfa.

Regarding the soil organic carbon (SOC) content, lower values were also obtained when soybean was included in a short crop rotation with corn compared to a corn monoculture because soybean produce lower residue and those are decomposed more quickly. Even if SOC was higher in corn monoculture than in corn-soybean crop rotation, more complex rotations can lead to a greater SOC accumulation (Zuber et al., 2015).

Within an experiment carried out in Poland on a luvisoil including 2 long-term crop rotations with potato-oat-flax-winter/rye-faba bean-winter triticale and sugar beet-maize-spring/barley-pea-winter rape-winter wheat as well as a long-term monoculture with each species included in crop rotation, the organic carbon content slightly increased in both crop rotations, being greater in the first one. In monoculture conditions, the lowest amount of organic carbon was registered in maize (0.57%), pea (0.63%) and potato (0.66%) and the highest content was registered in winter triticale (0.81%), winter wheat (0.80%) and faba bean (0.80%) (Rychcik et al., 2006).

Liu et al. (2006) reported that 11-year continuous maize, soybean, and wheat led to soil organic carbon decline, compared to all crops in rotation. SOC decline in deeper soil profile in continuous soybean may be due to the tap root system of this or the impact of monoculture on

soybean root nodules and nitrogen fixation. Also, under continuous cropping, the SOC loss reported by Salvo et al. (2010) in the Ap horizon was in average of 540 kg/ha/year while in crop-pasture rotation, the average loss was only of 80 kg/ha/year.

Gregorish et al. (2001) found a large increase in SOC level in maize-oat-alfalfa-alfalfa crop rotation than under maize monoculture. However, the species included in the crop rotation may have different effects on the quantity and quality of C inputs and further on the mineralisation rates and the growth of subsequent crops (Huggins et al., 2007). Plant species that return greater amounts of residues to the soil can be included into crop rotation and can be associated with greater SOC contents (Page et al., 2020).

Rotations which include grain legumes such as mung bean, pigeon pea, cowpea, chickpea, soybean etc., can maintain higher organic matter levels in the soil than non-leguminous crops grown in monoculture systems (Kamanga et al., 2014). After long-term continuous cropping of cereals, the inclusion of grain leguminous species (i.e. pigeon-pea, mung-bean or chickpea in crop rotations with maize or wheat brought significant changes in SOC, the increase being probably due to the addition of C-input through above- and belowground crop biomass (Venkatesh et al., 2017). Also, wheat-lentil crop rotation resulted in higher soil C levels than other wheat cropping systems, which is attributed to more efficient conversion of residue C to soil C in grain legume rotation systems than in monoculture wheat (Campbell et al., 1999).

Crop rotations that include perennial forage crops can be considered very important for SOC because, compared to annual crops, there is a lower soil disturbance from tillage and they have a higher root biomass (Bolinder et al., 2012). A 38-years experiment carried out on a silty clay loam soil in Uruguay was used to observe the effect of a 4-year cycle annual crop rotation with sorghum-flax-wheat-sunflower followed by 4-year periods of pasture on TOC at 20-40 and 40-60 cm soil depths. In both depths, TOC was higher in pasture (15.39 and 9.26 g C/kg soil) than in annual crop rotation (11.39 and 7.26 g C/kg soil) (Gentile et al., 2004).

### *Tillage systems and soil organic matter*

Land management practices including different tillage systems influence the quantity and composition of SOM (Šimon et al., 2009) and its turnover due to different quantity and quality of plant residues, ratio between above- and belowground inputs and changes in soil disturbance (Machado-Pinheiro et al., 2015).

Conventional tillage can increase soil erosion process and carbon mineralization rate which further can cause significant losses of SOM content (Salvo et al., 2010; Pantani et al., 2022). Under intense tillage, SOM is exposed to oxidation, this process stimulating its decomposition by soil microbes (Hussain et al., 2021).

The loss of organic matter and structure degradation that is potentially produced by CT (ploughing) could be prevented using conservation tillage (Hazarika et al., 2009). Adopting CvsT or NT could be a solution for farmers to preserve soil fertility (Pantani et al., 2022) and to reduce the soil carbon loss (Haddaway et al., 2017).

Using different tillage methods (i.e. CvsT, CT, NT, MT) on a clay-loam texture soil Šimon et al. (2009) noted that SOM content was greater under CvsT where plant residues remained on the soil surface compared with CT, where SOM was distributed in the soil profile. Hussain et al. (2021) noted also that CvsT can improve SOC and organic matter content and can have a big contribution to SOC sequestration.

Under NT system, the percentage of SOM of a sandy loam soil from central Italy was higher (3.31%) than under CT (2.19%) (Sapkota et al., 2012). Also, SOC gains under NT were about 250 kg/ha/year higher than in tilled systems regardless of the cropping frequency in semiarid climate conditions. In the surface layer, NT system had 7.28 Mg/ha more SOC and 4.98 more particulate organic matter carbon than CT (Liu et al., 2006).

In NT system, the crop residues are left on the soil surface and thus a higher amount of organic matter mineralization rate (Hussein et al., 2021). Compared to CT, RT or NT increased SOC in the topsoil (9.8% in NT and 16.7% in RT). This increase could be explained through the retention of crop residues on the soil surface which created a barrier and reduced the contact

with soil microorganisms, thus protecting the microbial decomposition (Allam et al., 2022). The adaptation of long-term conservation tillage practices, including NT or RT favours higher organic carbon concentrations, especially in upper soil profiles but a continuous monitoring of soil quality and SOC changes is essential (Liu et al., 2006).

### ***Fertilisation methods and soil properties***

Fertilisation contributes to crop yield increase, soil fertility improvement and agricultural ecosystem functioning (Jaskulska et al., 2020; Wen et al., 2020), but also can produce changes in soil properties soon after their application or after many years. The effect on soil depends on the type and dose of fertilisers, the methods of application, the climate conditions, or other agricultural technologies (Jaskulska et al., 2020).

### ***Fertilisation and soil physical properties***

The impact of mineral or organic fertilizers on soil environment can be assessed by quantifying the modifications of soil structure (Naveed et al., 2014). Nitrogen fertilization could increase (Bronick et al., 2005; Naveed et al., 2014) or reduce the soil aggregate stability (Plaza-Bonilla et al., 2013) or there is no effect of inorganic fertilizer on soil structure (Plaza-Bonilla et al., 2013; Zhou et al., 2013).

Zhang et al. (2021) noted that fertilisation with N and P separately didn't significantly affect the aggregate stability as compared to the combination of N and P fertilizers (at high doses of N - 540 kg N/ha/year and P - 67.5 kg P/ha/year). Also, the decrease of aggregate stability was found to be larger when higher amounts of N and P fertilizers were applied (e.g. at rates bigger than 100 kg N/ha/year or than 40 kg P/ha/year) than when N and P were applied alone or in smaller doses (Blanco-Canqui et al., 2013). In an experiment carried out by Tuo et al. (2016), the mean weight diameter (MWD) values of soil aggregates were lower when chemical fertilizers with N and P were applied alone or in combination as compared to non-fertilised variant.

Applying organic fertilizers could be an option to reduce the negative impacts of chemical fertilizers by stagnating soil degradation (Li et al., 2021). Some authors reported that organic manure can increase aggregate stability and soil porosity and decrease the bulk density (Haynes et al., 1998; Pagliari et al., 2004). However, Yu et al. (2012) found out that farmyard manure significantly reduced the proportion of microaggregates, while Zhang et al. (2018) found out that this type of fertiliser had no significant effects on the microaggregates. Those different effects of organic fertiliser on the distribution of microaggregates may be associated to the specific soil characteristics and climatic conditions (Yu et al., 2012).

Research carried out in a semi-humid or arid region in China revealed that swine manure has a negative impact on soil aggregation, which can be explained by the accumulation of exchangeable  $\text{Na}^+$  on the topsoil in these areas (Guo et al., 2019). Compared to chemical fertilisation, manure or the combination of manure and chemical fertilizers increased the proportion of small macroaggregates and decreased the proportion of microaggregates (Xie et al., 2015). Naveed et al. (2014) reported an improvement of soil structure and related soil functions (water holding capacity, total porosity, wider pore size distribution, higher pore connectivity) with increasing animal manure in combination with NPK fertilizer applications.

Compost application increase soil properties like porosity, available water, organic matter, and decrease bulk density (Ejjigu et al., 2021). For instance, Bouajila and Sanaa (2011) showed that the application of 120 t/ha manure and household waste compost led to an increase of structural stability, a better soil permeability, an increase of organic carbon and organic matter in the soil when compared to control. Those results can be due to the presence of a great amount of organic matter which is associated to a greater microbiological activity.

The effect of chemical and organic fertilizers on soil structure (Table 1) is a complex process which can be affected by many factors (soil type, crops, fertilizer rates, etc.).

Table 1. The impact of chemical and organic fertilization on soil structure

Soil type	Crop	Fertilization rate/year	Impact on soil structure	References
ns	ns	N fertilisation - no rates specified	Increased soil aggregation	Bronick and Lal, 2005 - review; Naveed et al., 2014
Loess	Soybean-maize rotation	Manure - 500 kg/ha, N -100 kg/ha and P <sub>2</sub> O <sub>5</sub> - 50 kg ha alone or in combination	Manure alone or with N and/or P can increase WSA, MWD and AS. Long-term application of N and P alone or NP results in lower values of properties above.	Tuo et al., 2016
Mollic Andosol Luvisol	Grassland Maize-wheat rotation	164-184 kg N/ha/ year  0, 60, 120 mg N/kg dry soil Crop residue input	Higher SOC mineralisation and worse structural state of soils	Shimizu et al., 2009  Le Guillou et al., 2011
Aridic Haplustoll	Maize	0, 45, 90, 134, 179, 224 kg N/ha; 0, 20, and 40 kg P <sub>2</sub> O <sub>5</sub> /ha	Decrease of aggregate stability and macropore reduction	Blanco-Canqui et al., 2013
Brown soil	Maize	Composted swine manure: 13.5 and 27 Mg hm <sup>-2</sup> yr <sup>-1</sup> ;  NPK fertiliser (urea, multiple phosphate, and potassium sulphate) rates: 135, 29, 56 kg hm <sup>-2</sup> yr <sup>-1</sup>	NPK treatment decreased the proportion of small macroaggregates, but manure or manure plus chemical fertilizers increased the same size aggregate. NPK increased the proportion of microaggregates, but manure or manure plus chemical fertilizers decreased the same size aggregate.	Xie et al., 2015
Vertisol	Wheat-maize crop rotation	12-year N fertilisation with 0, 360, 450, 540, 630, 720 kg/ha/year	Reduced soil aggregate stability by 12-18% at rates of 0-720 kg/ha/year (because of the increases in monovalent ions (H <sup>+</sup> and NH <sub>4</sub> <sup>+</sup> ).	Guo et al., 2022
Vertisols Luvisols	Maize	Urea-0, 50 and 100 kg/ha Compost - 0, 5 and 10 t/ha	Compost alone or combined with mineral fertilizer decreased soil BD hence improving total porosity, water infiltration and aeration of the soil. Low BD value (1.22 g/cm <sup>3</sup> ) -in compost 10 t/ha	Ejigu et al., 2021
Ns Loam soil	Ns Maize	Organic manure  Compost: 0, 40 and 10 Mg/ha and livestock manure – 10 Mg/ha	Increased water holding capacity, porosity, infiltration capacity, WSA and decreased BD	Haynes et al., 1998 - review;  Pagliai et al., 2004

Ns - unspecified; MWD - mean weight diameter; WSA - water stable aggregates; AS - aggregate state; BD - bulk density

Fertilisation methods can also influence the soil organic matter content. Synthetic N fertilizer can reduce the SOM stocks due to a greater mineralization (Russel et al., 2009) or can increase the amount of SOM, mainly at optimum N fertilizer rate because it increases net primary productivity (Poffenbarger et al., 2017). The application of mineral fertilizer in combination with composted farmyard manure improved SOM contents compared to non-fertilised treatment (Guo et al., 2014). Also, one way of restoring SOM content is to increase the organic inputs by applying organic fertilisers. The effect

of organic fertilisers may be related to the amount and quality of the organic matter applied and to the stabilisation capacity of the soil. The stabilisation of SOM depends on its interaction with mineral surfaces, which make it less available to microorganisms. Thus, long-term utilisation of compost as fertiliser led to higher amounts of soil organic matter in the 30 cm layer than in soils treated with mineral fertilisers, with an average difference of 46% in soil C stocks (Garcia-Pausas et al., 2017).

The excessive use of chemical fertilizers can result in 7 to 11% SOC loss from soil (Zhang et

al., 2021). Long-term organic fertilisation can lead to higher organic C content in soils than in those that are receiving only mineral fertilisers, which is observed mainly in the upper soil (Garcia-Pausas et al., 2017; Zhang et al., 2018). A positive impact on SOC was observed when organic fertilizers were applied alone or in combination with mineral fertilizer (Allam et al., 2022). Organic fertilizers supply the substrate for soil microorganisms which are converting it into soil organic matter (Yang et al., 2016). Even if the high rates of nitrogen fertilisers stimulate the decomposition process and determine the depletion of SOM, their application may lead to a SOC increase, favouring the accumulation of plant biomass (Allam et al., 2022).

## CONCLUSIONS

Soils represent an important component of the ecosystem, and its functions are strongly interrelated. Soil structure and fertility provide essential ecosystem services to agroecosystems. Changes in soil quality are gradual and the measurements regarding the effect of different cropping systems cannot be observed on short-term. Thus, the long-term experiments are of great importance.

Some management practices have negative or no effects on soil properties (i.e monoculture, intensive tillage, or chemical fertilisation). Differences in the tillage practices can determine changes in soil physical properties and its fertility, thus affecting the soil function and its capacity to provide ecosystem services (Bai et al., 2018).

Soil organic matter is related to various key soil functions that are relevant to soil ecosystem resilience and recovery (Grandy et al., 2012). Organic fertilisers can improve soil structure and soil organic matter content, both alone and in combination with mineral fertilisers, but increased soil organic matter contents depend on the amount and type of organic matter applied as well as on the period of application. Agricultural management practices are important elements in obtaining the benefits of ecosystem services and reducing disservices from agricultural activities, but future research is needed to estimate the value of various ecosystem services related to agriculture and to analyse the interactions between soil functions.

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## EFFECTS OF CLIMATIC CONDITIONS, ORGANIC AMENDMENTS AND PLANT CULTIVATION SYSTEM ON SOIL WATER CONTENT

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### Abstract

*The soil water availability is essential for soil organisms activity, for the growth and development of cultivated plants as well as for soil tillage. It can affect the sustainability of agricultural production and the resilience of agro-ecosystems. This paper refers to soil water behavior during autumn-summer cropping cycle within a study conducted between October 2021 and September 2022 in a field experiment set up at Moara Domnească Teaching and Research Station of the USAMV of Bucharest, Romania. The study is part of a research topic aiming to evaluate the sustainability of agricultural production and the resilience of agro-ecosystems under the effects of climate change. The study is carried out on red preluvosoil in Sylvosteppe area of Romanian Plain. In order to achieve the study objectives monthly measurements regarding temperature and precipitation were performed. Starting with March 2022, determinations of soil moisture were made, and its evolution was followed under the effect of the mineral fertilizers, manure compost and of agricultural crops (wheat, maize, soybean and mixtures of perennial grasses).*

**Key words:** organic amendments, soil moisture, plant cultivation system, red preluvosoil, wilting point.

### INTRODUCTION

Water is of primary importance for the growth of plants and at the same time a limiting factor at the global level considering the climatic conditions. In many water-scarce areas, crop production is very limited (Vereecken et al., 2008). But climate change makes that even in temperate areas, where water used to be provided by rainfall, the absence of water is felt especially in the critical phenological phases of cultivated plants, and the impact on production is increasing, sometimes even compromising production. The availability of water in the soil is important for crop production, including tillage, but also for the physico-chemical properties of the soil and for the activity of macro and micro-organisms in the soil (Mujdeci et al., 2017). Plants uptake the water for their growth and development through their root system, only a small quantity of it being taken by leaves, stem and branches (Mujdeci et al., 2017).

“During their growth, most annual plants absorb from soil in temperate climatic conditions 300 to 500 units of water for each unit dry matter formed” (Russell and Sir, 1937).

The amount of water from soils could affect the sustainability of agricultural production and the

resilience of agroecosystems (Seneviratne et al., 2010).

Crop production in agricultural systems is inextricably linked to the presence of water. The impact of soil moisture in the climate system is related to its role for evapotranspiration in soil moisture-limited regimes. The soil moisture is directly linked to the soil water potential or soil suction (how tightly the water is bound to the soil matrix) (Seneviratne et al., 2010).

Through the actions undertaken for the purpose of growing plants, through the intensity and timing of certain works, man can contribute both to the improvement of soil water-holding capacity and to the degradation of its physical properties. The soil tillage methods influence the soil characteristics (pore size distribution, soil porosity, bulk density) which are related to moisture and that determines the amount of plant available water holding capacity of soil (Gao et al., 2017; Bai et al., 2016; Mujdeci et al., 2017). There are many differences between soils regarding their water-holding capacity, which is determined by the physical properties, the content in organic matter, the climatic characteristics of the area etc. One of the most important strategies for increasing the soil water-holding capacity and for improving its

water regime is the application of organic fertilizers (Seyedsadr et al., 2022). “Fertilization has a strong influence on soil water content, because it stimulates plant growth and thus the plant use of soil water and its distributions” (Ritchie and Johnson, 1990). The organic amendments influence the wet range of the soil water retention characteristics differently (Zhou et al., 2020). “Organic amendments may (Zibilske et al., 2000) or may not (Gupta et al. 1977) increase available water in soil, most of this increase resulting from the water adsorbed by the organic matter” (Larney and Angers, 2012).

The influence of organic amendments on soil water content is very important in degraded sandy soils than fine-textured soils, the latter having greater intrinsic water-holding capacity (Larney and Angers, 2012). In sandy loam and clay soils, the application of organic composts had different effects on the soil moisture. For example, treatments with high rates of organic composts applied after crop harvest show a positive impact on soil water content in sandy loam soil in 0-15 cm depth but, on clay soil, various treatments with organic composts had no significant effect on soil moisture in 0- 20 cm depth (Gagnon et al., 1998). Under 8-year manure amendments, the soil water content in the 0-10 cm soil layer increased compared with the sole application of mineral fertilizer (Yang et al., 2011). Also, through the application of ammoniated straw (ammonification of crop straw through adding urea), during a 3-year period, the soil water storage and crop water productivity increased significantly in a summer maize - winter wheat rotation (Yu et al., 2017). Again, the differences in organic amendments composition could have different effects on soil water content and crop water productivity through the changes in soil functions (Hossain et al., 2017; Barzegar et al., 2002; Yazdanpanah et al., 2016).

The main objective of this study is to assess the effect of climatic conditions, organic amendments applied to soil and plant cultivation system on soil water moisture during an annual crop production cycle.

## MATERIALS AND METHODS

The experiments were carried out in Moara Domnească Teaching and Research Station of

the University of Agronomic Sciences and Veterinary Medicine (USAMV) of Bucharest situated in a Sylvosteppe ecological area of Romanian Plain, with characteristic type of soil, which is red preluvosoil. The preceding crop was alfalfa. In the fall of 2021, due to the drought, the soil was worked with the scarifier at 40 cm depth. Before sowing period, it rained enough so that the seed bed could be prepared by two passes with the cultivator, soil having an average moisture of 19.5%. The physico-chemical characteristics of soil are presented in Table 1.

Table 1. The physico-chemical characteristics of the red preluvosoil from Moara Domneasca Experimental Field

Soil parameters	Mean values
pH	5.89
C <sub>org</sub> (%)	2.02
N <sub>total</sub> (%)	0.216
N-NO <sub>3</sub> (mg/kg d.m.)*	28.67
N-NH <sub>4</sub> (mg/kg d.m.)	9.12
P <sub>AL</sub> (mg/kg d.m.)	86.04
K <sub>AL</sub> (mg/kg d.m.)	289.5

\* mg kg<sup>-1</sup> dry matter

The experimental field was organized into 4 blocks, and each block was organized into 8 plots (32 in total), each one with an area of 15 square meters. Eight (8) experimental variants (V<sub>1</sub>.....V<sub>8</sub>) in 4 replicates have been organized with the following treatments: V<sub>1</sub>-control (soil); V<sub>2</sub> - fertilized only with complex chemical fertilizers (NPK); V<sub>3</sub> - 15 t/ha compost; V<sub>4</sub> - 15 t/ha compost + complex chemical fertilizers (NPK); V<sub>5</sub> - 30 t/ha compost; V<sub>6</sub> - 30 t/ha compost + complex chemical fertilizers (NPK); V<sub>7</sub> - 60 t/ha compost; V<sub>8</sub> - 60 t/ha compost + complex chemical fertilizers (NPK). The complex chemical fertilizers were applied fractionally (Table 2; Photo 1).

The experiment included the following crops: 1) winter wheat (*Triticum aestivum* L.), 2) mixture of grasses (rye grass - *Lolium perenne* L., smooth meadow-grass - *Poa pratensis* L. and orchard grass - *Festuca pratensis* L.) and perennial legumes (white clover - *Trifolium repens* L., birdsfoot trefoil - *Lotus corniculatus* L.), 3) soybean (*Glycine max* L.) and 4) maize (*Zea mays* L.).

Table 2. Fraction of mineral fertilizers applied to crops during the study

Treatment	Wheat			Mixture of grasses			Maize			Soybean			
	Fraction 1 (24-03-2022)	Fraction 2 (15-04-2022)	Fraction 1 (24-03-2022)	Fraction 2 (15-04-2022)	Fraction 1 (15-04-2022)	Fraction 2 (13-05-2022)	Fraction 3 (30-05-2022)	Fraction 1 (15-04-2022)	Fraction 2 (13-05-2022)	Fraction 3 (30-05-2022)	Fraction 1 (15-04-2022)	Fraction 2 (13-05-2022)	Fraction 3 (30-05-2022)
V1 - control (soil)	-	-	-	-	-	-	-	-	-	-	-	-	-
V2 - NPK	57 kg/ha N + 57 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	28 kg/ha N + 28 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	40 kg/ha N + 40 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	40 kg/ha N + 40 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	40 kg/ha N + 40 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	46 kg/ha N + 46 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	29 kg/ha N + 29 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	29 kg/ha N + 29 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	18 kg/ha N + 18 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	20 kg/ha N + 20 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	20 kg/ha N + 20 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	13 kg/ha N + 13 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	13 kg/ha N + 13 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O
V3 - 15 t/ha compost	-	-	-	-	-	-	-	-	-	-	-	-	-
V4 - 15 t/ha compost + NPK	42 kg/ha N + 42 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	21 kg/ha N + 21 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	29 kg/ha N + 29 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	29 kg/ha N + 29 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	29 kg/ha N + 29 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	34 kg/ha N + 34 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	21 kg/ha N + 21 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	21 kg/ha N + 21 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	16 kg/ha N + 16 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	18 kg/ha N + 18 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	18 kg/ha N + 18 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	11 kg/ha N + 11 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	11 kg/ha N + 11 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O
V5 - 30 t/ha compost	-	-	-	-	-	-	-	-	-	-	-	-	-
V6 - 30 t/ha compost + NPK	27 kg/ha N + 27 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	13 kg/ha N + 13 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	18 kg/ha N + 18 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	18 kg/ha N + 18 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	19 kg/ha N + 19 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	22 kg/ha N + 22 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	14 kg/ha N + 14 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	14 kg/ha N + 14 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	13 kg/ha N + 13 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	15 kg/ha N + 15 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	15 kg/ha N + 15 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	9 kg/ha N + 9 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O	9 kg/ha N + 9 kg/ha P <sub>2</sub> O <sub>5</sub> + 0 K <sub>2</sub> O
V7 - 30 t/ha compost	-	-	-	-	-	-	-	-	-	-	-	-	-
V8 - 30 t/ha compost + NPK	-	-	-	-	-	-	-	-	-	-	-	-	-

In V<sub>8</sub>, for winter wheat, mixture of perennial grasses and maize, according to the calculation, the amount of manure compost should have ensured the nutrient requirements (NPK) and it was decided not to supplement it with chemical fertilizers.



Photo 1. Application of different doses of manure compost in Moara Domneasca Experimental Field plots

Measurements regarding precipitation and temperature were performed monthly as well as determinations of soil moisture (Figure 1). After 20<sup>th</sup> of October 2021, the quantity of precipitation (57.2 mm) and the soil moisture allowed sowing winter wheat in the optimal time and in good conditions. Unfortunately, the prolonged drought did not allow sowing the mixture of perennial grasses and legumes during September. The delay in sowing the mixture of perennial grasses by almost a month made the sprouting of the plants to be deficient, even if the favorable climatic conditions in autumn lasted until November. The perennial grasses had a good germination but did not germinate, and the perennial legumes generally did not germinate. Thus, in the spring of 2022, an over seeding was done to obtain a more consistent vegetation cover.

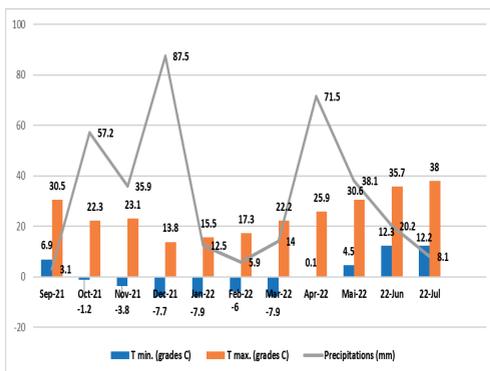


Figure 1. Climatic conditions in Moara Domneasca Experimental Field, September 2021-July 2022 period

In January-July, the minimum temperatures varied between -7.9 and 12.2°C and the maximum temperatures between 15.5 and 38°C. The precipitation regime was deficient during the first three month of 2022, the amount of precipitations varying between 5.9 mm (February) and 14 mm (March). April, corresponding to the sowing period for maize and soybean, was characterized by minimum temperatures of 0.1°C and maximum temperatures of 25.9°C, and the monthly amount of precipitation was 71.5 mm.

Starting with March 2022, soil samples were taken from each plot and determinations of soil moisture and wilting point (WP) were made and their evolution was followed under the effect of organic matter (manure compost) for all crops (winter wheat, maize, soybean and mixtures of perennial grasses and legumes). Soil samples were collected at a depth of 0-20 cm. To determine soil moisture, the gravimetric method was used with the drying of soil samples in aluminum ampoules at a temperature of 105°C for 8 h. The wilting point was calculated indirectly based on hygrosopicity coefficient. This one was determined by exposing the soil in a thin layer to an atmosphere of definite humidity under conditions of constant temperature and pressure.

## RESULTS AND DISCUSSIONS

Water acts as a solvent and as a medium for the transfer of nutrients from the soil to plants. As a nutrient, water becomes part of the cell contents without changing or is broken down into its elements and used in the production of new compounds. Thus, in proper amounts, soil moisture becomes one of the controlling factors in crop growth. The amount of water held within the plant is not much in comparison with the amount that is lost by evapotranspiration (Lyon and Buckman, 1922). In the conventional agriculture system, 50-60% of the water from rainfall is lost through evaporation during one year (Rusu et al., 2015).

During March-July 2022, the soil water content in 0-20 cm depth has fluctuated depending on climatic conditions and cultivated plants. Thus, in March, the lowest value of soil moisture was determined in the plots where maize and soybean were to be sown (17.11% in maize plots

and 17.3% in soybean plots) and the highest values were registered in the soil cultivated with winter wheat (19.3%) followed by the soil covered by the mixture of perennial grasses and legumes (18.5%) (Figure 2). The denser the vegetation cover, the higher the degree of water conservation in the soil (Photo 2).

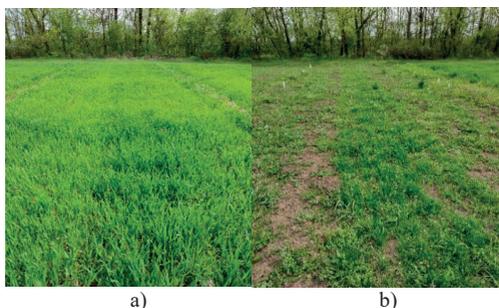


Photo 2. The vegetation cover in winter wheat (a) and mixture of perennial grasses and legumes plots (b) in March, 2022

The lower values of soil water content measured in the plots where maize and soybean were to be sown were due to the dry spring but also to the absence of the vegetation cover.

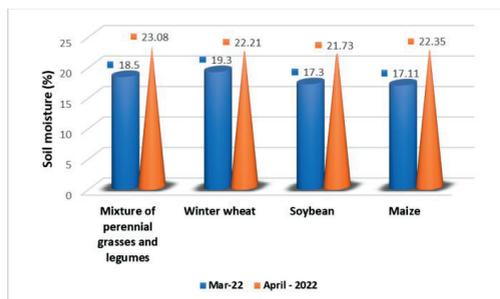


Figure 2. The soil moisture content during March-April period within soil cultivated with winter wheat, mixture of perennial grasses and legumes, soybean, and maize

In April, the amount of precipitation was good and thus the soil moisture values were higher in all plots, varying from 21.73% in soil covered by soybean to 23.08% in soil cultivated with mixture of perennial grasses and legumes. Rational fertilizer application could facilitate sustainable and effective exploitation of available rainfall (Liu et al., 2020). Moreover, organic matter content in soil has an influence on its physico-chemical properties, being both a binder of particles and a permanent source of

nutrients for plants (Carter, 2002). In this sense, our experiments show that the soil moisture content varies among crops and fertilization regime.

May 2022 was characterized by higher temperatures and a smaller quantity of precipitations (38.1 mm), which were also reflected by the soil moisture values. Thus, in winter wheat crop, the highest value of soil moisture was observed in the unfertilized variant (17.58%) followed by V3 (15 t/ha manure compost) with 16.29% and V5 (30 t/ha manure compost) with 15.59%. The lowest value was determined in V7 (60 t/ha manure compost) i.e. 12.81%.

In maize crop, the highest value of soil moisture was registered in variant V2 where only chemical fertilizer was added (21.46%) and the lowest soil moisture (18.76%) was determined in V3, with 15 t/ha compost (Figure 3).

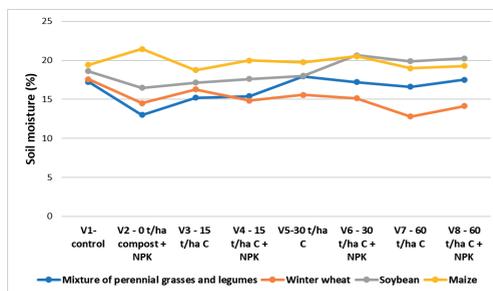


Figure 3. Soil moisture (%) under different fertilization regime and crops – May period, Moara Domneasca Experimental Field, 2022

In the mixture of perennial grasses and legumes, soil moisture had the highest values in the variants where 30 t/ha manure compost was applied, in the control and in the variant with 30 t/ha manure compost and NPK fertilizer (17.96%, 17.26% and 17.21%) and low values in the variant with chemical fertilizers only (13%). Also, in soybean plots, the lowest value of soil moisture was registered in V2 (16.48%) but the highest value in variant V6 (20.66%) (Figure 3).

In June, the pluviometric regime was deficient and the temperatures were higher than the normal registered for this period. The rising temperatures lead to an increase of evaporation at soil level and a decrease of soil moisture. However, the highest values of soil moisture were registered in soybean plots, in the variants V1 (14.89%), V8 (14.87%) and V4 (14.81%). In

soil cultivated with maize, the highest soil moisture value was determined in V1 (13.95%), followed by V5 (30 t/ha compost) (13.77%) and V7 (60 t/ha compost) with 13.32% (Figure 4).

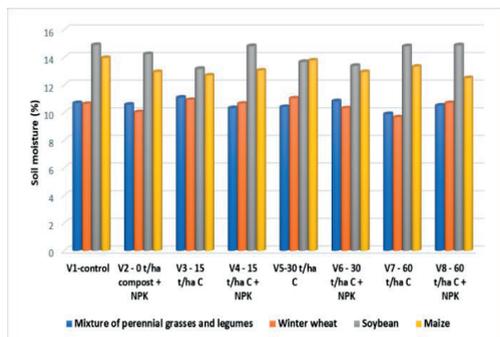


Figure 4. Soil moisture (%) under different fertilization regime and crops - June period, Moara Domneasca Experimental Field, 2022

In winter wheat and in the mixture of perennial grasses and legumes plots, soil moisture values were much lower than in soil covered by maize and soybean. Thus, in the soil cultivated with winter wheat, the moisture values varied from 11.02% (V5) to 9.65% (V7) and in the soil with mixture of perennial herbs, from 11.08% (V3) and 9.89% (V7) (Figure 4). It can be observed that in these two experiments, the lowest values for soil water content were determined in the variants where 60 t/ha compost was applied. An explanation could be that as it was the first year of compost application, the porosity of soil was higher so the bond between soil particles and organic matter was weak which is in accord with Derdour et al. (1993). Thus, the effect of different doses of compost on soil water content could be better observed on long-term. Another explanation of this decrease of soil moisture could be associated with the increase of crop water demand for usage of the available nutrients from the applied fertilizers.

In July, the soil moisture fluctuated in relation with the deficient rainfall regime (8.1 mm) and registered in all experiments the lowest values from entire vegetation period. So, in drought conditions, the highest values of soil moisture were registered in soybean plots, these varying between 10.84% (V1) and 9.5% (V4). In case of soil covered by maize crop, the highest value was determined also in V1 (9.18%) and the lowest value, in variant with 15 t/ha compost

(7.43%). In these two experiments it can be observed that soil moisture values are higher in variants where compost is applied in combination with mineral fertilizers.

In winter wheat plots, soil moisture was lower than in soybean plots, the highest value being determined in V8 (60 t/ha compost and NPK fertiliser treatment), i.e. 10.14%. This result can be explained by the fact that in periods with low precipitations, the increased soil organic matter content and a good vegetation cover led to an increased soil water retention. The lowest values of soil moisture in this period were registered in the soil covered by mixture of perennial grasses and legumes, i.e. between 11.08% (V3) and 9.89% (V7). This may be because the land was not covered very well by vegetation and high temperatures led to an increase of evaporation losses (Figure 5).

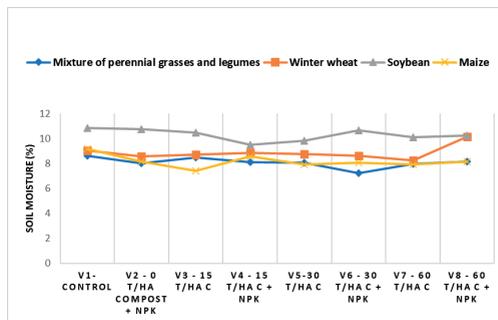


Figure 5. Soil moisture (%) under different fertilization regime and crops - July period, Moara Domneasca Experimental Field, 2022

The capacity of a soil to regulate the freshwater supply is a fundamental ecosystem service. The water percolated through soil is filtered, stored for plant utilization, and redistributed to groundwater and surface water bodies. Thus, the sustainability of water resources is directly influenced by soil (O'Geen et al., 2010).

The water from the soil is taken by the plant roots or evaporated from the topsoil into the atmosphere. If there is not an additional water supply to the soil, it gradually dries out and if the soil become very dry, the remaining water is retained more tightly, and plant roots are not capable to extract it. At a certain point, the water uptake is not sufficient to meet the plant's needs, so they are losing their freshness and wilts, they change the colour of leaves and finally the plant dies (Brower et al., 1985).

Our research showed that in plots cultivated with winter wheat, mixture of perennial grasses and legumes, soybean and maize, there was a strong correlation ( $R^2$  was between 0.73 at winter wheat and 0.83 at soybean) between soil moisture values and the wilting point. As much as soil moisture decrease to WP, the greater the effort of the plants to absorb the water (Figure 6).

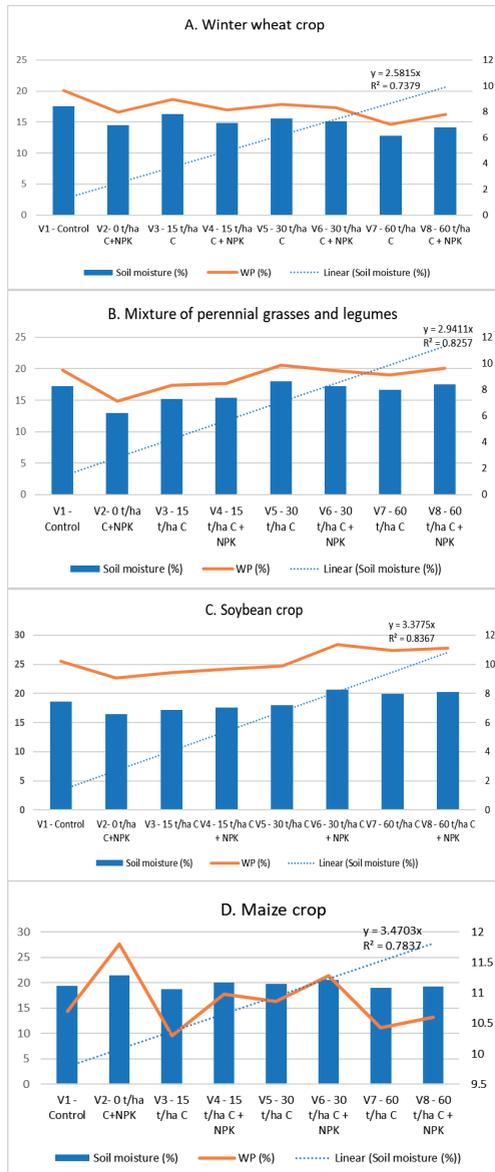


Figure 6 (A., B., C., D.). The correlation between soil moisture and wilting point in the winter wheat, mixture of perennial grasses and legumes, soybean, and maize, under different fertilization regimes, Moara Domneasca Experimental Field, May 2022

In winter wheat plots, the WP determined in May period showed a variation from 9.65% in the control and 7.78 % in V8 where 60 t/ha manure compost + NPK were applied. In the mixture of perennial grasses and legumes, the lowest value of WP was in V2 (7.14%) and the highest, in V5 (9.86%).

In soybean plots, the WP values were between 11.35% in V6 (30 t/ha C + NPK) and 9.05% in V2 (only NPK fertilizer) and in the soil cultivated with maize, the lowest value of WP was registered in V3 (10.3%) and the highest value, in V2 (11.8%).

## CONCLUSIONS

The soil moisture varied under the influence of climatic conditions and crops, and with the fertilization regime. So, during the vegetation period, complex relationships are established between soil, water and plants, which are strongly influenced by the evolution of climatic conditions, especially by the rainfall regime.

Climatic factors have a strong influence on plant-water relationships, but the soil is the key for water regulation acting as a sponge to hold water against gravitational forces in forms that are available for plants. So, maintaining a good structure of the soil through organic matter addition can lead to an increase of plant available water holding capacity. Also, understanding the soil-water relation is fundamental for the most land use decisions.

In the conditions where only the water from the precipitation would ensure soil moisture and therefore the water necessary for the growth and development of plants, a good water capacity of the soil would be essential for the sustainability of the agroecosystem. At the same time, a good correlation of the natural supply of precipitation with the water needs of the cultivated plant species would be necessary and useful. The elements of cultural technology such as the distances between plant rows and between plants in a row, respectively the density of the vegetation cover, are also important for an efficient use of soil water and for achieving crop production.

Research on the effects of organic fertilization must continue in the coming years to be able to reveal the contribution of organic matter from compost in the medium term on the physical

properties of the red preluvosoil and on its water regime.

## ACKNOWLEDGEMENTS

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## EFFICIENCY OF HERBICIDES IN THE TECHNOLOGY OF CULTURATION OF *Miscanthus giganteus*

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### Abstract

Among the elements of *Miscanthus giganteus* cultivation technology, the most important link is the protection of plants from weeds. Weed component control is necessary in the most critical periods of plant development. For *Miscanthus giganteus*, mainly plots are allocated on low-fertility previously uncultivated lands with high weediness. In this regard, it became necessary to study the methods of weed control in the *Miscanthus giganteus* agroecosystem and their effect on the yield of plantations in the second year of life. Research to examine the aftereffect of herbicides on the formation of the productivity of *Miscanthus giganteus* was carried out in 2016-2018 on the experimental site of Penza State Agrarian University (Russia) on light gray forest sandy loamy soil. The most favorable weather conditions were in 2016. With optimal thermal conditions and sufficient precipitation, by the end of the growing season, the plants reached a height of 239.0-300.0 cm with the number of stems 12.0-22.0 pcs/plant. The maximum yield of *Miscanthus giganteus* dry mass of 11.35 t/ha was obtained by applying the herbicide based on metsulfuron-methyl against the background of glyphosate. The return of frosts in May 2017 to minus 1.8°C led to the death of regrown plants and the number of stems after overwintering decreased to 4-8 pcs/m<sup>2</sup>, against 18-38 pcs/m in 2016. The highest stem density of 24 pcs/m<sup>2</sup> on average over three years was noted in the agroecosystem, where in the year of laying the plantation of *Miscanthus giganteus*, against the background of glyphosate-containing herbicide, they were treated with preparations based on metsulfuron-methyl and 2.4D + florasulam.

**Key words:** *Miscanthus giganteus*, weed vegetation, herbicides.

### INTRODUCTION

Improvement of crop cultivation technologies and their adaptation, in relation to the soil and climatic features of the land use area, is the most important task in the development of modern farming systems. Among the elements of cultivation technology, the most important link is plant protection from weeds. Depending on the weather conditions and the location of the crop on the territory, the species composition of weeds varies significantly over the years. These changes are associated with the biological characteristics of weeds, the strategy for combating them, the predominant range of herbicides used, the method of tillage, the weed control system throughout the crop rotation, and the general crop culture. (Atkinson, 2009; Xuea et al., 2015; Winkler et al., 2020)

Weed damage is extensive and varied. In agroecosystem, they enter into a competitive relationship with cultivated plants for the use of moisture, nutrients, light and other life factors.

As a result, weeds worsen the conditions for their growth and development (Lewandowski, 1998; Gushina et al., 2021).

Weakly competes with weeds and a new technical plant - *Miscanthus giganteus*, especially in the first two years of cultivation. Weed component control is necessary in the most critical periods of plant development (Chuansheng et al., 2021). For *Miscanthus giganteus* mainly areas are allocated on low-fertility previously uncultivated lands with high weediness. Ways to control weeds in plantings of *Miscanthus giganteus* have been little studied, since today this crop occupies small areas. In this regard, it became necessary to study the methods of weed control in the *Miscanthus giganteus* agroecosystem and their effect on the yield of plantations of the second year of life.

### MATERIALS AND METHODS

Herbicide after-effect researches on agrotechnical and chemical measures to combat

the weed component in the *Miscanthus giganteus* agrocenosis were carried out in 2016-2018, humus content in the arable horizon - 2.7% (State Standard (SS) 26213-91), alkaline hydrolysable nitrogen - 102.8 mg/kg (according to Kornfield), mobile phosphorus and exchangeable potassium - 188 and 110 mg/kg, respectively (SS 26204-91), pH<sub>Cl</sub> - 5.7 (SS 26483-75).

Experiment scheme:

1. Absolute control (control 1);
2. Production control (control 2 - two inter-row treatments);
3. Treatment with glyphosate-containing herbicide (Tornado 500 4 l/ha);
4. Herbicide treatment based on 2.4 D + florasulam (Ballerina 0.6 l/ha);
5. Treatment with metsulfuron-methyl herbicide (Magnum 0.01 kg/ha);
6. Treatment with herbicide Tornado 500 (4 l/ha) + treatment with Balerina herbicide (0.6 l/ha);
7. Treatment with Tornado 500 herbicide (4 l/ha) + treatment with Magnum herbicide (0.01 kg/ha).

The repetition is fourfold, the placement of plots is systematic.

Systemic herbicides Magnum and Ballerina were applied in the phase of two to four leaves in annual weeds and rosettes in perennials with a water consumption rate of 300 l/ha using a knapsack sprayer.

Soil preparation included autumn plowing to a depth of 22-25 cm three weeks after the application of the herbicide Tornado 500 at the end of the second decade of August, harrowing with tooth harrows in the spring, and cultivation before planting. Planting of *Miscanthus giganteus* was carried out with rhizomes - rhizomes 8-10 cm long as early as possible to a depth of 10 cm according to the scheme 75 × 70 cm. Observations, records and analyzes were carried out according to generally accepted methods.

The weather conditions during the years of the research were different, which made it possible to give an objective assessment of the studied cultivation methods. Favorable for the development of plants was 2016, when the hydrothermal coefficient at the beginning of regrowth - cleaning was 1.17, which characterizes it as sufficiently moistened.

During the growing season in 2017, 280 mm of precipitation fell with a sum of active temperatures of 2116.6°C. But, frosts in May to minus 1.8°C led to the death of regrown plants and the number of stems after overwintering decreased. In the dry year of 2018. However, during the period of intensive increase in the above-ground mass of precipitation, more than the norm fell at optimal positive temperatures.

## RESULTS AND DISCUSSIONS

The success of the cultivation of wintering crops is determined not only by the weather conditions of the growing season, but also by the conditions prevailing in winter (Barksdale et al., 2020; Doronin et al., 2017). Overwintering of agricultural crops is also determined by the biological characteristics of plants and their condition during the period of cessation of the autumn vegetation. In the first year of wintering, they can be severely damaged by frost, resulting in their partial death (Jones, Walsh, 2001; Płazeka et al., 2011).

The early spring regrowth of *Miscanthus giganteus* plants in 2016 (April 14) was facilitated by the increased temperatures of the month and the period of the beginning of regrowth - harvesting was 165 days. 18 days later, the regrowth of culture was noted in 2017, full - on May 10, which affected the duration of the growing season, the length of which was 147 days. In 2018, the elevated temperature regime (16.6°C) and the lack of precipitation (27.8 mm) were extremely unfavorable for the initial growth of *Miscanthus giganteus*, so full regrowth was observed only on May 28. The duration of the period of the beginning of regrowth - harvesting was 125 days.

The problem of winter hardiness of *Miscanthus giganteus* occupies a special place in cultural studies. Favorable overwintering conditions for the plantation developed in 2016 and 2018, which had a positive effect on plant survival. In the initial period of growth, the number of *Miscanthus giganteus* stems of the second year of life was within 16-38 pcs/m<sup>2</sup>, which is 1.3-3.0 times more than before leaving for winter. Their smallest number was noted in the absolute and production controls. With chemical methods of weed control, the stem per

square meter exceeded 34 pieces in 2016 and 20 pieces in 2018. This indicates a good overwintering of *Miscanthus giganteus*, whose survival rate in these years was 100%.

In mid-May 2017, the regrown plants suffered from frost, which led to the thinning of landings. As a result, the number of stems was 4-6 times less than before winter, and 4.5-8.5 times less than in the previous year, and their number in the experiment did not exceed 8 pieces/m<sup>2</sup>. Plant survival decreased by 19-25%. On average, over three years, the highest stem density after overwintering, 26 pcs/m<sup>2</sup>, was noted in the variants with double herbicide treatment, the survival rate of plants varied from 73.0 to 75.0%.

The value of the yield of perennial crops largely depends on the density of the stem. Close crops significantly reduce unproductive moisture consumption due to good soil shading and do not leave an ecological niche for weeds. As a result of three years of research, it was found that the studied agricultural practices, as well as the weather conditions of the growing season, influenced the formation of *Miscanthus giganteus*.

The productivity of agrocenosis is formed due to the main indicators of the crop structure, which include the height of the stems and their

number. So, in 2016, due to the early spring growth of *Miscanthus giganteus*, the number of stems in early June increased to 9-19 pieces with a height of 120-140 cm (Figure 1). They were most developed in plantings with double herbicide treatment. On the plantation with the introduction of the herbicide Tornado 500, with well-developed 18 shoots, the height of the plants did not exceed 133 cm weight - up to 358-458 g. In the control variants, the height of the plants was 120-123 cm, the number of shoots was 9-10 pcs/plant. The mass of the above-ground part was within 168-658 g/plant. In August, the height of plants increased by 116-147 cm compared to the previous determination in June 236 cm with developed 12 shoots. Mechanical weed control contributed to an increase in the number of stems up to 12 pcs with a height of 279 cm. The use of herbicide Tornado 500, compared with absolute control, led to an increase in plant height by 1.18 times, the number of stems - by 1.57 times, the use of systemic herbicides - 1.16-1.17 times and 1.67-1.75 times, respectively.

The greatest effect was noted on plantations where a double herbicide treatment was carried out. At the same time, the height of plants was 280-295 cm, the number of stems was 22 pcs, weight 2094- 2336 g/plant (Figure 1).

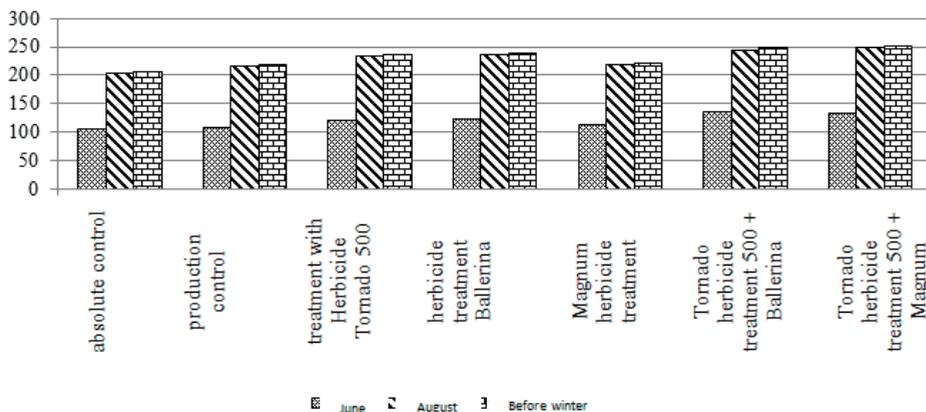


Figure 1. The height of *Miscanthus giganteus* second year of life (2016-2018), cm

Before leaving for winter, the height of the shoots increased by 3-6 cm and reached 300 cm, while their number did not change. The mass of the aerial part increased by 16-22 g and the largest (2112-2358 g) it was on the plantations where a double herbicidal treatment was carried out.

Spring frosts after the growth of *Miscanthus giganteus* in 2017 had a negative impact on its development. As a result, at the time of the first determination, compared with the previous year, the number of stems decreased by 4.5-8.5 times, the height of plants was 1.05-1.30 cm

less, and their weight did not exceed 115 g/plant.

At the end of August, the plants reached a height of 176-212 cm, the number of stems increased to 4-11 pieces, and their weight - up to 106-595 g. In plantings where care work was not carried out, the *Miscanthus giganteus* was weaker, the shoots had a height of 176 cm, and their number did not exceed four pieces. On the plantation treated only with Tornado 500, the number of shoots was 10 pieces 200 cm high. In the production control, the plant height reached 178 cm with well-developed 7 shoots. Herbicides of systemic action contributed to an increase in the number of stems up to 8...10 pieces, and their height up to 179-205 cm, weight 586-595 g.

Before harvesting, the growth of *Miscanthus giganteus* was insignificant 2-4 cm, the number of shoots did not change. The highest yield of wet weight (596-604 g) was obtained on plantations after double herbicide treatment. In absolute and production control, the wet weight was 116 and 289 g, respectively. The use of only Tornado 500 contributed to an increase in the mass of the aerial part up to 431 g, only systemic herbicides - up to 296-555 g/plant.

The optimal air temperature and precipitation in June 2018 evened out the development of plants of the second year of life, the height of which was 99-135 cm with the number of shoots 8-13 pcs/plant. The raw weight of the above-ground part of *Miscanthus giganteus* was 1.5-4.5 times more than in the same period of previous years. The use of herbicides Ballerina and Magnum against the background of Tornado 500 contributed to an increase in the number of shoots up to 13, plant height up to 132-135 cm, wet weight yield up to 238-240 g, which is 1.3-1.6 times higher than the absolute control.

Slight increase in plant height in August (up to 195-240 cm) is associated with the biological characteristics of the culture and about severe rainfall deficit during this period. The number of shoots, in this case, amounted to 8-12 pcs/plant. The yield of raw mass in relation to the previous definition increased by 454-1159 g/plant.

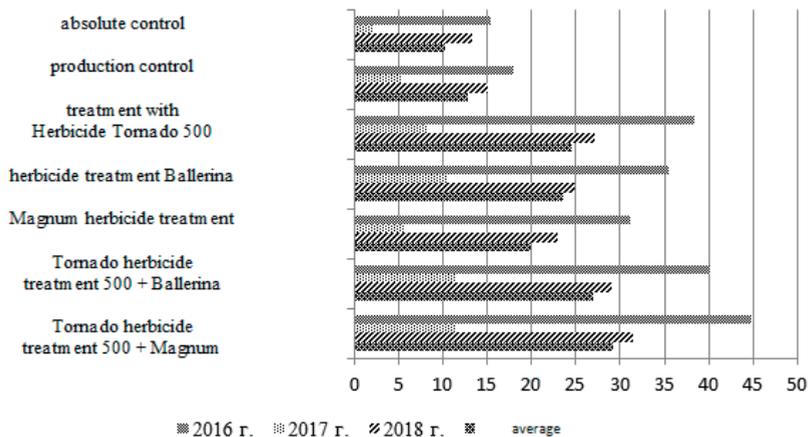
By harvesting, the plants reached a height of 199-245 cm, with a weight of 670-1578 g,

shoot formation stopped. The highest rates of growth and development of *Miscanthus giganteus* plants were noted on plantations with double herbicide treatment.

On average over three years, overwintering conditions had a direct impact on the development of *Miscanthus giganteus*, but plants suffered most in 2017 from frosts that were observed during their mass growth. At the same time, depending on the methods of weed control, the greatest effect was obtained from the use of systemic herbicides Balerina and Magnum against the background of Tornado 500. An increase in the number of stems and their height is accompanied by a regular increase in the mass of the *Miscanthus giganteus* plant.

In the conditions of 2016, the yield of fresh mass in absolute control reached 15.41 t/ha, in the variants where weed control was carried out, it increased by 2.61-29.51 t/ha or 1.17-2.91 times (Figure 2). Favorable overwintering conditions and early regrowth of culture contributed to the formation of powerful plants. Compared to the absolute control, the yield of wet mass after inter-row treatment increased to 18.02 t/ha. An increase in the treatment of plantations with herbicides Magnum and Ballerina by 15.79-20.10 t/ha was noted. The highest yield 40.23-44.92 t/ha was obtained after a double herbicide treatment. In the version where only the herbicide Tornado 500 was used, the wet weight yield was 38.48 t/ha.

The spring frosts of 2017 had an impact on the development of *Miscanthus giganteus* plants. As a result, the yield of green mass was 3.28-6.97 times lower than in the previous year, and the lowest (2.21 t/ha) was noted on the plantation, where maintenance work was not provided. With inter-row processing, the yield of wet mass increased to 5.50 t/ha. The use of systemic herbicides contributed to an increase in yield by 2.55-4.78 times, and when plantations were treated with a continuous herbicide, by 3.71 times compared to absolute control. The greatest effect was noted when the herbicides Magnum and Ballerina were applied against the background of Tornado 500. On this agricultural background, the most powerful plants with a fresh weight of 11.35-11.50 t/ha were obtained.



NDS<sub>05</sub>, t/ha: 2016 - 0.68, 2017 - 0.17, 2018 - 0.47.

Figure 2. Yield of wet mass of *Miscanthus giganteus* second growth year, t/ha

The yield of the above-ground mass of the *Miscanthus giganteus* second year of life in 2018 was 1.2-1.4 times less than in the favorable year 2016, but more by 9.56-20.21 t/ha than in the previous year. The maximum yield of wet weight 29.1-31.56 t/ha was obtained when herbicides Magnum and Ballerina were used in the year of planting against the background of Tornado 500, against 13.4 t/ha under absolute control.

The same trend can be traced in the collection of dry mass of plants. On average, over three years, its highest yield (9.71-11.35 t/ha) was obtained from plantations where a double herbicide treatment was carried out, the lowest 3.73 t/ha - from plots where maintenance work was not provided. The use of herbicides of systemic action contributed to an increase in the yield of dry matter in comparison with the absolute control by 1.92-2.28 times, and the use of the herbicide Tornado 500 - by 2.48 times.

Consequently, the methods of weed control in different ways allow *Miscanthus giganteus* to realize its biological capabilities. Thus, in 2017, overwintering conditions were less favorable for plants, which negatively affected the yield of the crop, the wet and dry weight of which, on average, according to the experiment, was 7.85 and 2.31 t/ha, respectively. In the first and third years of research, the conditions for the

formation of yields were much better, therefore, on average, according to experience in 2016, the yield of green mass was 4.0 times higher, in 2018 - 3.0 times higher, and the dry mass yield was 19.56 and 8.37 t/ha, respectively.

## CONCLUSIONS

In this way, the most favorable weather conditions were in 2016. With optimal thermal conditions and sufficient precipitation, by the end of the growing season, the plants reached a height of 239.0-300.0 cm with the number of stems 12.0-22.0 pcs/plant. The return of frosts in May 2017 to minus 1.8°C led to the death of regrown plants and the number of stems after overwintering decreased to 4-8 pcs/m<sup>2</sup>, against 18-38 pcs/m<sup>2</sup> in 2016. The highest stem density of 24 pcs/m<sup>2</sup> on average over three years was noted in agroecosis, where in the year of laying the *Miscanthus giganteus* plantation, systemic herbicides were treated against the background of Tornado 500. The maximum yield of wet and dry mass of *Miscanthus giganteus* was 29.28 and 11.35 t/ha respectively, obtained by applying the herbicide Magnum on the background of glyphosate.

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## INFLUENCE OF ORGANOMINERAL FERTILIZER BASED ON FERMENTED POULTRY WASTE ON THE PHYSICO-CHEMICAL PARAMETERS OF AGRICULTURAL SOILS

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### Abstract

*The paper discusses aspects of the influence of organo-mineral fertilizer, which is poultry waste fermented with the help of a complex of microorganisms, on the structure of the soil. The technology for the preparation of organo-mineral fertilizer is complex and involves cascade fermentation of spent litter masses, their integration with mineral components under conditions of correction of the environmental reaction. This ensures the minimization of losses of biogenic elements (nitrogen, phosphorus, sulfur, and others) in the process of destruction. This approach, on the one hand, reduces the volume of emissions of gas fractions into the atmosphere, and, on the other hand, determines the transition of biogenic elements into mobile forms available for plants in the fertilizer composition. A reliable positive effect of the developed fertilizer on the chemical composition of the soil and its characteristics such as air and water permeability. The use of organo-mineral fertilizer on leached chernozem contributes to the preservation and increase of humus reserves, enrichment of the soil with its mobile components. A change in the characteristics of the physical state of the arable soil layer is shown, which manifested itself in soil decompaction, an increase in the proportion of structural components of an agro-valuable size and the number of water-stable aggregates. The application of fertilizer provides the best agro-physical properties of the soil by reducing the bulk density to 0.25 g/cm<sup>3</sup>, increasing ACF by 4.5% and VA by 10.6% compared to the unfertilized background.*

**Key words:** poultry waste, waste bioconversion, organo-mineral fertilizers, soil structure.

### INTRODUCTION

An urgent problem of agriculture is the protection of the natural environment from pollution by organic production waste. The most dynamically developing branch of animal husbandry is poultry farming. The placement of environmentally hazardous waste in the form of manure can act as a limiting factor in scaling and expanding production. The solution to this problem can be the conversion of such waste into organic and organo-mineral fertilizers (OMF). About 640 million tons of manure and litter are produced annually in agricultural enterprises of the Russian Federation, which, in terms of fertilizer value, is equivalent to 62% of the total production of mineral fertilizers in the country (Subbotina et al., 2014).

Organic fertilizers are not only an important source of nutrients and carbon for plants and soil microorganisms, but also a means of improving the agronomic properties of the soil and replenishing the humus reserve in it, one of the main factors of soil fertility. There are reports in the literature about testing innovative

fertilizers obtained by microbial fermentation of waste (Jara-Samaniego et al., 2017; Sigurnjak et al., 2019; Ahuja et al., 2020; Fernández-Delgado et al., 2021; Saleme et al., 2021). The works show the positive effect of such fertilizers on plant productivity and soil fertility, its agrochemical and agro-physical indicators. In this regard, the relevance of developing a technology for the bioconversion of poultry waste into organo-mineral effective and economical fertilizers is beyond doubt.

The aim of this work was to evaluate the effect of organic-mineral fertilizer, which is a poultry waste fermented with the help of a complex of microorganisms, integrated with mineral components, on the soil structure.

### MATERIALS AND METHODS

The studies were carried out on the basis of the Penza State Agrarian University. Poultry waste was obtained from the waste storage site of the Damate Group of Companies, located in the Nizhnelomovsky district of the Penza region (Russia). These are spent litter masses

containing turkey manure and straw-sawdust materials (Figure 1).



Figure 1. A sample of poultry waste - spent manure and bedding mass

The litter-litter mass was brought to 60% moisture content and placed in plastic containers in an amount of 5.0 kg. The substrates were exposed under standard conditions (20°C, 760 mm Hg) for 2 weeks, during which, an occasional stirring was done. During this period, fermentation of substrates occurred due to the enzymatic activity of the native microflora.

For the degradation of hardly decomposable materials, after a two-week period, a functional complex of microorganisms, which is a culture liquid with the remains of a nutrient medium, was introduced into the manure-litter mass that underwent primary fermentation (Figure 2).



Figure 2. Microorganism inoculum prepared for waste fermentation

As a functional group of ammonifiers, bacteria of the genus *Bacillus* were used; as nitrifiers - representatives of the genus *Nitrosomonas*; for the implementation of the destruction of cellulose and lignin components, cultures of filamentous fungi of the genera *Thelavia*, *Cellulomonas* and *Myceliophthora* and actinomycetes of the genus *Nocardia* were used.

The functional complex of microorganisms is a deep culture, namely a suspension of cells with a titer of 10.0-12.0 million cells per liter of culture fluid. For use, the concentrated culture is diluted with tap water in a ratio of 1:10 and introduced with stirring into the mass of the mineral carrier, glauconite, crushed to a fraction of 0.01-0.5 mm in the amount of 1 liter per 10.0 kg. The microbial complex immobilized on a mineral carrier is added to the mass of waste at the rate of 5:100. The fermentation process takes place in piles of 1.5-2.0 m high. Raising the temperature in piles to 50-60°C is not a critical moment, since thermophilic species of micromycetes are involved in the fermentation processes.

The resulting organo-mineral fertilizer was applied to the soil in the conditions of a peasant farm located in the Lopatinsky district of the Penza region, in the autumn period under clean fallow. The soil is leached heavy loamy chernozem, the arable layer of which contains 6.0-6.4% humus; exchangeable bases 50.0-36.0 meq/100 g; pH KCL<sub>1</sub> 5.9-6.2. The area of the plots was laid by 5 m<sup>2</sup>, the repetition of the experiment was four times, the variants in the experiment were placed by the method of randomized repetitions. The scheme of the experiment included the following options: 1) control (without fertilizers) (C); 2) manure and litter materials without microbial fermentation (MLM); 3) organo-mineral fertilizer (OMF); 4) mineral fertilizer equivalent to OMF in terms of the content of biogenic elements (nitrogen, phosphorus, potassium) (MF).

Soil samples were taken in July of the following year after two cultivations (April, June) from a layer of 0-20 cm. Humus carbon was determined according to Tyurin (Arinushkina, 1970). The bulk density was determined according to Kachinsky, the humidity was determined by the thermoweight method (Alexandrova, 1986); structural composition according to Savvinov (Vadunina

et al., 1978); water resistance of the structure - on the Baksheev (1969) device (Methodological guide). The results of the study were processed by the analysis of variance (Dospheov, 1969).

## RESULTS AND DISCUSSIONS

On the basis of pre-selected functional cultures of microorganisms, the physiological and biochemical potential of which is capable of providing high efficiency of composting of manure-litter mass, functional microbial compositions were compiled. The composting process is similar to the rotting process. It is characterized by a cascade of stages that are implemented in a certain chronological order. At the first stages of destruction, readily available components are decomposed: easily degradable polysaccharides, low molecular weight carbohydrates, proteins, nitrogenous substances, which, as a rule, is accompanied by heating of the substrate, as well as a shift in the overall biochemical balance towards ammonification. At later stages, more difficult to utilize components become substrates, localized mainly in the bedding material (wood chips, sawdust, cereal straw). By their chemical nature, the main substances of these materials are polymers such as cellulose, hemicelluloses and lignin. In view of the foregoing, at the initial stages of fertilizer preparation, cultures providing ammonification were used, and at later stages, cellulose- and lignolytic complexes of microorganisms were used. In establishing the timing of the introduction of the appropriate complexes of microorganisms throughout the process, an important role is played by the pH index, which can be subject to significant dynamic changes. At the initial stages of active fermentation, the pH is in the alkaline region, and as biodegradation progresses, it shifts to the acid side. This fact is explained by the fact that in the process of ammonification, the released ammonia, interacting with water, forms a solution of ammonium hydroxide, with alkaline properties. At later stages, the hardly decomposable compounds are sequentially oxidized, resulting in products with acidic properties (carboxylic acids and phenolic compounds). Thus, the change in the pH value is largely determined by the activity of the

microorganisms themselves and the gradual change in their formations. In addition to changes in the species composition of microorganisms, shifts in pH values can also contribute to the transformation of the forms of substances, the mobility of elements, and, as a result, the degree of assimilation of such important biogenic elements as phosphorus and nitrogen. However, to accelerate these changes, it seems possible to correct the pH of the substrate with acidic solutions, which can provide better adaptation of certain microorganisms and the fullest possible realization of the physiological and biochemical potential. Thus, since the final product - an organomineral fertilizer - is an integral result of the activity of all microorganisms at all stages of biodegradation of a degradable material, then the regulation of the process through the sequential release of the corresponding microbial complexes from the mineral carrier with simultaneous correction of the pH value can ensure the production of a high-quality product in the most compressed terms.

The source material - manure and bedding mass, which is a common poultry waste, also contains biogenic elements in the following quantities: 2-4% N, 3-3.5% P<sub>2</sub>O<sub>5</sub> and 1.5-2.0% K<sub>2</sub>O, humic substances were not detected.

The product obtained as a result of fermentation (OMF) is a friable mass with a moisture content of 30%, color from light brown to rich brown, with a characteristic earthy smell (Figure 3).



Figure 3. Material of organo-mineral fertilizer obtained as a result of microbial fermentation poultry waste

Physico-chemical characteristics of the product are shown in Table 1.

Table 1. Results of chemical analysis of the product (OMF),  $p < 0.05$

Indicator	Result
Particle size, mm	$1.6 \pm 0.4$
Moisture content, %	$12.6 \pm 0.9$
Indicator of activity of hydrogen ions in an aqueous suspension, pH	$7.8 \pm 0.3$
Mass fraction of organic matter, % for dry product	$82.6 \pm 1.7$
Mass fraction of biogenic elements, % on dry matter:	
N	$51.8 \pm 2.4$
P <sub>2</sub> O <sub>5</sub>	$4.8 \pm 0.7$
K <sub>2</sub> O	$4.2 \pm 0.3$
humic substances	>15.0
Nitrate nitrogen content, mg/kg dry matter	$270.4 \pm 11.4$

The data obtained indicate the feasibility of using WMD to improve the structure of the soil and enrich it with biogenic elements.

The fertilizers used in the agrochemical experiment showed differences in quantitative estimates of the intensity of the humification process in terms of humus carbon ( $C_{\text{hum}}$ ) (Table 2). A predominant effect on the content of mobile carbon forms of organic compounds ( $S_{\text{mob}}$ ) and, to a lesser extent, on the parameters of a stable forms of carbon ( $C_{\text{stab}}$ ) was found.

Table 2. Influence of agromeliorants on humus parameters, mgC/100 g ( $p > 0.05$ )

Option	$S_{\text{hum}}$	$S_{\text{mob}}$	$S_{\text{stab}}$	% $S_{\text{mob}}$ from $S_{\text{hum}}$
Control	3581	874	2707	24.4
MLM	3714	812	2902	21.9
OMF	3983	1042	2941	26.1
MF (eq. N <sub>52</sub> P <sub>48</sub> K <sub>42</sub> )	3218	716	2502	22.2

The analysis of the obtained results indicates that the introduction of organo-mineral fertilizer obtained by fermentation of manure-litter materials under fallow provides a significant increase in the content of mobile forms of carbon. This can be explained by the increased enzymatic activity of the introduced microorganisms and the role of glauconite as a phase that immobilizes microorganisms and

biogenic substances, including mobile forms of carbon.

Leached chernozems themselves are distinguished by good agro-physical properties, and fertilizers are not always able to significantly improve them (Kurachenko et al., 2008). Moreover, long-term systematic use of fertilizers can have a negative impact on the bulk density and structural composition of soils (Pyatkovsky et al., 1983; Kretinina, 1989; Perveeva et al., 2004). The agromeliorants studied in the experiments change the density of the arable layer, which was established by evaluating soil samples taken after the first (spring) and second (summer) cultivations of bare fallow (Figure 4). After the first cultivation on the control variant, it was  $1.1 \text{ g/cm}^3$ . Despite a fertilized background, the density of the soil was reduced by  $0.1\text{-}0.25 \text{ g/cm}^3$  which could be explained by the presence of organic substances at different stages of microbial destruction. After the second cultivation, a decrease in density was noted in all variants, but again the most pronounced decrease was noted in the variant with OMF, which confirms the assumption about the role of organic compounds, as well as microbial degradation processes.

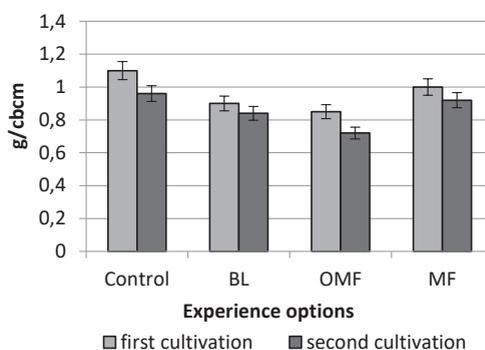


Figure 4. Density of addition of leached chernozem in the variants of the experiment: Control - control; BL - manure-litter mass; OMF - organomineral fertilizer; 4 - MF - mineral fertilizer (equiv. N<sub>52</sub>P<sub>48</sub>K<sub>42</sub>) ( $p < 0.05$ , error bars - mean error)

The introduction of organo-mineral fertilizer into the soil increases the content of agro-valuable fractions (AVF) with aggregate sizes from 0.25 to 10 mm, which have porosity and water resistance, both after the first and after

the second cultivation. This structure determines the most favorable water-air soil content. Despite, the background of mineral fertilizer, an increase in this indicator is also found, the introduction of bird litter did not have a significant effect on the share of AVF. A statistically significant increase in AVF by 4.5% after the first and second cultivations was found in the variants with the application of organic mineral fertilizer and by 3.5% - with the application of bedding materials.

An increase in the number of water-stable aggregates by 7-10.6% after the first cultivation was revealed in all variants of the experiment. Organo-mineral fertilizer (OMF) had the greatest impact on the aggregate composition. Such data can be explained by the activity of soil microbiocenosis, activated by the introduced biogens.

A variety of microbial waste products (biofilms, mucus capsules, cell wall material and other biofactors), due to their adhesive and gel-forming properties, can have a beneficial effect on soil structure and its water-holding capacity. After the second cultivation, the content of water-stable aggregates in the soil increased by 3-10.5%, and during this period, the most noticeable effect of organo-mineral fertilizer was shown.

## CONCLUSIONS

A reliable positive effect of the developed organo-mineral fertilizer obtained by microbial fermentation of poultry waste on the chemical composition of the soil and its characteristics such as air and water permeability has been established.

The use of organo-mineral fertilizer on leached chernozem contributes to the preservation and increase of humus reserves, enrichment of the soil with its mobile components.

The experiment established a change in the characteristics of the physical state of the arable soil layer, which manifested itself in soil decompaction, an increase in the proportion of structural components of an agronomically valuable size and the number of water-stable aggregates.

The application of fertilizer provides the best agro-physical properties of the soil by reducing the bulk density to 0.25 g/cm<sup>3</sup>, increasing agro-

valuable fractions by 4.5% and water-stable aggregates by 10.6% compared to unfertilized background.

The obtained data testify to the expediency of using the studied organo-mineral fertilizer to improve the soil structure.

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## HUMUS BALANCE AND NUTRIENT REGIME OF IRRIGATED SOIL UNDER DIFFERENT SYSTEMS OF BASIC TILLAGE AND FERTILIZER

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### Abstract

*The article presents an analysis of the results of experimental research in a stationary field experiment to establish the peculiarities of the formation of humus reserves and nutrient regime in the arable layer of dark chestnut soil under the influence of different fertilizer systems and basic tillage. The purpose of research: to establish patterns of conversion of crop by-products of crop rotation into humus and the main elements of mineral nutrition of plants by organic and two organic-mineral fertilizer systems against the background of five systems of main cultivation in 4-field row crop rotation irrigation system in the area of the Ingulets irrigation system. The most favorable conditions for accumulation of leaf mass of crop rotation crops, the formation of a positive balance of humus with an average annual growth of 2.02 t/ha provided organic-mineral fertilizer system using by-products of crop rotations and doses of mineral fertilizers N<sub>120</sub> P<sub>60</sub> against the background of differentiated main cultivation with one slit to a depth of 38-40 cm per rotation.*

**Key words:** row crop rotation, main tillage, fertilizers, humus, post-harvest residues.

### INTRODUCTION

The ability of the soil to create the necessary conditions for the development of crops and the formation of high yields is inextricably linked with the reserves of organic matter in it. It is known that humus determines favorable nutritional, water-air, thermal and biological regimes, soil structure, accumulation of physiologically active substances (Baliuk & Medvediev, 2012; Slepeliene & Slepetytys, 2005).

In recent decades, the entry of organic matter into the soil is mainly due to post-harvest crop residues, which are energy material for microbiological processes, the formation of nutrients and the accumulation of humus. After all, each crop rotation, after fertilization, leaves in the irrigated soil a certain amount of roots, which becomes a source of humus - the most important indicator of fertility (Heitkamp et al., 2011).

As noted by GA Mazur, in recent years, due to the decline of the livestock industry, less than 1 t/ha of organic fertilizers have been applied

annually, and humus losses have reached such proportions that 132 t/ha of litter manure is required to compensate for them without taking into account current humus mineralization processes (Mazur, 2013).

From one ton of the most nutrient-poor crop residues in the soil remains about 5 kg nitrogen, 1-1.5 - phosphorus, 8-10 - potassium and 3-4 kg - calcium. The remnants of green cereals contain the same amount of ash elements, but more nitrogen (10-15 kg/t). In general, it is 3-4 times less than required for a new harvest. Post-harvest residues of legumes enrich the soil the most (Kanivets, 2001).

Perennial grasses are able to leave behind 100 kg/ha or more of dry organic matter in the form of roots and aboveground post-harvest residues. Therefore, with the expansion of sown areas of these crops and especially alfalfa, the balance of humus will be positive, and a sharp deterioration of this balance will lead to an increase in the structure of sown areas of pure steam and row crops, in the fields of which and plant remains may be completely absent, as in the field of pure steam, or in most cases their

number may be insignificant (Yeshchenko et al., 2012).

Prolonged (26 years) agricultural use of typical chernozem without fertilizers has led to a decrease in humus content in the arable soil layer by 11% compared to baseline. The application of only mineral fertilizers to the soil was accompanied by a violation of the mineralization-immobilization balance in the direction of the predominance of mineralization and as a result the organic matter content decreased by 7.5% compared to the control. The use of manure in organic and organic-mineral fertilizer systems contributed to the maintenance of higher humus content (Skrylnyk et al., 2019).

According to a long experiment L Shedei on chernozem podzolic heavy loam application of mineral fertilizers together with cattle manure for a ten-year period did not ensure the accumulation of humus in the arable soil layer. Moreover, there was a tendency to reduce its content compared to baseline by 0.07% (Shedey, 2005).

The results of 27-year observations of changes in humus status in sod-podzolic sandy soil in the experimental 9-field crop rotation, depending on the use of different methods of basic tillage and fertilization systems proved that the use of by-products of grain and postharvest fertilizers in the background halved dose of fertilizers compared to conventional contributes to the accumulation of organic matter in the arable layer and can be a reserve for maintaining and reproducing soil fertility (Kochik, 2011).

Studies show that partial replacement of mineral fertilizers in crop rotation with alternative sources of nutrients allows you to create a resource-saving fertilizer system. Thus, reducing the dose of mineral fertilizers to 60% of the recommended against the background of plowing the post-harvest residues of previous crops crop rotation (straw of cereals, leaf mass of corn, soybeans, sunflowers) provides relatively high crop productivity and fertilizer yield (Maliarchuk et al., 2021).

Under the current conditions, the influence of methods and depth of basic cultivation, when using crop rotation by-products of crop rotation, on the direction of the processes of transformation, redistribution, mineralization

and humification of fresh organic matter is insufficiently studied.

The influence of irrigation on the humus condition of soils is determined by the content and reserves of humus in the arable and 0-100 cm layer. The total balance of humus on irrigated and non-irrigated lands, judging by its reserves ranges from 100 tons on dark chestnut medium loamy soils to 230 and 520 t/ha on southern and ordinary chernozems, respectively (Saiko, 2006; Khruslova et al., 1991).

83% of its irrigated fund is concentrated in the southern region of Ukraine. However, in recent years the irrigation potential is not fully used and less efficiently (Vozhehova et al., 2014).

Under conditions of strong irrigation impact on soils, such forms of degradation as dehumidification, decalcification, compaction, reduction of water permeability and water content, increase of moisture loss due to runoff, reduction of soil biogenicity, crop quality and pollution of water sources with harmful elements and compounds (Tweeten & Thompson, 2002; Pavlichenko et al., 2014).

During irrigation with mineralized waters in the steppe agricultural zone of Ukraine, soil depletion of carbon dioxide and calcium is often observed, the content of exchangeable sodium increases and the leptization of soil colloids, compaction, formation of lumpy structure is revealed (Tarariko, 2011).

Irrigation water affects the soil, changes the rate and direction of chemical and microbiological processes, as well as the conditions of decomposition of organic matter (Pavlichenko et al., 2014).

According to scientific conclusions, many scientists irrigation with mineralized water, practiced on the Inguets irrigation massif, has a negative effect on the soil, in connection with which its treatment under irrigated conditions plays an important role in improving the physical and chemical condition of the soil and related properties (Rusu et al., 2013).

Irrigation with a significant increase in water inflow into the geographical landscape, especially into the soil, leads to the formation of a number of new properties and reduce their potential fertility (Markovska et al., 2016; Matsko et al., 2002).

In connection with the above, the development of new and improvement of existing systems of

fertilizers and basic tillage when using crop by-products of crop rotations on irrigated lands is relevant and requires in-depth experimental research.

The aim of the research is to establish the regularities of crop by-products conversion of crop rotation to humus and basic elements of mineral nutrition of plants under organic and organic-mineral fertilizer systems against different main tillage systems in 4-field row crop rotation on irrigated lands in the Ingulets irrigation system.

## MATERIALS AND METHODS

The research was conducted in a stationary experiment of the Institute of Irrigated Agriculture of NAAS of Ukraine during 2016-2020 in a 4-field row crop rotation on irrigation in the area of the Ingulets irrigation system.

The soil of the experimental field is dark chestnut medium loam; the arable layer contains humus 2.06%, total nitrogen content - 20.0, mobile phosphorus 30.0, potassium - 300.0 mg/kg of soil, the lowest moisture content - 21.2%, wilting moisture - 9.1%, the equilibrium density of the structure is 1.41 g/cm<sup>3</sup>.

To characterize the nutrient regime (NPK content), soil samples were taken twice during the growing season – at the time of emergence and before harvest. The experiments determined the content of nitrates according to Kravkov, mobile phosphorus was determined according to Machigin, exchangeable potassium on the flame photometry, the humus content was determined by the method of IV Tyupin-Kononova (DSTU 4114-02, 2002; DSTU 4362: 2004, 2006).

The 4-field crop rotation included: corn for grain, winter wheat, grain sorghum and soybeans. In field experiments, soybean varieties and hybrids of agricultural crops, entered in the "State Register of Plant Varieties Suitable for Distribution in Ukraine", were sown (State Register of Plant Varieties Suitable for Distribution in Ukraine for 2021, 2021).

Crop rotation technologies are generally accepted for irrigated conditions, except for the factors studied. The irrigation regime ensured the maintenance of the pre-irrigation moisture threshold under the crops of all crop rotation

crops at the level of 70% of the lowest soil moisture content in the soil layer 0-50 cm.

In crop rotation, five systems of basic tillage (Factor A) with different methods, techniques and depth of loosening on the background of organic and two organic-mineral fertilizer systems (Factor B) were studied.

Factor A - tillage:

1. Different-depth main tillage with a plow PLN-5-35 with rotation of the slice to a depth of 14-16 to 25-27 cm depending on the crop rotation - CONTROL;
2. Different-depth main tillage with the plow PCH-2,5 without rotation of the slice with chisel loosening of the soil to a depth of 14-16 to 25-27 cm, depending on the crop rotation;
3. Single-depth shallow tillage with a heavy disc harrow BDVP-4,2 without rotation of the slice with loosening to a depth of 12-14 cm for all crop rotations;
4. Differentiated-1 tillage with shallow disc loosening for corn for grain and winter wheat to a depth of 8-10 cm using a disc light harrow BDLP-4, soybean heavy disc harrow BDVP-4.2 to a depth of 14-16 cm, and for sorghum grain combined tillage with the use of disco-chisel harrow BDVP-3,0-0,1 with a depth of loosening of 12-14 + 38-40 cm;
5. Differentiated-2 tillage with plowing plow PLN-5-35 for corn to a depth of 18-20 cm, chisel tillage for sorghum grain plow PCH-2.5 to a depth of 16-18 cm and shallow disc loosening BDLP-4 for wheat winter (10-12 cm) and BDVP-4.2 under soybeans (14-16 cm)

Factor B - fertilizer system:

- Organic - using only by-products of crops crop rotation (background) - CONTROL;
- Organic-mineral – mineral fertilizers with a dose of N<sub>82.5</sub>P<sub>60</sub> + by-products;
- Organo-mineral – mineral fertilizers with a dose of N<sub>120</sub>P<sub>60</sub> + by-products.

During the experiment, field, quantitative-weight, visual, laboratory, calculation-comparative, mathematical-statistical methods were used using generally accepted in Ukraine methods and methodological recommendations (Vozhegova et al., 2014).

To analyze the effectiveness of the combined use of fertilizer systems and basic tillage, the

following indicators were used: the mass of post-harvest residues for each crop rotation; stocks of humus in the arable layer; humus balance; mass of nitrogen, phosphorus and potassium received in the soil with plant residues; ecological and economic effect (UAH/ha); energy increase (GJ/ha).

Receipts of plant residues, stem and root, were determined by the regression equation from the level of yield of the main product.

Changes in humus stock were calculated depending on the mass of by-products and mass of roots, their humification coefficient, humus mineralization coefficient.

The calculation of the supply of basic nutrients to the soil, depending on the fertilizer systems, was carried out taking into account the amount of nutrients received from mineral fertilizers and root and leaf residues.

The humus balance was calculated by the difference between the total weighted average of the newly created, due to the humification of postharvest residues (leaf and root) and mineralization of humus under crops of crop rotation.

Ecological and economic efficiency of organo-mineral fertilizer systems used in the cultivation of crops was determined taking into account production costs, the yield of fertilizers, the cost of their application and harvesting separately for each variant of the

experiment, taking into account the growth of humus (Filip'ev I.D. et al., 2001).

## RESULTS AND DISCUSSIONS

Organic and organic-mineral fertilizer systems using fertilizer for post-harvest crop residues in modern conditions have become the main source of organic matter in the soil. Due to the methods and depth of the main tillage, post-harvest residues fall into different hydrothermal conditions of the arable layer, which changes the nature and intensity of their decomposition. According to the results of research it is established that on average for one year of crop rotation the inflow of postharvest residues (leaf-stem and root) under the organic fertilizer system per 1 hectare of crop rotation area in the variant of different depths of the main tillage system 4.8 t, with different depth tillage without rotation of the chip (2nd) - 4.5 t, single- depth shallow disk (3rd) - 3.9 t, differentiated-1 and differentiated-2 - 4.9 and 4.2 tons, respectively.

The organic-mineral fertilizer system with the application of mineral fertilizers with a dose of  $N_{82.5}P_{60}$  in the soil earned an average of 7.4 to 8.6 tons of post-harvest residues per year, and the organic-mineral fertilizer system with a dose of mineral fertilizer  $N_{120}P_{60}$  from 7.5 to 9.7 t/ha (Table 1).

Table 1. Receipt of post-harvest residues of agricultural crops in the soil under different systems of fertilization and cultivation in crop rotation under irrigation, on average for 2016-2020

Basic tillage system	Mass of postharvest residues, t/ha per year			
	grain corn	winter wheat	grain sorghum	soybeans
Organic fertilizer system (post-harvest residues - background) - control				
1- With the rotation of the slices, different depths	5.2	5.7	5.2	2.9
2- Without rotation of the slice, different depths	4.8	5.6	4.6	2.8
3- Without rotation of the slice, single- depth (12-14 cm)	3.8	5.2	4.1	2.3
4- Differentiated-1	5.4	5.8	5.4	3.0
5- Differentiated-2	4.0	5.4	4.6	2.8
Organic-mineral fertilizer system ( $N_{82.5}P_{60}$ + background)				
1- With the rotation of the slices, different depths	13.4	8.1	8.2	3.5
2- Without rotation of the slice, different depths	12.5	7.8	8.0	3.3
3- Without rotation of the slice, single- depth (12-14 cm)	11.9	7.6	6.8	3.1
4- Differentiated-1	13.7	8.4	8.7	3.6
5- Differentiated-2	10.7	7.8	7.8	3.1
Organic-mineral fertilizer system ( $N_{120}P_{60}$ + background)				
1- With the rotation of the slices, different depths	16.2	9.0	8.5	3.9
2- Without rotation of the slice, different depths	14.9	8.6	8.3	3.7
3- Without rotation of the slice, single- depth (12-14 cm)	11.6	8.1	6.9	3.3
4- Differentiated-1	16.7	9.2	9.0	3.7
5- Differentiated-2	12.3	7.9	8.1	3.4

Calculations of humus formation in the soil from wrapped post-harvest residues under different systems of basic tillage and fertilizer show that against the background of the organic fertilizer system a negative balance of humus is

formed, while the largest deficit was found for single- depth shallow (12-14 cm) without rotation of the slice - 0.37 t/ha, and the smallest - 0.14 t/ha for differentiated-1 (Table 2).

Table 2. Estimated balance of humus for different fertilizer systems and basic tillage in crop rotation, average data for 2016-2020 in a layer of 0-40 cm

Components of the balance sheet	Variants of the main tillage system				
	with the rotation of the slices, different depths (control)	without rotation of the slice, different depths	without rotation of the slice, single- depth (12-14 cm)	differentiated-1	differentiated-2
Organic fertilizer system (post-harvest residues - background) - control					
Mass of residues, t/ha	4.8	4.5	3.9	4.9	4.2
Increase in humus, t/ha	1.0	0.94	0.81	1.02	0.88
Humus mineralization, t/ha	1.18	1.18	1.18	1.18	1.18
Humus balance, t/ha	-0.18	-0.24	-0.37	-0.16	-0.30
Organic-mineral fertilizer system (N <sub>82.5</sub> P <sub>60</sub> + background)					
Mass of residues, t/ha	8.3	7.9	7.4	8.6	7.4
Increase in humus, t/ha	1.73	1.65	1.54	1.79	1.54
Humus mineralization, t/ha	1.18	1.18	1.18	1.18	1.18
Humus balance, t/ha	+0.55	+0.47	+0.36	+0.61	+0.36
Organic-mineral fertilizer system (N <sub>120</sub> P <sub>60</sub> + background)					
Mass of residues, t/ha	9.4	8.9	7.5	9.7	7.9
Increase in humus, t/ha	1.96	1.85	1.56	2.02	1.65
Humus mineralization, t/ha	1.18	1.18	1.18	1.18	1.18
Humus balance, t/ha	+0.78	+0.67	+0.38	+0.84	+0.47

According to the organic-mineral fertilizer system with the introduction of N<sub>82.5</sub>P<sub>60</sub> there is an increase in humus. In the variant of differentiated-1 cultivation, the increase in humus reserves compared to the control was 10.9%, while in different depths without rotation of the slice, it was lower by 14.5%. With the systems of single-depth shallow and differential-2, the increase in humus reserves was lower compared to the control by 34.5%.

Increasing the dose of mineral fertilizer to N<sub>120</sub>P<sub>60</sub> against the background of by-products provided a further increase in the growth of humus reserves; at the same time, the regularity observed for the doses of mineral fertilizer N<sub>82.5</sub>P<sub>60</sub> was preserved. It should be noted that at the increased dose of mineral nutrition in variants of different depths with rotation of the slice, and without and differentiated-1 cultivation, the average annual increase in humus reserves increased by 13.2; 12.0; 12.3%, and for differentiated-2 and single-depth without rotation shallow slices - only 7.1 and 1.2%, respectively.

Based on the mass of post-harvest residues, the inflow of nitrogen, phosphorus and potassium

into the soil with root and leaf residues was calculated, which is a compensation for the removal of mineral nutrients from the crop yield. Thus, with the organic fertilizer system in the variant of differentiated-1 tillage with one slit by 38-40 cm per crop rotation there is an increase in the supply of mineral nutrients compared to the system of different depths of main tillage with rotation of the slice (control) on N and K<sub>2</sub>O by 3.4%, and P<sub>2</sub>O<sub>5</sub> at 3.7%.

A similar pattern, but with higher values, is noticeable in organo-mineral fertilizer systems. Under the organic-mineral fertilizer system with the application of a dose of mineral fertilizers N<sub>82.5</sub>P<sub>60</sub> with post-harvest residues in the soil received nitrogen, phosphorus and potassium by 68-80% more than organic.

The inflow of nitrogen, phosphorus and potassium into the soil under the organo-mineral fertilizer system with the application of mineral fertilizers N<sub>120</sub>P<sub>60</sub> was the highest for differentiated-1 tillage and increased compared to different depths of plowing by 6.0-11.1%.

Only in the variant of single-depth shallow without rotation of the slice of the tillage system with its long-term use in crop rotation

for organic and organic-mineral fertilizer systems there was a significant decrease in all

mineral nutrients compared to different depth tillage with rotation of the slice (Table 3)

Table 3. Nutrient uptake into the soil with post-harvest crop residues of crop rotation under different fertilizer systems and basic tillage

Basic tillage system	Mass of nutrients in post-harvest residues, kg/ha per year		
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
Organic fertilizer system (post-harvest residues - background) - control			
1- With the rotation of the slices, different depths	21.7	10.9	26.1
2- Without rotation of the slice, different depths	20.3	10.2	24.4
3- Without rotation of the slice, single-depth (12-14 cm)	17.6	8.8	21.1
4- Differentiated-1	22.5	11.3	27.0
5- Differentiated-2	19.3	9.6	23.1
Organic-mineral fertilizer system (N <sub>82.5</sub> P <sub>60</sub> + background)			
1- With the rotation of the slices, different depths	38.7	19.4	46.4
2- Without rotation of the slice, different depths	37.1	18.6	44.5
3- Without rotation of the slice, single-depth (12-14 cm)	29.5	14.8	35.4
4- Differentiated-1	41.2	20.8	49.5
5- Differentiated-2	36.0	18.0	43.2
Organo-mineral fertilizer system (N <sub>120</sub> P <sub>60</sub> + background)			
1- With the rotation of the slices, different depths	43.4	21.7	52.1
2- Without rotation of the slice, different depths	41.8	20.9	50.1
3- Without rotation of the slice, single-depth (12-14 cm)	33.0	16.5	39.2
4- Differentiated-1	46.1	24.1	55.2
5- Differentiated-2	41.3	20.7	49.6

In general, the application of mineral fertilizers and the use of post-harvest (leaf and root) residues on fertilizers contributed to the creation of different levels of available forms of mineral nutrients at the beginning of the spring winter vegetation and the emergence of spring cereals and industrial crops.

In order to assess the environmental and economic efficiency (EEE) of basic tillage systems, energy and monetary equivalents were assessed not only on the components of crop production technologies and material and technical means for their implementation, but also on crop and humus growth. The calculation is made on the variant of organic-mineral fertilizer system with doses of mineral fertilizers N<sub>120</sub>P<sub>60</sub>.

It was found that due to increased crop yields and increased humus reserves in the soil layer 0-40 cm energy increase compared to the system of different depths of main tillage with rotation of the slice (used as a control option), provided only differentiated-1 system of basic tillage, in which one splitting for crop rotation alternated with shallow loosening without rotation of the slice under the grain and soybeans. The increase in energy in this variant due to yield and humus was higher than in the control by 8.9% (Table 4).

Systems of different depth and single-depth main tillage without rotation of the chips on the contrary led to a reduction in energy growth compared to control primarily due to poor yields, and in the case of long-term use of shallow single-depth shelfless loosening also due to lower rates of humus accumulation. The decrease in the average annual increase in energy due to harvest and humus was 7.8 and 37.8%, respectively. The system of differentiated-2 main cultivation with one plowing per crop rotation also led to a reduction in energy growth due to yield and humus.

As a result of calculations it is established that the highest total ecological and economic effect was provided by the differentiated-1 system of the main cultivation with one crack on depth of 38-40 cm once for rotation and organo-mineral system of fertilizer with N<sub>120</sub>P<sub>60</sub> and use of postharvest residues, which is higher than the control by 8.9%. In the variant of single-depth shallow (12-14 cm) cultivation without rotation of the chip, the reduction of the total ecological and economic effect reached 37.8%, and with different depth without rotation of the chip and differentiated-2 – 7.8 and 7.4% (Table 4).

Table 4. Ecological and economic efficiency of 4-field row crop rotation on irrigation under different systems of basic cultivation of dark chestnut soil, fertilizer dose N<sub>120</sub>P<sub>60</sub>

Basic tillage system	Energy increase, GJ/ha			EEE, UAH/ha	
	total	including due to:		total average	± to control
		harvest	humus		
1- With the rotation of the slices, different depths	132.0	120.6	11.4	63650.4	-
2- Without rotation of the slice, different depths	121.7	111.9	9.8	58683.7	- 4966.7
3- Without rotation of the slice, single-depth (12-14 cm)	82.1	77.4	4.7	39588.6	- 24061.8
4- Differentiated-1	143.8	130.7	13.1	69340.4	+ 5690.0
5- Differentiated-2	122.3	112.3	10.0	58973.1	- 4677.3

Note. EEE - environmental and economic effect

Estimation of yield and humus growth in monetary terms made it possible to determine that the highest total ecological and economic effect is provided by differentiated-1 system of basic tillage against the background of organo-mineral fertilizer system using all by-products of crop rotation and application of mineral fertilizers N<sub>120</sub>P<sub>60</sub>

## CONCLUSIONS

As a result of research, it was found that the most favorable conditions for the accumulation of leaf mass of crop rotation crops, the formation of a positive balance of humus with an average annual growth of 2.02 t/ha provided organic-mineral fertilizer system using by-products of crop rotation and fertilizer dose N<sub>120</sub>P<sub>60</sub> background of differentiated main tillage with one for rotation slitting to a depth of 38-40 cm.

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## DYNAMIC PREGL-DUMAS TECHNIQUE APPLIED IN NITROGEN DETERMINATION FROM INPUTS USED IN ORGANIC AGRICULTURE

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### Abstract

*Nitrogen is one of the most important component of fertilizers. Quantifying nitrogen, along with other elements, is an essential operation for calculating the efficiency of these inputs. Because Romanian regulations doesn't provide any methodology for determining nitrogen from inputs used in organic agriculture, each laboratory uses its own method for these determinations. This study presents a fast and efficient dry combustion method of determining the total nitrogen from organic inputs, using a CHNS elemental analyzer (Eurovector EA 3100). This Automated Pregl-Dumas technique is a very good alternative to the classic Kjeldahl method, which has several drawbacks: it does not quantify nitrogen from nitrates (although fertilizers contain a large amount of nitrogen in this form), needs a longer time for the sample to be analysed, it is not environmental friendly, and has lower accuracy of the results. To ensure the quality of the results, the necessary parameters for the validation of this method were calculated according to international guidelines, and the acceptance criteria of results were verified based on certified reference materials.*

**Key words:** dry combustion, method validation, nitrogen, organic input.

### INTRODUCTION

Ordinary laboratory determinations tend to become more and more automated, computer-coordinated, environmentally friendly, and with shorter execution times. Although old-fashioned methods of analysis are still widely used, technology is gaining ground and new devices are being developed which greatly facilitate the work of the operators.

Nitrogen determination is a routine analysis and a quick and accurate method is required (Beljkas et al., 2010). There are many ways to determine nitrogen, depending on the nature of the sample. Some of these methods are standardized and can be applied directly, with the certainty of suitable results. Most methods have been developed for the determination of protein in food and feed. Methods for determining soil nitrogen and fertilizers have also been developed for agriculture, but on a much smaller scale.

Two of the most widely used methods for determining nitrogen are based on principles discovered more than 100 years ago: Kjeldahl method (published in 1883) and Dumas method (published in 1831).

The limitations of both methods have been studied intensively and a lot of improvements

have been made to each of them over the past century (Sapan & Lundblad, 2015; Tomé et al., 2019; Sáez-Plaza et al., 2013). High precision and good reproducibility, along with the simplicity of use, have made the Kjeldahl method the standard method for comparison against all other methods. Starting with the 1990's, due to the development of technology (high purity gases, high precision detectors, high precision microbalances), the Dumas method gained recognition versus the traditional Kjeldahl method that was the dominating method for nitrogen and protein analysis for more than 100 years (Müller, 2017).

Some differences between Kjeldahl and Dumas methods are presented in the Table 1 (Mihaljev et al., 2015; Walter, 2018; Moore et al., 2010).

Due to the fact that Kjeldahl method does not quantify nitrogen from nitrates, the use of Dumas method is a suitable alternative for the analysis of fertilizers, which contain large amounts of nitrates. There are also some modified Kjeldahl method that can be used. There are a few official methods for total N determination in fertilizers recommended by International Fertilizer Industry Association (IFA), presented on Table 2.

Table 1. Comparison of Kjeldahl and Dumas methods

<b>Kjeldahl</b>	<b>Dumas</b>
Manual	Automatic
Time consuming (hours)	Fast (minutes)
Very dependent on the operator	Slightly dependent on the operator
Partial N determination (NOT NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> , azo)	All N components determination
Use toxic reagents	No toxic reagents
Low cost/sample	Low cost/sample
Low price equipment	Expensive equipment
Medium sample quantity required (1-5 grams)	Small sample quantity required (1-20 mg)
Precision influenced by operator and preparation	High precision provided by standards
Suitable for protein determination	Suitable for protein/total N in soil/fertilizers

Table 2. Official methods for total N determination in fertilizers

<b>Standard</b>	<b>Method type</b>
ISO 5315:1984	Kjeldahl
AOAC 978.02	Kjeldahl
AOAC 955.04	Kjeldahl
AOAC 993.13	Dumas

As the number of certified producers in organic agriculture is growing, the need of fertilizers testing is increasing as well. In order to keep their certification, they need to use fertilizers that respect regulation EC 2008/889 (Ion et al., 2021). As the above methods refer to fertilizers in general, the development of specific methods for inputs used in organic farming is a requirement.

The N determination using combustion method was successfully tested for the determination of proteins in food (VELP Scientifica, 2018; Cortes-Herrera et al., 2021) and soils (Matejovic, 1993). Regarding fertilizers, studies on the advantages and disadvantages of the combustion method began more than 50 years ago when Morris et al. (1969) made a comparison between the Kjeldahl method and the dry combustion method for conventional fertilizers. In 2016 Incorporated Administrative Agency, Food and Agricultural Materials Inspection Center (FAMIC) Japan, published a guide “Testing Methods for Fertilizers 2016” where an impressive number of different types of fertilizer samples were analyzed by several laboratories both by the Kjeldahl method and by the dry combustion method. The results showed

that both methods can be successfully used for the fertilizers analysis.

In order to be sure that a newly developed method can be used successfully, a series of steps are necessary to validate the method, to demonstrate that its performance characteristics are adequate for use for a particular purpose. According to EURACHEM Guide, these steps usually refer to precision (repeatability, reproducibility, precision limits) and accuracy/bias estimates (Magnusson & Örnemark, 2014). The aim of this paper is to develop and to validate a method based on automated Pregl-Dumas technique for the quantification of total nitrogen in organic fertilisers. This method can be also optimised for other type of organic fertilisers based on biomass waste material, soils and food.

## MATERIALS AND METHODS

### *Chemicals and reagents*

The combustion tube used by the elemental analyzer Eurovector EA 3100 at high temperature (950°C) oxidizes the samples in the presence of pure O<sub>2</sub> gas (Messer Oxigen 5.0, 99.999% purity). Helium (Messer He 6.00, 99.999% purity) gas is used as carrier and flushing gas.

Tin capsules (8 x 5 mm) were used for weighing and packing the samples and also to enhance the combustion. The samples were weighted in the tarred capsules and tweezers were used to close them to exclude atmospheric air. High purity acetanilide (Certif. no. B2061-1) from Elemental Microanalysis Ltd was used as calibration standard (N content % = 10.34%).

### *Samples*

Three organic fertilizers and a standard reference material (SRM) were used as test matrices:

- Commercial liquid water-soluble fertilizer which contains trace elements chelated with citric acid and lignosulfonic acids - F1;
- Commercial semi-solid organic fertilizer obtained from soy and seaweed protein hydrolysis - F2;
- Commercial semi-solid organic fertilizer obtained from soy and seaweed protein hydrolysis fortified with micronutrients - F3;

- NIST® SRM® 695 (National Institute of Standards and Technology).

The samples were used without any treatments and were stored at room temperature during the analysis.

### **Basic principles**

In the combustion process (furnace at 950-1100°C), C is converted to CO<sub>2</sub>; H to H<sub>2</sub>O; N to N gas/oxides of N and S to SO<sub>2</sub>. If other elements such as Cl are present, they will also be converted to combustion products, such as HCl gas. To remove these additional combustion products, a variety of traps and absorbents are used, as well as some of the main elements, like water or sulphur, if the determination of these elements is not required.

The combustion products are carried through the combustion tube by a carrier gas (usually helium) and passed over heated high purity copper, situated at the base of the combustion tube. The function of this copper is to convert all forms of N to N<sub>2</sub>, C to CO<sub>2</sub> and S to SO<sub>2</sub> gases and to remove any oxygen not consumed in the initial combustion. This copper can be situated at the base of the combustion chamber or in a separate furnace.

The gases are then passed through the absorbent traps in order to leave only carbon dioxide, water, nitrogen, and sulphur dioxide.

Detection of the gases are carried out by a GC separation followed by quantification using a thermal conductivity detector (TCD). Quantification of these elements need calibration for each element by using high purity standards such as cystine, acetanilide, benzoic acid, ethylenediaminetetraacetic acid (ETDA), etc. (AMC TB, 2008).

### **Elemental analysis instrument and parameters**

The development and validation of the analytical method was performed using an automated CHNS elemental analyzer (Eurovector EA 3100) with the following accessories:

- Eurovector CHN combustion tube for 250 sample analysis of C, N and H;
- double autosampler (carousel) with 40+40 positions;
- Mettler Toledo microbalance (precision 0,000001 g);
- H<sub>2</sub>O trap for water elimination;

- PTFE GC Column, outer diameter 8 mm, length 2 m;
- Weaver.NET 1.8.0.0 software.

The parameters used for method development are described in the Table 3.

Table 3. Method parameters for N determination using elemental analyzer Eurovector EA3100

Parameter	Unit	Value
Carrier Pressure	kPa	90
Reference Pressure	kPa	10
Furnace #1	°C	950
GC Oven	°C	90
Transfer Line	°C	100
Run Time	s	400
Sample Delay	s	6
O <sub>2</sub> Volume	mL	20
O <sub>2</sub> Injection Rate		slow

This analysis lasts for 400 seconds per sample and is fully automated.

### **Method validation**

The following method-performance parameters were determined: linearity, accuracy, intra-day and inter-day precision.

#### *Linearity*

Linearity is recommended to be determined using a minimum of 6 standards whose concentration spans from 80% to 120% of expected concentration levels. Linearity report should include the slope, linear range and correlation coefficient data. Correlation coefficient must be greater or equal to 0.999 in the working range.

Calibration curve, made with acetanilide as a calibration standard, was constructed from six points and covered a wide range of nitrogen masses, corresponding from 0.5 to 2.5 mg of standard material.

#### *Intra-day (RSD<sub>r</sub> %) and the inter-day (RSD<sub>R</sub> %) precision coefficients*

Precision expresses closeness of a series of measurements of the same sample under identical conditions. A high precision does not necessarily mean high accuracy. Precision can be expressed as variance, standard deviation or as coefficient of variation of a series of measurements. Minimum of five replicate

sample determinations should be carried out for accurate results.

The precision of the method was evaluated based on the relative standard deviation (RSD) of the intra-day precision (RSD<sub>I</sub> %) and the inter-day precision (RSD<sub>R</sub> %).

For determination of repeatability precision, acetanilide (Microelemental Analysis), SRM 695 and three organic fertilizers was successively analyzed 10 times as 10 different sample weights in the same day, by one analyst. Intra-laboratory reproducibility precision was determined by analyzing the same sample in 10 replicates during 5 days by two different analysts.

### Accuracy and BIAS

Accuracy indicates the closeness of the measured value to the true value. BIAS is determined as the difference between the mean obtained from a large number of replicate measurements with a sample having a reference value.

For determination of accuracy two standard materials were used. The obtained results were compared with the certificate value. Bias was expressed as the difference between the mean of the 10 samples and the real value from certificate.

$$\text{Accuracy (\%)} = \frac{\bar{x}}{\mu} \times 100 \quad (1)$$

where:

$\bar{x}$  = the mean of MRS samples

$\mu$  = "real" value from MRS certificate

$$\text{Bias (\%)} = \frac{\bar{x} - \mu}{\mu} \times 100 \quad (2)$$

### Measurement Uncertainty

In order to assess the measurement uncertainty for each matrix, several sources were taken into consideration. From these sources, the following have been considered to contribute the most when combined uncertainty is calculated: uncertainty from weighing, uncertainty from method calibration data, uncertainty derived from inter-day repeatability on the matrix, and uncertainty from accuracy and bias tests.

## RESULTS AND DISCUSSIONS

The software enables a real-time view of the analytical process and it can be used for running

the equipment, storing the data, and for post-run analysis.

Once that a peak has been detected and its area has been calculated using the integration parameters, it labels the element in order to identify the detected peak as N, C, H, S or O, based on time around which the peak is expected to appear at the detector (Retention Time). For the integration parameters used in the development of the method, a chromatogram shows as in Figure 1, where the nitrogen retention time is at 54 s.

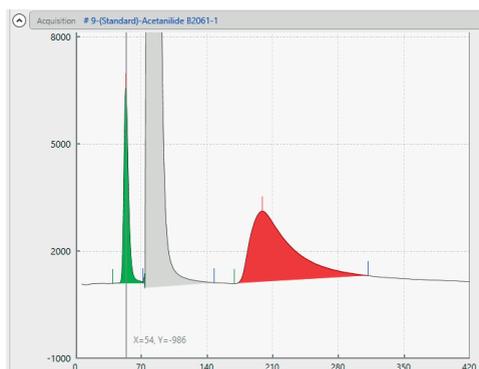


Figure 1. A typical chromatogram for CHNS analysis generated by Weaver.NET software (green peak - N; grey peak - Carbon; red peak - Hydrogen)

### Linearity

Calibration curve, made with acetanilide as a calibration standard, was constructed from six points and a correlation coefficient  $R^2 = 0.9999$  was obtained (Figure 2).

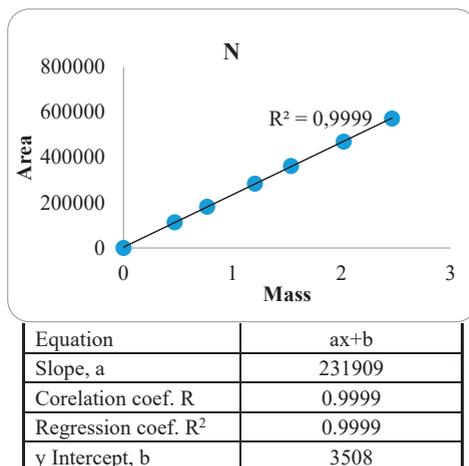


Figure 2. Calibration curve for N determination

### Repeatability and reproducibility precision

The intra-day and inter-day precision was evaluated using calibration standard (acetanilide - Microelemental Analysis), SRM 695 and the above-mentioned three organic fertilizers (F1, F2, F3).

The results obtained for both standard materials and fertilizer samples are showed in Table 4.

Table 4. Intra-day and inter-day precision

Sample	Quantity used (mg)	Mean Conc (%)	RSD <sub>r</sub> (%)	RSD <sub>R</sub> (%)
Acetanilide	0.5-2.5	10.376	0.486	0.667
SRM 695	0.5-2.5	13.139	0.218	0.349
F1	2.5-7	0.213	3.744	11.641
F2	1-3.5	3.712	1.505	3.409
F3	0.5-3.5	3.439	2.316	4.879

It was observed that the method has good results for the sample quantities used above. Smaller amounts will either suffer from sample heterogeneity or will not be detected by TCD. Larger quantities can lead to superposition of the peaks and the wrong integration of the areas. Also, large amounts can lead to incomplete combustion or oversaturation of the TCD.

The obtained RSD<sub>r</sub> values for standard materials are situated under 1%, from 0.218% for SRM 695 to 0.486% for Acetanilide, which indicated that the equipment method is highly repeatable. The RSD<sub>R</sub>% (reproducibility precision) values for all three organic fertilizers were less than 20% which means a good precision of reproducibility according to EURACHEM Guide.

Except F1 which still falls within the limits imposed by the validation guidelines, the values are comparable with published values of relative repeatability and reproducibility standard deviations (RSD<sub>r</sub> range 0.3-2.8% and RSD<sub>R</sub> range 0.8-4.3%) for 12 different types of analyzed fertilizers (FAMIC, 2016).

### Accuracy and Bias

For determination of accuracy, 10 samples of SRM (NIST 695) and 10 samples of acetanilide were successively analyzed. The obtained results were compared with the certificate value (Table 5).

Both standard materials obtained a good accuracy, in the interval of 94-101%, in

accordance with EURACHEM Guide (Magnusson & Örnemark, 2014), which recommends that the accuracy should fall within the range 70-110%. BIAS (trueness) also obtained good values, 0.35% for Acetanilide and -5.47% for SRM 695 (Table 5).

Table 5. Accuracy and BIAS of standard materials

Material	Acetanilide	SRM 695
Used quantity (mg)	0.5-3.0	0.5-3.5
Mean value	10.376	13.139
SD	0.087	0.029
Certificate value (%N)	10.34	13.9
Accuracy (%)	100.35	94.53
BIAS (%)	0.35	-5.47

### Measurement Uncertainty

Good results were obtained for measurement performances when using Dumas method to determine the total nitrogen. As expected, the expanded uncertainty  $U_{ext}$  (%) is higher at lower concentrations, as seen in Table 6 ( $U_{ext}$  - 23.43% for a concentration of 0.213% N<sub>2</sub>). The smallest uncertainty was obtained for the reference material ( $U_{ext}$  - 2.68%), which had the largest concentration and also the best sample homogeneity.

Table 6. Uncertainty results for matrix and reference materials

Sample	Mean Conc (%)	Ustd %	Uext %
SRM 695	13.139	1.34	2.68
F1	0.213	11.71	23.43
F2	3.712	3.65	7.29
F3	3.439	5.05	10.10

## CONCLUSIONS

The method development highlighted good results for:

- linearity,
- accuracy,
- precision of repeatability,
- precision of reproducibility.

The method was successfully applied to organic fertilizers, obtaining fast results, in accordance with the certificates of used materials.

The method can be optimized for other type of samples, as soils, plants or food, after a proper preparation of them.

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## THE SUITABILITY OF SOUTHEASTERN AREAS OF ROMANIA FOR THE ESTABLISHMENT OF SHELTERBELTS

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### Abstract

*Shelterbelts proved to be important structures that provide water preservation and wind protection for humans, their activities, and crops but they also promote biodiversity, by providing a diversity of habitats for wildlife, contributing to a natural balance of harmful and useful species, and promoting biological control of pests. Shelterbelts were used since the 17<sup>th</sup> century, but their extensive use began in the 19<sup>th</sup> century, following numerous scientific studies that proved their beneficial effects on soil and environment. As climatic and soil conditions are very specific for different regions, the impact of these factors on plants used to build the shelterbelts must be carefully analyzed prior to shelterbelts installation. The southern and southeastern plains of Romania are the regions most affected by climate change, especially drought. Because the area is outspread, with a very diverse soil cover, classified in different soil classes as cernisols (typical chernozem), luvisols (preluvosol), hydriols (gleiosol), protisols (alluvial), etc., soil surveys are mandatory. In the present paper we try to highlight the importance of determining the soil suitability for the establishment of shelterbelts, especially for those agricultural lands prone to frequent droughts, as those in steppe areas of the Roman Plain. Soil profiles were opened, and surveys were performed, the soil being characterized morphologically and physico-chemically. For each type of soil, the soils were divided according to the main criteria for grouping the lands according to the forest suitability as: soil volume, soil thickness up to compact rock, skeleton content, texture, compaction, salinization / alkalization, humus content, slope category, surface and depth erosion, landslides, groundwater level, etc. The current study may be a model for the suitability of land in lowland areas frequently affected by drought, with similar physical and geographical conditions.*

**Key words:** shelterbelts, mollic gleysol, chromic luvisol, typical chernozem, calcareous fluvisol.

### INTRODUCTION

Shelterbelts protect the agricultural lands against the prevailing winds, but in fact these forest constructions offer a much larger range of ecosystem services (Marais et al., 2022). The intense agricultural practices used in the last century caused serious land degradation and a gradual loss of essential ecosystem services (Jiang et al., 2022). Agroforestry recently gained an increasing interest among the scientific community as a sustainable farming system, as it combines perennial woody plants with crops or livestock, chronologically and spatially distributed, for a balanced production and improved ecology (EU, 2017).

Among the ecosystem services, the water conservation in soil is one of the most important (Cârciu et al., 2019), as the wind reduction has a

direct impact on reducing the evaporation of water from the soil surface and on lowering the transpiration of plants while in the winter the wind reduction translates into higher amounts of snow deposited on the lands adjacent to the shelterbelts and a lower soil erosion. Another positive aspect is creation of a specific microclimate, beneficial to cultivated plants and biodiversity. A complete network of shelterbelts, main and secondary, determined the reduction of wind speed by up to 50%, a significant decrease of evapotranspiration and the conservation of water in the soil (Andreu et al., 2008). Some studies proved that by setting up protective shelterbelts, the productive land decrease by 4% but this compensates with an increase of production by up to 35% or more, depending on the year.

Russia is considered the pioneer in fighting extreme drought using shelterbelts, as the first forests with protective role were established in 1696 in southern Ukraine, planted at the command of Tsar Peter the Great. In 1883, 80 hectares with shelterbelts were established, on the N-S direction, in the Kamennaya steppe (Vasilescu, 2004). Based on the scientific assessment Dokuchaev, agroforestry systematically expanded in the steppe zones (Chendev et al., 2015).

Shelterbelts have expanded in European countries, such as Denmark, Germany, Italy, Hungary, Bulgaria, then in the USA, Canada, Japan, etc., but never so expanded as in Russia (Musat et al., 2021).

In our country, the opportunity of using and establish shelterbelts was first mentioned by Ion Ionescu de la Brad, in 1866, who planted the first forests on the land of his farm in Neamt County, in the period 1870-1872. Ten years later, in 1880, in Mărculești, Ialomita county, new shelterbelts were planted, followed by some in Braila County, in the period 1933-1937 on about 90 hectares (Vasilescu, 2004).

In 1960, in Dobrogea and Bărăgan Plain, one million hectares of land were protected by shelterbelts, while in 1961, 7000 km of forest protected the agricultural fields and 1400 km protected the communication routes (Costachescu et al., 2012).

As the importance of shelterbelts is worldwide recognized, the research today must propose the best adapted solutions for areas with different pedo-climatic conditions, by conducting studies of land suitability when setting up the shelterbelts, especially the suitable trees species (recommended by forestry specialists).

In the last decade, Romania made important steps in revigorating the shelterbelts establishment. As the measures implemented by the Payments and Intervention Agency for Agriculture (APIA) from PNDR 2014-2020 did not had the expected results, the current Romania's Recovery and Resilience Plan (PNRR) aims to implement forested areas on 25000 ha by the end of 2023 and promise to reach another 31000 ha in the period 2024-2026. In the current paper the conditions of four shelterbelts locations were investigated and characterized morphologically and physico-

chemically, including the types and subtypes of soils, according to the main indicators.

## MATERIALS AND METHODS

### *Physico-geographical conditions*

The experiment was conducted in four different locations, in Southeastern part of Romania, in Orbeasca area, Teleorman County on a chromic luvisols (Figure 1), in Cunești area, Călărași County, on a calcaric fluvisols (Figure 2), in Perișoru area, Călărași County, on a tipycal chernozem (Figure 3) and Gulianca area, Brăila County, on a mollic gleysol (Figure 4).

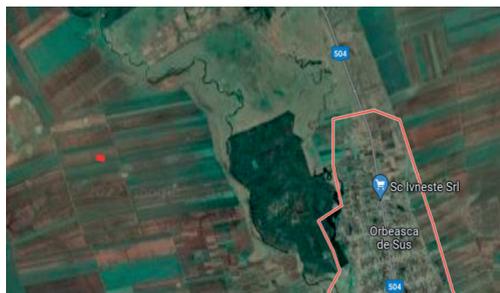


Figure 1. Orbeasca area

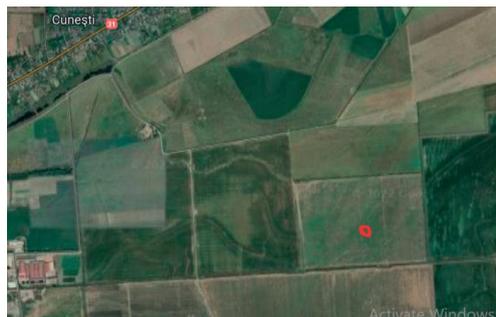


Figure 2. Cunești area

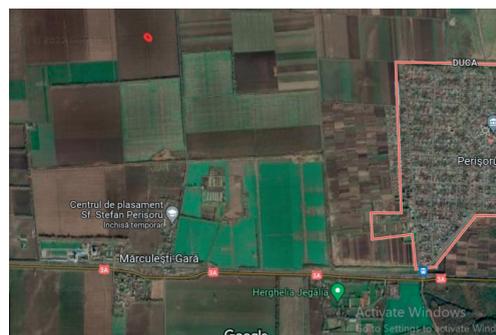


Figure 3. Perișoru area



Figure 4. Gulianca area

### Soil analysis

Soil samples from the four locations were dried at room temperature and the soil subsamples for each of the analysis were first homogenized, then milled, and sieved with the help of a 250  $\mu\text{m}$  sieve. The analyses were performed by specialized laboratories of the National Research and Development Institute for Soil Science, Agrochemistry and Environment - ICPA Bucharest.

### Soil chemical properties

Soil chemical properties were determined by the following analytical methods:

- organic matter (humus): volumetric determination (Walkley-Black humidification method, STAS 7184/21-82);
- $\text{CaCO}_3$  (carbonates): gasometrical method (Scheibler calcimeter, SR ISO 10693: 1998, %);
- the nitrogen content, by calculation, based on the humus content and the degree of saturation with bases ( $\text{IN} = \text{humus} \times \text{V}/100$ );
- mobile phosphorus content (Egner-Riehm-Domingo method and colorimetric molybdenum blue, Murphy-Riley method ascorbic acid reduction);
- mobile potassium content (Egner-Riehm-Domingo extraction and flame photometry);
- pH (potentiometric method in aqueous suspension at soil/water ratio of 1/2.5 - SR 7184/13-2001);
- hydrolytic acidity, extraction with sodium acetate at pH 8.2;
- degree of bases saturation V% (Kappen Schoffield method).

The following physical characteristics were determined:

- determination of granulometric fractions:

- pipette method, for fractions  $\leq 0.002$  mm;
- wet grinding method for fractions of 0.002-0.2 mm and dry grinding method for fractions  $> 0.2$  mm. The results are expressed as a percentage of the material remaining after pretreatment.

- bulk density (BD): The known volume of metal cylinders ( $100 \text{ cm}^3$ ) at the instant soil moisture ( $\text{g}/\text{cm}^3$ ) - total porosity (PT): by calculation (% by volume -% v/v);

- aeration porosity (PA): by calculation (% volume -% v/v);

- compaction degree (GT): by calculation (% by volume -% v/v), where: PMN - minimum required porosity, clay of the sample is calculated with the formula  $\text{PMN} = 45 + 0.163 \text{ A}$  (% by volume -% v/v); PT = total porosity (% v/v); A - clay content (% w/w),

- hygroscopicity coefficient (HC): drying at  $105^\circ\text{C}$  of a pre-moistened soil sample at equilibrium with a saturated atmosphere with water vapor (in the presence of 10%  $\text{H}_2\text{SO}_4$  solution) - % by weight (% w/g);

- wilting coefficient (WC, %, g/g), calculated based on hygroscopicity coefficient.

- field water capacity (FWC, % w/w), calculated based on Dumitru et al. (2011) formula, considering clay content (%), silt content (%), bulk density ( $\text{g}/\text{cm}^3$ ), and layer depth (cm);

- useful water capacity (UWC, % w/w) is calculated as the difference between field capacity (% w/w) and wilting coefficient (% w/w);

- total water capacity (TC, % w/w) is determined as the report between total porosity (% v/v) and bulk density ( $\text{g}/\text{cm}^3$ ).

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

## RESULTS AND DISCUSSIONS

### SOIL CHARACTERIZATION

#### A. Orbeasca de Sus, Teleorman County

GPS:  $44^\circ 5' 42''$  - N and  $25^\circ 22' 21''$  - E

Soil type: chromic luvisol

Landscape: plain

Use: arable

Parent material: loessoid deposits  
Groundwater: > 10 m



Figure 5. Orbeasca de Sus, soil profile

***Morphological characterization of the Orbeasca de Sus soil profile*** (Figure 5)

***Ao (0-30 cm)***, dusty clay, with well-developed grainy structure, with shades of 10 YR 3/2 in wet and 10 yr 4/3 in dry, frequent fine roots from cultivated vegetation, non-plastic, non-adhesive, weak compact, does not effervesce;

***AB (30-52 cm)***, clay-dusty clay, with shades of 7.5 YR 3/3 for the wet material and 7.5 YR 4/4 for the dry material, with moderately developed polyhedral structure, moderately tamped, hard in the wet state, hard in the dry state, moderately plastic and adherent, compact and moderately cemented, does not effervesce, clear straight transition;

***Bt<sub>1</sub> (52-90 cm)***, clay-clay, with shades of 7.5 YR 4/3 to the wet material and 7.5 YR 5/4 to the dry material, the structure is medium and large prismatic; the material is very hard in the wet state and very hard in the dry state, very plastic and adherent, very compact and strongly cemented.

***Bt<sub>2</sub> (90-130 cm)***, clay-clay, with shades of 7.5 YR 4/4 to the material in the wet state and 7.5 YR 5/6 to the material in the dry state, the structure is medium and large prismatic, frequent fine cracks; the material is very hard in the wet state and very hard in the dry state, very plastic and adherent, very compact, clear, straight;

***BC (130-174 cm)***, medium clay loam, with shades of 10 YR 5/6 for the wet material and 10 YR 7/6 for the dry material, weakly structured, slightly friable in the wet state, moderately cohesive in the dry state, presents grains of sand, with frequent spots and concretions of CaCO<sub>3</sub>, weak effervescence.

**B. Cunesti, Calarasi County**

Soil type: calcaric fluvisols  
Landscape: meadow  
Use: arable  
Parent material: alluvial deposits  
Groundwater: > 2 m



Figure 6. Cunesti soil profile

***Morphological characterization of the Cunesti soil profile*** (Figure 6)

***Ao (0-38 cm)***, dusty sandy loam, light brown (2.5 y 3/2 in wet and 2.5 y 4/3 in dry), moderately developed glomerular structure, regrowth, weak biological activity, non-plastic, non-adhesive, frequent fine pores, very frequent thin roots from cultivated vegetation, gradual wavy transition;

***AC (38-56 cm)***, medium sandy loam, yellowish brown (2.5 y 3/3 in wet and 2.5 y 4/4 in dry), moderately developed polyhedral structure, moderately tamped, with oxidation-reduction spots at the base of the horizon, frequent fine roots, clear wavy transition;

***C<sub>1</sub> (56-82 cm)***, fine clay sand, yellowish (2.5 y 4/4 at wet and yellowish brown 2.5 y 5/3 at Dry), friable, unstructured, non-plastic, non-adhesive, frequent coarse pores, frequent fine roots,

moderate effervescence in the lower half of the horizon, clear straight transition;

**C<sub>2</sub> (82-124 cm)**, coarse clay sand, light yellowish, marbled (5 y 5/3 in wet and 5 y 6/4 in dry), unstructured, re-loose, very friable, frequent CaCO<sub>3</sub> pseudomycelia, strong effervescence, clear straight transition;

**C<sub>3</sub> (124-165 cm)**, fine clay sand, light gray (7.5 y 5/4 at wet and 7.5 y 6/3 at Dry), unstructured, wet, very friable, frequent pseudomycetes of CaCO<sub>3</sub>, the presence of aquatic fauna (shells of snails and shells), very strong effervescence, clear straight transition.

### C. Perisoru, Calarasi County

Soil type: tipic chernozem

Landscape: plain

Use: arable

Parent material: loessoid deposits

Groundwater: >10 m



Figure 7. Perişoru, soil profile

### *Morphological characterization of the Perisoru soil profile* (Figure 7)

**Am (0-36 cm)**, dusty clay, dark brown (10 yr 2/1 in wet and 10 yr 3/2 in dry), glomerular structure well developed, porous, permeable, frequent fine roots from cultivated vegetation, weak effervescence at the base of the 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, aphanate,

with accumulations of carbonates in the form of pseudomycelia, moderate effervescence;

**Cca (68-120 cm)**, sandy loam dusty, yellowish (2.5 y 5/4 in wet and 2.5 y 6/6 in dry), unstructured, very friable, porous, aphanate, with accumulations of carbonates in the form of pseudomycelia and small crumbly concretions, strong effervescence.

### D. Gulianca, Braila County

Soil type: Gleiosol cernic (GS-ce)

Coordinate: 45°26'3" - N and 27°03'12" - E

Parent material: alluvial deposits

Landscape: meadow

Groundwater: - 2.5 m

Use: Arable



Figure 8. Gulianca soil profile

### *Morphological characterization of the Gulianca soil profile* (Figure 8)

**Am (0-26 cm)**, dusty clay, with shades of 7.5 YR 2/1 at wet and 7.5 YR 2/3 at dry, moderately developed polyhedral structure, medium aggregates, wet, moderately adhesive, moderately compact, gradually wavy transition;

**AC (26-52 cm)**, medium clay, with shades of 7.5 YR 2/3 at wet and 7.5 YR 3/4 at dry, with weakly developed polyhedral structure, small and medium aggregates, wet, weakly adhesive, weakly compact, clear straight transition;

**C (52-90 cm)**, medium sandy loam, unstructured, with shades of 7.5 YR 4/3 at wet and 7.5 YR 5/6 at dry, clay, weak plastic, weak adhesive, clear wavy transition;

**CGo (90-160 cm)**, dusty sandy loam, unstructured, with marbled shades of 7.5 YR 4/3 at wet and 7.5 YR 5/6 at dry, moderately plastic, moderately adhesive, wavy gradual transition;  
**CGr (160-200 cm)**, fine sandy loam, unstructured, with eggplant shades of 7.5 YR 4/3 in wet and 7.5 YR 5/6 in dry, non-plastic, non-adhesive.

Based on the morphological and physico-chemical characters of the soil types and subtypes in each of the four studied areas, their suitability for shelterbelts use was established. The following observations were made:

- regarding the edaphic volume and soil thickness up to the compact rock, all soil types identified and characterized morphologically and physico-chemically, are suitable for the first favorability class;
- the granulometric composition is generally loamy in the soils in the terrace, respectively typical chernozem and chromic luvisol, so it is suitable for Class I of favorability and those in the meadow, where the texture is clay-sandy, they are suitable for Class II;
- the physical properties of the soil in the four locations are less favorable (poor compaction), therefore suitability for Class II;
- in terms of salts, except for the area of Gulianca, Brăila County, where it was found poor saturation in high depth (over 135 cm), suitability for Class II, in the other locations, the land is suitable for Class I;
- the organic matter content is low only on the alluviosol in Grădiștea area, so the II Class of pretability and in the other three locations, the land is suitable for Class I;
- the skeletal material content, the slope and the ground cover frame the soil of the four locations to the first class of suitability;
- the phreatic level is very low (over 10 m) in the upper blind area and Perisoru, so the land is suitable for Class I and higher in the respective floodplain areas Grădiștea and Gulianca, where the train falls to Class II of suitability;
- the gleization process was found only in Gulianca, Braila County (low gleization in depth), the soil being classified at Class II of suitability;
- stagnogleization processes, surface excess and floodability, were not found on any type of soil, these being classified in the first favorability class.

## CONCLUSIONS

As a conclusion, at Orbeasca de Sus, in Teleorman county, the type of soil identified is suitable for the Class II of favorability only due to soil compaction, which can be intervened by pedomeliorative works (deep loosening, scarification, etc.).

At Cunești, in Călărași County, the soil type presents some particularities (compaction, low organic matter content, generally coarse texture, weak-moderately alkaline reaction, floodability, etc.), which is why the soil falls to the Class II for Forestry favorability.

At Perisoru, in Călărași County, the type of soil being of the best quality, lends itself to the first class of favorability, for all indicators.

At Gulianca, in Brăila County, ameliorative aspects (high phreatic level, weak-moderate gleization, compaction, etc.) were found, so the land is suitable for the Class II of favorability for forest use.

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## GENETIC PARTICULARS OF LEVIGATED CHERNOZEM IN THE NORTHERN MOLDOVA PLATEAU AREA

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### Abstract

*Nature gives the farmer a variety of soils in terms of agronomic fertility, depending on the type of natural vegetation. Different ways of soil formation and their fertility begin to merge into a single channel under agrocenoses due to the impact of cultivated plants on the soil and with the same direction of the biological cycle of substances. The results of research on the genetic peculiarities of leached chernozem in the Northern Plateau Area of Moldova are reported in this article. The authors describe the quality indicators of the researched soils and indicate the variety of physical, physico-chemical and chemical parameters. These soils cover an area of over 380 thousand ha. According to field research, the thickness of the humus profile is 93 cm, which is characterized as strong and deep. The results of laboratory analyzes indicate a humus content in the profile of arable leached chernozems varies from 3.90% in the Ahp horizon to the moderately humiferous class and decreases to 0.49% in the Ck horizon. Also, values of bulk density, texture, carbonates, pH, composition of exchangeable cations on the profile of the researched soil, etc. are determined. Leached chernozems are formed in the conditions of the mesophytic steppes of the forest-steppe zone. The profile is generally molic, leached, that is, completely devoid of carbonates (AmBm/l). Usually, the effervescence starts a little below the lower limit of the B horizon.*

**Key words:** genetic features, leached chernozem, Moldovan Northern Plateau Area.

### INTRODUCTION

The soil, according to Dokuchaev (1952) refers to open systems that exist under the conditions of an influx of matter and energy from outside. The stability of such a system largely depends on external conditions, despite the fact that soils are buffering. The soil is characterized by openness and the ability to produce bioproducts and specific organic matter, which determines its fertility. The destruction and creation of organic matter are the essence of soil formation. A fundamentally important consequence follows from this well-known position - the ratio between the processes of mineralization and humification determines the balance in the soil. The balance of these processes reflects the essence of soil stability, and therefore the agroecosystem as a whole (Крупеников, 2005). Involving the soil in agricultural production, man has always sought to cultivate the most valuable in terms of consumption, annual, mainly grain crops, which in turn led to soil plowing and a decrease in its fertility. The past

three centuries of active land use have significantly affected the landscape: ecosystems have simplified everywhere, transforming into agro-ecosystems with a small set of cultivated and weed plants. This situation had a negative impact on the most fertile and ecologically stable soil - chernozem.

The modern development of soil science is characterized by an increase in interest in the processes of transformation of the soil cover. Soil degradation caused by agricultural impact has become one of the urgent problems of mankind (Козловский, 1991; Щеглов, 2004). Agricultural use of chernozems without appropriate compensation measures leads to significant, mostly negative changes, causes degradation and reduces fertility (Щеглов, 2004).

Long-term studies of chernozems were carried out in accordance with their agricultural use. At the same time, the route field study of chernozems was carried out on the basis of the combined use of profile-genetic and comparative-geographical methods. The obtained materials made it possible to establish

the main morphological features, to find out the spatial patterns in the distribution of chernozems and to trace their functional subordination with other soils (Гринченко, 1964).

Thus, chernozem soils, widely and for a long time used as part of arable land, deteriorate, primarily as a result of erosion, plowing and steppe processes (Лебедева, 1983; 1985; Танасиенко, 2003). Also, processes undesirable for land use are especially intensified, as is known, in conditions of monocultural (extensive) agriculture (Сиухина, 2011).

Under the influence of the washout of the upper soil horizons by melt and rain waters, the content and reserves of humus consistently decrease, its qualitative composition worsens (Танасиенко, 2003). In addition, the physical indicators of chernozems are being transformed. The content of clay and silty fractions of the granulometric composition decreases, the lumpiness of the soil structure increases, granular and lumpy aggregates are destroyed, replenishing the fraction of microaggregates due to their fragments. The number of water-resistant aggregates is reduced. Along with the deterioration of the structural state of soils, density naturally and in accordance with the degree of development of erosion processes increases, the porosity of aggregates and interaggregate space decreases and, as a result, the water permeability and water-retaining capacity of chernozems decrease (Саввинов, 1931; Сиухина, 2011).

Conducted modern research (Крупкин, 1991; Крупеников, 2000; Сиухина, 2009; 2010; 2011) confirm significant changes in morphological features, physicochemical, physical properties of chernozems during long-term agricultural use. When analyzing the literature data, the following features of the transformation of chernozem attract attention:

1. Changes in the morphological features of the soil profile.
2. Reducing the amount of humus, changing its composition.
3. Deterioration of physical and chemical properties.
4. Degradation of the structural-aggregate state.
5. Change in water-physical properties.

In different areas of the study, these changes occur differently and at different rates, which depends on the facies and provincial

characteristics of the soil, the time of its use and the level of agricultural technology.

## MATERIALS AND METHODS

The object of the research is a levigated chernozem from the area of the Northern Moldavian Plateau, 93 cm thick, which is characterized as strong deep, moderately humic, clay-loam. Based on the soil research, the sites of the soil profiles were established. The office work planned for the stage was carried out by applying the systemic physical-geographical methods of spatial positioning of the ground cover and the location of the profiles (Герасимов и др., 1960). Archival materials available from the Institute of Pedology were used as information "Nicolae Dimo" Agrochemistry and Soil Protection (IPAPS "N. Dimo"), its Institute of Land Use Planning and Organization (IPOT), such as digital maps of the soil cover, files of previous and recent pedological research in various fields. Morphological and analytical data on genetic horizons were introduced in the geoinformation system of the Soil Quality Database within the "Data of the Pedological Center" of IPAPS "N. Dimo" ([http://gis.soil.msu.ru/soil\\_db/moldova/](http://gis.soil.msu.ru/soil_db/moldova/) and the SoilDB CPanel web application, [http://gis.soil.msu.ru/soil\\_db/assessment/](http://gis.soil.msu.ru/soil_db/assessment/)) of the Euro-Asian Soil Partnership under DO IT, office, field and laboratory research methods were used.

The works were carried out according to the "Methodology for the elaboration of pedological studies. Part I - Collection and systematization of pedological data. Part III - Ecopedological indicators" (Florea et al., 1987). The methods of investigations are accepted in ecopedological research.

The work program included the collection of soil samples in the field and analyzes in the laboratory, according to the methods known and widely exposed in published works: hygroscopic water - the method of drying the soil sample in the oven at temperature - 105°C for 5-6 hours; humus - Tiurin method in Simacov's modification; carbonates - gas volumetric method; adsorbed cations Ca, Mg - trilonometric method; pH, potentiometric method and other analyzes.

## RESULTS AND DISCUSSIONS

Rational use of soil resources is based on detailed knowledge of the main natural and anthropogenic factors that influence their effective fertility and quality status.

The soil is a natural body, which was formed under the action of pedogenetic factors such as climate, relief, parent rocks, flora, fauna and time. Recently, the anthropogenic factor has a great influence on the properties of soils. Human society, now endowed with advanced technical means, advanced methodologies, contributes substantially to the increase or decrease of fertility.

The initiation of sustainable agroecosystems can be done by noting the factors and functional components at different levels, forms of organization and agricultural management. Research and evaluation of the fundamental component of agroecosystems - the biotope, represented by the soil, allows the identification of degradations caused by the application of agricultural technologies with the adaptation of soil remediation measures.

As a result of the work performed, new data were obtained regarding the current state of natural factors that influence the economic situation within the researched sector.

The agricultural lands that occupy the largest area of the researched territory exert an increased influence on the environment.

The results refer to the research of the ecological natural environment - the soil cover, the specificity of the clay-loamy levigated chernozems on the Northern Moldavian Plateau, a case study in Grinăuți commune, Ocnîța district. In this area on the key polygon was researched profile No. 2. The profile of the arable land is located on a quasi-horizontal surface in the area of the Northern Moldavian Plateau. These soils cover an area of over 380 thousand ha. Morphometric indices include data on the thickness of soil genetic horizons. The researched arable levigated chernozem (Photo 1) are characterized by a profile of the type: Ahp1 - Ahp2 - Ah - Bhw1 - Bhw2 - BCK1 - BCK2 - Ck. In 20-30 percent of cases there are deeply leached profiles of these soils and then between the horizons Bhw2 and BCK1 the horizon BCw leached by carbonates is detected. With a humus content below 1.0% and brown

color. The morphological description on the genetic horizons of the profile is given below.

### ***Morphological description of Profile no. 2 - levigated chernozems, arable:***

*Ahp1 (0-29 cm)* - recently dark gray arable layer with a faint brown tinge, clay-loamy, glomerular-lumpy structure, loose, very frequent medium and large pores, many organic residues, clear passage.

*Ahp2 (29-37 cm)* - dark gray postarable layer with a faint brown tinge, residue of the deep plowed layer in the 90<sup>s</sup>, clay-loamy, glomerular - lumpy or prismatic, compact, medium and small pores, prismatic aggregates are very compact, no pores, frequent medium and thin roots, insect holes, clear passage.

*Ah (37-51 cm)* - the underlying layer of the arable, the continuation of the horizon of maximum accumulation of humus, dark gray with a slight brown hue, clay-loam, glomerular structure, weakly compact to compact, medium and small frequent pores, frequent thin roots and medium, larval nests, coprolites, gradual passage.

*Bhw1 (51-71 cm)* - continuation of the humiferous profile, the beginning of the cambic horizon and the transition to the parent rock after the humus content, dark brown with reddish hue, clay-loam, glomerular structure, large, compact aggregates, small and medium frequency pores, frequent thin roots, larval nests, coprolites, gradual passage.

*Bhw2 (71-93 cm)* - continuation of the humiferous profile, the lower part of the horizon of passage to the parent rock, brown - reddish, clay-loam, glomerular structure - poorly developed lump, compact, frequent small and fine pores, sparse thin roots, shrubs of larvae, gradual passage.

*BCK1 (95-130 cm)* - the upper part of the parent rock slightly modified by the pedogenesis process, yellow with a brown tinge, clay-loam, very poorly developed structure, compact, frequent fine pores, carbonates are present in the form of pseudomycella, rare roots very thin, larval nests, rarely crotovine, gradual passage.

*BCK2 (130-160 cm)* - the lower part of the parent rock slightly modified by the process of pedogenesis, yellow with a slight brown tinge, clay-loam, massive structure, compact, frequent fine pores, the horizon of maximum

accumulation of carbonate neoformations in the form of pseudomycetes, crotovines are rare, gradual passage.

*Ck* (>164 cm) - yellow parent rock practically unchanged by the process of pedogenesis, compact, frequent fine pores.

The effervescence is recorded from 93 cm. In the profile of the researched soil, the carbonates are leached from the town. Ah and Bhw and accumulate in the horizon Bck and Ck, where they form an iluvial carbonate horizon.

The carbonate content in the Bck and Ck horizons varies within 10-22% (Figures 1-3).



Photo 1. Profile no. 2 of the levigated chernozem

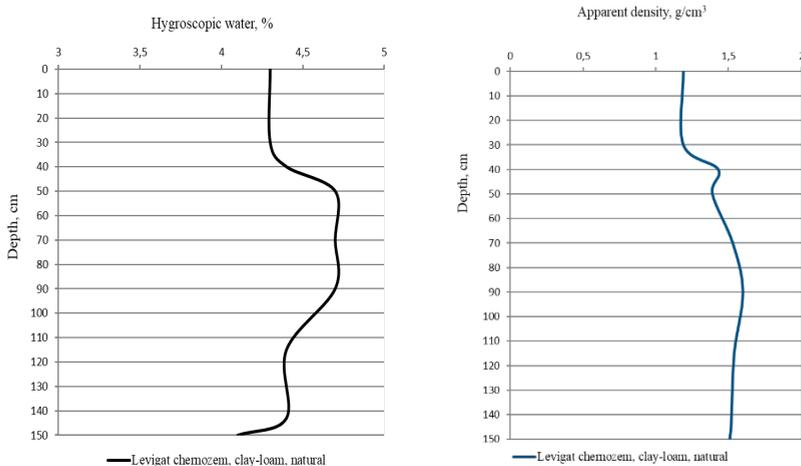


Figure 1. The values of the hygroscopic water, (%) and bulk density (g/cm³)

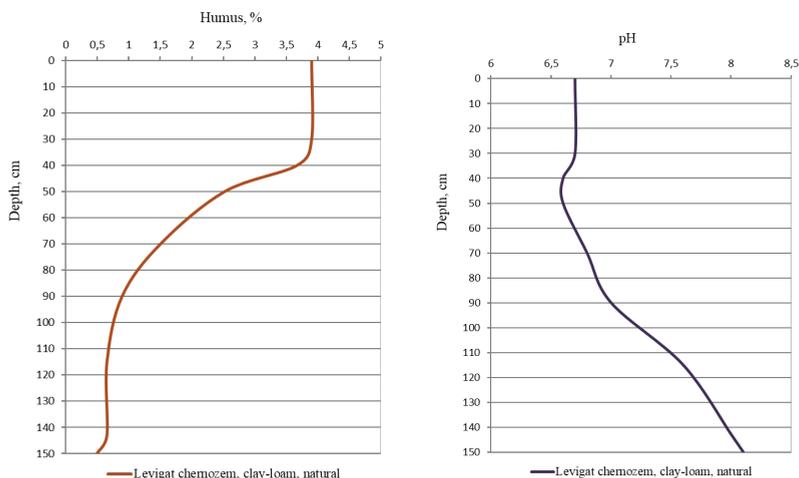


Figure 2. The values of the humus (%) and pH

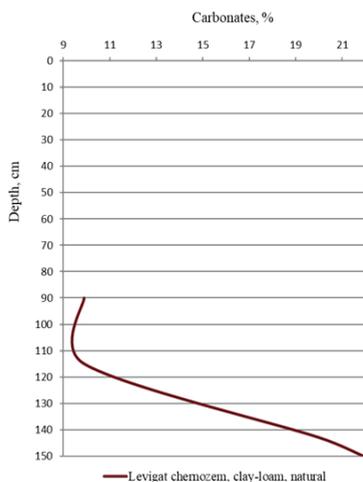


Figure 3. The values of the carbonates (%)

The thickness of the humiferous profile is 93 cm which is characterized as strong deep. The humus content in the profile of arabic leached

chernozems varies from 3.90% in the Ahp horizon to the moderately humiferous class and decreases to 0.49% in the Ck horizon.

The pH values on the studied soil profile change from 6.6-6.8 with a weakly acidic reagent in the Ah and Bhw horizon, neutral 7.0 in the Bhw2 horizon, to weakly alkaline 7.6-8.1 in the Bck and Ck horizon characteristic of chernozems smooth.

The bulk density in the chernozem profile of arable leachate varies from 1.19 g/cm<sup>3</sup> in the arable layer to 1.60 g/cm<sup>3</sup> in the Bhw horizon. The arable layer is characterized by optimal values of bulk density, but the underlying postarable layer is compacted - 1.43 g/cm<sup>3</sup>.

The composition of exchangeable cations in the adsorbent complex of the researched soils is typical for the chernozems of Moldova. The amount of exchange capacity for cations varies from 34 me/100 g of soil in the Ahp1 horizon to 27 me/100 g of soil in the Ck horizon corresponding to the large class (Table 1).

Table 1. Exchangeable cation content of the researched soils

No. profile	Name of the soil	Index	Depth, cm	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Sum	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>
				me/100 g sol			% of sum			
P2	Levigated chernozems, clay-loamy, arable	Ahp <sub>1</sub>	0-29	29.4	4.2	0.8	34.4	86	12	2
		Ahp <sub>2</sub>	29-37	27.8	4.1	0.7	32.5	85	13	2
		Ah	37-51	27.4	4.4	0.7	32.3	85	13	2
		Bhw <sub>1</sub>	51-71	27.6	4.2	0.7	32.8	85	13	2
		Bhw <sub>2</sub>	71-93	26.3	4.2	0.7	31.2	84	14	2
		Bck <sub>1</sub>	93-115	25.2	4.0	0.7	29.9	84	14	2
		Bck <sub>2</sub>	115-143	24.3	3.9	0.6	28.8	84	14	2
Ck	>143	23.6	3.5	0.6	27.5	85	13	2		

The researched soils are characterized by a comparatively homogeneous texture on the profile. On average, the content of physical clay is 60-62%, and of fine clay 37-39%. The high percentage of fine clay in the researched soils indicates that they have a defective object for performing the soil work. The soils are characterized by a fine medium texture and in case of low humus content they are arranged for destructuring and compaction (slitting). This phenomenon is widespread in the lower part of the arable layer (Ap2 - 29-37 cm) which is not currently plowed.

## CONCLUSIONS

According to the data obtained, the humus content in the Ah horizon varies between 3.9% and 0.5% in the Ck horizon of natural soils.

The textural composition of the leached chernozem is characterized by a comparatively homogeneous texture on the profile.

On average, the content of physical clay is 60-62%, and of fine clay - 37-39%.

The high percentage of fine clay in the researched soils indicates that they have a defective object for performing the soil work.

This phenomenon is widespread in the lower part of the arable layer (Ap<sub>2</sub> - 29-37 cm) which is not currently plowed.

The composition of exchangeable cations in the adsorbent complex of the researched soils is typical for the chernozems of Moldova.

## ACKNOWLEDGEMENTS

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## MICROBIAL BIOMASS IN CHERNOZEMS OF NATURAL AND AGRICULTURAL ECOSYSTEMS

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### Abstract

*Microbial biomass in calcareous, leached and podzolic chernozems has been studied in connection with the evaluation of soil degradation processes as a result of a long-term arable use. Experimental sites located in the southern and northern zone of the Republic of Moldova have been tested in August and September 2021. Microbial biomass sampling was carried out in 6 profiles per soil horizons to a depth of 150-240 cm. The highest values of microorganisms' abundance were registered in the upper horizon in chernozems of the natural ecosystem. Microbial biomass content constituted 535.0-1168.6  $\mu\text{g C g}^{-1}$  soil and was much more than in arable chernozems. Microbiological indices in soil profiles decreased with the depth. Microbial biomass reserves in chernozems in the 0-100 cm layer has been reducing from natural chernozems (4925.2-11816.4 kg ha<sup>-1</sup>) to old-arable chernozems (2547.3-6659.2 kg ha<sup>-1</sup>). The microbial biomass was connected with humus content. Correlation coefficients constitute 0.94. The binding effect between microbial biomass and humus content decreases from natural chernozems ( $R^2 = 0.94$ ) to arable ones ( $R^2 = 0.64$ ).*

**Key words:** microbial biomass, chernozem, degradation, natural and agricultural ecosystem.

### INTRODUCTION

Microbial indicators are at present suggested for the estimation of soil quality (Kennedy & Papendick, 1995; Hargreaves et al., 2003; Maharjan et al., 2017). Soil microbial biomass indicator is often used, as it is more susceptible and can therefore clearly indicate changes in the environment more responsively than physicochemical attributes (Masto et al., 2009). Microbial biomass is determined by the quantity and quality of organic matter that, at the same time, depends on the soil management. Therefore, when microbial biomass is high, it indicates microbial diversity and an optimal environment. However, if they are at low levels, then this may be a sign of negative changes in land use (Maharja et al., 2017). Thus, the processes of destruction of natural ecosystems and intensification of the biological degradation of soils are interdependent. In this regard, studies of microbial biomass in degraded chernozems and soils of reference plots (which are in balance, retain all the main parameters and have not lost their ecological and genetic links with landscape components) are of particular importance.

The objective was to obtain an indication of the microbial biomass status of the chernozems and to assess the impact of the long-term use of soils on the microbial carbon in the connection of the soil carbon sequestration and the diagnostics of degradation processes.

### MATERIALS AND METHODS

**Experimental sites and soils.** Our comparative study has been performed in the southern and northern zone of the Republic of Moldova. *The first site* was located in the southern zone, on the South Plains steppe area, in the district no. 13 of ordinary and calcareous chernozems of the South Bessarabian steppe plains (Figure 1). The plot with calcareous chernozem (profile 11 under fallow; profile 12 under arable) and the plot with ordinary chernozem (profile 13 under fallow; profile 14 under arable) were situated in the Ursoaia village of the Lebedenco district, Cahul region. *The second site* was located in the zone of the hilly wooded steppe of the Northern Plain (1), in the district of wooded steppe of the middle Prut (2) with gray forest soils, podzolic and leached chernozems (Figure 2). The plot with podzolic chernozem (profile 17 under fallow; profile 18 under arable) was

situated in the Shaptebani village, Ryshkani region.

The content, reserves and profile distributions of the microbial biomass of zonal undisturbed chernozems in natural ecosystems were investigated in comparison with the arable chernozems. Investigations were performed on the calcareous, ordinary and podzolic chernozems. Microbial biomass sampling was carried out in 6 profiles per soil horizons to a depth of 150-240 cm.



Figure 1. Fragments of natural and agricultural landscapes located in the southern zone of the Republic of Moldova



Figure 2. Fragments of natural and agricultural landscapes located in the northern zone of the Republic of Moldova

The microbial biomass carbon (C) was measured by the rehydration method based on the difference between C extracted with 0.5 M  $K_2SO_4$  from dried soil at 65-70°C for 24 h and fresh soil samples with  $K_c$  coefficient of 0.25 (Blagodatsky, Blagodatskaya et al., 1987).  $K_2SO_4$  - extractable organic C concentrations in the dried and fresh soil samples were simultaneously measured by dichromate oxidation. The ratio between microbial and

organic carbon was determined according to Kennedy & Papendick (1995). Reserves of microbial biomass have been calculated taking into account the carbon content of the microbial cell and the bulk density of soils (Senicovscaia et. al., 2012). Organic C was analysed by the dichromate oxidation method (Arinushkina, 1970). The microbial biomass index and humus content was evaluated statistically by the correlation analysis.

## RESULTS AND DISCUSSIONS

The distribution of microorganisms on the genetic horizons in soils of natural and anthropogenic ecosystems is different. The highest level of the microbial biomass and organic carbon content have been determined in the  $A_1$  horizon (layer 0-10 cm, 0-8 cm, 0-9 cm) of the calcareous, ordinary and podzolic chernozems which have been formed in natural ecosystems. The quantity of the microbial biomass reaches to 535.0  $\mu\text{g C g}^{-1}$  soil, in the ordinary chernozem - to 819.4  $\mu\text{g C g}^{-1}$  soil, in the podzolic chernozem - to 1168.6  $\mu\text{g C g}^{-1}$  soil (Table 1). A similar trend has been noticed in the humus content, the value of which in these chernozems constitutes 2.65%, 3.63% and 4.99% respectively. Microorganisms in natural soils are concentrated in the A genetic horizon (70.3-77.3%), the biomass index decreases in the soil profile to a depth of 96-220 cm.

The long-term use of arable management leads to the decrease of the content and reserves of microbial biomass in arable chernozems as in the upper horizons, and as a whole in the soil profile. The concentration of microorganisms in horizon A in chernozems is much lower and amounts to 47.6-658%. Microbial biomass index gradually decreases in the soil profile to a depth of 72-151 cm.

The share of microbial carbon in the total carbon content of natural chernozems constitutes 2.02-2.34% in the  $A_1$  genetic horizon, in arable chernozems - 0.98%, 1.11% and 1.15% in the soil layer 0-40 cm, 0-31 cm and 0-38 cm ( $A_p$  genetic horizon).

Thus, the share of microbial carbon in its total content in natural chernozems is higher than in arable ones.

Table 1. The content of microbial and carbon content in chernozems in conditions of natural and agricultural ecosystems

Genetic horizon	Depth, cm	Organic C (C <sub>org</sub> ), %	Microbial biomass (MB), $\mu\text{g C g}^{-1}$ soil	C <sub>MB</sub> /C <sub>org</sub> , %	Reserves of MB, kg ha <sup>-1</sup>
Natural calcareous chernozem (P11)					
A <sub>1</sub>	0-10	2.65	535.0	2.02	1348.2
A <sub>k</sub>	10-49	1.60	212.9	1.33	2192.0
B <sub>1k</sub>	49-71	1.25	184.5	1.48	1104.1
B <sub>2k</sub>	71-102	0.55	34.1	0.62	300.2
B <sub>Ck</sub>	102-151	0.30	0	0	0
C <sub>k</sub>	151-170	0.27	83.1	3.07	448.4
Arable calcareous chernozem (P12)					
A <sub>pca</sub>	0-40	1.59	155.7	0.98	1756.3
B <sub>1k</sub>	40-59	0.92	101.7	1.11	533.3
B <sub>2k</sub>	59-72	0.49	69.8	1.42	257.7
B <sub>Ck</sub>	72-114	0.30	0	0	0
C <sub>k</sub>	114-150	0.18	0	0	0
Natural ordinary chernozem (P13)					
A <sub>1</sub>	0-8	3.63	819.4	2.26	1455.3
A	8-47	1.89	208.4	1.10	2015.7
B <sub>1k</sub>	47-64	1.43	228.8	1.60	1081.3
B <sub>2k</sub>	64-96	0.78	40.5	0.52	344.7
B <sub>Ck</sub>	96-160	0.51	0	0	0
C <sub>k</sub>	160-175	0.21	32.2	1.53	132.3
Arable ordinary chernozem (P14)					
A <sub>p</sub>	0-31	1.81	200.7	1.11	1418.6
A	31-48	1.67	157.0	0.94	693.9
B <sub>1k</sub>	48-66	1.34	109.5	0.82	520.3
B <sub>2k</sub>	66-93	0.77	61.0	0.79	457.9
B <sub>Ck</sub>	93-151	0.38	15.2	0.40	250.4
C <sub>k</sub>	151-170	0.33	0	0	0
Natural podzolic chernozem (P17)					
A <sub>1</sub>	0-9	4.99	1168.6	2.34	1893.1
A	6-47	2.33	613.8	2.63	6341.8
B <sub>1</sub>	47-65	0.80	404.4	5.01	1950.8
B <sub>2</sub>	65-96	0.44	166.4	3.78	1454.7
B <sub>Ck</sub>	96-170	0.27	144.7	5.36	3255.2
C <sub>k</sub>	170-190	0.20	39.4	1.97	238.0
Arable podzolic chernozem (P18)					
A <sub>p</sub>	0-38	1.95	224.4	1.15	2285.3
A	38-53	1.93	398.2	2.06	1732.7
B <sub>1</sub>	53-82	0.79	281.1	3.56	2641.2
B <sub>2</sub>	82-100	0.26	248.7	9.57	1468.3
B <sub>Ck</sub>	100-220	0.20	134.8	6.74	not determined
C <sub>k</sub>	220-240	0.15	0	0	0

Microbial biomass reserves in natural chernozems in the 0-100 cm layer increase consecutively: natural calcareous chernozem (4925.2 kg ha<sup>-1</sup>) → natural ordinary chernozem (5029.3 kg ha<sup>-1</sup>) → natural podzolic chernozem (11816.4 kg ha<sup>-1</sup>) (Figure 3).

The biomass reserves of microorganisms in arable chernozems are 1.6-1.9 times lower than in undisturbed chernozems. Microbial biomass reserves in arable chernozems in the 0-100 cm layer increase consecutively: arable calcareous chernozem (2547.3 kg ha<sup>-1</sup>) → arable ordinary

chernozem (3120.9 kg ha<sup>-1</sup>) → arable podzolic chernozem (6659.2 kg ha<sup>-1</sup>).

Because of the long-term use of arable land, homogenization of the arable layer and decrease in the reserves of microbial carbon and humus, the natural stability of chernozems reduces. The microbial biomass is connected with the humus content. The correlation coefficient ( $R^2$ ) between the microbial biomass and humus content in the calcareous chernozem constitutes -  $R^2 = 0.94$  ( $n = 11$ ); in

the ordinary chernozem -  $R^2 = 0.94$  ( $n = 12$ ), in the podzolic chernozem -  $R^2 = 0.94$  ( $n = 12$ ).

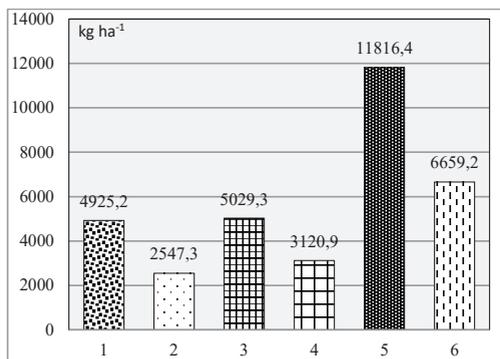


Figure 3. Reserves of microbial biomass in chernozems (0-100 cm)

The correlation coefficient between the microbial biomass and humus content in chernozems under natural vegetation is  $R^2 = 0.94$  ( $n = 18$ ); in arable chernozems -  $R^2 = 0.64$  ( $n = 17$ ).

## CONCLUSIONS

Assessment of microbial biomass resources in chernozems in conditions of natural and agricultural ecosystems showed significant differences between these soils. Microorganisms of natural soils live in conditions of the high supply of the organic matter and its conservation within the limits of the ecosystem. As a consequence, undisturbed chernozems in conditions of the natural ecosystems are characterized by a higher biomass and reserves of soil microorganisms in comparison with arable soils.

The abundance and reserves of microorganisms and the humus content in soil profiles decreased with its depth. The microbial biomass, being a part of the labile organic matter, was connected with the soil organic carbon content. Prolonged use of chernozems in the agricultural production led to the reduction of humus content and contributed to the degradation and decrease of soil stability. Profiles of the arable chernozems are covered by the degradation process as a whole. The low content of microorganisms in the upper layers is characterized by arable chernozems. The biomass reserves of microorganisms in arable chernozems are 1.6-1.9 times smaller compared

to undisturbed chernozems. The negative effects on soil microorganisms were observed as a result of mineralization processes and long-term land management practices without organic fertilizers. The results have proved that the interaction between microbial components and humus status is closer in soils of natural ecosystems. As a result, their resistance to natural and anthropogenic negative impacts is higher than that of the soils in agricultural ecosystems. The rupture and the attenuation of relations between the biotic and abiotic components of soils lead to the decrease in their natural stability and the development of degradation processes.

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## EFFECTS OF FERTILIZATION WITH ALCOHOLIC BEVERAGE PRODUCTION WASTE ON HUMUS AND BIOPHILIC ELEMENT BALANCE IN CAMBIC CHERNOZOME

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### Abstract

*As a result of any human activity an enormous amount of waste accumulates. Unused for various reasons (psychological, economic, legal, technological, etc.), they cause an ecological imbalance in nature, disrupting the normal functioning of the soil, the atmosphere and water resources. At the same time, they contain considerable quantities of elements necessary for plant nutrition and soil fertilization. In the current conditions when the application of industrial fertilizers has fallen to minimal levels, the widespread use of organic waste is of particular importance for Moldovan agriculture. An extensive study, recently carried out in the Institute of Pedology, Agrochemistry and Soil Protection "Nicolae Dimo", shows that the use of waste from the production of alcoholic beverages (wine lees, vinasse, grains borage) applied to various crops, provides a specific income between 90-900 lei/ton. One euro invested in the use of these wastes is recouped with 1.3-3.7 lei. Expenditure is fully recouped, with yield increases in 1-2 years for field and vine crops. With all the benefits listed and the increasing needs during the growing season, waste from the production of alcoholic beverages is practically not used, but left unused, causing mess, dirt and health problems. The above-mentioned wastes were tested in two long-term field experiments at the technological-experimental station "Codru" located in Codru commune, Chisinau municipality.*

*Wine lees and vinasse are discharged from wine production units, and grain borage from enterprises producing ethyl alcohol. Research has confirmed that wine waste increases the content of humified organic matter by 0.16-0.41%, mobile phosphorus by 0.41-1.12 mg/100 g of soil and exchangeable potassium by 11-15 mg/100 g of soil. Yields per hectare when wine waste is applied are 11-13 t/ha. Grape yield increase is 1.0-3.2 t/ha (10-31%). Application of cereal borage increases soil organic matter by 0.12-0.21% (3000-6500 kg/ha), mobile phosphorus by 0.31-0.54 mg/100 g (6-11 kg/ha) and exchangeable potassium by 4-9 mg/100 g soil (80-180 kg/ha). The yield increase when using cereal borage is 40-60%. The beneficial influence of fertilization with waste from alcoholic beverage production on humus and biophilic elements (NPK) is also demonstrated by calculating the balance of these indicators.*

**Key words:** chernozem, wastes, wine lees, vinasse, grain mashes, humus, biophilic element, balance, cambic chernozem.

### INTRODUCTION

Carbon and nutrient cycling in the soil-fertilizer-plant-environment system is of paramount importance, since soil fertility, crop yields, agricultural production quality, environmental purity and population health depend on their quantity.

One of the main laws of agriculture is to restore the humus and nutrients extracted from the soil with the harvests obtained and to compensate for their non-productive losses in order to maintain soil fertility at a satisfactory level and with a balanced humus and nutrient balance.

At present, when the application of organic fertilizers has been reduced to a minimum in Moldovan agriculture, the unproductive loss of humus and nutrients from the soil continues,

and urgent measures are needed to stop this phenomenon and increase the fertility of arable soil. This can be done on the basis of detailed research with the development of objective indices of all the values of input and consumption items in the humus and nutrient balance circuit in the soil.

Soil organic matter plays an extremely important role through its physico-chemical and biological functions, serving as an energy source for microbial flora and a factor on which the soil's fertility status largely depends. Humus is the most important store and at the same time the most important source of carbon and nutrients. It should be noted that the chernozems of Moldova have lost about 25 percent of their accumulated organic matter over 100 years (Crupenicov et al., 1989). This

phenomenon is also characteristic for other countries. For example, Canadian soils after 100 years of exploitation have lost 25 percent of their original carbon (Jausen et al., 1998).

In our country, waste from the production of alcoholic beverages (wine lees, vinasse and cereal borage) can partly serve as a source of carbon and nutrients in the soil. It should be noted that these wastes are a very important source for increasing carbon reserves, which serve as material for the formation of humus and huminic acids that improve the nutrient regime of agricultural crops and consequently soil fertility.

The aim of this work was to determine agro-chemical indices and to assess their balance in the soil when applying waste from alcoholic beverage production as organic fertilizer. On the basis of the research carried out, technological models for the application of these wastes were developed (Material and method, Siuris, 2017; Siuris, 2019).

As study material served the organogenic wastes from the production of alcoholic beverages: wine lees, vinasse and grain borage. The research was carried out at the Technological-Experimental Station (STE) "Codru", located in Codru commune, Chisinau municipality in two field experiments during 2011-2019. The investigated soil is a luto-clay levgated chernozem with humus content - 4.31%; P<sub>2</sub>O<sub>5</sub> - 3.42 mg/100 g soil; K<sub>2</sub>O - 43 mg/100 g soil (Macighin method); pH 6.8 units; hydrolytic acidity 2.71 me/100 g soil. Wine wastes are applied to vine cultivation (Sauvignon variety) and those from ethyl alcohol production to field crop cultivation. In 2012 sunflower was grown in the experimental field, followed by autumn wheat, sunflower, grain maize, autumn wheat, grain soya and in 2019 autumn wheat.

The determination of humus balance and biophilic elements was carried out respectively according to the methodical guidelines (Banaru, 2002) and instructions (Donos, 2001) developed in the Institute of Pedology, Agro-chemistry and Soil Protection "Nicolae Dimo".

## RESULTS AND DISCUSSIONS

### Characteristics of the waste investigated

**Wine lees** are formed, after dehydration by pressing of liquid lees. They are found to be

concentrated fertilizers that can economically justify their transport over long distances, more than 10 km from the wineries. Compared to conventional manure, solid wine lees contain 2.7 times more nitrogen, 1.6 times more phosphorus, 2.4 times more potassium and 2.7 times more organic matter.

It is characterized by an acidic environment. The average pH value is 3.5 units. Moisture content ranges from 42% to 59%, averaging 48%. The chemical composition shows that solid wine lees are an important source of organic matter for the soil and primary elements for agricultural plants. Calculated from the mass with natural moisture the organic matter content averages 47%. Of the primary elements, total potassium predominates, averaging 2.5%, followed by total nitrogen 1.5% and total phosphorus 0.70%. On average 1 ton of solid wine yeast with natural moisture contains 47 kg NPK, with a ratio of these elements of 1:0.5:1.7 which corresponds approximately to the nutrient requirements of the main crops.

**Vinasse (burnt wine)** is the liquid remaining after distillation of the alcohol from the wine and is a residue of boiled wine without alcohol, golden-brown in color, with a specific smell of heat treatment and a sour taste. It contains organic and mineral compounds, proteins, coloring compounds, nitrogenous substances, phenolic substances, which can positively influence the biological qualities of alcoholic beverages or serve together with other compounds as a sterile nutrient medium in the process of fermentation of molasses and production of refined ethyl alcohol. In the Republic of Moldova there are no methods of complex processing of molasses. Thus, it goes into the sewage system, increasing the degree of wastewater pollution, polluting the environment. Vinasse is characterized by an acidic environment (pH = 3.0-3.7 units). It has an average content of 98% water and 2% dry matter. It contains on average 13.3% organic matter, 0.02% total nitrogen, 0.02% total phosphorus and 0.12% total potassium. The aqueous extract is dominated by monovalent potassium cations (580 mg/l) and sodium (170 mg/l). The concentration of bivalent calcium and magnesium cations averages 106 mg/l and 84 mg/l. Among the anions, sulphates

predominate with an average value of 155 mg/l. The chlorine content averages 90 mg/l.

**Cereal borage** is formed as a waste product in ethyl alcohol production plants. It is characterized by an acidic environment (pH = 3.4-4.2 units). They have an average content of 93% water and 7% dry matter. They contain a considerable amount of organic matter (1.6-6.2%) and a varied content of primary elements: total nitrogen 0.21-0.33%, total phosphorus 0.06-0.19% and total potassium 0.09-0.13%.

From the above we can see that the waste from the production of alcoholic beverages can be included in the agricultural circuit by using it as an organic fertilizer.

**Influence of wastes on the main agrochemical indicators of levigated chernozem.** The application of waste from the production of alcoholic beverages had a beneficial influence on the humus, nitrogen, phosphorus and potassium content of levigated chernozem (Table 1).

Table 1. Influence of waste from alcoholic beverage production on humus and nutrient content in the levigated chernozem layer averaged over the years 2011-2019 (STE "Codru")

Experience variant	Humus		Nitrogen		Phosphorus		Potassium	
	%		mg/100 g sol					
	Average	Increase	Average	Increase	Average	Increase	Average	Increase
<b>Waste from wine factories</b>								
1. Unfertilized control	3.99	-	0.74	-	2.16	-	28	-
2. Wine lees (N <sub>100</sub> ), 13 t/ha	4.22	0.23	0.92	0.18	2.75	0.59	36	8
3. Wine lees (N <sub>200</sub> ), 26 t/ha	4.36	0.37	1.07	0.33	3.14	0.98	40	12
4. Vinasse (K <sub>450</sub> ), 300 m <sup>3</sup> /ha	4.17	0.18	1.22	0.48	2.43	0.27	38	10
5. Vinasse (K <sub>900</sub> ), 600 m <sup>3</sup> /ha	4.26	0.27	1.35	0.61	2.48	0.32	41	13
DL 0.5%	0.11	0.11	0.40	0.40	0.11	0.11	6.70	6.70
Sx, %	8.20	8.20	10.23	10.23	5.10	5.10	9.10	9.10
<b>Waste from the production of ethyl alcohol</b>								
1. Unfertilized control	2.91	-	1.17	-	2.08	-	26	-
2. Cereal borage (N <sub>120</sub> ), 47 m <sup>3</sup> /ha	3.03	0.12	1.34	0.17	2.32	0.24	29	3
2. Cereal borage (N <sub>240</sub> ), 94 m <sup>3</sup> /ha	3.13	0.22	1.38	0.21	2.58	0.50	32	6
DL 0.5%	0.10	0.10	0.60	0.60	0.19	0.19	2.80	2.80
Sx, %	7.80	7.80	7.40	7.40	12.20	12.20	10.70	10.70

**Humus in soil.** Average data showed that the administration of wine yeast doses (13 and 26 t/ha), equivalent to 100 and 200 kg N/ha per year, leads to a significant increase in humus content in the chernozem layer. The average increase over nine years (2011-2019) was 0.18 and 0.37% respectively. The application of vinasse at 300 and 600 m<sup>3</sup>/ha, equivalent to 450 and 900 K/ha per year, leads to statistically significant increases in the humus content values, where the increase compared to the baseline averaged over nine years was 0.18-0.27%. Cereal borage applied at rates equivalent to 120 and 240 kg N/ha led to significant increases in soil humus content. The values of humus content increase in eight years

of experimentation (2012-2019) averaged 0.12-0.22%, respectively.

**Mineral nitrogen.** The administration of waste from alcoholic beverage production into the aerial layer of levigated chernozem positively acted on the mineral nitrogen content. Fertilization with wine yeast at doses containing 100 and 200 kg N/ha per year led to a significant increase in mineral nitrogen content. Over eight years the average value of mineral nitrogen content compared to the control increased by 0.18-0.33 mg/ 100 g soil. Fertilization with vinasse at 300 and 600 m<sup>3</sup>/ha increased this index by 0.48-0.61 mg/100 g soil compared to unfertilized soil. The application of cereal borage at the earlier mentioned rates

led to statistically significant increases in the mineral nitrogen content during the eight years of experimentation, where compared to the control it averaged 0.17-0.21 mg/100 g soil.

**Accessible phosphorus and potassium.** Fertilization with wine yeast at rates of 13 and 26 t/ha led to statistically significant increases in accessible phosphorus content. Over nine years the mean value of accessible phosphorus content compared to the control increased by 0.59-0.98 mg/100 g soil. The application of vinasse at rates of 300 and 600 m<sup>3</sup>/ha led to statistically significant increases in accessible phosphorus content values in all nine years of experimentation (2011-2019). The average phosphorus gain compared to the reference variety was 0.27-0.32 mg/100 g soil. As for the potassium content when applying wine waste statistically assured increases over the control were recorded throughout the years. The value of the increase in exchangeable potassium content was respectively 8-12 mg/100 g soil and 10-13 mg/100 g soil compared to the control.

Statistically significant values of accessible phosphorus content were also found when cereal borage was applied at 47 and 94 m<sup>3</sup>/ha (equivalent to N120 and N240 kg N/ha). The difference of the eight-year mean value from the control was 0.24 and 0.50 mg/100 g soil. As regards the value of exchangeable potassium content when applying cereal borage, the statistically assured increase was 3-6 mg/100 g soil.

According to the methodical instructions (Andrieş et al., 2007) due to the application of waste from the production of alcoholic

beverages the levigated chernozem investigated by humus content is classified as high, by moderate nitrogen content, by optimal phosphorus content and by high potassium content.

**Humus and nutrient balance in the application of waste from alcoholic beverage production.** The beneficial influence of organogenic waste fertilization has also been demonstrated by calculating the humus and nutrient balance in vine and field crop cultivation (Table 2-4).

**Humus balance.** Humus is the most important store and at the same time the most significant source of carbon and nutrients. It should be noted that in recent years zonal soils in the country are practically not respected. The share of perennial grasses in field soils has decreased considerably. At the same time, the area occupied by biological nitrogen-fixing leguminous crops has tripled and the share of grassland crops has increased to 65% (Andrieş, 2017). In the last 20 years, small amounts of organic fertilizers have been applied in agriculture (0.02-0.03 t/ha), the optimal dose being 10 t/ha (Monitoring bulletin, 2000). Secondary agricultural production is not applied everywhere as organic fertilizer. As a result, the humus balance in Moldovan agriculture is negative (-0.7 t/ha), and due to erosion losses we have an even greater reduction (-1.1 t/ha), (Andrieş et al., 2002; Andrieş, 2005). Organogenic wastes from the production of alcoholic beverages serve an important source for increasing carbon stocks. The humus balance is negative in the reference scenario, with a deficit of 1203 kg/ha per year (Table 2).

Table 2. Annual humus and nutrient balance on levigated chernozem under vines, kg/ha. STE "Codru", 2011-2019

Experience variant	Humus	Nitrogen	Phosphorus	Potassium
1. Unfertilized control	-1203	-54	-18	-64
2. Wine lees (N <sub>100</sub> ), 13 t/ha	2514	126	70	273
3. Wine lees (N <sub>200</sub> ), 26 t/ha	4497	225	158	562
4. Vinasse (K <sub>450</sub> ), 300 m <sup>3</sup> /ha	184	9	41	296
5. Vinasse (K <sub>900</sub> ), 600 m <sup>3</sup> /ha	1153	56	101	645

For wine waste, the results of the balance were positive for the accumulation of humus content. When applying wine lees and vinasse the humus balance was respectively: 2514-4497 and 184-1153 kg/ha annually. It was

determined that when applying cereal borage (Table 3), the humus balance in the unfertilized control variant was negative, with a deficit of 2136 kg/ha annually. In the variants fertilized with 47-94 m<sup>3</sup>/ha cereal borage the average

annual humus balance (2012-2019) is respectively 857 and 1676 kg/ha. Research has shown that crops sown often in our case, autumn wheat are more favorable to

the soil in terms of humus loss. The humus balance in the reference variety is negative (Table 4).

Table 3. Humus and nutrient balance in soil on levigated chernozem fertilized with cereal borage, kg/ha on average for the years 2012-2019. STE "Codru"

Experience variant	Humus	Nitrogen	Phosphorus	Potassium
1. Unfertilized control	-2136	-107	-40	-103
2. Cereal borage (N <sub>120</sub> ), 47 m <sup>3</sup> /ha	857	18	31	238
2. Cereal borage (N <sub>240</sub> ), 94 m <sup>3</sup> /ha	1676	95	85	529

Table 4. Humus and nutrient balance in the soil per crop when fertilizing levigated chernozem with cereal borage, kg/ha. STE "Codru", 2012-2019

Experience variant	Crop			
	Autumn wheat	Sunflower	Grain maize	Soya beans
<b>Humus</b>				
1. Unfertilized control	-1930	-1173	-1408	+78
2. Cereal borage (N <sub>120</sub> ), 47 m <sup>3</sup> /ha	1211	625	456	308
2. Cereal borage (N <sub>240</sub> ), 94 m <sup>3</sup> /ha	1625	2820	2700	2536
<b>Nitrogen</b>				
1. Unfertilized control	-147	-59	-70	+20
2. Cereal borage (N <sub>120</sub> ), 47 m <sup>3</sup> /ha	16	31	23	63
2. Cereal borage (N <sub>240</sub> ), 94 m <sup>3</sup> /ha	31	141	135	127
<b>Phosphorus</b>				
1. Unfertilized control	-53	-22	-25	+10
2. Cereal borage (N <sub>120</sub> ), 47 m <sup>3</sup> /ha	23	23	40	54
2. Cereal borage (N <sub>240</sub> ), 94 m <sup>3</sup> /ha	61	114	112	101
<b>Potassium</b>				
1. Unfertilized control	-107	-120	-68	+16
2. Cereal borage (N <sub>120</sub> ), 47 m <sup>3</sup> /ha	245	195	282	257
2. Cereal borage (N <sub>240</sub> ), 94 m <sup>3</sup> /ha	529	486	587	560

A humus balance with positive values was found when applying cereal borage at rates of 47 and 94 m<sup>3</sup>/ha, equivalent to N100 and N200 annually. Positive values were 625-2820 and 456-2700 kg/ha annually.

The highest soil humus losses through mineralization are brought by the arable crops, in our case grain maize and sunflower. During the investigation period in the experimental field grain maize was grown for one year. Negative values of soil humus balance or established in the control variant, which makes up 1408 kg/ha. The application of borage at rates equivalent to N120 and N240 kg/ha offsets a good part of the mineralized humus. The amount of humus deficiency compensation is directly proportional to the applied rate of cereal borage and amounts to 456-2700 kg/ha. In sunflower cultivation a negative deep balance was established only in the unfertilized variety. Humus losses were -1173 kg/ha. In the

variants with cereal borage application the humus balance was positive, making up 625-2820 kg/ha.

The crops sown often are more favorable to the soil in terms of humus losses. The humus balance of the winter wheat crop in the control variant is negative. The four-year average value is - 930 kg/ha. When waste is applied, this index has positive values, averaging 1211-1625 kg/ha. So, more pronounced positive values was established when fertilizing with 94 m<sup>3</sup>/ha of borage (1625 kg/ha).

When growing soybean plants for grain (one year only) the humus balance in the reference variety was positive, making up 78 kg/ha of humus accumulated in the soil. When using waste, the humus balance was profoundly positive. Humus content values were 308- 2536 kg/ha respectively.

**Nitrogen balance.** Nitrogen is the most active nutrient, which plays an important role in plant

life. It is part of protoplasmic structural proteins, nucleic acids, chlorophyll pigments, some vitamins and enzymes.

The main amount of nitrogen is found in soil organic matter. Plant-accessible nitrogen is formed and accumulates in the soil due to the decomposition of humus by micro-organisms. It has been established over time that every 1% of humus in the ploughed layer provides plants with 24 kg/ha of accessible nitrogen.

It should be noted that the nitrogen balance in soils is negative. This phenomenon leads to a decrease in soil fertility and productivity of agricultural crops.

The investigated wastes had a beneficial influence on the nitrogen balance in leigated chernozem (Table 2). The nitrogen balance in the unfertilized version is negative, the deficit being -54 kg/ha (Table 2). When wine waste was applied in the named doses the nitrogen balance results were positive, making up respectively 126-225 and 9-56 kg/ha annually. The nitrogen balance in the reference variant was found to be negative, with a deficit of 107 kg/ha per year (Table 3). In the variants fertilized with 47-94 m<sup>3</sup>/ha of cereal borage the average annual humus balance was 18 and 95 kg/ha respectively.

It has been shown that the nitrogen balance at crop level in the control variant is characterized by positive values in grain soybean cultivation, making up 20 kg/ha (Table 4). More pronounced positive values of 63-127 kg/ha were established when applying cereal borage doses equivalent to N120 - N240 kg/ha. The nitrogen balance in autumn wheat cultivation in the control variety is deeply negative. The four-year average value is 147 kg/ha. The application of waste at rates of 47-94 m<sup>3</sup>/ha maintains a positive nitrogen balance with values between 16 and 31 kg/ha. Losses of nitrogen from the soil through mineralization are brought by the arable crops (grain maize, sunflower). In the control variant the nitrogen balance values are negative and amount to 59 and 70 kg/ha respectively. When borage was applied the nitrogen balance was 23-135 kg/ha for maize and 31-141 kg/ha for sunflower.

**Phosphorus balance.** The phosphorus problem is very acute for contemporary agriculture because it is required by crop plants in high quantities and in low concentrations in the soil.

It should be noted that phosphorus deposits in the country are few, with limited reserves. The soil contains on average 1.3 times more nitrogen and 17.0 times more potassium than phosphorus (Zarin et al., 1963). At the same time, the soluble salts of phosphorus have the property of reacting with soil consistencies, forming insoluble compounds that are difficult for plants to access. Of the total amount (3000-5000 kg P<sub>2</sub>O<sub>5</sub>/ha) contained in the unfertilized plough layer, only 24-36 kg/ha is maintained in a form accessible to plants - 1.0% of the total (Lixandru, 1990; Andrieș, 2000). To improve plants with phosphorus it is necessary to apply fertilizers, especially organic ones.

Fertilization with organogenic wastes from alcoholic beverage production had a positive influence on the accessible phosphorus balance. When wine waste was applied, the phosphorus balance was 70-158 and 41-101 kg/ha per year, respectively, with a deficit of 18 kg/ha. It was determined that the phosphorus balance was negative when applying cereal borage (Table 3), with a deficit of 40 kg/ha per year. When waste was incorporated this index made up 31-85 kg/ha annually.

The accessible phosphorus deficit in winter wheat, sunflower and grain maize was 53, 22 and 25 kg/ha per year respectively.

In the cultivation of soybean plants for grain the phosphorus balance in the non-fertilized variety has positive (balanced) values of +10 kg/ha.

**Potassium balance.** The exchangeable potassium in the soil is determined to optimize the plants with this element by applying fertilizers. Soils of Moldova formed on rocks with potassium-rich minerals are characterized by a relatively high potassium content. It has been established that the potassium content in the soils of our country depends to a large extent on the mineralogical and granulometric composition. Potassium adsorbed on the surface of the colloidal clay-humic complex readily passes into the soil solution by exchange reaction with another monovalent cation (NH<sub>4</sub><sup>+</sup>, Na<sup>+</sup>). It should be noted that in clay-textured soils, the exchangeable potassium content is 20-22 mg/100 g soil, equivalent to 1.4-2.3% of the total. To keep the potassium content at an optimum level, fertilizer application is recommended. In our case we

used organogenic waste from the production of alcoholic beverages as organic fertilizer. By calculating the potassium balance in the soil, it was shown that this index in the unfertilized version was negative with annual values of 62 kg/ha. The application of wine waste favored the exchangeable potassium balance in the fertilized variants positively. The positive values of the accessible potassium balance were respectively 314-570 and 293-652 kg/ha annually. According to the calculations made when determining the potassium balance in crop rotation per crop when fertilizing levigated chernozem with cereal borage for the years 2012-2019 is shown in Table 4. It was determined that the potassium balance in unfertilized field crops was negative: 107 kg/ha in autumn wheat, 120 kg/ha in sunflower and 68 kg/ha in grain maize. Application of cereal borage compensated for the exchangeable potassium deficit by crop: 245-529; 195-486 and 282-587 kg/ha respectively. The exchangeable potash balance in grain soybean cultivation in the unfertilized variety was balanced, constituting 16 kg/ha. While in waste incorporation this index was deeply positive (257-560 kg/ha).

## CONCLUSIONS

Research conducted during 2011-2019 confirmed that wine and ethyl alcohol production wastes increased the content of humified organic matter in soil by 0.12-0.37%. A significant increase of mineral nitrogen by 0.17-0.61 mg/100 g soil, mobile phosphorus by 0.19-0.98 mg/100 g soil and exchangeable potassium by 3-13 mg/100 g soil was found. The results obtained from the determination of the humus balance and their nutrients in the soil showed that the unfertilized soil during nine years lost annually 1203-2136 kg/ha humus, 54-107 kg/ha nitrogen, 18-40 kg/ha phosphorus and 64-103 kg/ha potassium. Waste application compensated the losses of these indices respectively by 857-4497, 9-225, 31-158 and 296-645 kg/ha. The results of the determination of the balance of humus, nitrogen, mobile phosphorus and exchangeable potassium at crop level showed that the application of the mentioned waste to autumn wheat, sunflower, grain maize and soya grain maintained a balanced and positive balance of these indices.

More pronounced positive values of agrochemical indices were established when fertilizing levigated chernozem with wine yeast at the rate of 26 t/ha, vinasse at the rate of 600 m<sup>3</sup>/ha and grain borage at the rate of 94 m<sup>3</sup>/ha.

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## SOIL SOLUTION IS A KEY FACTOR OF SOIL NUTRITION

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### *Abstract*

*It is highlighted the dynamics features and the importance of soil solution in plant nutrition in the main genetically contrasted soil kinds of Ukraine. It is shown that soil solution is the most sensitive indicator of plant nutrition and changes in reaction and in concentration of nutrient elements in it make quick impact on plant growth. Optimal concentration of phosphorus and potassium ions in soil solution was revealed in set of vegetation experiments with oat and barley. Soils with high buffer mobilization capacity can compensate the deficit of a large part of these ions while mineral nutrition of plants is ongoing. In contrast, soil solution becomes poor on biogenic elements in soils with low buffer capacity. Processes of immobilization and mobilization of biogenic elements are regulated by bio-organomineral complex, which specifies a dynamics pattern of the fertility element concentration in soil solution. This pattern is described by buffer curve in relation to the curve of bufferless substrate. The more distance between these curves the higher buffer capacity in investigated soil.*

**Key words:** *buffer ability, models of fertility.*

### INTRODUCTION

Due to a wide range of scientific works (Gynzburg, 1975; Kanunnikova et al., 1981; Khristenko, 2016; Paul et al., 2010) it was proved that diagnostics and agroecological assessment of soil nutrient regime only by the level content of mobile forms of biogenic elements at the moment of measurement is not enough. Therefore, there is a necessity to determine a criteria for functional diagnostics, which would take into account the dynamics of the concentration (activity) of the most accessible forms of the main plant nutrients in soil ("intensity factor" - IF).

There are many different methodological approaches and techniques for the diagnostics of nutrient regime. However, a close correlation between the content of mobile forms of nutrients in soils and yield, as a rule, was not observed (Nay et al., 1980; Khristenko, 2016). Low correlation or its absence is caused by not only the antagonism-synergism between nutrients in soil solution during the process of water-mineral nutrition of plants (Fateyev et al., 2020), but also by the functioning of soil processes of natural regulation - natural and acquired soil ability to place and maintain the IF value at a constant level. This ability is

considered (Truskavetsky, 2003; Truskavetsky et al., 2016) one of the most important factors that determine the soil conditions for mineral nutrition of plants.

The patterns of IF changes are reflected by the buffer curve, the shape of which depends on the functioning of soil mechanisms of natural regulation.

Unfortunately, "buffer" issues are poorly understood in soil science, except of the acid-base buffer ability of soils (pH-buffer), which is covered in a number of scientific papers (Nadochiy, 1993; Philep et al., 1989). But only several scientific works are devoted to other types of soil buffer ability, such as nitrogen, phosphate, potassium, hydro-, thermo- and redox-buffering as well as soil buffer ability to heavy metals and microelements (Kanunnikova et al., 1981; Savich et al., 1980; Beckett, 1964). Analysis of literature sources (Nay et al., 1980; Shakhparonov, 1976) shows that soil solution is the most influential factor on crop formation. In laboratory and analytical practice, soil solution is studied by simulating it with various soil extracts - water, acid-free, as well as with soil pastes, suspensions, centrifuges etc. The closest to the natural conditions ones are such with the "soft" impact on soil - water and acid-free extracts (Gynzburg, 1975; Nay et al., 1980;

Beckett, 1964), which do not ravage the soil solid phase and thus do not cause significant changes in chemical composition of the soil solution. However, using methods with acid-free and water extracts you cannot determine the ability of soil to provide plants with nutrients, which was called "capacity factor" (CF) (Beckett, 1964). In agrochemical practice, CF is determined using more aggressive extragents - acid and alkaline solutions, but it is not usually possible to assess the actual agroecological condition of the soil solution because of its compositional disorder caused by the destructive (aggressive) action of acid and alkaline to the soil substrate.

A great number of researchers (Khristenko, 2016; Nosko, 2017) say in their methodological works about the futility of using "hard" chemical reagents for the functional diagnostics and optimization of soil fertility. Currently, methods with acid and alkaline soil extracts could determine CF, which is the first reserve for the reduction of IF deficit during the process of plant nutrition. These factors (CF and IF) are in thermodynamic equilibrium (Cheshko, 2015), which is varied in parameters and depends on the buffer properties of each soil kind.

The ability of soil to compensate partly the content of a nutrient in the soil solution during plant nutrition was defined as its potential buffer capacity (PBC) (Beckett et al., 1964). These authors worked out methods for determination PBC of soil to both phosphorus (PBC<sub>P</sub>) and potassium (PBC<sub>K</sub>) and mentioned them as important diagnostic criteria.

Thus, it should be used two main diagnostic indicators as criteria for the optimization of the fertility elements - the concentration (activity) of nutrients in soil solution (IF) and the ability of soil to store and maintain IF in optimal parameters (buffer capacity). This theoretical conclusion formed the basis of our research.

Motivation for the writing this article was an attempt by its authors to put scientists of foreign countries next to the results of author's long-term research on soil "buffer" issues and their use in the practice of soil nutrient management.

## MATERIALS AND METHODS

The methodology of functional diagnostics and optimization of soil fertility was worked out by studying the behavior of phosphate and potassium ions in soil solution of genetically contrasted soils in Ukraine.

For the study, samples were taken from the 0-25 cm arable layer of the main soil types in the Polesye and Forest-Steppe zones of Ukraine, the characteristics of which are given in Table 1. Samples were taken on control (long-term unfertilized) variants of stationary field trials of research institutions of Ukraine. Soil mass was also taken from the arable layer of unfertilized variants for vegetation experiments.

Soil samples were analyzed for: pH of the soil-water extract (according to UNS ISO 10390:2007), granulometric texture (according to UNS 4730:2007), bulk density (according to UNS ISO 11272:2001), organic carbon content (according to UNS 4289:2004), mobile forms of phosphates and potassium (according to UNS 4405:2005); phosphate and potassium buffer ability according to our modernized methods (UNS 4724:2007 and UNS 4375:2005, respectively). In peat samples it was determined the ash content and peat decay degree according to UNS 7942:2015 and UNS 7829:2015, respectively.

Phosphate (IF<sub>P</sub>) and potassium (IF<sub>K</sub>) intensity factors and their dynamics under the increasing additives of calcium monophosphate [Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>] and potassium chloride (KCl) in soil samples were determined by the method of Scofield using 0.01n and 0.001n CaCl<sub>2</sub> solutions according to the instructions (Beckett et al., 1964).

Optimal parameters of phosphate and potassium IF were determined earlier (Truskavetsky, 2003) by conducting a series of vegetation experiments with cereals. Experimental schemes included variants with progressive increased doses of phosphorus and potassium fertilizers single (pre-sowing) application at the time when moisture and nutrients are optimized.

Table 1. Characteristics of the studied soils in the 0-25 cm arable layer

MINERAL SOILS				
		Chernozem podzolized heavy-loamy (Left-bank Forest-Steppe zone of Ukraine)	Light-gray forest light-loamy soil (West Forest-Steppe zone of Ukraine)	Sod-podzolic sandy-loamy soil (Central Polesse zone of Ukraine)
pH <sub>water</sub>		6.2	5.2	4.9
C <sub>org.</sub> , %		2.2	1.1	0.7
Total content, %	Nitrogen	0.21	0.23	0.06
	Phosphorus	0.10	0.12	0.07
	Potassium	2.10	1.70	1.10
Content of particles, %	<0,01 mm	42.2	23.3	16.4
	<0,001 mm	12.8	7.8	7.8
PEAT SOILS				
		Peat eutrophic mesotrophic soil	Silty-peat ash-rich soil	Peat alcalitrophic ash-rich soil
pH <sub>water</sub> .		5.4	6.5	7.1
Ash content, %		12.0	41.0	25.0
Peat decay degree, %		30.0	46.5	57.0

Optimal minimum of IF was at a dose of fertilizer, above which the efficiency of fertilizer decreases rapidly, and the optimal maximum of it was when negative impact of fertilizer on yield could be seen with its total

decrease of 15-25%. Optimal limits (minimum and maximum) of phosphate and potassium ions concentration in soil solutions of different soils (Table 2) were determined in vegetation experiments.

Table 2. Soil gradation by phosphate and potassium buffer capacity (BCp) and by mini-optimum of IF

Soil gradation	BCp, points		Mini-optimum of IF	
	phosphate	potassium	pP	pK
Low buffer capacity	< 5	< 3	< 4.35	< 3.85
Medium buffer capacity	5-15	3-8	4.35-4.65	3.85-4.25
High buffer capacity	15-35	8-16	4.65-4.90	4.25-4.45
Very high buffer capacity	> 35	> 16	> 4.90	> 4.45

The upper limit of the concentration of nutrients in soil solution with a single application of increasing doses of fertilizers was basically the same apart from the buffer capacity of studied soils: for phosphate and potassium ions it was at the level of 3.25 and 3.15, respectively (pP and pK) and fluctuations did not exceed 0.20 units (delta pP and pK).

The mini-optimum for pP and pK in soil solutions is the most important parameter that should be achieved and kept in the soil root layer for the maximum yield. There was found a significant correlation between soil immobilization buffer capacity and the optimal minimum of pP and pK in soil solutions with the coefficients for phosphate ions -  $R_p = 0.88$  and potassium -  $R_k = 0.72$  and described by the following equations:

$$pP = 3.77 + 0.066 * X_p - 0.001 * X_p^2$$

$$pK = 3.79 + 0.023 * X_k - 0.0003 * X_k^2$$

where:  $X_p$  and  $X_k$  are immobilization buffer capacities to both phosphate and potassium ions, points (on a 100-point scale).

The more immobilization buffer capacity of the soil the less use efficiency of fertilizers because soil absorbs nutrients from soil solution and they become unavailable for efficient plant nutrition.

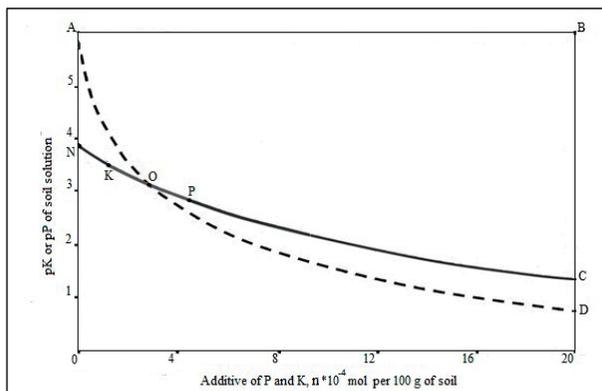
## RESULTS AND DISCUSSIONS

Soil buffering to nutrients is its ability to resist changes in concentrations (activities) of these elements in the soil solution (IF). Buffer curve characterizes the dynamics of IF changes in different kinds of soils under the influence of

increasing fertilizer additives and shows in fact the strength of fertility elements fixation by the soil bio-organo-mineral complex, and on the other hand, the easiness of their release from it. The concentration (activity) of nutrients in the soil solution (IF) may be low, but optimal functioning of soil buffering mechanisms makes favorable conditions for mineral nutrition of plants as opposed to high, but unstable IF, which is in soils with low buffer capacity. It is clear that instability of plants mineral nutrition is due to the deficit of IF in the soil solution and to the inability of the soil mass to make it up in time from the potential

reserves of the fertility element in the soil solution.

Buffer capacity is the rate of deviation of the soil-buffering curve from the "zero-buffering" curve. "Zero-buffering" curve is the curve of change in the concentration (activity) of phosphate and potassium ions with their increasing additives in the bufferless substrate or in pure aqueous solution (hydroponics). General view of the graphical model for the diagnostics and optimization of soil phosphate and potassium regimes is shown in Figure 1. This author's model is presented for the first time in foreign publications.



--- buffer curve of bufferless substrate (pure sand) - AD; — buffer curve of some soil - NC  
 N - initial condition of fertility element; O - thermodynamic equilibrium of turnover;  
 ABD - buffer capacity standard area (100 points); OCD - mobilization buffer capacity area;  
 AON - immobilization buffer capacity area; KP - optimal parameters of intensity factor

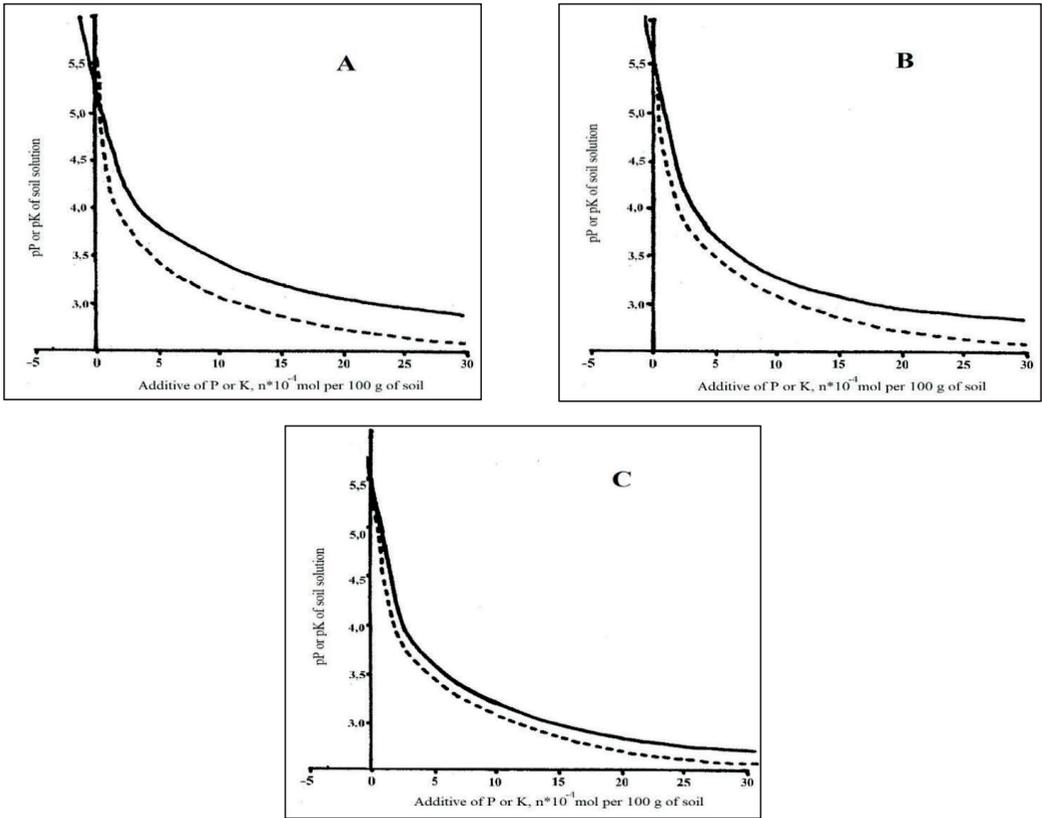
Figure 1. Graphical model of diagnostics and optimization of phosphate and potassium condition of some soil

Thus, our research on "buffer" issues was aimed at improving the methodology of functional diagnostics. It is important to assess the level of deviation of the actual state of the fertility element from its genetic parameter as well as the dynamics of the transition and stable retention of the fertility element in optimal parameters for plant growth (segment KP on the buffer curve, Figure 1). External influences significantly change soil functioning and the line of the flow of biological cycle processes. However, due to buffer mechanisms, these changes are in the appropriate parameters for a particular soil.

Therefore, we formalized obtained results of experimental studies related to the determination of soil buffer parameters into integrated diagnostic and optimization

(management) graphical models. In summary, optimal IF values (minimum and maximum) obtained for each studied soil are plotted on their buffer curves and, thus, we obtain an integrated graphical model that optimizes phosphate and potassium regimes of the studied soils and assesses the dynamics of their state.

Unfortunately, it is not possible to disclose all our created models for different soils of Ukraine in this article. At the same time, we consider it necessary to show and describe phosphate and potassium models for three soils that sharply contrasted by buffer properties and fertility and dominate in the Polesse and in the Forest-Steppe zone of Ukraine, such as sod-podzolic sandy, light-gray forest light-loamy and chernozem podzolized heavy loamy (Figure 2).



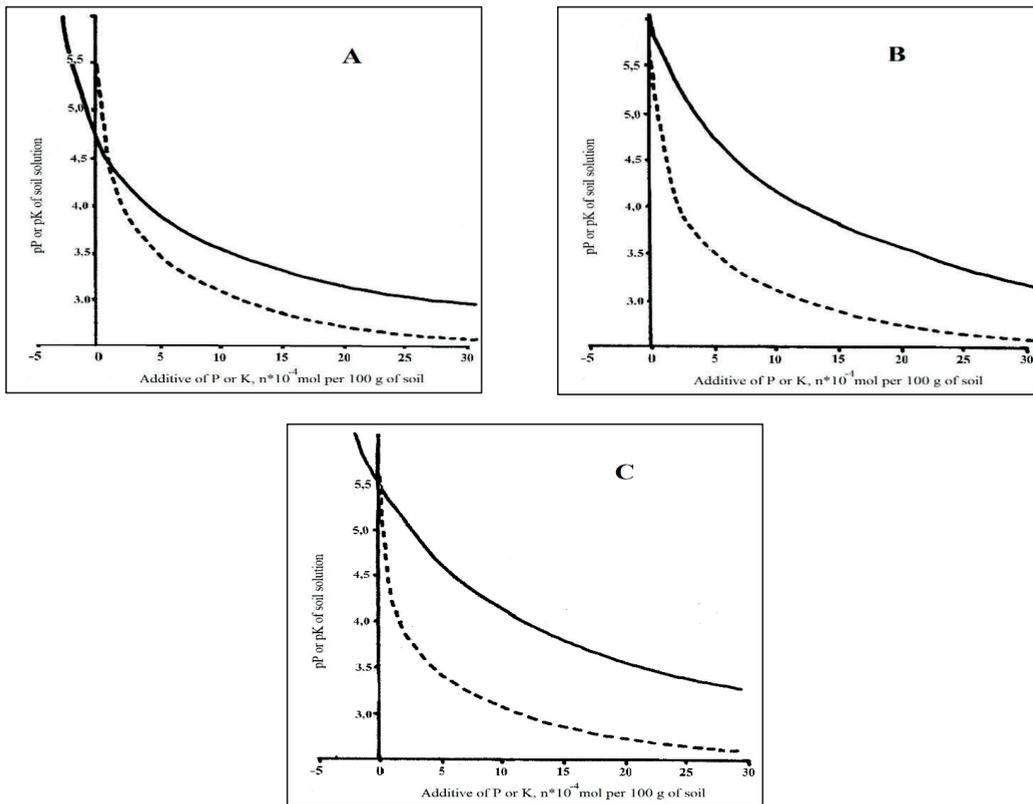
----- buffer curve of bufferless substrate (pure sand); A - buffer curve of chernozem podzolized heavy loamy; B - buffer curve of light-gray forest light-loamy soil; C - buffer curve of sod-podzolic sandy soil

Figure 2. Graphical model of phosphate and potassium buffer ability of different mineral soil types

As shown in Figure 2 buffer curve of chernozem significantly deviate from the buffer curve of bufferless substrate (“zero-buffering” curve). Instead, a deviation of buffer curve of sod-podzolic soil from “zero-buffering” curve is not significant and indicates its low buffer capacity, which is expressed in points on a 100-point scale of the author's computer

programs (pP-buff and pK-buff). At the moment, buffer curve of light-gray soil is in the mean position between mentioned soils, which is a natural phenomenon.

Our models for three types of peat soils (peat eutrophic mesotrophic soil, peat alcalitrophic (carbonate) ash-rich soil and silty-peat ash-rich soil) are also demonstrative (Figure 3).



----- buffer curve of bufferless substrate (pure sand); A - buffer curve of peat eutrophic mesotrophic soil; B - buffer curve of peat alcalitrophic (carbonate) ash-rich soil; C - buffer curve of silty-peat ash-rich soil

Figure 3. Graphical model of phosphate and potassium buffer ability of different peat soils

Indeed, buffer curve of peat eutrophic mesotrophic soil should be etalon for the susceptibility to optimization of the phosphate regime and the easiness of its control and silty-peat ash-rich soil one - should be the same in relation to the management of plant potassium nutrition. Instead, alkalitrophic (carbonate) peat soils require appropriate reclamation techniques (including gypsum and acid clay application) to improve their phosphate and potassium regimes.

## CONCLUSIONS

Soil solution is the most influential factor in crop formation and sensitive to changes in the soil environment. Objective agroecological diagnostics of soil solution is impossible without taking into account its dynamics. The effectiveness of the main sources of making up for the deficiency of nutrients in the

soil solution (capacity factor and fertilizers) is closely related to the functioning of soil buffer mechanisms.

Methods of "hard" chemical diagnostics (acid and alkaline extracts) cannot be criteria for optimization of soil nutrient regime.

Author's graphical models of soil nutrient management (on the example of phosphate and potassium regimes) integrate diagnostics and optimization into one whole and are based on the patterns of intensity factor dynamics (indicators of concentration (activity) of phosphate and potassium ions in the soil solution) under the increased doses of fertilizers and experimentally proved optimal parameters of IF.

Author's graphical diagnostic and optimization models allow to assess the initial state and conditions of phosphate and potassium nutrition of plants in soils; set doses of fertilizers to achieve optimal parameters of concentration (activity) of phosphate and

potassium ions in the soil solution, depending on the methods of their application and the duration of their aftereffects; ground the choice of effective ways to use phosphorus and potassium fertilizers and reclamation techniques aimed at improving the conditions of mineral nutrition of plants.

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## METHODOLOGY FOR COMPLEX AMELIORATIVE EFFECT ON THE ACID-ALKALINE BALANCES IN THE SOILS

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### *Abstract*

*For several years have developed and tested specific methods for soil sampling and interpretation of the results regarding the assessment of soil heterogeneity in the vineyard terroir. The heterogeneity of the soil in terms of the indicators characterizing the harmful acidity in the soil must be taken into account not only in the area horizontally, but also in the vertical direction - i.e. the change in the depth of the soil profile. The role of acid-alkaline balance due to the structure of soil acidity is a complex soil component of the terroir. In terms of its relative influence, it is comparable to the importance of the chemical composition of the soil as it determines the dynamics of its components in the soil-plant system.*

**Key words:** acid-alkaline balance, liming model, soil sampling model.

### INTRODUCTION

The productivity and fertility of the soil when it is part of the vineyard terroir should be considered in a different way compared to that one used for determination the suitability and fertility of all other crops. The world's viticultural terroirs are built on low-yielding lands. The main thing in the formation of a good and perspective terroir is that high productivity is not necessarily pursued, but it is important that the soil cover has a very high degree of equality not only in terms of acidity, but also the geological basis and natural hydrology of the terrain. In order to neutralize the harmful acidity and to cover the cost levels in the calcium balance and ultimately to increase the yield, a balance rate must be calculated, leading not to complete neutralization of the permanent sorption positions, but to reduction of the toxic action of mobile aluminum, hydrogen and manganese (Almaliev, 2020).

In Bulgaria, the leveling of the geological and hydrological conditions of the terrain can rarely be observed, as there are large vineyards in which there is a high diversity in terms of soil, topographic, geological and hydrological conditions within one and same vineyards.

From this point of view, our work is an attempt to parameterize the existing heterogeneity in our country in terms of location.

For several years, we have developed and tested specific methods for soil sampling and

interpretation of the results regarding the assessment of soil heterogeneity in vineyard terroir.

Vineyards have been shown to vary spatially in terms of soil, vine nutrition (Bramley, 2001; Davenport and Bramley, 2007; Reynolds and Hakimi Rezaei, 2014a), vegetative growth (Baldy et al., 1996; Bramley et al., 2011), yield, and fruit composition (Bramley, 2001; Reynolds et al., 2007; Bramley et al., 2011).

Precision viticulture techniques including global positioning systems (GPS) and geographic information systems (GIS) have become powerful tools to study vineyard terroir (Reynolds et al., 2007) and variability (Bramley and Hamilton, 2004; Bramley, 2005) while keeping key environmental factors constant. Other studies that have utilized precision viticulture to explain interactions between soil characteristics and vine growth and/or fruit composition.

Bramley (2001) found that soil texture had an impact on yield in Australian vineyards. Areas within the vineyard that had higher percentage of clay contained lower yielding vines. Strong spatial and temporal distribution patterns were found within vineyards for many nutrients in various tissue types of vines in Coonawarra vineyards (Davenport and Bramley, 2007).

The aim of the presented publication was to describe methodically - step by step how to approach the melioration of acid soils in order to neutralize the acid-alkaline balance.

## MATERIALS AND METHODS

The study was conducted on terrain occupied by old vineyard on strongly acidic eroded Eutric regosols. Due to the strong development of the processes erosion and acidity, the vineyard should be uprooted and replanted. The task of the melioration study was to equalize the terrain in terms of acidity. When surveying terrains with heterogeneous soils, we used different soil sampling methods, including for the same terrain we collected soil samples in different ways so that we could determine which of them is the most representative to describe the terrain most accurately. The most representative was soil sampling method in a square grid in accordance with modern GIS systems. Based on this, in the course of the present study we adopted a model for soil sampling, in which each of the samples is taken from the field using a soil probe, and the sampling points are located within the field in a square grid, regardless of the boundaries of soil differences and topography of the terrain.

Four sections, which have different degrees of erosion, were inspected. The soil sampling was taken at two depths 0-25 and 25-50 cm.

After standard preparation, the soil samples were analyzed to establish the following parameters: pH, potentiometric in KCl (Arinushkina, 1970); easily mobile exchangeable  $\text{Al}^{3+}$  and  $\text{H}^+$ , titrimetric according to (Sokolov, 1939); easily mobile exchange  $\text{Mn}^{2+}$  in extract with 1 m KCl, as the preparation of the extract was carried out according to the Laboratory system for liming (Palaveev and Totev, 1970a), (LSVPT-64), and the determination of  $\text{Mn}^{2+}$  in the extract by AAS (BDS11047, 1995); easily mobile exchange  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , complexometric by the method of Mazaeva, Neugodova and Khovanskaya (Palaveev and Totev, 1970b), the integrating index V3% and the lime rate was calculated.

## RESULTS AND DISCUSSIONS

There are very few scientific publications in Bulgaria about the mathematical apparatus of geostatistics and its application for characterization of the variables in the environmental and precision agriculture.

Heterogeneity is rarely used and is often ignored due to lack of appropriate technology or lack of time and knowledge to work with it, especially in technological processes in our agriculture.

The main question is how to quantify it. Soil sampling from the study area is difficult and expensive, and it is often necessary to judge the condition of the entire study area by one sample, which is obviously extremely inaccurate. Geostatistics solves this problem by giving us the variation in the places from which no samples were taken as evaluates the error that is being handled (Kutev, 2013).

In the last two decades, the method has received wide acclaim and has been applied to analyze and map data from various fields such as agriculture, fisheries, geology, hydrology, climatology, oil industry, remote sensing, soil science. Geostatistics entered the field of precision farming in the 1990s with the development of technologies such as Yield monitors, Global Positioning System (GPS), Remote Sensing (RS), and Variable rate technologies (VRT) and Geographic Information Systems (GIS) (Sahoo, 2014).

Soil sampling is the first step in generating field-specific information to make lime and fertilizer decisions. Selecting an appropriate sampling strategy ensures that the soil in a field is collected in a manner that produces the most accurate and reliable soil test results. Because soils in agricultural fields can vary significantly, use a sampling strategy that best captures that variation. Proper sampling is particularly important when a site-specific management approach is embraced.

Modern technologies including GPS (Global Positioning System), GIS (Geographic Information Systems), FMIS (Farm Management Information Systems), and Variable Rate Technology (VRT) allow producers to manage soils and amendments with greater precision. Site-specific soil sampling provides the foundation for many lime and fertilizer decisions enabled by these technologies. Site-specific soil sampling is the basis for:

- identifying the spatial distribution of nutrient deficiency and sufficiency within fields;
- increasing lime and fertilizer use efficiency by variably distributing lime, nutrients, and

other amendments based on the spatial distribution of soil properties and crop requirements;

- minimizing potential for nutrient loss from fields by overapplication;
- optimizing production through the targeted use of agricultural amendments.

To best utilize site-specific soil sampling, you need a clear understanding of the benefits and limitations of each sampling strategy, as well as knowledge surrounding the tools and process used to develop the sampling plans.

This publication provides a methodological approach to assist in the selection and development of soil sampling strategies, based on:

1. Describes how to position the sampling points.
2. The method of soil sampling is described - separately for each depth, and not by mixing them.
3. Because the work is methodical, and in order to be clearer, the values and spatial location of the individual points are shown only on the horizon A. Scilicet from now on, each depth selection is approached in this way (as in horizon A).
4. Using this approach, the color indicators of the different indicators characterizing the acid-base balance, as well as the integrating indicator V3 in the different parts of the terrain are very well depicted.
5. The decision taken in the development of norms of demand needs is fully consistent with the design and sequence described in this study, namely to create a buffer background-which is absolutely mandatory when considering and interpreting values only from single receivables, here in this case horizon A, and in most cases from practice as an average sample of all horizons.

The outlines of the terrain, which are on a cadastral map, are presented in Figure 1. The terrain is developed in the northwest-southeast direction.



Figure 1. Boundaries of the terrain on a cadastral map

The logic is that the survey grid should be in the same direction. Figure 2 shows how we built the soil sampling grid and how it lies on real terrain.



Figure 2. Soil sampling grid

Each side of the square is 90 m in size. The intersected points of each square indicate the place from which a single soil sample should be taken with a probe at two depths 0-25 and 25-50 cm (Figure 3). These points are stored in the GPS memory, each of which is numbered, and this number corresponds to the number of the laboratory sample. Samples from different depths are not mixed but are indicated as A and B. The subject of our study is only the data of the samples at a depth of 0-25 cm (A-horizon).

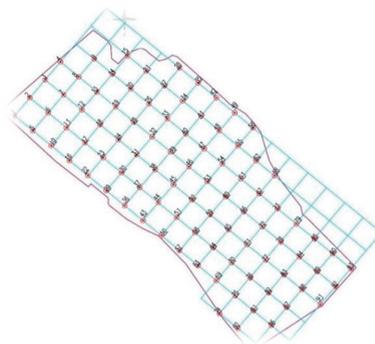


Figure 3. GPS soil sampling points

Table 1 presents the results of analyzes of some of the soil samples as showing the laboratory numbers, each number corresponding to a specific point with UTM coordinates. The following columns show the pH values, the indicators of harmful acidity ( $\text{Al}^{3+}$ ,  $\text{H}^+$  and  $\text{Mn}^{2+}$ ) and their antagonists ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ), the integrating indicator ( $\text{V}_3$ ) and the lime rate (NN).

The obtained values of all indicators are plotted on the map. Figure 4 shows the pH values. The

most acidic areas (in red) were in the northwestern parts of the terrain, and in the southeast the acidity was not so widespread. The very acidic spots in the examination area became very clear. A spot of well-defined acidity is noticed in the south-eastern part, which was very different from the neighboring sections, these were points 83-84 and 88. If this had occurred in only one sample, then

according to the methodology would have condensed the soil sampling grid in smaller squares around this point to determine if it was an analytical error or accidentally a small area of acidic soil that was found during the sampling with the probe. However, this acidity was found in three adjacent samples, it is considered as correct.

Table 1. Results of laboratory analyzes

No.	ox (UTM)	oy (UTM)	pH	Al meq/100	H meq/100	Mn meq/100	Ca meq/100	Mg meq/100	V3 %	NN kg/da
1	428522	4591963	3.94	1.14	0.08	0.45	4.84	1.70	79.7	300
2	428554	4592001	3.95	1.27	0.08	0.41	3.60	1.90	75.8	500
3	428588	4592039	3.95	1.28	0.09	0.57	3.95	2.25	76.2	500
4	428619	4592078	3.94	1.02	0.07	0.58	4.98	1.85	80.4	300
5	428563	4591930	3.97	0.82	0.05	0.52	5.22	1.65	83.2	200
6	428596	4591970	3.92	0.79	0.05	0.54	3.44	1.70	78.8	300
7	428625	4592006	3.95	1.12	0.07	0.38	4.50	2.00	80.5	300
8	428653	4592043	4.02	1.08	0.07	0.39	4.02	1.95	79.5	300
9	428692	4592082	3.96	0.72	0.05	0.37	4.24	1.65	83.8	200
10	428605	4591898	3.98	0.86	0.06	0.47	3.98	2.05	81.3	300
11	428634	4591938	4.04	1.25	0.08	0.40	4.04	1.70	76.8	500
12	428664	4591978	3.93	1.01	0.07	0.48	4.27	1.60	79.0	300
13	428693	4592009	4.04	0.87	0.06	0.33	4.61	1.80	83.6	200
16	428637	4591865	3.99	1.21	0.08	0.38	4.34	2.10	79.4	300
17	428672	4591902	4.01	1.19	0.08	0.45	4.29	1.95	78.4	300
18	428702	4591939	3.97	1.09	0.07	0.56	3.69	2.20	77.4	300
22	428672	4591837	3.98	0.99	0.07	0.36	4.89	2.00	82.9	200
23	428706	4591872	3.99	1.11	0.07	0.57	4.90	2.00	79.8	300

Figures 5, 6 and 7 show the content and spatial distribution of the different soil sampling points, respectively: exchangeable aluminum, manganese, calcium and magnesium in different parts of the terrain, as in a color scale, the values are displayed on the side.

Figure 8 shows the soil saturation degree with bases in different parts of the terrain, as a result of which the lime necessity of the soil was determined.

Despite the low pH found in relatively large areas, the strong lime demand is limited in very small areas. In principle, sections with less than 90% should be limed, but the solution is to lime all sections below 95% to create a buffer background.

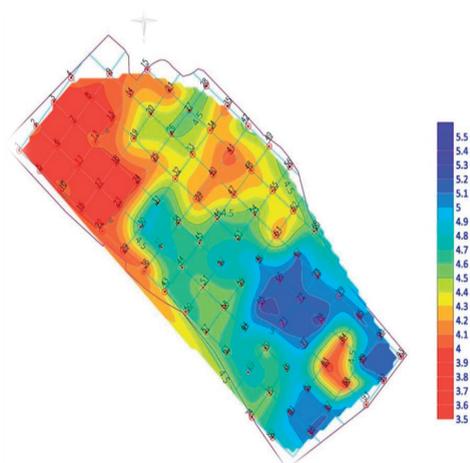


Figure 4. Spatial pH distribution

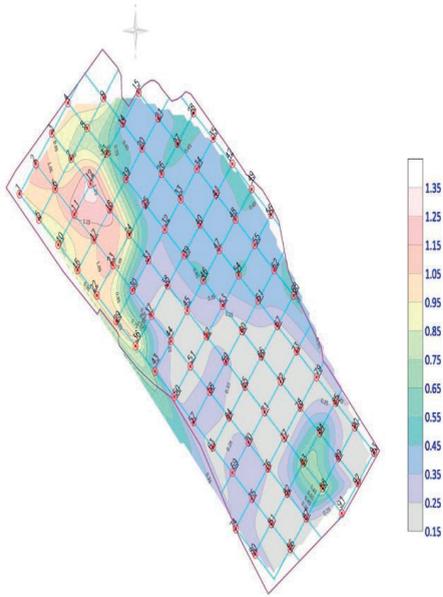


Figure 5. Spatial distribution of exchange aluminum

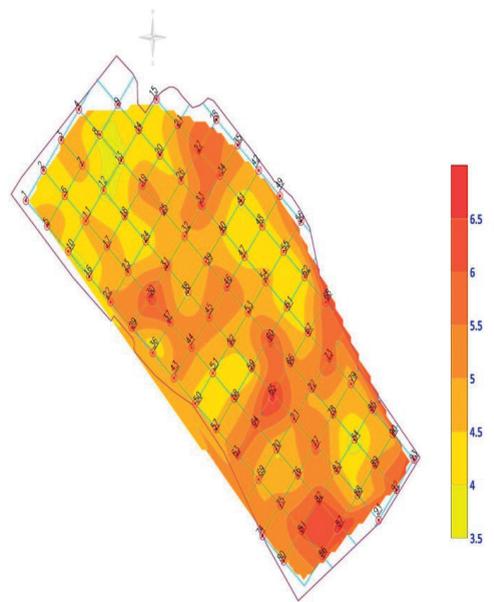


Figure 7. Spatial distribution of exchange calcium and magnesium

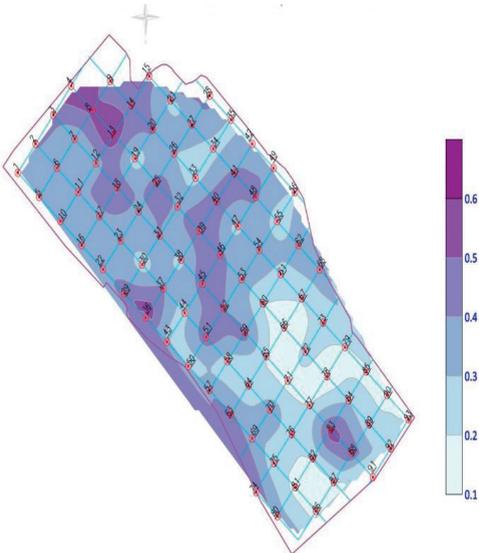


Figure 6. Spatial distribution of exchange manganese

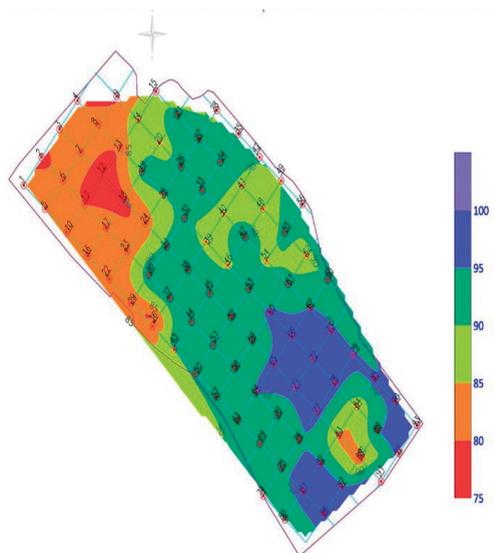


Figure 8. Spatial distribution of the soil saturation degree with bases

In order to achieve equalization of the acidity indicators in the whole area, it is necessary to lime as shown in Figure 9. However, as the different lime necessity sections are outlined, they are not convenient and not efficient for the movement of machines and therefore the solution is to develop a model in approximately the same contours, but already as meliorative areas - Figure 10.

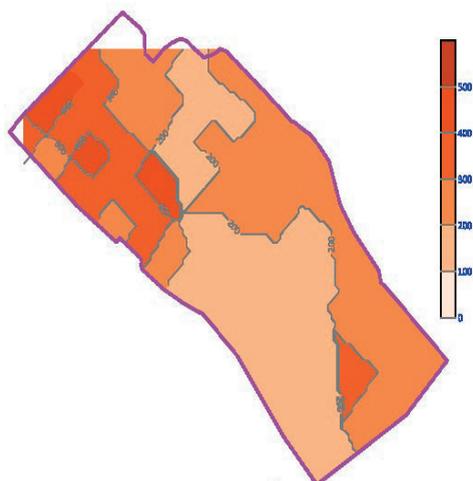


Figure 9. Distribution of the areas of lime necessity

Then the terrain would look like this. Here the contours are twisted so that it can be seen better. The machine will move from left to right and will apply a different amount of ameliorant for each of the already outlined rectangular chemical meliorative subareas. The interrupted line indicates the track of the machine, and the solid line indicates the boundaries of the individual areas and reflects the rate of ameliorant that must be applied for each sector.

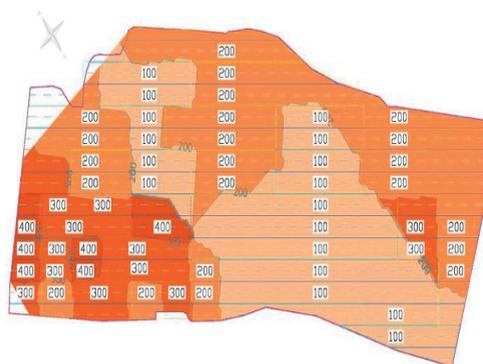


Figure 10. Model of meliorative plots

## CONCLUSIONS

The described method is part of a methodology for complex meliorative effect on the acid-alkaline balance of soils that have the potential of viticulture terroirs, but are characterized by two main disadvantages:

- Contain harmful acidity to the main crops, expressed by the degree of constant sorption positions in the soil with easily mobile exchange bases.
- Characterized by a high degree of heterogeneity in terms of genesis, composition and properties and particularly in terms of acidity.

The heterogeneity of the soil in terms of the indicators characterizing the harmful acidity in the soil must be taken into account not only in the area horizontally, but also in the vertical direction - i.e. the change in the depth of the soil profile. The role of acid-base balance due to the structure of soil acidity is a complex soil component of the terroir. In terms of its relative significance, it is comparable to the importance of the chemical composition of the soil as it determines the dynamics of its components in the soil-plant system.

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## INCREASING IRRIGATION EFFICIENCY AND SOIL PROTECTION BY REUSING EXCESS WATER USING THE CLOSED DRAINAGE TECHNIQUE

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### Abstract

*Elaborating this paper, the authors took into account previous research that highlighted the following: Romania falls into the category of countries with modest exploitable water resources; over 85% of the arable land arranged with irrigation works is fragmented in small plots; soil water deficit is the most important risk factor in agriculture. Watering efficiency depends on the watering method; sprinkling is the main watering method; the concept of water monitoring provides for the complex recovery, and quantitative and qualitative control of water sources; sustainable use of irrigation water requires watering at the optimum time, depending on the optimal real evapotranspiration ( $ET_{Roptim} = K_t \times E_v$ ). It is necessary to know the physical evaporation of water,  $E_v$  and the monthly value of the indicator  $K_t$ , as an average over a series of at least 30 years, for a certain plant. The proposed method captures the irrigation water, so that once the water has passed the root zone it gets absorbed and stored in a modular underground tank. The excess water collected in the modular basin will be reused when the soil and plant sensors will signal the presence of the humidity deficit.*

**Key words:** soil, irrigation, draining.

### INTRODUCTION

As early as 1924, on the occasion of the drought of that year, Gheorghe Ionescu-Șișești, referring to the frequency of drought years in our country, he showed that out of 100 years, 3 were very dry, 58 were dry, 24 were rainy, 5 were very rainy, only 10 were normal (Botzan, 1984). The worst situation is when the soil drought is concomitant with atmospheric drought, as happened in 1946 (Staicu et al., 1977). The negative effects of the drought in July and August are amplified high temperatures, low relative humidity and hot and dry winds (Pleșa, 1979).

In other countries, such as the United States, there are concerns about water supply to agriculture and horticultural crops are of vital importance. Due to the rising costs of water irrigation, it is increasingly necessary to use technological means, management and plant genotypes that can reduce water use in irrigated agriculture (Howell, 2001). There are years when only irrigation saves crops, like it happened in 2007 in the southern part of the country. The purpose of this paper is to present the need for the efficient use of irrigation water

by storing it in retention basins on a land arranged with closed modulated drainage.

### MATERIALS AND METHODS

The influence of full-time and part-time irrigation on the lawn and roses in a dendrological park is sought.

Periodically, the soil moisture reserve is determined from the entrance to the vegetation until the end of October, collecting soil samples which are compared with the measurements given by the weather station, which in addition to gravimetric humidity measures the N, P, K content.

Watering is applied by spraying on the lawn and by dripping on the roses when the soil moisture reserve decreases, a decrease signaled by the sensors with which the weather station is equipped, near the minimum ceiling of 50% of the IUA, at a depth of 80 cm. The water administered in the plots is measured by the meteorological station, watering stopping is when the full norm is achieved on the optimally irrigated variant.

As pedological drought is often associated with atmospheric drought, unfavorable conditions are created for the growth and development of lawns and roses, requiring watering to be applied in April, May, June and July. In the summer months, on the 0-40 cm layer, the soil moisture deficit (% from the IUA) can reach the level of wilting coefficient, therefore a dual irrigation-drainage system with the possibility of storing excess water is an optimal solution. It is also mandatory to assess the quality of the water periodically because water quality is changes over time, under the influence of various environmental and anthropogenic factors. The soluble salt content of irrigation water is between 0.15-3 g/l. If the soluble salt content is 4 g/l, the water becomes harmful to plants. The most harmful salts are carbonate and sodium chloride not exceeding 1 g/l.

The temperature of the irrigation water must be as close as possible to the temperature of the environment in which the plants grow. The irrigation water for the experimental perimeter in Figure 1 comes from a borehole at a depth of about 40 meters, with PREMO type tubes. In the experimental site where the risk factors are represented by both excess and/or water deficit, the hydro-ameliorative arrangement being autonomous, it adapts to the given conditions, excess or deficit of humidity, being reversible. The research of the water recovery solution from the drainage system is first of all a resource research and then an arrangement scheme, more and more used in the countries with hydro-admirable tradition.

The water quality monitoring equipment for lawn and rose cultivation is made at the experimental model phase. It has the following composition:

- hydraulic measuring circuit laid on a type fixing system panel, on which the sensors are located for determination physical and chemical parameters of water: turbidity, electrical conductivity at 25 degrees C, Na<sup>+</sup> ions, Cl<sup>-</sup> ions; a sensor must be included for nitrates;
- Devices for connecting the sensors to the circuit measurement: turbidity sensor mounting device; pH sensor glass; vat electrical conductivity sensor, thermostatic bath for ion sensors selective chlorine and sodium;
- Collection tank with drain pipe;
- Filtration system;

- Solenoid valve for purging the filtration system;
- Fittings and passage valves.

From the turbidity transducer, water passes through a filtration system which removes solid suspensions based on the attraction exerted by granules of filter media and the effect of surface stresses created at passing water over the granular filtration bed. In case of water recirculation for laboratory analysis the water is stored in a vessel that must provide a time of stationary both to ensure the amount of water needed for cleaning filter as well as to ensure a long system life filtering. The filtration system goes through a cleaning process once per day. The cleaning process can be started automatically or by manual control. The vessel in which the water is recirculated is fed by an electric pump (Q = 60 l/h; H = 10 m H<sub>2</sub>O).

Automatic compensation of temperature influence on the measurement of pH and electrical conductivity is done by software means taking into account the measured value of the temperature. The concentration of sodium and chlorine ions is measured at constant temperature, their mounts being inserted in a tank with a thermostat with continuous stirring. The drainage system that ensures reversibility is modular and has in its componence (Figure 1):

- draining prisms (height = 1.0 m, width = 0.25 m);
- reflated absorbent drains (diameter = 110 mm);
- PVC collector drains (diameter = 250 mm);
- Fittings, plugs, dampers.

## RESULTS AND DISCUSSIONS

The parameters below allow us to easily appreciate water quality, which in order to be good for irrigation, must fall within the limits shown below:

- Mineralization, g/l <1;
- $Mg = [Mg / (Ca + Mg)] \times 100\% < 50\%$ ;
- $Na = [Na / (Ca + Mg)] \times 100\% < 70\%$ ;
- $Na = [Na / (Ca + Mg + Na)] \times 100\% < 50\%$ ;
- Na/Ca ratio <1;
- Potential sodium absorption coefficient (SAR) <10.

The quality of water for irrigation depends on the total content of salts, the nature of the salts

present in the solution and the proportion of Na of Ca, Mg, bicarbonates and other cations.

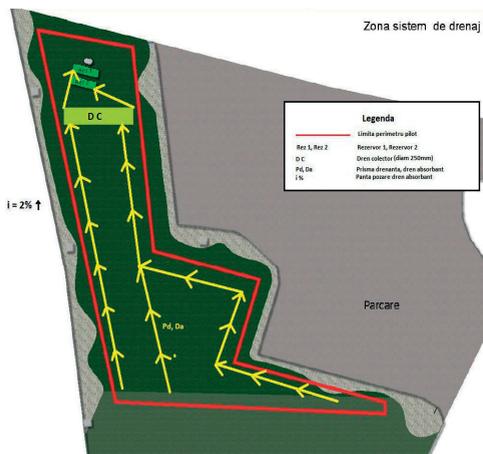


Figure 1. Drainage system

The water source used to irrigate the experimental perimeter comes from a borehole dug at a depth of about 40.0 m. The site under study is equipped with dual irrigation-drainage system in order to achieve a local arrangement, with groundwater supply (drilling), characterized as follows:

- **Geographical and geomorphological characterization**

The studied perimeter is located within the city of Arad located in the northwestern part of our country and is part of the Tisza Plain, named after the main collector, which flows into the rivers in northwestern and western Romania. The northern plain of the Tisza is bounded on the west by the Hungarian border, and on the east by the hills that make up western Piedmont, stretching in some parts to the mountains.

- **Climate characterization**

The northern plain of the Tisza, which also includes the city of Arad, is characterized by a moderate continental climate, due to the Carpathian chain that prevents the entry of cold air from the north and east in winter. Thermal regime: The average annual temperature is 10.8°C, in the Arad area, and the average winter temperature is around -1.1°C, one month of the year registering negative values. Precipitation regime: The amount of precipitation falling annually in the northern Tisza Plain decreases

from north to south (630 mm in Satu Mare, 483 mm in Chişinău-Criş) and from east to west (640 mm in Ineu, 483 mm in Chişinău-Criş). The analysis of climatic factors shows the tendency of arid soils due to climatic excesses, on large areas in the southern part of the country and moderate in the rest of the territory (Figure 2).

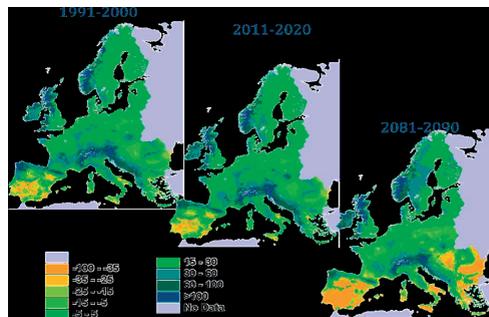


Figure 2. The trend of the average annual precipitation over three decades: historical data (1901-2000) and modeling over two periods: 2011-2020 and 2081-2090 through the Indicator “Cumulative average annual water deficit” (ICPA source)

The configuration of these changes is similar to that observed during the twentieth century. It is "very likely" that the trend of increasing the values of extreme maximum temperatures and increasing the frequency of heat waves will continue.

- **Hydrographic characterization**

The northern Tisza Plain is bordered by a network of waters running from east to west. This whole network is tributary to the river Tisza. The hydrographic network has a medium density. Most of the plain has a density of 0.1-0.3 km/km<sup>2</sup>. The studied area is located in the Mureş river basin, the average slope is 1.5‰, and in the plain 0.2‰, the length of the river is 718.5 km, the surface of the hydrographic basin on the territory of Romania is 27,919 km<sup>2</sup> and the altitude of 90 m (Figure 3).

- **Hydrographic system**

In the studied area, the studied hydrographic system is represented by the Mureş River. It flows in the plain at a distance of about 90 km. It has abandoned riverbeds that spread like a fan both north and south of the current riverbed. It has no major tributaries in this sector. The average flow of Mureş is 169 m<sup>3</sup>/s in the Arad

area. During the summer, the volume of water decreases considerably, reaching a minimum flow of 13 m<sup>3</sup>/s. The hydrological characterization of the Northern Tisza Plain consists of clayey alluvium in the low plain, where the Mureş Plain is located, which also has the greatest hydrogeological differentiation. Groundwater is found near the soil surface, at 5-10 m and is poorly mineralized (salts <1 g/l). In Arad Park, where the soil has a large amount of colloidal clay, the groundwater is at a depth of 3-5 m and is poorly mineralized, but with salts > 1 g/l, and drainage is good.

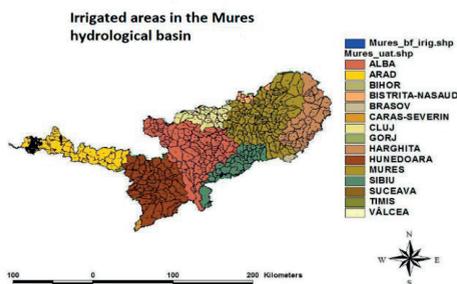


Figure 3. Irrigation potential of the Mureş river basin (ICPA source)

### • Hydrogeological characterization

The most important groundwater aquifers in the Arad area are located in the Holocene deposits (represented by sandy clays, silts, sands, gravels) in the Mureş meadow and in the upper Pleistocene-Holocene deposits in the Mureş alluvial cone. The groundwater aquifer in these deposits are local sources of water supply. The direction of groundwater flow in the area is oriented, from west to east, to the Mureş River. We also considered the improvement of the properties of the saline soil known for the fact that saline soils have physical, chemical and biological properties unfavorable to the growth and development of plants in general and horticultural plants in particular.

Due to the high content of exchangeable sodium, there is a dispersion of soil colloids, which leads to the destruction of the structure. This entails an extremely low permeability for air and water, the halomorphic soils being compact, difficult to work. The high content of soluble salts of these soils increases the osmotic pressure of the solution beyond the limits of water accessibility for plants, which leads to a deficit in their water supply.

The suction force of the plant roots can be a maximum of 14 atmospheres, and the suction force of a strongly salinized soil can reach 200 atmospheres, far beyond the possibility of supplying the plants.

The chemical properties are influenced by the high content of slightly soluble salts, sodium chlorides and sulfates, carbonates and bicarbonates of Na<sup>+</sup>, Ca<sup>+</sup>, and Mg<sup>+</sup>, which in the quantities in the soil can only be supported by a very small number of adapted plants.

Salinity and alkaline reaction also cause changes in the processes of absorption of nutrients from the soil by plants.

Under these conditions the nitric form of nitrogen is absorbed to a lesser extent compared to the ammoniac form. The explanation for this selectivity is the increase in the activity of OH<sup>-</sup> ions in the soil solution, which prevents the exchange of anions from the surface of the root hairs. High concentrations of Na<sup>+</sup>, disrupt plant nutrition and prevent the absorption of other species of cations, Ca<sup>2+</sup> and K<sup>+</sup>, partially replacing them.

Plants have a different capacity to withstand soluble salts in the soil: some are very sensitive, others more resistant, and some of them, salt plants, are well adapted morphologically and physiologically, to excess salts. Roses have a low tolerance to soluble salts in the soil. This plant trait is called "salinity tolerance."

The following indices are used to characterize the salinity tolerance of plants:

- lower tolerance limit for salinity 60-90 mg soluble salts/100 g soil;
- upper limit of tolerance to salinity 200-700 mg soluble salts/100 g soil;
- tolerance range;
- agronomic tolerance.

Roses have the highest sensitivity to the negative effect of salts immediately after planting. Seedlings that do not possess the ability to soak in water under conditions of high osmotic pressure cannot grow.

### Criteria for correcting the alkaline reaction of the soil

In order to establish the need to amend the saline and alkaline soils, the following criteria are used:

- pH, in aqueous suspension - pH values greater than 8.5 indicate the presence of

NaCO<sub>3</sub> or a high exchangeable sodium content in the colloidal complex;

- the total content of soluble salts - determined conductometrically in aqueous extract with soil ratio: 1:100 solution, is a criterion in establishing measures to improve saline soils, and the content of carbonates and bicarbonates (CO<sub>3</sub> + HCO<sub>3</sub>), greater than 1 me/100 g soil, is used to calculate the dose of amendments, along with the sodium saturation of the adsorbent complex;
- sodium saturation of the adsorbent complex - or the percentage of adsorbent sodium (PSA) is the main criterion in assessing the opportunity to correct the alkaline reaction, while serving to calculate the dose of amendments:

$$PSA = \frac{meNA \cdot sch}{meT} \cdot 100$$

Soils with an adsorbent sodium content greater than 10% of the total exchange capacity (T), the sum of alkaline carbonates and bicarbonates greater than 1 me/100 g soil, need to be amended to correct the alkaline reaction.

- urgency to apply gypsum amendments (GU) - It can be evaluated with the help of the index, the percentage of adsorbing sodium (P.S.A.) for all cultures according to the relation:  $UG = 4.0 - 0.1 \times PSA$ .

The first soils that will be subject to the fine are those with the lowest UG values: the value "0" (zero) indicates the highest urgency; value 1-2 = high urgency; 3 = medium urgency; 4 = amendment not required.

### Materials used to correct the alkaline reaction

Depending on their origin, the materials used to improve the properties of saline and alkaline soils are grouped as follows:

- a. actual amendments: gypsum, sulfur, lignite, limestone;
- b. chemical preparations: H<sub>2</sub>SO<sub>4</sub>, aluminum sulphate, iron sulphate;
- c. industrial waste: phospho-gypsum, defecation foam, industrial residues containing iron sulphate and aluminum sulphate, residues from the manufacture of furfural, residual waters from the manufacture of sulfuric acid.

**Native gypsum**, CaSO<sub>4</sub>•2H<sub>2</sub>O, is found in nature in an amorphous or crystallized state, having a white-yellow or gray color. It is used to improve solonchaks and sodium solonchaks. The material is finely ground so that 70-80% of the particles pass through the sieve with 0.15 mm mesh and the rest through that with 1 mm mesh. Contains 15-18% S and about 31% CaO. It is sparingly soluble in water. It solubilizes slowly in the soil and participates in the replacement reactions of exchangeable Na in the calcium adsorbent complex and in the neutralization of the alkaline reaction.

The replacement of Na from the colloidal adsorbent complex with Ca favors the coagulation of the soil colloids, and the calcium humate that is formed and CaCO<sub>3</sub> produce the leaching of the particles into stable aggregates. This improves the circulation of air and water in the soil. Sodium sulfate is a neutral salt, not harmful to cultivated plants when it is in small amounts. When large amounts result, Na<sub>2</sub>SO<sub>4</sub> must be removed by freshwater irrigation.

**Gypsum** can also be used to improve salts that contain a lot of Mg in exchangeable form or as salts in toxic concentrations. In Romania, the natural gypsum reserves are very high.

**Phosphogypsum** is a residue that results in wet phosphoric acid production and the manufacture of trisodium phosphate. Contains 75-80% CaSO<sub>4</sub>•2H<sub>2</sub>O and 3-8% P<sub>2</sub>O<sub>5</sub>. Due to the phosphorus in its constitution, it gives superior results to gypsum when applied as an amendment on saline soils. The disadvantage of phosphogypsum is that, after evacuation from the installations, it presents itself as a sludge that is difficult to wilt if it is not placed in small piles. For field application it must contain at most 10% humidity.

Compared to gypsum, whose acidification value is 100%, phosphogypsum has an acidification value of 75-80%.

**Calcium chloride**, CaCl<sub>2</sub>•6H<sub>2</sub>O, obtained as waste at soda factories. After application to the soil, calcium replaces sodium in the adsorbent complex, giving rise to NaCl which can be removed from the soil profile by washing with fresh water.

**Native sulfur** is rarely used as an amendment because it is expensive but has good efficacy. By oxidizing it under the action of thiobacteria, sulfuric acid results, which, reacting with NaHCO<sub>3</sub> or Ca (HCO<sub>3</sub>)<sub>2</sub>, gives rise to salts.

### **Formulas for calculating the doses of gypsum amendments**

The amount of amendments applied to alkaline and saline soils must ensure that the sodium ion moves out of the colloidal complex, so that it represents less than 10% of the cation exchange capacity. Dose amendments are established using calculation ratios applied to soils with a carbonate and bicarbonate content <1 m/100 g soil.

$DAG = 0.086 (Na^+ - 0.1 * T) * h * DA * 100 / CGA$   
DAG - dose of plaster amendments

0.086 grams per gypsum

$Na^+$  - changeable Na content in the soil complex

0.1 - Na content tolerated by plants

T - total cation exchange capacity

h - the thickness of the soil layer desired to be improved

DA - bulk density

CGA - the plaster content of the amendment

For soils with carbonate and bicarbonate content > 1 me/100 g soil the  $DAG = 0.086 (Na^+ - 0.1 * HCO_3^- - CO_3^{2-} * T) * h * DA * 100 / CGA$

### **Soil improvement in the experimental perimeter**

The soils in the dendrological parks are subjected to intense salinization and alkalization processes as a result of the accumulation of salts coming from the irrigation water as well as from the organic fertilizers that are administered in high doses. Because of this, in the short time since the commissioning of the parks, even if the initial reaction of the soil was weakly acid-neutral, it tends to become neutral-alkaline and even alkaline which leads to poor crop development.

When the pH value, determined in aqueous suspension, in the ratio soil: water of 1:5, exceeds the value of 7.5, it is necessary to apply the amendments.

In order to research the alkaline reaction of park soils, strong acid oligotrophic peatlands with a pH between 3 and 4.5, and hydrolytic acidity around 90 m per 100 g soil, as well as mesotrophic peatlands with pH=5 are used as amendments. 0-5.5, from Miercurea Ciuc, Paraul Rosu, Mandra.

The doses of peat administered are conditioned by the sum of the exchange bases, the degree of saturation in the bases, the hydrolytic acidity of the peat and the thickness of the soil layer

desired to be improved. Also in order to correct the alkaline reaction of the soils in the parks, the forest compost can be used, resulting from the bark of softwood or beech, it has a pH of 4.5-5.5. The doses required for the amendment can be calculated using the same reactions as in the case of peat.

### **Fertilization of degraded soils in parks**

Organic fertilization is the main agrotechnical measure by which the soil humus regime is positively influenced. Organic fertilizers with a solid consequence, as well as plant residues left in the soil from flower crops, are sources of raw material for nutritious humus, but also for the synthesis of stable humus. Both contribute, along with other links in plant cultivation technologies, to maintaining or increasing the humus content of cultivated soils.

### **Organic fertilizers with fertilizing and/or ameliorating value**

There are many organic fertilizers that can be used in our country. The group of vegetable fertilizers includes composts, green manures and peat fertilizers. Animal manure, produced in the household and in the industrial animal husbandry system, consists of manure, urine, manure and sludge, compost, turbidity and wastewater, respectively. The residual origin has sludge from urban and industrial wastewater treatment plants, composts resulting from them as well as household waste. Currently in our country, as well as in other countries, organic fertilizers of animal origin are the most widely used.

Peat and composts of animal and vegetable origin are used to stimulate the growth of rose cuttings. The introduction of plant residues into the soil, for improvement purposes, is not a common practice. The use of sludge and compost from various treatment plants on agricultural land has gone beyond the experimental stage.

*The ameliorating effect* of organic fertilizers is due to the appreciable contribution of organic matter, which consists of both easily and hardly degradable compounds. The more stable fraction of organic matter, consisting mainly of lignin, persists longer in the soil, causing the lasting effect of organic fertilizers and soil improvement, including the humus regime.

With the exception of semi-liquid (turbid) and liquid organic fertilizers (urine, manure, sewage), whose organic matter is completely readily biodegradable, all organic fertilizers with solid consistency constitute to a greater or lesser extent in soil improvement. Comparatively the efficiency of equal doses of organic substances introduced into the soil such as manure, roots, cereal straw, green manure, leaves, wood sawdust and Sphagnum peat in increasing the humus content is inserted in order 1; 0.55; 0.45; 0.35; 0.25; 2; 2.5. Numerous experiments have shown that 1/5 of the dry mass of traditional manure and only 1/8 - 1/9 of the mass of straw is converted into humic substances.

Of the organic fertilizers of animal origin, cattle manure contributes the most to the formation of stable humus, as it contains the highest amount of lignin relative to the organic substance. In relation to the animal species, the proportion of hardly biodegradable compounds in manure increases from animals fed with concentrates (birds, pigs) to animals fed with coarse manure (horses, sheep, cattle); In the same sense, the effect of organic fertilizers with solid consistency from these species in the long-term improvement of the soil also increases. The ameliorating effect of sludge from industrial livestock complexes also depends on the presence or absence of bedding and the total amount of unused feed. Composts from sludge of non-zero origin, plant residues and other additives, subjected to aerobic fermentation directed for several years, bring into the soil a significant amount of humic substances already formed during the composting process, contributing substantially to the complex improvement of soil properties. At present we have in the experimental phase the use of biocompost obtained from food residues that also contributes to the improvement of the soil structure and to the increase of permeability for water and air, an extremely important property to consider in the cultivation of roses and ornamental plants.

## CONCLUSIONS

The advantages of using groundwater for irrigation are the following:

- low costs for pumping water (wells are drilled near the agricultural areas to be irrigated);
- low electricity costs for water pumping;
- the possibility of operating on a diesel generator;
- automation and control of flow and pumping pressure;
- low maintenance costs.

## Recommendations for the use of groundwater for irrigation

In order to guarantee the real prospects for the use of groundwater for irrigation, it is necessary to carry out a feasibility study based on the results of hydrogeological research, groundwater monitoring and the physico-chemical and physical characteristics of the soils. In order to have an efficient and suitable irrigation system, the farmer must choose the right system for the given situation in the field, must take into account the costs of purchasing and installing the system, as well as pumping costs, operation and maintenance and periodic inspections.

When choosing the irrigation system, the following are taken into account: type of crop, water requirements, soil type, energy source, location of the water source and financial availability (both for purchase and for operation and maintenance). Small irrigation systems, powered by groundwater, are more efficient in terms of consumption because they are adapted to the needs of each farmer, the main advantages being the savings of electricity and water. Thus, these individual groundwater irrigation systems are more profitable for vegetable farms (vegetable crops in solariums, but also in the field), parks, vine farms and small vegetable farms.

In the conditions of a dry year, by irrigation with rationally dimensioned irrigation norm, good results are obtained for lawns and roses in conditions of maximum efficiency by storing water in retention basins and reusing it.

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## SOIL-SAVING TECHNOLOGIES AND THEIR INFLUENCE ON AGROPHYSICAL AND COLLOID-CHEMICAL PARAMETERS OF CHERNOZEM

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### Abstract

*According to the criteria of destructuring and compaction of agricultural soils, it is possible to establish and improve a set of agricultural measures that will increase their productivity and reduce the cost of growing crops to producers in agricultural enterprises. The structure helps to inhibit the mineralization of organic matter, which is better stored in agronomically valuable units with limited air supply. The more agronomically valuable of structure units, the higher the manifestation of physical factors of soil fertility. Physical condition is one of the most important factors for determining the conditions of growth and development of plants and the value of their productivity, as it determines the formation of water-air and thermal regime of the soil. We offer method of expert assessment of soils according to the criteria of destructuring and compaction of soils in which are in agricultural use.*

**Key words:** soil-saving technologies, agrophysical parameters, colloid-chemical parameters, chernozem.

### INTRODUCTION

All over the world there is an increasing attention to ensuring the proper state of ecosystems, soil fertility, agriculture on the basis of maximum recycling of all farm waste. At the same time, soil conservation technologies play a leading role, combining traditions, innovations and science to improve the environment, prevent soil degradation, develop and implement environmentally friendly farming systems, which together determine the relevance of our research.

Soil-saving technologies help to improve the nutritional conditions of cultivated plants, soil fertility, product quality, increase arable land productivity, have a positive impact on the environment. Implementing ways to produce quality and safe agricultural products in agricultural practice ensures stable production of high quality and safe food and reduces the risk of life and health hazards of food consumers (Makarenko, 2010).

Fertile soil must correspond in its properties to the ecological characteristics of cultivated crops; have in an accessible form the necessary nutrients for plants; have an optimal and stable

supply of moisture; be sufficiently loose and structural, ensure free and deep development of the root system of plants, as well as be air and water permeable; to have optimal heat capacity and thermal conductivity, to be warm enough to ensure the viability of relevant plants; to perceive, accumulate, store and simultaneously and evenly provide plants with water, nutrients, provide plants with conditions of air-thermal and redox regimes (Medvedev, 2017).

The introduction of chernozems in agricultural use has led to dramatic changes and the ratio of almost all soil processes and properties: the entry of organic matter into the soil and its mineralization, physical performance (deterioration of structure) and water regime, acidification and decalcification. Physical indicators as well as humus condition of the soil significantly affect a number of agrophysical properties of the soil, including moisture capacity, heat regime, etc. Therefore, they are the most important factors in soil fertility. In turn, organic farming helps to increase the flow of organic matter into the soil, thus forming favorable indicators in the soil (Zhernova, 2017; Dehtyarov, 2017).

Studying the influence of different phytocenoses on the physical characteristics of typical chernozems, it was found that typical chernozems of the virgin steppe have the most favorable physical characteristics for plants. Plowing of soils leads to deterioration of almost all studied indicators. In particular, in comparison with the absolute virgin land, the density of composition increases by 15-20% and the density of the solid phase of the soil by 2-5%. The ability of soils to withstand the destructive effects of water is also declining (especially in the arable horizon) (Miroshnychenko, 2013).

Typical chernozems of absolute virgin land and fallow land are characterized by the highest content of agronomically valuable water-resistant units. Agricultural use of chernozems for 77 years leads to the dispersal of structural aggregates. Agrogenic chernozems contain four times less agronomically valuable (3-1 mm) water-resistant units and twice as many aggregates <0.25 mm in size in 0-20 cm layer compared to virgin soil (Panasenko, 2015).

Thus, during the long-term agricultural use of chernozems, their agrophysical properties deteriorate, there is a weak degree of compaction in the subsoil horizons, there is a decrease in the number of agronomically valuable aggregates against the background of accumulation of big aggregates. Intensification of agricultural production leads to changes in the particle size distribution of chernozems. The number of silty particles in a number of soils increases. This indicates that the growth of anthropogenic load and high doses of mineral fertilizers lead to increased gleying processes and increase the sludge content by reducing the share of fine and medium dust fractions (Bezuhlova, 2006).

Shikula (2004) and other scientists have scientifically substantiated ways to abandon chemicalization, but with the use of organic farming, which can not only keep yields at the previous level, but also significantly increase it. Organic farming system, on the other hand, helps to improve agrophysical and agrochemical indicators of soil fertility, reduce weeds by 25-40% and the number of pests, increase soil biological activity by 6.5-7.5%, reduce reduction of nitrate content in agricultural products by 10-12% compared to

products grown by traditional technology (Antonets', 2010).

The aim of the work was to study some physical characteristics, the content of humus and its colloidal forms in chernozems typical of soil-saving technologies.

## MATERIALS AND METHODS

The farm "Agroecology" is located in the village Mikhailliki of the Poltava region (49°29'52.2"N 34°18'50.6"E). The total land use area is 8026 ha. Of these, agricultural land - 3290 hectares, including: arable land - 2909 hectares, hayfields - 50.1 hectares, pastures - 201 hectares. Plowing of agricultural land is about 92%.

At the heart of the unique technology of private enterprise (PE) "Agroecology" - shelf less tillage, which does not disturb the soil structure, retains moisture, maintains temperature, creates conditions for the life of soil biota. The farm is working on the creation of tillage units that would best meet the needs of such agriculture. In "Agroecology" introduced a unique system of organic farming, the foundation of which was laid in the 70-s of the twentieth century. Thus, for more than 40 years it has been managed in cooperation and harmony with nature, using only organic technologies. Due to this, the company has clean, healthy, fertile soils and produces organic products.

The soil cover of the farm is represented by typical medium-loam chernozem. 5 variants were selected for the study: fallow land, control (without fertilizers), organic fertilizer system, green manure system and mineral fertilizer system. Individual samples were taken every 10 cm to a depth of 50 cm.

Variant 1 - fallow - 30 years without tillage.

Variant 2 - control (without fertilizers). Culture: winter wheat crops ("Vidrada" 250 kg/ha). Predecessor: sainfoin of the third year of cultivation, yield 10 t/ha. Tillage: the predecessor was harvested for haylage, stubble peeling with 3-4 cm disc harrows, cultivation 4 cm, moisture closure 5 cm, winter wheat sowing 250 kg/ha.

Variant 3 - mineral fertilizer system. Culture: sugar beet. Predecessor: winter wheat, yield 3.2 t/ha. Tillage: plowing to a depth of 30 cm.

Variant 4 - organic fertilizer system. Culture: corn MIA. Predecessor: radish and oats for fodder, yield 15 t/ha. Tillage: moisture closure 5 cm, pre-sowing cultivation by 8 cm, sowing - 7 cm.

Variant 5 - green manure system. Culture: winter wheat crops ("Vidrada" 250 kg/ha). Predecessor: vetch on green manure, yield 15 t/ha. Tillage: closing of moisture by 4 cm, sowing of winter wheat without tillage (Tarasen complex seeder 8 m).

The study was conducted by the following methods:

- the density of the composition of the intact soil by the method of the cutting cylinder according to N.A. Kaczynski;
- density of the solid phase of the soil by pycnometric method;
- porosity - calculated (according to the density of the composition and the density of the solid phase of the soil);
- structural-aggregate composition and water resistance of structural aggregates by the method of fractionation on sieves according to M.I. Savvinov;
- coefficients of structure and water resistance - calculated;
- the content of general humus according to I.V. Tyurin in the modification of V.M. Simakova;
- content of colloidal forms of humus (active and passive) by the method of O.N. Sokolovskyy.

## RESULTS AND DISCUSSIONS

**Soil density.** The results of the study of soil density (Table 1) showed that fallow as a natural ecosystem has optimal performance.

Table 1. Soil density, g/cm<sup>3</sup>

Depth of soil, cm	Variant				
	without fertilizers		fertilizer system		
	fallow (30 years)	control	mineral	organic	green manure
0-10	1.03	1.01	1.18	1.00	1.09
10-20	1.11	1.18	1.19	1.08	1.14
20-30	1.18	1.22	1.18	1.11	1.17
30-40	1.23	1.25	1.24	1.21	1.21
40-50	1.23	1.25	1.25	1.23	1.24

Thus, in the layer of 0-10 centimeters, the density is 1.03 g/cm<sup>3</sup>, and with depth this figure

increases to 1.23 g/cm<sup>3</sup>. This is due to the loosening effect of the root systems of natural grass vegetation in the conditions of fallow.

The use of organic fertilizer system helps to reduce the density of 0.01-0.11 g/cm<sup>3</sup> of chernozem typical of this variant compared to the control soil. Chernozem in the organic system of fertilizer in terms of density is close to the variant of fallow throughout the study thickness.

When applying the sidereal fertilizer system, there is an increase in the density of chernozem from a depth of only 30 centimeters to 1.21 g/cm<sup>3</sup>. Thus, the use of green manures has a positive effect on the soil density of typical chernozem and brings the value closer to the soil in natural conditions (fallow).

Under the conditions of the mineral fertilizer system, the arable layer is compacted in comparison with the organic and green manure systems, but the greatest compaction is undermined by the subsoil horizon (1.24-1.25 g/cm<sup>3</sup>). This is due to the formation of the plow sole.

### Density of the solid phase of the soil.

Examining the density of the solid phase of the soil of typical chernozem (Table 2), it was found that the fallow land as a natural ecosystem has the lowest values for the entire soil thickness (2.45-2.58 g/cm<sup>3</sup>). This is due to the greater humus content of the soil of the fallow area.

Table 2. Density of the solid phase of the soil, g/cm<sup>3</sup>

Depth of soil, cm	Variant				
	without fertilizers		fertilizer system		
	fallow (30 years)	control	mineral	organic	green manure
0-10	2.45	2.48	2.48	2.46	2.48
10-20	2.45	2.52	2.48	2.46	2.48
20-30	2.45	2.52	2.48	2.46	2.50
30-40	2.53	2.56	2.56	2.53	2.53
40-50	2.58	2.60	2.60	2.58	2.60

In the organic and green manure fertilization system, the density values are close to the values of the fallow area, but they are slightly higher, especially in the green manure fertilization system (2.46-2.58 and 2.48-2.60 g/cm<sup>3</sup>). Thus, the action of organic fertilizers and green manures has a positive effect on the

density of the solid phase of the soil. The control variant has the highest values (2.48-2.60 g/cm<sup>3</sup>) in comparison with all studied variants. This is due to the low humus content in this study.

**Porosity.** Porosity depends on the particle size distribution, structure, content of organic matter. In arable soils porosity is due to cultivation and cultivation techniques. As a result of any loosening of the soil, the porosity increases, and in the case of compaction decreases. The more structured the soil, the greater the overall porosity (Hakansson, 2000; Makarenko, 2010).

Studies show that on a scale of Kaczynski's duty cycle in all variants of the study is assessed as "satisfactory" and ranges from 51-60% (Table 3).

Table 3. Porosity, %

Depth of soil, cm	Variant				
	without fertilizers		fertilizer system		
	fallow (30 years)	control	mineral	organic	green manure
0-10	58	59	52	59	56
10-20	55	53	52	56	54
20-30	52	52	52	55	53
30-40	51	51	52	51	52
40-50	52	52	52	52	52

In the layer of 0-10 centimeters of all studied variants (except for the mineral fertilizer system, which has a porosity index of 52%), the highest soil porosity index, which is in the range of 56-59% and is assessed as more satisfactory.

Variant control does not have a high rate of porosity, except for 0-10 cm layer, where the porosity is 59%. This value is close to the variant of the mineral fertilizer system, which is due to the root system of cultivated crops (sowing of winter wheat).

Fallow and organic fertilizer system have the highest rate of soil porosity to other studied variants, and with depth the indicator tends to decrease insignificantly.

Sidereal fertilizer system in terms of porosity is almost not inferior to organic fertilizer system (52-56%).

When using the mineral fertilizer system, the porosity index is low and the value of 52% of

the entire studied thickness is 52%, which is a less satisfactory estimate compared to all the studied variants.

**Soil structure.** Analysis of the obtained data on the aggregate composition of typical medium loam chernozems for different fertilization systems showed that the content of agronomically valuable aggregates (0.25-10 mm) is maximum in the soil of the fallow compared to control and fertilizer variants and is 93.3-91.0%. This is due to the high content of humus, the presence of a high proportion of detritus in its composition, as well as the structure-forming effect of the root system of plants in the natural ecosystem. At the same time, structural aggregates >10 mm in size make up a very small share, which is the lowest figure of all the studied variants. The reason for this is the dense, very branched root system of herbaceous vegetation, which mechanically prevents the formation of aggregates >10 mm. With a depth (20-50 cm) the number of units >7 mm increases almost twice.

Agricultural use of chernozems causes an increase in the content of structural units in the soil >7 mm and a decrease in the content of aggregates 1-5 mm. This is especially evident in the upper part of the soil control profile (0-20 cm), in the part that is most exposed to plowing. It should also be noted that the upper part of the profile of the control variant is characterized by a higher content of structural units <0.25 mm in the upper part of the profile (0-20 cm).

The application of organic and green manure systems brings typical chernozem of the structural condition of the soil of the natural ecosystem (fallow). This contributes to an increase in the number of agronomically valuable aggregates (10-0.25 mm) compared to chernozem control to 89.20 and 88.61%, respectively (layer 0-10 cm).

In chernozem under the mineral fertilizer system there is a decrease in trends in improving soil structure. The number of structural units with a size of 1-5 mm has not increased significantly, especially for the upper (0-20 cm) part of the soil profile. The content of agronomically valuable aggregates (0.25-10 mm) here is 77.94%. This is due to the method of fertilization and tillage technology.

Thus, with soil-saving agricultural technologies (variants with organic and green manure systems) plowed chernozems of PE "Agroecology" have a content of agronomically valuable aggregates (0.25-10 mm) almost 10% more than chernozem with mineral fertilizer system compared to the variant of control - without application of fertilizers (Figure 1).

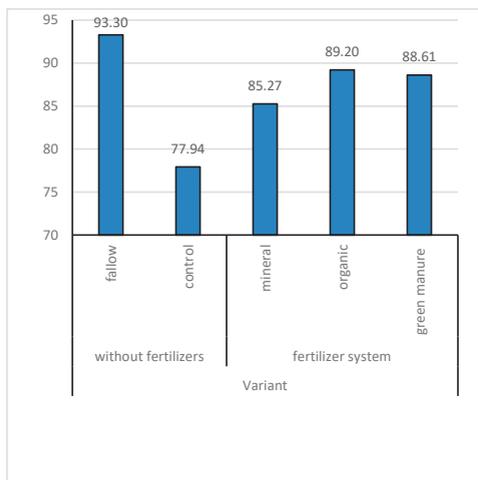


Figure 1. Content of agronomically valuable aggregates (depth 0-20 cm), %

Having studied the soils of the Kharkiv region Medvedev V.V. (2015), based on many years of research, notes that the structural-aggregate composition of typical and common chernozem under the action of high doses of mineral fertilizers becomes stable, despite its deterioration in the first years. Moreover, after 6 years there was even a tendency to improve the structural-aggregate composition of chernozems.

Plowing and agricultural use of typical chernozems causes a sharp decrease in the structural factor, especially in the upper part of the studied soil thickness. Thus, in the 0-20 cm layer of soil control, compared to the soil of the natural ecosystem, the coefficient of structural is 3.5, which is ten times lower than in the soil under fallow (Figure 2).

The use of organic and green manure systems significantly increase the structural factor of typical chernozem. Here the coefficient of structural is 8.20 and 7.77, respectively. This contributes to an increase in the number of agronomically valuable aggregates (10-0.25

mm) compared to chernozem control, where in the layer of 0-20 cm the structural factors 5.78. The degree of structure in agrogenic phytocenoses varies considerably, the physical properties of the soil change.

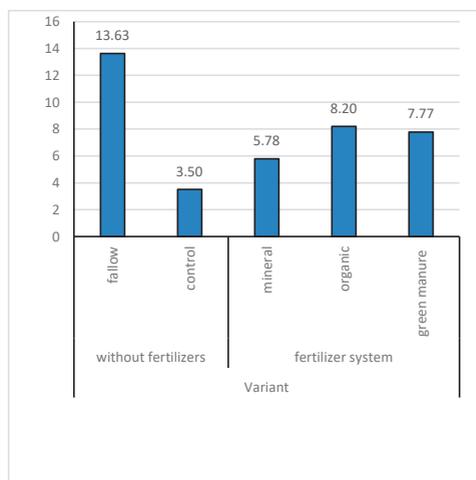


Figure 2. Structural coefficient (depth 0-20 cm)

The quality of the soil structure is determined by its size, porosity, mechanical strength and water permeability. The most agronomical valuable are macroaggregates 0.25-10 mm in size, which have high porosity (over 45%), mechanical strength and water permeability. The formation of a water-resistant structure of chernozem requires a relatively small amount of organic matter, but the differences in the composition of humus in macro and microaggregates are quite significant (Medvedev, 2008).

Content of water-resistant aggregates of chernozems typical of different fertilizer systems shows that the use of organic and green manure systems increases the number of water-resistant structural units compared to the variant without fertilizers.

The 0-10 cm layer of the control variant is dominated by structural units with a size of 1-0.25 mm and <0.25 mm. The smallest number of structural units >3 mm in size, in a layer of 20-30 cm is only 2.3.

In the variant with a mineral fertilizer system in the soil layer 0-10 cm, the largest number of structural units with a size of <1 mm is available. With depth, their number decreases,

and the number of units with a size >1 mm increases.

With an organic fertilizer system, the largest number of structural units with a size of <2 mm in a layer of 0-10 cm. Structural units with a size of >3 mm quantitatively predominate at a depth of 0-20 cm.

In the variant with sidereal fertilization system, the number of structural units of 1-0.25 and <0.25 mm is the largest in the entire studied soil thickness. The smallest number of structural units measuring >3 mm at a depth of 30-40 cm and 40-50 cm.

The highest water resistance is characterized by structural aggregates in the variant of fallow (0-10 cm). In the variant of the mineral fertilizer system, in comparison with all the others variants, the smallest number of aggregates is >3 mm in size (2 times less than in the fallow area).

With an organic fertilizer system, the largest number of structural aggregates with a size of <2 mm in a layer of 0-10 cm. Structural aggregates with a size of >3 mm quantitatively predominate at a depth of 0-20 cm.

In the variant with sidereal fertilization system, the number of structural aggregates of 1-0.25 and <0.25 mm is the largest in the entire studied soil thickness. The smallest indicators are of structural aggregates of size >3 mm at a depth of 30-40 cm and 40-50 cm.

The highest water resistance is characterized by aggregates in the variant of fallow in 0-10 cm soil thickness. In the variant with the mineral fertilizer system, in comparison with all the others, the smallest number of aggregates is >3 mm in size (2 times less than in the fallow area).

The highest coefficient of water resistance is observed in fallow chernozem. In the control variant, the indicators are sharply reduced, especially in the lower part of the soil. A similar decrease in water resistance is observed in the variant with a mineral fertilizer system. Sidereal and organic fertilizer system in the 0-20 cm layer helps to improve the soil structure in contrast to the mineral fertilizer system and compared to the control.

On the cultivated lands the intensity of soil processes intensifies and the soil structure is destroyed. Therefore, the content of agronomically valuable aggregates in

agrocenoses is lower than in the fallow. Thus, under the influence of soil-saving technologies, the physical condition of arable land is significantly improved and becomes more favorable for growing field crops.

The destruction of the soil structure on the control is accompanied by a decrease in the number of units 0.25-10 mm. On annually cultivated lands with mineral fertilization system there is less significant improvement of agronomically valuable structure, fewer structural water-resistant units compared to organic and green manure systems.

**Humus content.** One of the most important diagnostic signs of soil degradation is the reduction of organic matter and its main component - humus. This is due to many reasons, the main of which is the lack of constant compensation of plant residues and organic fertilizers for current costs of organic matter, mainly due to their biological mineralization and changes in the relationship between mineralization of fresh organic matter, formation and stabilization of new humus in soil.

Studies of the content of total humus in typical medium loam chernozems showed that the highest rate on the variant of fallow compared to the control, as well as with all other studied variants. In the 0-10 cm layer it is 6.70%, with depth the humus content decreases, and already at a depth of 40-50 cm the humus content is 4.23%, which is 25% less (Table 4).

Table 4. Content of total humus, %

Depth of soil, cm	Variant				
	without fertilizers		fertilizer system		
	fallow (30 years)	control	mineral	organic	green manure
0-10	6.70	4.20	4.60	5.59	5.41
10-20	5.54	4.14	4.60	5.50	5.25
20-30	5.04	4.10	4.12	4.86	4.45
30-40	4.97	3.90	3.80	3.86	3.84
40-50	4.23	3.61	3.57	3.59	3.55

In the variant without the use of fertilizers (control) there is a tendency to reduce the content of total humus compared to all studied variants.

The results of our research show that the application of organic fertilizers in typical

chernozem increases the content of total humus in the 0-10 cm layer of soil to 5.59%, and with depth its content decreases and is 3.59%.

When applying the mineral fertilizer there is an intensive mineralization, so humus in 0-10 cm 4.60%. This applies to the entire soil profile studied, compared to the control.

The use of sidereal fertilizer system helps to increase the content of total humus, relative to chernozem control. But compared to the organic fertilizer system, the values of total humus content are lower.

Thus, sidereal crops not only increase soil fertility and crop yields, but also reliably protect the soil from erosion. The main advantage of green manures is the high content of organic substances, and they should be considered as a biological catalyst for soil transformations that improve the mineral nutrition of plants (Korzhev, 2011).

**The content of colloidal forms of humus.** The conducted research showed (Table 5) that in typical chernozems under fallow the share of passive forms of humus in the total humus is the highest 2.6%, which is 1.6% more than the control and with depth it decreases to 1.4%.

Table 5. Content of passive forms of humus, %

Depth of soil, cm	Variant				
	without fertilizers		fertilizer system		
	fallow (30 years)	control	mineral	organic	green manure
0-10	2.6	1.0	1.1	1.4	1.3
10-20	2.0	1.0	1.0	0.9	1.1
20-30	1.8	0.9	0.9	1.6	0.8
30-40	1.5	0.7	1.0	0.8	0.9
40-50	1.4	0.6	1.0	1.2	0.9

This is due to the constant influx and accumulation of organic residues that are not alienated from year to year compared to the agrochernozems of the four other studied variants and the accumulation of detritus as one of the most stable components of the organic in soil.

The chernozem of control over the content of passive humus in the studied agrophytocenoses has the lowest values of all variants. Thus, in the 0-10 cm layer of chernozem of this variant,

the passive humus index is 1.0% and decreases with depth to 0.6%.

The application of an organic fertilizer system helps to increase the content of passive humus in typical chernozem compared to the control soil. This is especially true for the upper 0-10 and 20-30 centimeter part of the studied soil thickness where the content of passive humus is 1.4% and 1.6%, which is 0.4% and 0.7% more, respectively, compared to the variant of control. In general, chernozem with the organic fertilizer system is almost close in terms of passive humus to the soil of the fallow area.

When applying the sidereal fertilizer system, there is also an increase in the content of passive humus compared to the chernozem control area, because green manure, like manure is one of the main measures to increase the passive form of humus in the soil. In the 0-10 cm layer of soil, the passive humus index is 1.3%, which is 0.3% more than in the control variant. With the depth of the studied variant tends to decrease the passive humus and at a depth of 40-50 centimeters the content is 0.9%. The application of the mineral fertilizer system of typical chernozem leads to the depletion of the soil of the content of passive humus, where the indicators are close to the control variant (without fertilizers).

According to the results of the study, we can say that variant of fallow and variant of organic fertilizer system the share of passive form of humus in the total humus is the highest. This is due to the constant influx and accumulation of organic residues and the formation of detritus, as one of the most stable components of the organic part of the soil. Sidereal fertilizer system is almost not inferior to the organic system. In variant of mineral fertilizer system, we have the lowest rate of passive humus compared to other fertilizer variants.

Examining the active form of humus (Table 6), we can conclude that in the typical chernozem under fallow, which is a natural ecosystem, the share of active form of humus in the total humus is the lowest.

Variant of control at a depth of 0-10 centimeters has an indicator of active humus 3.20%, and with depth the share decreases and already at a depth of 40-50 centimeters of soil thickness is 3.01%.

Table 6. Content of active forms of humus, %

Depth of soil, cm	Variant				
	without fertilizers		fertilizer system		
	fallow (30 years)	control	mineral	organic	green manure
0-10	4.10	3.20	4.19	3.50	4.11
10-20	3.54	3.14	4.60	3.60	4.15
20-30	3.24	3.20	3.26	3.22	3.65
30-40	3.47	3.20	3.06	2.80	2.97
40-50	2.83	3.01	2.39	2.57	2.65

The mineral fertilizer system in the 0-10 cm layer has active humus - 4.19%, which is 1% more than the control variant. The use of mineral fertilizer system causes less of passive humus and including increases the active form of humus.

During the application of organic and green manure fertilizer system increases the content of active humus chernozem typical, especially in the upper horizons, compared with the control variant. In depth of 30-40 cm, there is a tendency to reduce the active humus compared to the control area.

**Influence of soil-saving technologies on agrophysical and colloid-chemical indicators of chernozem.** Adverse conditions for the preservation and reproduction of humus are the traditional system of agriculture, which is designed to use large amounts of manure and industrial fertilizers. Recently, as a soil protection alternative, a system of sustainable agriculture has been implemented, which provides for the minimization of technical and chemical loads on the soil, with an emphasis on perennial sources and means of managing soil regimes. The latter circumstance determines the restoration of research interest in the involvement of fresh plant material in the renewal of soil organic matter (Tarariko, 2007; Miroshnichenko, 2016).

The results of studies of the qualitative composition of humus in the process of humus formation in virgin and arable soils are contradictory.

Thus, some researchers (Arbyzov, 2001) point to the fundamental preservation of the nature of humus formation and the quality of humus in arable lands in accordance with the original zonal type of soil formation. At the same time, other scientists note a clear dependence of the

quality of humus on the intensity of the impact on the soil of agricultural crops, in particular there are significant changes in the organic part of the soil, namely the formation of more valuable in agronomic terms humus (Dehtyar'ov, 2011; Medvedev, 2017).

Preventing soil degradation and reducing the risk of environmental disturbances in the process of growing crops, comprehensive biologization of agricultural technologies and the priority of organic means of optimizing soil fertility - the hallmarks of modern farming systems. Great importance in solving these problems is given to the use of crop residues, by-products of crops, increasing the share of intermediate and green manure crops, as well as perennial grasses in the structure of crop rotations. Plant residues contribute to the comprehensive - both direct and indirect effects on the physical, chemical and biological properties of the soil, its air, temperature and nutrient regimes (Mukha, 2004). The entry of plant residues into the soil and their transformation into humic substances and organo-mineral complexes is a way of sequestering carbon and reducing the concentration of CO<sub>2</sub> in the atmosphere (Medvedev, 2017). Plant residues are a source of organic matter in the soil, a means of forming aggregates and improving the structure, a factor in regulating the mobility of mineral nutrients, nutrient and energy substrate for microorganisms, producer of low molecular weight soluble organic compounds that are essential for soil metabolism (Demydenko, 2021).

This is confirmed by our research results based on the study of some physical and colloid-chemical parameters of typical chernozem, when using variants of soil-saving technologies compared to other studied variants.

Thus, the density of chernozem typical of the mineral fertilizer system deteriorates compared to the control, there is a compaction of the arable layer, but the greatest compaction is the subsoil horizon, as a result of the formation of the plow sole. The use of organic and green manure systems on agrochernozems in organic farming, helps to improve density of soil.

The most optimal indicators of the solid phase of the soil belong to chernozem under natural vegetation (fallow). Variants of agrochernozems

are characterized approximately equally by the density of the solid phase of the soil, throughout the studied soil thickness. Some differences are noticeable only on the variants of chernozem under fallow and chernozem with organic fertilizer system. This is due to the increase in humus in these variants.

Indicators of porosity on the studied variants vary within optimal limits. With depth, the porosity decreases. In the 0-10 cm layer, the variant with an organic fertilizer system is slightly higher due to the application of organic fertilizers, in contrast to the variant with a mineral fertilizer system. The upper layer of chernozem control also has a high porosity, which is associated with the cultivation (winter wheat).

The maximum content of agronomically valuable units (0.25-1 mm) is characterized by a 0-20 cm layer of the fallow area, as a variant of natural phytocenosis, due to the high content of humus and the presence of roots of natural vegetation. Plowing virgin chernozems leads to an increase in the content of fine units (<0.25 mm) and a decrease in the content of agronomically valuable units - a control variant.

This is especially true of the upper part of the soil profile, which is subject to the action of tillage implements. Fertilization certainly improves the structural condition of agricultural chernozems, but not equally. The application of organic fertilizers and green manure crops brings the studied soil closer to the soil of natural phytocenosis in terms of structural condition. And intensive use of mineral fertilizers has less impact on the reproduction of the structural state of typical chernozem. Plowing and agricultural use of typical chernozems is accompanied by a sharp decrease in the coefficient of structure, especially in the upper soil layers. The introduction of the fallow regime contributes to the growth of the structural factor.

Water resistance of chernozem structure, depending on agricultural technologies, has significant differences. Compared to the control in agricultural chernozems, the best indicators of water resistance of the structure are characterized by variants with sidereal and organic fertilizer systems. Chernozem with a mineral fertilizer system has the lowest water resistance coefficient of all the studied variants.

The structure of chernozem typical under fallow (natural phytocenosis) has the greatest water resistance.

The formation of the structure of typical medium loam chernozem depending on different fertilizer systems has some differences. On the cultivated lands the intensity of soil processes intensifies and the soil structure is destroyed. Therefore, the content of agronomically valuable aggregates in agrocenoses is less than in the fallow. Thus, under the influence of soil-saving technologies, the structural condition of arable land is significantly improved and becomes more favorable for growing crops.

The highest humus content in the farm is in chernozem of the fallow area, in the 0-10 cm layer - 6.70%. The use of an organic fertilizer system helps to increase the content of total humus in typical chernozem. Sidereal fertilizer system also increases the content of total humus, but compared to the organic fertilizer system values are it slightly lower. When applying the mineral fertilizer, the humus in the soil decreases compared to all variants.

Studies of colloidal forms of humus have shown that the variant of chernozem under the natural phytocenosis in the total humus has a higher percentage of passive form of humus in contrast to the variants under the influence of agricultural use. Among agrochernozems more passive form in chernozem humus by organic and sidereal fertilizer system, especially for the upper 0-10 cm layer of soil. At the same time, having studied the active form of humus, we can say that in agricultural chernozems the content of the active form of humus in the total humus is almost 2 times higher than the share of passive humus.

## CONCLUSIONS

The variant with mineral fertilizer system showed an increase in the density of composition, the density of the solid phase of the soil, and as a result of reduced porosity, deterioration of the structural condition (number of agronomically valuable units) and water resistance of structural aggregates. In contrast to the previous variant, the physical indicators under fallow, as a natural

phytocenosis and variants for organic and green manure are more optimal.

The use of an organic fertilizer system helps to increase the content of total humus in typical chernozem. The use of sidereal fertilization system leads to an increase in the content of total humus, but compared to the organic fertilization system, the values of the content of total humus are slightly lower. Sidereal crops not only increase soil fertility and crop yields, but also reliably protect the soil from erosion. The largest amount of passive humus is contained in the variants of fallow, and among the variants of agricultural use in sidereal and organic fertilizer systems.

Thus, soil-saving technologies, namely variants with the use of organic and green manure fertilizers contribute to some improvement of physical and colloid-chemical parameters of the soil compared to the variant of mineral fertilization and variant control - without the use of fertilizers.

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# CROP SCIENCES



## AMELIORATIVE EFFECTS OF *Calotropis procera* AMENDED SOIL ON *Fusarium* WILT DISEASE, ENHANCEMENT IN GROWTH AND NUTRITIONAL QUALITIES IN PEA (*Pisum sativum*)

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### Abstract

Commercial fungicides are effective to control fungal pathogens in agriculture but all are associated with ill effects. In multi-years pot and field trials, we investigated the disease suppressing efficacy of *Calotropis procera* against *Fusarium oxysporum*, the causal agent of wilt disease in pea. *C. procera* reduced negative effects of disease and resulted in 41.1 and 52.8% increase in shoot dry weight besides 94.8 and 84% improvement in root dry weight of pea plant, during years 1 and 2, respectively, in pot bioassays. Similarly, *C. procera* amendment increased 25.5% and 17.4% green pod yield under field conditions, in years 1 and 2, respectively. Incorporation of *C. procera* mulches in pea also improved proteins up to 64% and carbohydrates content up to 37.2%. Moreover, iron, calcium and potassium also showed an increased concentration in response to *C. procera* addition. The present study concluded that *C. procera* mulches can be used to manage *Fusarium* wilt disease and to improve nutritional traits of pea.

**Key words:** nutritional, *calotropis*, *fusarium*, *pisum*, wilt.

### INTRODUCTION

Fungi that spoil foods or infect crops can have major socioeconomic impacts, posing threats to food security (Davies et al., 2021). Major food safety issues are related to fungal pathogens (Fisher et al., 2012). Pea (*Pisum sativum*) is a member of family fabaceae. Proteins and amino acid lysine are abundant in pea (Nawab et al., 2008). Higher amount of proteins, vitamins, dietary fibers, antioxidant and carbohydrates have made pea an excellent food source. Pea is among the major cultivated crops in Pakistan with 71,792 tons production over 10478 hectares, due to which it is considered as third most important crop in Pakistan (Achakzai et al., 2006). In Pakistan, pea production is facing many biotic and abiotic threats (Khan et al., 2016). Wilt is a crucial disease of peas in Pakistan (Nawab & Rashid, 2014). Fungal problems in pea are crucial and yield decline ranges between 50-75% (Fisher et al., 2012; Amian et al., 2011). Especially, the root diseases severely impede grain legume cultivation

worldwide (Wille et al., 2019). *Fusarium oxysporum* is responsible for causing vascular wilt in many crops (Dean et al., 2012). *Fusarium* wilt, caused by *F. oxysporum* is a pervasive disease of pea in all pea cultivation fields resulting in complete failure of crop under conducive environmental conditions for the pathogen (Hafez et al., 2014; Aslam et al., 2019).

It is one of the most common fungal threats to pea production in Pakistan leading to substantial economic losses (Hafez et al., 2014).

Disease can be controlled by the use of several management schemes including biological, cultural, and chemical as well as by planting resistant varieties. The quickest and active method to control *Fusarium* wilt disease is with application of synthetic fungicides (Khan et al., 2016). The need of current increased food production to feed a fast-growing human populace is creating a pressure on the extreme use of pesticides but these agrochemicals are contaminating the environment and poisoning food products. Use of synthetic chemicals and

fungicides has been reported to cause serious threats to human life. Moreover, there are reports of fungal resistance against these fungicides. Environmental pollution and toxicity in food products posed by these chemical fungicides is another serious issue.

Use of weeds to overcome plant disease caused by fungi seems the economical and very promising strategy. In an investigation, compost tea and a commercial fungicide (Vitavax-T) was evaluated on controlling soil borne diseases such as wilt of pea plants (cv. Master-B) caused by *F. solani*, *F. moniliform*, *F. oxysporum* and *Macrophomina phaseolina*. These treatments significantly enhanced yield in greenhouse and field assays and protected the pea plants from fungal wilt diseases (Taha et al., 2017). *Calotropis procera* (Aiton) (Vern. apple of sodom, calotrope, and giant milkweed), family Apocynaceae, is an evergreen, perennial shrub, mainly found in arid and semi-arid regions. It is a multipurpose plant, which can be utilized for medicine, and phytoremediation. It has been widely used in traditional medicines (Kaur et al., 2021; Oliveira et al., 2021). Cysteine peptidases from *C. procera* latex were inhibitory (IC<sub>50</sub> of 50 µg/mL) to *F. oxysporum* spores. These peptidases enhanced membrane permeabilization, changes in morphology, leakage of cellular contents, and induction of reactive oxygen species (ROS) in *F. oxysporum* spores (Freitas et al., 2020). *C. procera* contains glycosides (mostly cardenolides), flavonoids, triterpenes, alkaloids, steroids, saponins, proteins and enzymes. These phytochemicals have antioxidant, wound healing and antimicrobial activities (Amini et al., 2021). The *n*-hexane extract of *C. procera* controlled *M. phaseolina*, the cause of charcoal rot in *Vigna radiata*. Spectroscopic analysis of the extract of *C. procera* revealed the presence of chlorocarbon, aromatic hydrocarbon, azocompounds, aromatic carboxylic acids and fatty acids (Waheed et al., 2016). In another investigation, plant extract of *C. procera* at 25% concentration inhibited the mycelium growth of *F. oxysporum* to 87% (Nasrin et al., 2018). There are number of reports describing the antifungal activity of *C. procera* against *F. oxysporum* under *in vitro* conditions, but, there is no report available that describes *C. procera* disease suppressing ability under pot

and field conditions as well as its impact on nutritional values of pea grains. Therefore, the present study was planned to evaluate of disease suppressing ability of *C. procera* under pot and field conditions as well as nutritional enhancement in pea by an eco-friendly method.

## MATERIALS AND METHODS

### *Test plant, fungal pathogen and crop*

*C. procera*, member of family Asclepiadaceae was selected to evaluate the antifungal activity against selected fungal species, *F. oxysporum*. One pea variety “Meteor Faisalabad” was selected as test crop to evaluate the disease suppressing ability of *C. procera* as well as impact on the nutritional quality of pea grains.

### *Culturing of fungal isolate, storage and preparation of inoculum*

The fungal culture was sub cultured on Potato Dextrose Agar (PDA) medium and kept in a refrigerator at 4°C till further use. Fungal inoculum was prepared on dried peas. These peas were washed in running tap water to remove dust or any other impurity with a final rinse with dH<sub>2</sub>O. After washing, these peas were packed in plastic bags at 1 kg/bag. These peas were then soaked in water overnight and then autoclaved at 121°C for 20 minutes. Each bag of chickpea was inoculated with bits of fungal mycelia and spores and mixed well. The inoculated peas were left at 25°C for 21 days.

### *Processing of allelopathic weed*

The *C. procera* plants growing wildy were uprooted at flowering stage. After collection, *C. procera* plants were cleaned under tap water to remove soil and other impurities and then put under fan to remove extra moisture. Then these plants were cut into smaller pieces (~ 3-4 cm) and sun dried for 1 week and then stored in paper bags for further use in pot and field experiments.

### *Pot experiments*

Five treatments were made as T<sub>1</sub> (negative control; where neither fungal inoculum nor *C. procera* powder was added), T<sub>2</sub> (positive control; where only fungal inoculum was added), treatment T<sub>3</sub> comprised dead inoculum of *F. oxysporum*, while T<sub>4</sub> and T<sub>5</sub> comprised fungal inoculum along with 0.75% and 1.5%

(w/w) mulches of *C. procera*, respectively. Total 15 pots were made and for every treatment there were three replicates arranged in a Completely Randomized Design (CRD). 10 kgs of loam soil was put into each pot. Pots were frequently watered and left for two weeks before sowing of pea with hand hoeing twice. 10 seeds of pea were sown in each pot at equal distance from each other. After germination, 3 uniform seedlings were kept in each pot for growth and data collection and 10 chickpea seeds coated with fungal inoculum were agitated with 250 mL of autoclaved water for 5 minutes and this spore suspension was mixed with pot soil yielding  $\sim 2.1 \times 10^4$  colony forming units  $g^{-1}$  of soil as determined by dilution method.

### **Field experiments**

Field experiments were carried out in two growing seasons. For field experiments, area with loam soil was chosen. Plots were prepared adopting all agronomic procedures. Seeds of pea were sown on hills of each plot at 2 seeds/hill. After germination, thinning was carried out for 1 pea plant/hill and total 25 seedlings in each plot were maintained. After maintaining equal number of pea seedlings in each plot, *F. oxysporum* inoculum at 1 kgs chickpeas inoculated with pathogen, *F. oxysporum* were agitated with 10 liters of autoclaved water for 5 minutes and this spore suspension was mixed with soil yielding approximately  $3.6 \times 10^4$  colony forming units  $g^{-1}$  of pathogen as determined by dilution method. 2 kg of fungal inoculated soil was introduced in each plot and *C. procera* mulches were also gently mixed in soil through hand hoeing. There were 3 treatments: T<sub>1</sub> (negative control; where neither fungal inoculum nor *C. procera* was added), T<sub>2</sub> (positive control; where only fungal inoculum was added), and T<sub>3</sub> comprised fungal inoculum along with mulches of *C. procera* at 400 grams/plot. Treatments were arranged in Randomized Complete Block Design (RCBD) and each treatment was replicated thrice. Plots were watered as per requirement by visual observations. No fertilizer was added in any plot and weeds were removed by hand hoeing.

### **Data harvesting**

Data were computed for after removing soil particles, rinsing in tap water and evaporation of

excessive moisture. After this, all the plants were dried in sunlight for 3 days followed by final drying in an electrical oven at 65°C till constant weight and then dry biomass was recorded. Following parameters were recorded in pot experiments; shoot length (cm), root length (cm), shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), and root dry weight (g); while in field experiments, following parameters were recorded viz. plant height (cm), Green pod yield (Mt. ha<sup>-1</sup>), and 100 seed weight. The chemical composition of wheat grains was calculated following AOAC (1970). Carbohydrate content was determined by using the protocol as described by Watson et al. (1975). Concentrations of mineral elements were calculated by atomic absorption spectrophotometer. At harvest, composite soil samples for analysis were taken from every replicate/treatment.

### **Confirmation of Koch's postulates**

From pathogenicity tests conducted in pot and field experiments, pathogen was reisolated and identified again with the help of colony morphology, texture, color as well as conidial size and shape.

### **Statistical analysis**

Statistical analyses (ANOVA & Tukey's Test) were computed by using Minitab-19.

## **RESULTS AND DISCUSSIONS**

### **Pot experiments**

#### **Shoot and root length**

There was 37.5 and 39.4% decline in shoot length of pea in pot experiments during year 1 and 2, respectively. Soil amendment with *C. procera* plant powder significantly overcome the fungal attack and subsequently increased the shoot length of both pea varieties. There was a significant increase of 53, 62% increase in shoot length of pea by the introduction of *C. procera* mulches. There were non-significant effects by the application of dead *F. oxysporum* inoculum. However, the higher conc. of *C. procera* was found inhibitory to the growth of pea plants (Figure 1 A).

Effect of different treatments on root length of pea plant was also found significant as there was reduction of 40.1 and 40.8% in pots where

fungal inoculation was not accompanied by soil amendment with powder of *C. procera*. However, soil incorporation with different concentrations of *C. procera* significantly increased the growth parameter of pea. There was a significant increase of 69.3 and 78.3% in shoot length of pea plants during the year 1 and 2, respectively. While, inhibitory effects were encountered where higher concentration of *C. procera* mulches was investigated (Figure 1 B).

### Shoot fresh and dry weight

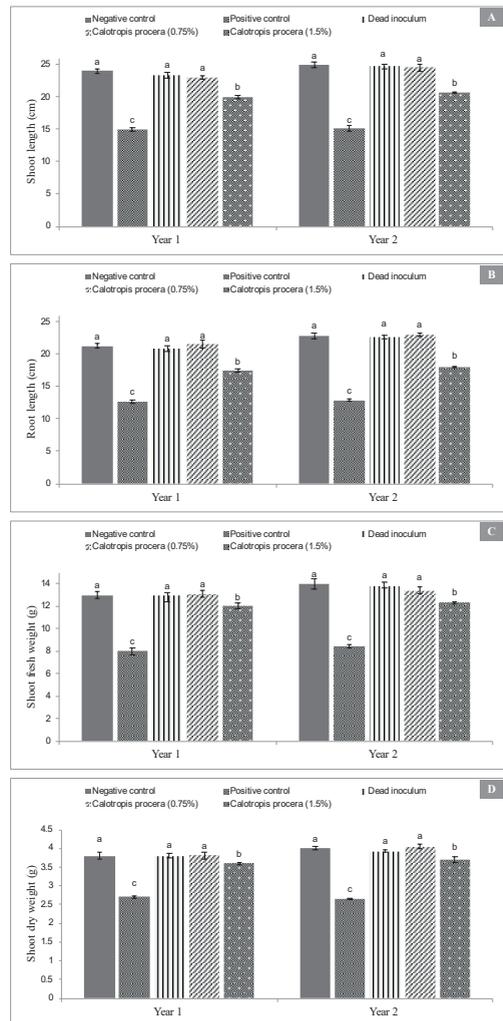
There was significant decrease of 38.5 and 40% in shoot fresh weight of pea in years 1 and 2, respectively. Soil treatment with *C. procera* significantly controlled *F. oxysporum* and ultimately increased the shoot fresh weight by 63.7 and 59.5% in pea plants, in study years 1 and 2, respectively. On the contrary, higher concentrations of *C. procera* were found inhibitory to the growth of pea plants (Figure 1 C).

Similarly, shoot dry weight also decreased in fungal inoculated pots where no *C. procera* treatment was employed and this decrease was 28.9 and 33.7% in years 1 & 2, respectively. Application of *C. procera* significantly increased the growth parameter of pea crop by 41 and 52.8%, in experiment years 1 & 2, respectively. The higher concentrations were found inhibitory to the growth of pea variety as there was a decrease of 5.6 and 8% in the shoot dry weight of pea against 1.5% *C. procera* conc. in years 1 & 2, respectively (Figure 1 D).

### Root fresh and dry weight

There was significant decrease of 51.8 and 43% in root fresh weight of pea plants by the introduction of *F. oxysporum* inoculum, in years 1 & 2, respectively. Soil treatment with *C. procera* powder significantly controlled *F. oxysporum* and ultimately increased the root fresh weight by 111.3 and 72% in pea plant against conc. of 0.75% *C. procera* mulches. On the other hand, higher concentrations of *C. procera* were found inhibitory to the root growth of both pea plants. There was 22 and 41.2% decrease in root fresh weight of pea where 1.5% concentration of mulches was investigated and compared with negative control (Figure 1 E).

Likewise root dry weight of pea plants was reduced because of fungal inoculation. There was significant decrease of 48.9 and 46.8% in root dry weight in fungal inoculated pea plants, when compared with negative control. The harmful effect in pea plants was improved by soil application of *C. procera* plant powder. 0.75% *C. procera* significantly controlled wilt disease in both pea varieties and enhanced root dry weight by 94.8 and 84%, during years 1 and 2, respectively, but inhibitory trend was noted at higher employed concentrations of 1.5% where this concentration resulted in 24.4 and 19.1% decline in root dry weight, when compared with negative control (Figure 1 F).



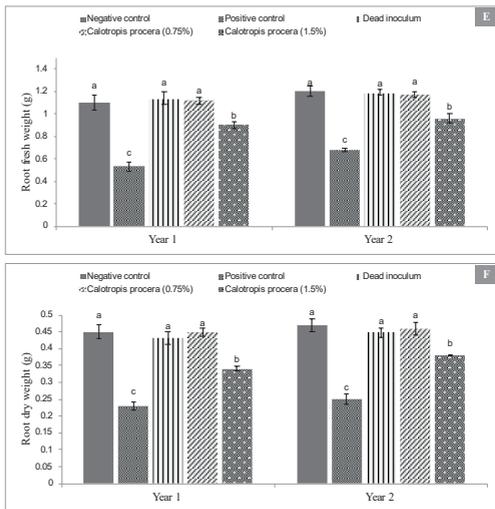


Figure 1 (A-F). Effect of inoculation of *Fusarium oxysporum* and different concentrations of *Calotropis procera* on pea plants, in pot experiments. Bars with similar letters show insignificant difference ( $P \leq 0.05$ ), as computed by Tukey's test using, Minitab 19. Y error bars represent standard errors of means of 3 replications

## Field experiments

### Plant height

*F. oxysporum* significantly reduced the plant height of pea crop up to 35 and 31.3%, during field experiments of year 1 and 2, respectively. Application of *C. procera* mulches overcome the deleterious effects caused by fungal pathogen in pea crop. There was significant rise of 43.2 and 37.5% increase in plant height by the application of *C. procera* mulches, during the year 1 and 2, respectively (Figure 2 A).

### Green pod yield

*F. oxysporum* significantly reduced the green pod yield of pea crop up to 21.6 % and 17.3%, in study years 1 and 2, respectively. Application of *C. procera* mulches overcome the deleterious effect caused by fungal pathogen in pea crop. There was significant increase of 25.5 and 17.4% in green pod weight of pea crop by the application of *C. procera*, during investigation years 1 and 2, respectively (Figure 2 B).

### 100 seed weight

*F. oxysporum* significantly reduced the 100 seed weight of pea crop up to 21.9 % and 15.8%, in study years 1 and 2, respectively. Application of *C. procera* mulches overcome the deleterious effect caused by fungal pathogen in pea crop.

There was significant increase of 23.3 and 28.3% in 100 seed weight of pea crop by the application of *C. procera*, during investigation years 1 and 2, respectively (Figure 2 C).

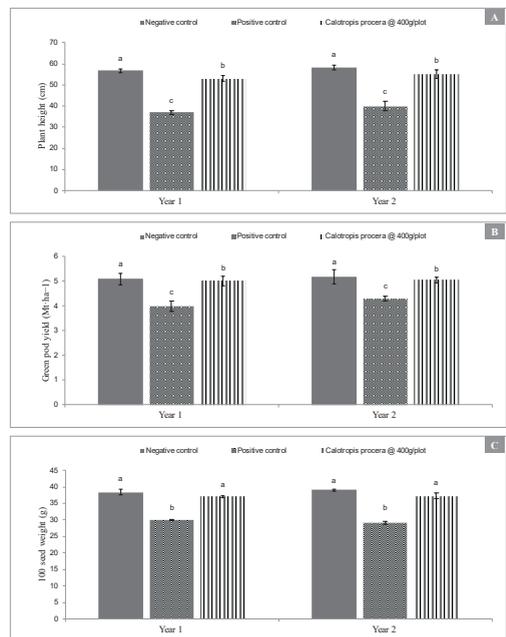


Figure 2 (A-C): Effect of inoculation of *Fusarium oxysporum* and *Calotropis procera* on pea crop, in field experiments. Bars with similar letters show insignificant difference ( $P \leq 0.05$ ), as determined by Tukey's test using, Minitab 19. Y error bars represent standard errors of means of 3 replications

## Nutritional analysis of pea grains

In this study, incorporation of *C. procera* mulches in pea also improved the nutritional characteristics of pea grains. Protein content was significantly increased to 55.8 and 64% when compared with plots having inoculation of *F. oxysporum*, during year 1 and year 2, respectively. Carbohydrate content also depicted a significant rise due to amendment of *C. procera* mulches in pea fields. There was 37.2 and 35.8% increase in the carbohydrate contents when compared with positive control plots, in years 1 and 2, respectively. Iron, calcium and potassium also showed an increased concentration in response to mycorrhizal inoculation. There was 20.3% and 15.4% rise in the concentration of iron and 44.4 and 48.3% rise in the concentration of calcium while there was 25 and 25.5% enhancement in the concentration of potassium, at site 1 and site 2,

respectively. The above mentioned values were calculated by comparing the rise in concentrations of proteins, carbohydrates, iron, calcium and potassium obtained in plots where *C. procera* was amended with diseased plant samples of positive control. However, the increase in concentrations of proteins, carbohydrates, iron, calcium and potassium of

grains obtained from plots with *C. procera* amendments was less pronounced when compared with grains obtained from plants in negative control treatments, where neither *C. procera* amendments were made neither these plots were inoculated with *F. oxysporum* pathogen (Table 1).

Table 1. Nutritional analysis of pea grains in different treatments in field experiments

Treatments	Proteins (g/100 g)		Carbohydrates (g/100 g)		Iron (mg/100 g)		Calcium (mg/100 g)		Potassium (mg/100 g)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
<b>Negative control</b>	5.1± 0.09b	5.3± 0.07b	13.6± 0.37b	14.2± 0.9b	1.39± 0.09a	1.43± 0.08a	22± 1.7b	23± 2.2b	232± 5.7c	240±5.0c
<b>Positive control</b>	4.3± 0.09c	4.2± 0.07c	11.3± 0.17b	12.0± 0.7c	1.23± 0.08b	1.3± 0.07b	18± 1.3c	18.2± 1.2c	212± 7.2b	216±9.1b
<b><i>Calotropis procera</i> at 400 g/plot</b>	6.7± 0.11a	6.9± 0.14a	15.5± 0.81b	16.3± 1.1a	1.48± 0.05a	1.5± 0.1a	26± 1.3a	27± 1.2a	265± 13.5bc	271±8.3b

Abbreviations used in Table 1: g. grams, mg. milli grams.

Note: Values are means of 3 replicates ± standard error. Standard error values were rounded off up to second decimal. Values sharing same letter do not differ at  $P \leq 0.05$  as computed by ANOVA & Tukey's Test, using Minitab-19.

Fungal disease caused a significant decrease in shoot length of pea plants. Our results are in agreement with the findings of Abdel-Monaim et al. (2011) showing that the organic solvent extracts had reduced damping off and wilt disease in lupine plant. Likewise, the mulches of *C. procera* had improved the plant shoot and root length. Shoot fresh weight decreased significantly in pea plants, during the years 1 and 2, respectively. Lower concentration (0.75%) of *C. procera* increased the shoot fresh weight in years 1 and 2, respectively. On the other hand, higher concentration of *C. procera* negatively affected pea plants. Similar behavior was recorded in case of shoot dry weight of pea plants in both study years. This decline in growth of pea plants at higher concentration of *C. procera* can be attributed to allelopathic inhibition by *C. procera* and this effect was also reported by other workers.

Fungal inoculation also decreased root fresh and dry weight in pea plants. The allelopathic inhibition was observed at the highest concentration of 1.5% as this concentration significantly decreased the root growth as compared to fungal inoculated control. In pot experiments, a significant reduction in the growth of pea plants was recorded by the application of higher concentrations of *C. procera*. Gulzar and Siddiqui (2017) also

reported that *C. procera* extracts reduced germination and growth of *Brassica oleracea*. The inhibitory effects increased with the increase in the concentration of extract. Similarly, another study showed that 7% and 10% aqueous extract of *C. procera* significantly declined seed germination and early seedling growth of *Triticum aestivum* under laboratory conditions. In contrast, the extract had no inhibitory effect on seed germination of *Hordeum vulgare* but significantly repressed its early seedling growth (Radwan et al., 2019). In field assays, plant height of pea crop was significantly declined due to inoculation with *F. oxysporum*. In another study, it was observed that *Solidago canadensis* L. suppressed tomato crop pathogens like *Pythium ultimum* and *Rhizoctonia solani* (Zhang et al., 2009). Our result is in agreement with the findings of Abdel-Monaim et al. (2011) who revealed that organic solvent extracts had reduced damping off and wilt disease in lupine plants. *Lycium arabicum* is a source of antifungal compounds which suppressed *F. oxysporum* f. sp. *radicis-lycopersici*. Besides this, *L. arabicum* extracts enhanced tomato growth. Equally, water and organic solvent extracts of *L. arabicum* also reduced mycelial growth of pathogen (Nefzi et al., 2017). Root dry weight of pea plants probably reduced because of fungal inoculation.

In a previous study, crude water, ethanol and acetone extracts of *Adhatoda vasica*, *Jatropha curcas*, *Sapindus emarginatus* and *Vitex negundo* are reported to inhibit mycelial growth of *F. oxysporum* during *in vitro* experiments. Amongst these, *A. vasica* at 40% conc. exhibited complete inhibition of mycelial growth. Likewise, in pot experiments, water extract of six plant species reduced the disease symptoms of the eggplant and consequently improved the root and shoot growth of eggplant (Siva et al., 2008). Lupine (*Lupinus termis* Forsik) seeds treated with extracts of different plant species including *C. procera* overcome the effect of pathogens causing damping-off and wilt diseases. Moreover, under field conditions, the extracts of *Nerium oleander*, *Eugenia jambolana* and *Citrullus colocynthis* significantly reduced wilt disease as well as improved crop growth parameters (Abdel-Monaim et al., 2011). Peptide fraction from *C. procera* (PepCp) latex showed a low effect against the spore germination of both fungi *Colletotrichum gloeosporioides* and *Fusarium solani*. However, PepCp (1.25 mg mL<sup>-1</sup>) inhibited the mycelial growth of *C. gloeosporioides* by 80% (Amaral et al., 2021). In another study,  $\alpha$ -helical propeptides (SnuCalCpIs) from *C. procera* are antifungal against yeasts. The peptide, SnuCalCpI15, exhibited minimum inhibitory activity of 0.20±0.01, 0.20±0.01, 0.26±0.01, and 0.26±0.01 mM against *Candida albicans*, *Saccharomyces cerevisiae*, *Pichia anomala*, and *Rhodotorula mucilaginosa*, respectively. SnuCalCpI15 initially bound to yeast cell surfaces and then enter the cells and cause increased cell membrane permeability and alter cell wall thickness of yeast cells (Han et al., 2022). Similarly, the essential oils of *C. procera* exhibited significant antifungal activity against fungal species, *Trichophyton shoelenii* and *Aspergillus fumigatus* (Al-Rowaily et al., 2020). *C. procera* extract is used as an alternative to fungicides due to its effectiveness on several pathogens, including *Fusarium oxysporum* f. sp. *lycopersici*. Ten isolates of the pathogens were obtained from diseased tomato plants. These isolates exhibited tomato wilt disease symptoms to variable extents, and isolate 5 exhibited the highest disease severity of 73%. Aqueous extract of *C. procera* was effective against

*Fusarium wilt* disease of tomato. This extract exhibited antifungal activity and also induced systemic resistance in tomato plants. All concentrations of *C. procera* extracts suppressed the growth of *F. oxysporum*. The 15% aqueous extract exhibited antifungal activity of 70%. In greenhouse experiments, the aqueous *C. procera* extract at 15% significantly reduced fusarium wilt disease of the tomato by 83.6%. This conc. of extract also significantly increased fresh and dry weight of tomato plants (g plant<sup>-1</sup>) compared to inoculated plants to 86.6 and 120%, respectively. Treatment with extracts of *C. procera* also enhanced total phenolics, flavonoids and antioxidant enzymes in inoculated and non-inoculated tomato plants (Abo-Elyousr et al 2022).

## CONCLUSIONS

Fusarium wilt disease substantially decreased the growth of pea plants. However, soil amendment with *C. procera* ameliorated the adverse effects of fungal attack resulting enhanced growth of applied plants. *C. procera* amendment increased 25% and 17.4% green pod yield under field conditions. Incorporation of *C. procera* mulches in pea also improved the nutritional characteristics of pea grains as proteins were significantly increased up to 64% and carbohydrates content also depicted a significant rise 37.2%. Moreover, iron, calcium and potassium also showed an increased concentration in response to *C. procera* addition.

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## MEASURING OF YIELD AND OTHER TRAITS OF WINTER PEAS VARIETIES ON DIFFERENT PLANTING DATES

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### Abstract

*The objective of this study was to analyze at NARDI Fundulea (44°26'N latitude, 68 m altitude) the influence of time of planting on yield and other traits at winter peas, as support of the appropriate technological decision. A split plot layout with in randomized block design, with three replications has been applied in this study. Four dates of planting were considered as main plots, respectively: October 11, 2019/October 21, 2020 (autumn) and February 19, 2020/March 03, 2021 (spring), while three replication subplots include twelve winter peas varieties and breeding lines (Lavinia F, Ghittia F, 13008MT28-1, 13020MT, 12018MT1, 12023MT1-1, 13002MT, 12038MT2, 13008MT42-2, 12025MT4, 13020MT1-2, 12004MT2). According to the analyzed variables such plant height, seed yield, TGW and protein content, results suggest that planting in autumn leads to a higher performance of the both TGW and Yield capacity (4838 kg/ha), as compared to the spring one, in this case being registered an yield of 1796 kg/ha.*

**Key words:** winter peas, grain yield, protein content, plant height.

### INTRODUCTION

Peas (*Pisum sativum* L.) are an ancient crop with extensive ecological and production potential, cultivated for their seeds in the majority of countries around the globe. Peas seeds are used as food, in the processing industry and as feed. Seeds value cherished for their biochemical content, resides in their high content of proteins (27.8%), starch (43.2%) and fats (1.2%) (Muntean et al., 2001; Ungureanu et al., 2018). Due to several valuable agronomic characteristics pea has been preferably introduced in the main agricultural crop rotations: tolerance to various environment conditions; good adaptability to different soil conditions; fixing of atmospheric nitrogen into the soil; short period of vegetation (Simion et al., 2017).

Peas are important grain legumes. Dry pea cropping has major advantages in sustainable farming systems because of it's low requirement for water, chemicals and fossil energy; ability to symbiotically fix atmospheric nitrogen which precludes the need for N-fertilizer; and reduced emissions of N<sub>2</sub>O, NO<sub>3</sub> and CH<sub>4</sub>, which, in addition to CO<sub>2</sub> savings, significantly reduce the greenhouse effect of agricultural activities

(Mukherjee et al., 2013; Munier and Carrouee, 2003).

In cultivated pea a considerable genetic variation was found and numerous varieties characterized by a broad spectrum of desirable agronomic traits have been released from many breeding programs across the world.

Main factors considered to establish the specific technology of cropping pea in terms of varietal productivity, planting date, seeding rate, harvesting conditions, include the local pedo-climatic conditions, end use and marketing options.

Among these, pea cultivar and seeding rate are the most determinant factors on yield parameters. Decreased yield is a sign that seeding rates are above or below the optimum rates (Asik et al., 2020).

Sowing rate influences plant establishment, growth, seed yield, and the profitability of a crop (Loss et al., 1998). Olle and Tamm (2021) affirm that sowing rate of field peas is affected by environmental factors (rainfall, irrigation, temperature, or soil type). The more favourable the environment, the higher will be the optimum density of the sowing rate. Higher densities will also suppress weed growth and this was observed in other research.

This study presents the results obtained at the National Agricultural Research and Development Institute Fundulea (44°26' N latitude, 68 m altitude), regarding the characterization of yield, protein content, TGW, plant height, as response to different planting dates in 12 Romanian pea.

## MATERIALS AND METHODS

The experiments were conducted during the agronomic cycle 2019/2021 at the National Agricultural Research and Development Institute Fundulea (44°26' N latitude, 68 m altitude). Soil texture was cambic cernoziom with the moderate amount of organic matter and nutrient elements. Each experimental plot included six planting rows with the parameters: length (L) = 6 meters, harvested area (HA) = 4 m<sup>2</sup>, distance among rows = 12.5 cm and planting density = 130 plants/ m<sup>2</sup>.

Winter peas cultivars (*Lavinia F* and *Ghittia F*) and the breeding lines *13008MT28-1*, *13020MT*, *12018MT1*, *12023MT1-1*, *13002MT*, *12038MT2*, *13008MT42-2*, *12025MT4*, *13020MT1-2*, *12004MT2*) were analyzed, as

subplot factor in a split plot experiment based on randomized block design, with three replications, according to four sowing dates considered as the main plot factor, respectively October 11, 2019/ October 21, 2020 (autumn) and February 19, 2020/March 03, 2021 (spring). The experimental plots were mechanically harvested with Wintersteiger equipment.

Parameters analyzed in this study included: grain yield (kg/ha at 14% humidity), seed protein content (%), thousand grains weight (TGW) and plant height (cm).

Seed protein concentration was determined by near-infrared (NIR) method using a Grain Analyzer (Infratech 1241, Foss Tecator).

The obtained data were statistically calculated by ANOVA.

Climatic conditions, at NARDI Fundulea area are characterized by a continental temperate climate, with uneven distribution of rainfall by months. Temperature (°C) and rainfall (mm) registered across the experimental period, by the Weather station of NARDI Fundulea, are presented in Figures 1 and 2. Weather conditions of the both agronomic cycles during vegetation period of winter peas and especially the grain filling period, were very different.

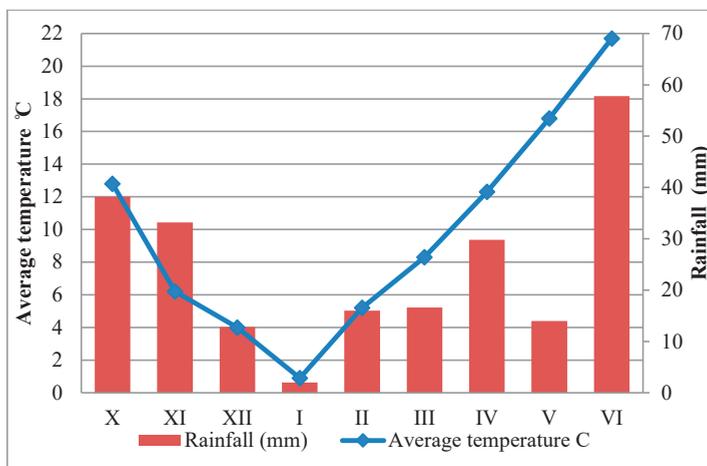


Figure 1. Weather conditions of field pea vegetation period 2019-2020 according to Meteorological Station of NARDI Fundulea

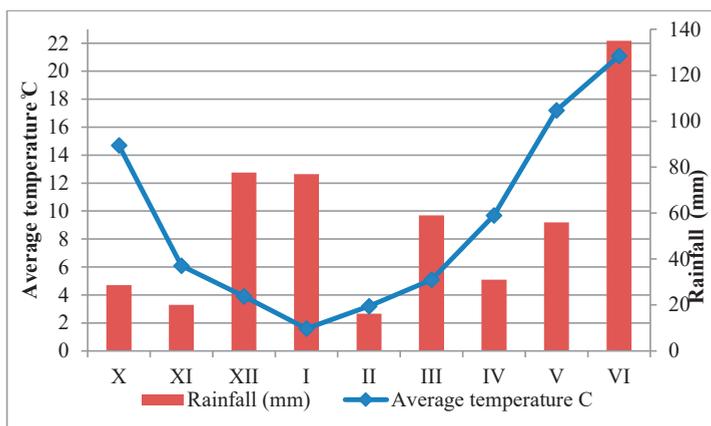


Figure 2. Weather conditions of field pea vegetation period 2020-2021 according to Meteorological Station of NARDI Fundulea

## RESULTS AND DISCUSSIONS

The results showed that the time of planting is determinant on yield and other traits in winter peas (Table 1). Results obtained indicate the strong influence of different sowing dates on plant height. The highest value of the plant height (71 cm) was recorded on October 21, 2020, whereas the shortest plant height (51 cm) was recorded on February 17, 2020 (Table 1). Differences among the pea genotypes related to response to the sowing date on the plant height has been also observed.

*Lavinia F*, *Ghittia F*, *13002MT* and *13020MT1-2* express their high plant type as compared to *12038MT2*, *12025MT4*, that could be characterized as short.

Another finding refers to the relation planting date/TGW. Data obtained on TGW varies from 145 g to 170 g (spring sowing system) and from 160 to 200 g (autumn sowing system).

Planting of winter pea in spring system revealed a negative influence on seed yield that was

significantly reduced in our study. The both planting dates, respectively February 17 and March 3, caused up to 50% reduced yields (Tables 1 and 2). The highest seed yield has been registered for the genotypes *13020MT* and *13020MT1-2* respectively 4838 kg/ha and 4538 kg/ha.

The protein content also significantly varied among the pea cultivars, as effect of genetic differences related to the potential of biological N fixation (Abi-Ghanem et al., 2013).

Genotypic differences of protein content were also highlighted under field conditions in our trials (Tables 1 and 2).

The lowest values on average for this parameter were registered for the winter peas cultivars sown in autumn (X = 22.98%), in comparison to the values obtained by sowing in spring time (X = 25.62%). The genotypes sown in spring characterized by a high protein content were: *13008MT42-2* (27.8%), *13008MT28-1* (27.3%) and *13020MT1-2* (27%).

Table 1. Effect of planting date on winter peas characteristics, NARDI Fundulea

Planting date	Plant height (cm)	TGW (g)	Seed yield (kg/ha)	Protein content (%)
Oct. 11 <sup>st</sup> 2019	60.8	183	2980	23
Oct. 21 <sup>st</sup> 2020	75.0	178	5448	22.7
Feb. 17 <sup>st</sup> 2020	51.8	155	2695	25.6
Mar. 3 <sup>th</sup> 2021	63.2	168	1710	24.8

Table 2. The mean of agronomical traits of twelve varieties of winter pea

No.	Variety	Planting date	Plant Height (cm)	TGW (g)	Seed yield (kg/ha)	Protein content (%)
1.	Lavinia F	winter	64.5	160	4394	21.2
		spring	62	145	2496	23.8
2.	Ghittia F	winter	71	200	4397	23.4
		spring	60.5	175	2272	25.5
3.	13008MT28-1	winter	64	200	4104	23.7
		spring	57.5	175	2325	27.3
4.	13020MT	winter	62	200	4838	22.5
		spring	61	180	1796	24.9
5.	12018MT1	winter	62	200	3480	22.4
		spring	60	185	2106	25.9
6.	12023MT1-1	winter	59	200	3739	22.7
		spring	56	183	2307	23.5
7.	13002MT	winter	69.5	170	3954	21.6
		spring	57.5	165	2418	23.5
8.	12038MT2	winter	57	173	3580	23.2
		spring	51	165	2105	26.6
9.	13008MT42-2	winter	62	180	3871	23.5
		spring	55	165	1968	27.8
10.	12025MT4	winter	58.5	200	4521	24.5
		spring	48.5	185	2225	26.1
11.	13020MT1-2	winter	76	160	4538	22.9
		spring	58	155	1821	27.0
12.	12004MT2	winter	54	180	4005	23
		spring	58.5	160	2567	25.1
<b>Average values</b>		<b>winter</b>	<b>67.9</b>	<b>185,3</b>	<b>4118</b>	<b>22.98</b>
		<b>spring</b>	<b>57.3</b>	<b>170</b>	<b>2201</b>	<b>25.62</b>

Analyses of variance were performed considering years as a random factor and genotypes as a fixed factor. ANOVA indicated a

significant effect of genotypes and years on the protein content, grain weight and plant height (Table 3).

Table 3. ANOVA for average seed yield, average protein, average TGW, and plant height for winter peas cultivars, NARDI Fundulea, 2019-2021 period

Source of variation	df	Seed yield		Protein content		TGW		Plant height	
		F	P-value	F	P-value	F	P-value	F	P-value
Genotypes	11	0.64	0.76	4.14*	0.013	18.21*	0.00001	3.01	0.04
Years (time of planting)	1	151.2	9.06	75.5**	2.94	62.63**	7.23	22.19**	0.0006
Interaction	11	-	-	-	-	-	-	-	-
Total	23	-	-	-	-	-	-	-	-

## CONCLUSIONS

Cultivation of winter pea in either autumn or spring planting systems is possible and could be decided depending on the immediate needs of farmers:

- Planting in autumn allows a more efficient use of humidity during the winter season, a less vulnerability to the spring droughts and a longer vegetation period that determine a higher productivity, grain yield and TGW, respectively;

- High protein content are achieved mainly when the winter pea crop is established in spring time.

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## THE APPLICATION OF TECHNOLOGICAL ELEMENTS IN THE CULTIVATION AND USE OF SEVERAL FORMS OF *Nigella damascena* L. IN THE REPUBLIC OF MOLDOVA

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### Abstract

Nowadays, non-traditional edible oils of sesame, flax, mustard, chia etc. are widely used in various industries. Among them, the oil extracted from the seeds of *Nigella damascena* species, which possesses unique medicinal properties, is in great demand. In our research, we applied methods of individual selection and chose several forms, which differ from the 'Azuriu' cultivar registered in the Catalogue of Plant Varieties and the initial population. One of the selected forms (ND-4) was characterised by higher seed and oil productivity, good adaptability, adequate response to the technological elements applied in Comparative Testing of Varieties, and then it was proposed to the farm that had requested the seeds, where the necessary tests were done. The given form (ND-4), being stable in terms of production of raw material, will be submitted for registration in the Catalogue of Plant Varieties of the Republic of Moldova.

**Key words:** seeds, breeding, fatty oil, food, germination, test weight (weight of 1000 seeds), production.

### INTRODUCTION

Modern society is becoming more cautious in choosing food products. Crops and products that have been known for a long time but have been forgotten are now becoming popular again. Non-traditional edible oils of sesame, flax, mustard, chia etc. are widely used in various industries (Kukhareva & Pashina 1986; Lavrov, 1995; Kostadinović Veličkowska et al., 2018; Prokhorov, 2021; Suárez et al., 2021). Among them, the oil extracted from the seeds of the *Nigella* species is in great demand. Over the years, we have been studying some oil-producing species, including love-in-a-mist, the fatty oil of which has unique medicinal properties (Khan, 1999; Korableva & Rakhmetov, 2011; Salehi et al., 2021).

Love-in-a-mist or devil in the bush *Nigella damascene* L., *Ranunculaceae* family, native to southern Europe, north Africa and southwest Asia has been known and used since ancient times as a spice and medicinal plant in the treatment of various diseases (Kukhareva & Pashina, 1986; Lavrov, 1995; Korableva & Rakhmetov, 2011; Salehi et al., 2021; Prokhorov, 2021; Zaky et al., 2021).

Modern phytotherapy, based on the active ingredients extracted from the seeds of *Nigella*.

species, as well as volatile and fatty oils, indicates the following properties: antimicrobial, antiviral, antifungal, anticancer, bronchodilator, blood glucose balancing, energizing, vasoreparative, metabolic, detoxifying, antiallergic, immunostimulatory and aphrodisiac (Gali-Muhtasib et al., 2006; Kazemi, 2014; Mashirova & Orlovskaya, 2012; Prokhorov, 2021). The seeds contain carbohydrates, lipids (35.5-41.6%), proteins, resins, tannins, volatile oil (1.5%), glycosides, alkaloids, amino acids, triterpenes, flavones, saponins, ergostane-type steroids (obtusifoliol), omega 3 and omega 6, essential fatty acids, significant amounts of minerals and vitamins (Kudinov, 1986; Dudcenko et al., 1989; Dauksas et al., 2006; Kudinov, 2007; Sieniawska et al., 2018; Prokhorov, 2021).

### MATERIALS AND METHODS

The valuable medicinal and nutritional properties of this species contributed to the initiation of the study on its adaptation, breeding and testing, with the aim of further introduction and implementation of the given species. In our research, we applied methods of individual selection and chose several forms, which differ from the 'Azuriu' cultivar registered in the

Catalogue of Plant Varieties. One of the selected forms (ND-4) was characterised by higher seed and oil productivity, good adaptability, adequate response to the technological elements applied in CCC tests. The given form (ND-4), being stable in terms of production of raw material (seeds), will be submitted for registration in the Catalogue of Plant Varieties of the Republic of Moldova.

For the study, seed samples of two forms of *Nigella damascena* were used, one as a control - 'Azuriu' and the selected form (ND - 4). The seeds were separated according to the test weight (the weight of 1000 seeds) and tested for this index to determine its degree of influence on grain production. The seeds of *Nigella damascena* were passed through a sieve and categorized as follows: I batch - 1.2 mm in diameter (small), II - 1.5 mm (medium), III - 2.0 mm (large), this operation was performed every year, on freshly harvested seeds. We chose the method of propagation by direct sowing in open ground, according to the scheme (45 cm x 5 cm) at a depth of 1.5-2 cm, always followed by rolling. The optimal time for harvesting the seeds has

been determined, considering that they may scatter when ripe, considerably diminishing the yield. Besides, phenological observations, morphological analyzes and biometric measurements were performed during the growing season.

## RESULTS AND DISCUSSIONS

During the three-year period when the germination capacity and growth energy of seeds were tested, seed samples were selected (divided in a seed selection machine) according to the test weight (the weight of 1000 seeds), into three categories for each of the tested forms. The small seeds had a low growth energy, namely, 22.4% in the control and 26.2% in the new ND-4 cultivar. In the case of the samples with large seeds, the energy was equal to 33.4 (control) and 30.2%, respectively, but the seeds of medium size had the most considerable value, constituting the largest fraction of the total amount and being characterized by a germination capacity of 42.4 and 46.2% in the given tests (Table 1).

Table 1. The impact of the test weight on the germination capacity of *Nigella damascena* seeds

Tested cultivars	Test weight of seeds, g			Germination capacity, %		
	small	medium	large	small	medium	large
'Azuriu' control cultivar	0.92	1.8	2.2	22.4	42.4	33.4
ND-4, new cultivar	1.2	2.1	2.6	26.2	46.2	30.2

The test weight is also one of the essential characteristics that influences seed productivity. Analyzing batches of seeds with various test weight, we noticed that stronger plants and higher yields are not always obtained from the samples with greater weight of 1000 seeds. The seed fractions that have medium test weight are the most representative and valuable. They represent approximately 57-62% of any batch of seeds, which is also characteristic of the studied forms of *N. damascena*. The small seeds are often underdeveloped, irregularly shaped, and sometimes have an abnormal development in terms of germination capacity. This research was conducted to track the influence of the index - test weight on the seed production potential of plants, which consists of several elements. It has been established that seed productivity is directly correlated with the number of flowers per plant. In the forms of directly correlated with

the number of flowers per plant. In the forms of *Nigella* ssp., this index varied between 1 and 30; in the years of the research, there were on average from 14.5 to 29.2 flowers per plant (in the control) and from 22.7 to 34.6 in the ND-4 cultivar. The average number of rudimentary seeds in a pod has been, over the years, from 22.3 ('Azuriu' cultivar) to 26.8 (ND-4). The average number of rudimentary seeds per plant varied over the years within an amplitude from 1873.9 to 2977.9, and the productive potential of a plant was from 4.1 to 10.4 g. Based on the values of the test weight and the average number of plants growing per 1 m<sup>2</sup>, the seed production potential per unit area of the ('Azuriu' cultivar reached values of 533.1 g/m<sup>2</sup> and ND-4-910.4 g/m<sup>2</sup>. For a species of short, small plants, the values are quite good (Table 2). The actual seed productivity, for several reasons is lower than the calculated potential.

Table 2. Elements of the calculated productive potential of *Nigella damascena* L.

Tested cultivars	Average number: pcs.				Potential seed productivity	
	flowers per plant	Pods per plant	rudimentary seeds per pod	rudimentary seeds per plant	plant/g	g/m <sup>2</sup>
'Azuriu', control cultivar	14.5	4.9	22.3	1873.9	2.1	533.1
ND-4, new cultivar	21.2	5.8	26.8	2912.6	8.4	910.4

The difference between the number of flowers and the capsules with seeds is not essential, because practically every flower forms seeds. A more considerable difference was observed (Table 3) between the number of rudimentary seeds formed in a pod and the number of normally developed seeds, which also coincides with the data obtained by other researchers -

Makrushin et al. (2007), Kuznetsov (2015). The real seed productivity of a plant reached values of 4.5 g in the control cultivar and 6.4 g - in the new variety ND-4. Thus, the actual seed productivity per unit area was equal to 483.3 g in the control cultivar and 594.6 g in the new cultivar ND-4 (Table 3).

Table 3. Actual seed productivity of *Nigella damascena*, average per three years

Tested cultivars	Average number: pcs.				Potential seed productivity	
	capsules per plant	Pods per capsule	normal seeds per pod	seeds per plant	per plant, g	g/m <sup>2</sup>
'Azuriu', control cultivar	20.2	5.1	16.6	1642.4	4.5	483.3
ND-4, new cultivar	21.2	5.3	20.3	2084.6	6.4	594.6

There are several factors that influence the formation of fully developed seeds from the rudimentary ones. Some of them may be related to morphological features - the location of rudimentary seeds in the ovary, the quality of pollen or its insufficiency, the lack of pollinators, unfavorable growth conditions, the data being confirmed by other authors such as

Astafyeva (2008), Chunikhovska (2009), Orlovskaya & Masirova (2012), Margout et al. (2013), Kuznetsov (2015).

The data in Table 4 show that the level of development of actual seeds from the rudimentary ones - the coefficient of seed productivity has changed over the years of testing (Table 4).

Table 4. Seed productivity coefficient of *Nigella damascena* L.

Tested cultivars	Average number: pcs.		Seed productivity coefficient, %	Test weight, g
	Rudimentary seeds per plant, pcs.	Normally developed seeds per plant, pcs.		
'Azuriu', control cultivar	2049.9	1612.7	78.6	2.63
ND-4, new cultivar	3122.8	2517.3	80.6	2.82

The seed productivity coefficient of the control cultivar 'Azuriu' was 84.1 while - of the selected form - 86.6%. In terms of the test weight index, the selected forms in both variants had slightly higher values, from 2.61 to 2.82 g. The germination capacity evaluated under laboratory condition, in two repetitions, was equal to 78% in the control and 80% in new cultivar. The

seeds correspond to the quality class I. The growth energy was quite high, reaching values of 65 and 67% within three days from the beginning of germination. The test weight was 2.1-2.3 g, and the number of seeds per gram was 197 pcs. in the control and 201 in the new cultivar (Table 5).

Table 5. Quantitative indices in assessing the quality of *Nigella damascena* seeds, in the 2<sup>nd</sup> fraction

Tested cultivars	Average values	Germination capacity, %	Growth energy in three days, %	Test weight, g	Number of seeds in 1 g.
'Azuriu', control cultivar	X	78	65	2.1	219
	Sx	5.2	2.7	0.6	1.1
ND-4, new cultivar	X	80	67	2.3	224
	Sx	4.1	2.8	0.7	0.9

Several morphological and biological indices were assessed. The plant height was 64.3 cm in the control cultivar and 65.1 cm in the new cultivar. The number of branches was practically equal to the number of capsules, from 7.6 in the control cultivar to 8.05 in the new cultivar. The duration of the growing season

from seedling emergence to seed harvesting was from 86 to 98 days, in different years, being the same for both forms, because sowing and harvesting were done at the same time at seed ripening, so that we haven't noticed any difference.

Table 6. Morphological and productive indices of *Nigella damascena* L., average for three years

Tested cultivars	Average values	Plant height, cm	Number of branches	Number of capsules/plant	Seed productivity, kg/ha	Duration of the growing season, days
'Azuriu', control cultivar	X	64.3	7.6	7.7	531	86-98
	Sx	0.48	0.64	0.57	-	
ND-4, new cultivar	X	65.1	8.0	8.05	572	85-98
	Sx	0.63	0.52	0.53	-	

DL<sub>05</sub> to seed production constituted 0.03 t/ha

Seed productivity (recalculated per 1 hectare) of both *Nigella damascena* cultivars, for the three-year period, averaged 0.531 t/ha in the control and 0.572 t/ha in the new cultivar, which is with 0.041 t/ha more (Table 6).

## CONCLUSIONS

Based on the research conducted and the results obtained, we can conclude that: the selections made in the initial samples allowed us to highlight a form of *Nigella damascena* (DN-4), which under the climatic conditions of our country, achieved a rather high potential productivity, which constituted 80.6-86.6%. The average productivity over three years reached values in the range 446.5-594.6 g/m<sup>2</sup>, and recalculated per 1 ha, it was on average for three years 531 kg/ha in the control and 572 kg/ha in the new cultivar, being much higher than the biological potential for productivity, obtained in other neighboring regions.

The seed fractions that have medium test weight are the most representative and valuable. They represent approximately 57-62% of any seed batch, being used for sowing in industrial plantations and testing.

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## A COMPARATIVE STUDY OF THE FEEDING EFFECT WITH HAY FROM VARIOUS ALFALFA VARIETIES IN FATTENING LAMBS

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### Abstract

The main aim was to establish some differences in productivity, nutritional value but also in the efficiency in obtaining performances in growing and fattening animals. The following alfalfa varieties were studied: Pioneer PR55 V48, Sandra, Valahia (4AG08), Dobrogea (4AG07), and Dimitra. Five plots were established for the cultivation of alfalfa with an area of 1 hectare each on a farm in Balotesti village following the evolution of plant growth parameters and their chemical composition, especially in year 2, at the 2nd harvest. The green mass obtained was preserved in the form of hay. The animal experiment was organized on a sheep farm in Dambovita County where fattening young sheep from Turcana × Suffolk half-breed were fed with hay for a period of 3 months. Concerning the alfalfa hay, the dry matter ranged between 848 and 861 g, organic matter between 759 and 782 g, and crude protein 65 and 76 g, respectively. Gross energy was similar for all varieties ranging around 18 MJ. Considering the fattening performances, the average daily gain recorded by the fattening lambs, during the experiment ranged between 171.4 and 181.2 g/day. We recommend the use of hay from the Romanian varieties in fattening lamb diets, which demonstrated similar characteristics to the valuable foreign ones.

**Key words:** *Medicago sativa* L., dry matter, gross energy, crude protein, fattening lambs, gain.

### INTRODUCTION

The cultivation of blue alfalfa (*Medicago sativa* L.) has one of the essential roles in re-launching the livestock sector in Romania, which will not be possible without the development of a feed base with fodder to ensure optimal vital functions and the enhancement of the biological and productive potential of farm animals (Pelmuș et al., 2020). However, achieving the cultural performance of alfalfa (useful yield, persistence and high competitive capacity) depends on a multitude of technological, environmental and genetic factors (Dunea et al., 2015; Oprea and Dunea, 2011; Dincă et al., 2017). To these is added the selection of varieties suitable for pure cultivation or for obtaining more perennial and more productive mixtures with other perennial legumes and grasses (Cavero et al., 2017; Dincă et al., 2021). As a general rule, rations for fattening animals cannot even be designed without the participation of alfalfa in various

forms of preparation and preservation (Quick et al., 1986; Lloveras et al., 2008). Most farms are looking for dietary supplement solutions to feed fattening ruminants with different concentrate mixtures that generally have higher energy content by providing a balanced energy-protein ratio and balanced mineral vitamins and alfalfa may provide substantial benefits (Sanderson, 1992; Schitea, 2010).

The significant productive effects of fattening young sheep and other farm animal species are well known when legumes including alfalfa have been included in feed diets. In our case, it was demanded especially by the possibilities of experimental technique, to use a single ingredient (alfalfa hay) to more easily highlight the effect of the ration in feeding lambs, implicitly the effect of the varieties that were the subject of research. It would have been quite difficult to separate the effect of alfalfa hay from the other ingredients if we added a mixture of concentrates or only an assortment (e.g. corn). Certainly, the effect of the

productivity of a complete ration would have been greater (the daily gain).

Studies have shown that the qualitative performance of lambs grazing either legume or grass pasture was better than lambs receiving an all-concentrate diet in dry lot (Wildeus et al., 2007). McClure et al. (1994) observed higher daily gains in dry lot lambs, though the growth of lambs grazing alfalfa approached that of dry lot lambs, while producing leaner carcass with a more desirable yield grade.

Wildeus et al., 2007 noted that the growth rates of the forage-fed lambs were higher than those reported in lambs fed bermudagrass (*Cynodon dactylon*) pellets or in lambs fed tropical Guinea grass (*Megathyrsus maximus*).

The paper presents the results obtained from testing five Romanian and foreign alfalfa varieties to assess the lamb fattening performance fed with hay. Growth of the sheep lambs on an alfalfa hay diet without supplementation is required to be analyzed in the context of obtaining valuable ecological products.

## MATERIALS AND METHODS

The study was conducted between 2018 and 2020 with the aim of analyzing the forage quality of several Romanian and foreign alfalfa varieties highlighted the special quality of fodder produced by the tested Romanian alfalfa varieties.

A breeding program for new alfalfa varieties is promoted by S.C. Patru Agro S.R.L. (<https://samantalucerna.ro/>) in cooperation with academic researchers. It is expected that these varieties will be more adapted to the Romanian eco-pedoclimatic conditions (Dincă et al., 2021) having better winter hardiness due to the cold winters with a higher incidence and duration of frost days. The program provided two varieties i.e., Dobrogea and Valahia, which have been included in the national official list of varieties (<https://istis.ro/image/data/download/catalog-official/CATALOG%202020.pdf>), while Dobrogea is included in the OECD list of varieties eligible for seed certification.

The selected varieties presented in Table 1 were grown in a farm from Balotești according to a specific experimental protocol in distinct

plots with an area of 1 ha. Samples were taken from these plots to determine the quality of the feed.

The following alfalfa varieties were studied: Pioneer PR55 V48, Sandra, Valahia (4AG08), Dobrogea (4AG07), and Dimitra. Initially, 5 plots were established for the cultivation of alfalfa with an area of 1 hectare each, following the evolution of plant growth parameters and their chemical composition, especially in year 2, at the 2nd harvest.

The green mass obtained was preserved in the form of hay. The feeding experiment was organized for a period of 3 months in a sheep farm located in Dambovită County for fattening lambs from Turcana × Suffolk half breeds.

A group of 40 individuals was selected being divided into 5 subgroups of 8 lambs, each one being fed with hay originating from a single variety.

Table 1. Alfalfa varieties considered in the experiments (3 Romanian and 2 foreign varieties from U.S.A./Austria, and Italy)

Variety	Maintainer name	Maintainer code*	Country
Sandra	INCDA Fundulea	1562	Romania
Dobrogea	SC Patru Agro SRL	2782	Romania
Valahia	SC Patru Agro SRL	2782	Romania
Dimitra	Continental Semences	724	Italy
PR55V48	S&W Seed Company/Pioneer Hi-Bred Services GmbH	3133	U.S.A., Austria

\*<https://www.oecd.org/agriculture/seeds/documents/codes-and-schemes-list-of-varieties-eligible-for-seed-certification.pdf>

During the experimental period, green mass samples (3 replicates) were taken from the field from each plot cultivated with each alfalfa variety by identifying an area of one square meter with an average load of plants in the beginning phase of flowering on the second cut. Alfalfa hay samples were taken from warehouse stocks (3 replicates) to eliminate all factors that could have allowed errors regarding the homogeneity of the sample. For the analysis laboratory at the National Research and Development Institute for Animal Biology and Nutrition (INCDBNA-IBNA Balotești), the square method was applied, finally reaching the

quantities requested for laboratory and calorimetric analysis (1000 g).

The samples were first subjected to dry matter determinations at a temperature of 65 and 103°C to prepare them for the determination of organic matter with the respective ingredients by analytical TECATOR equipment ([https://www.fossanalytics.com/en/products/digestor\\_2508\\_2520](https://www.fossanalytics.com/en/products/digestor_2508_2520)). A series of parameters were determined for green mass and hay of each variety as follows: *DM* - Dry matter; *SO* - Organic substance; *CP* - Crude protein; *CF* - Crude Fat; *Cel. B* - Crude cellulose; *SEN* - Non-nitrogenous extractive substances; *Cen* - Ash; and *EB* - Gross energy. Data were analyzed for the effect of alfalfa hay intake on gain (kg/fattening period) for the five tested varieties. The differences between diets with various alfalfa varieties grouped in Romanian

and foreign groups were determined using the comparative statistics in SPSS software (SPSS Inc., Chicago, IL, 2011) in the presence of a significant *F* value and Levene's test, and values were presented as least squares means. More technical information regarding the project regarding the screening of alfalfa varieties' performances is available in Dincă and Dunea (2018) and Dincă et al. (2021).

## RESULTS AND DISCUSSIONS

Following the laboratory experiments, a screening of the chemical composition was performed for establishing important features regarding the forage quality of each tested alfalfa variety. Table 2 shows the average values of the parameters for green mass, and Table 3 for hay, respectively.

Table 2. Chemical composition of the alfalfa varieties (green mass harvested at the 2<sup>nd</sup> cut in the 2<sup>nd</sup> year of cropping)

Name of the variety	Dry Matter (DM) g	Organic Substance (SO) g	Crude Protein (CP) g	Crude Fat (CF) g	Crude Cellulose (Cel B) g	Non-nitrogenous extractive substances (SEN) g	Ash (Cen) g	Gross Energy (EB) MJ
PR55 V48	260 1000	229 859	65 281	5 23	38 170	121 385	31 141	17.70
Sandra	280 1000	245 862	70 269	6 24	45 174	124 395	35 138	17.73
Valahia (4AG08)	299 1000	260 870	74 247	8 27	50 167	128 429	39 130	17.28
Dimitra	300 1000	260 867	73 243	8 27	52 173	127 424	40 138	17.84
Dobrogea	310 1000	268 865	76 245	8 26	50 161	134 433	42 135	17.6

Table 3. Chemical composition of the alfalfa varieties (hay resulted from the 2<sup>nd</sup> cut in the 2<sup>nd</sup> year of cropping)

Name of the variety	Dry Matter (DM) g	Organic Substance (SO) g	Crude Protein (CP) g	Crude Fat (CF) g	Crude Cellulose (Cel B) g	Non-nitrogenous extractive substances (SEN) g	Ash (Cen) g	Gross Energy (EB) MJ
PR55 V48	861 1000	771 895	210 243	12 14	263 305	286 333	90 105	18.2
Valahia (4AG08)	848 1000	759 895	205 241	11 13	205 243	338 398	89 105	18.0
Sandra	850 1000	765 900	206 242	10 12	233 274	316 372	85 100	18.18
Dimitra	849 1000	772 909	200 235	11 13	219 258	342 403	77 91	18.26
Dobrogea	860 1000	782 909	203 236	13 15	210 244	356 414	78 91	18.3

Table 4. Statistical results from the comparative statistics between Romanian and foreign varieties groups using T-test for Independent Samples (statistical significance  $p < 0.05$  was not met)

Group 1 vs. Group 2	Mean	Mean	t-value	df	p	Valid	Valid	Std.D	Std.D	F-rati	p	Leven	df	p
	Group 1	Group 2	e			N	N	ev.	ev.	o	Varia	e	Leven	Leven
						Group 1	Group 2	Group 1	Group 2	Varia	nces	F(1,df)	e	e
PR55V48 vs. Sandra	16.79	16.66	0.11	14	0.92	8	8	2.40	2.31	1.08	0.93	0.05	14	0.83
PR55V48 vs. Valahia	16.79	16.60	0.19	14	0.85	8	8	2.40	1.51	2.52	0.25	2.88	14	0.11
PR55V48 vs. Dobrogea	16.79	16.10	0.48	14	0.64	8	8	2.40	3.28	1.87	0.43	1.30	14	0.27
Dimitra vs. Sandra	15.90	16.66	-0.61	14	0.55	8	8	2.64	2.31	1.31	0.73	0.31	14	0.59
Dimitra vs. Valahia	15.90	16.60	-0.65	14	0.53	8	8	2.64	1.51	3.07	0.16	3.98	14	0.07
Dimitra vs. Dobrogea	15.90	16.10	-0.13	14	0.90	8	8	2.64	3.28	1.53	0.59	0.63	14	0.44

Dry matter content in green mass varied between 260 and 310 g, *OM* between 229 and 268 g, and *CP* between 200 and 210 g, respectively. Gross energy was similar for all varieties ranging around 17 MJ (Average=17.63 MJ; *St.Dev.* = 0.21). Overall, Dobrogea and Dimitra varieties showed slightly better performances compared to the other varieties.

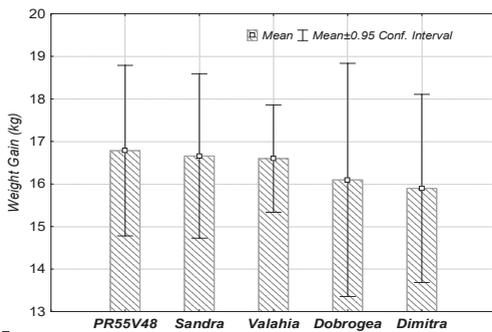


Figure. 1 Weight Gain average (kg) obtained by the lambs fed 93 days with hay from various alfalfa varieties (error bars show the spread within the group)

Concerning alfalfa hay, the *DM* ranged between 848 and 861 g, *OM* 759 and 782 g, and *CP* 65 and 76 g, respectively. Gross energy was similar for all varieties ranging around 18 MJ (Average = 18.18 MJ; *St. Dev.* = 0.11). In this case, PR55V48 and Dobrogea varieties seemed to show improved forage quality within the tested varieties. However, the differences between varieties were lower when considering hay compared to green fodder, and did not show statistical significance. The results regarding the hay quality are in agreement with data reported in the literature (e.g. Yanez-Ruiz and Molina-Alcaide, 2007).

After analyzing the chemical composition of alfalfa hay, the resulting amount of crude protein, which is an important qualitative indicator, was maintained at a good level in the case of the three Romanian varieties (Valahia, Sandra, and Dobrogea). These results support their cultivation in livestock farms from Romania and other regions with temperate climates.

However, an important test that qualitative fodder must pass is the performance of the animal to achieve a significant weight gain in the proper time. The quality of the diet can be considered satisfactory when the animal consuming that fodder achieves the performance desired by the breeder. The results regarding the production increase for the food rations based on alfalfa hay for young fattened sheep in a semi-intensive system were presented in Figure 1.

The weight gains per capita increases recorded by Sandra, Dobrogea, and Valahia varieties at the end of the testing period showed small differences compared to the increases made by the foreign varieties (PR55V48 and Dimitra). However, there were small differences without statistical significance -  $p > 0.05$  (Table 4). Valahia variety showed a constant response regarding the weight gain compared to the other varieties (the largest spread occurred for Dobrogea, followed by Dimitra). Based on the average value, the highest gain was reached by PR55V48 (16.8 kg; *St. dev.* = 2.4) followed by Sandra (16.7 kg; *St. dev.* = 2.3), Valahia (16.6 kg; *St. dev.* = 1.5) and Dobrogea (16.1 kg; *St. dev.* = 3.3). Dimitra showed the lowest average weight gain (15.9 kg; *St. dev.* = 2.6).

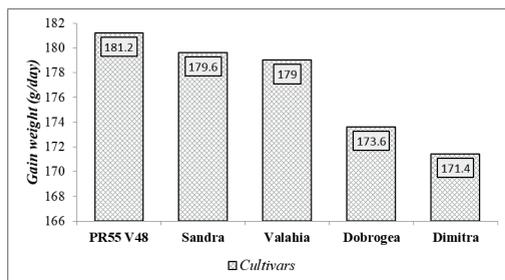


Figure. 2 Daily weight gain (g/day) obtained by the lambs fed 93 days with hay from various alfalfa varieties

Figure 2 shows the daily weight gain for all cultivars that ranged between 171.4 g/day (Dimitra) and 181.2 g/day (PR55V48). The hay from Romanian varieties provided good daily gains that demonstrate useful support in fattening lambs for obtaining ecological products.

## CONCLUSIONS

The results of the qualitative analysis of forage together with the weight gains of fattening lambs have positioned the Romanian alfalfa varieties at a very good level for using hay in young sheep diets, comparable to other well-established varieties such as the PR55V48 variety from Pioneer and the Dimitra variety from Continental Semences.

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## ESTABLISHMENT OF PHENOTYPIC CORRELATIONS AND GENETIC DISTANCE IN A DIALLEL CROSS OF DURUM WHEAT

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### Abstract

*The aim of this research is to study phenotypic correlations and establishing the genetic distance between parents and their hybrids by cluster analysis. Experiments were conducted in three consecutive years in the experimental field of the Field Crops Institute - Chirpan, Bulgaria. The trials were performed in a randomized block design in three replications. Nine quantitative traits were observed, seven related to productivity and two related to grain quality. Significant differences between genotypes were established for all studied traits. The traits grains number per spike and grains weight per spike had a high positive correlation. This relationship was of great importance in determining the right strategy for leading a selection for productivity. At the first level the cluster analysis revealed two clusters genetically distant from each other. The bigger cluster was divided into two clusters with significant distance. Three clusters with a significant distance between them were observed. Parents and their hybrids fall into different clusters, indicating that hybrid combinations are genetically distant from their parents.*

**Key words:** diallel cross, durum wheat, genetic distance, cluster analysis, correlations.

### INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is the most common type of tetraploid wheat. Its grain harvest is about 35 million tons of grain per year (Ranieri, 2015). Durum wheat has qualities that make it very special for on the world wheat market. It has a high vitreousness, strong gluten with high extensibility and low elasticity, high protein content and carotenoids and is used in pasta manufacturing. Durum wheat has the hardest grain of all types of wheat and is used to produce semolina (Dixon et al., 2009). It is a traditional crop that serves as a material for the production of pasta and is indispensable in this respect from common wheat.

The study of correlations between the individual characteristics of durum wheat plants and their practical use in the breeding of this crop is very important. The correlation analysis of traits makes it possible to establish the dependencies between them and especially those that determine productivity as the most important complex indicator and to look for opportunities for their change and obtaining stable and productive varieties (Dechev, 2004). Nayeem & Baig (2003) emphasize the grains' number per spike, test weight, and density. It has been reported that the number of grains per spike had a positive and significant correlation

with the yield and an increase in this trait would lead to an increase in productivity (Dogan, 2009). Rachovska & Uhr (2010) concluded that a significant increase in yield would be achieved when the weight of grains per spike is increased. It is known that the weight of the grains per spike is directly related to the yield and as its component, it is important to establish the presence of correlations with other elements of the yield. This would help in selecting highly productive plants in the early stages of the breeding process. Mollasadeghi et al. (2011) found that the increase in productivity would be achieved if the traits grains number per spike, grains weight per spike, and the thousand-grain weight increased at the same time. Cifri (2012) reported that the elements related to yield were spike length, grains number per spike, and grains weight per spike. According to Popova & Neykov (2013), the largest positive relationship with the yield had the characteristics of grains number per spike and grains weight per spike. Slafer et al. (2014) confirmed the statement that the yield per unit area was influenced to the greatest extent by the number of grains per spike and thousand-grain weight. Nofouzi (2018) revealed that plant height, productivity tillering, spike length

and grains number per spike had a positive and significant correlation with yield.

The genetic distance of genotypes determines their potential for breeding progress when applying the method of hybridization, according to the available genetic distance of genotypes. The more genetically distant the parental forms, the greater potential for gene interaction in the form of dominance and epistasis leading to an increase in the potential for heterosis and transgression (Falconer, 1989). The method of hybridization is leading to the creation of genetically distant genotypes at the beginning of the breeding process. The success of the breeding, to create high-yielding varieties of wheat, largely depends on the correct set of parents for crossing. There are no rules on the basis of which combinations for obtaining promising lines can be foreseen in advance. By studying the quantitative traits, it is possible to gain a greater understanding to combine the hereditary basis between genotypes in the hybridization process will be achieved. Increasing the number of genetically distant genotypes makes it possible to provide reliable sources of variation in different traits. By determining the genetic distance between genotypes, the correct selection of parental forms can be made in order to make significant progress in the yield potential in recombinant genotypes (Islam, 2004). The assessment of genetic distance between genotypes can be based on the phenotypic manifestation of quantitative and qualitative traits (Souza & Sorrells 1991). Most often, genetic distance is measured as phenotypic distance (Kabir et al., 2009). It is assumed that if the genotypes are different phenotypically by more traits, they are also genetically distant. Eivazi et al. (2007) successfully applied cluster analysis to determine the genetic distance in plant breeding. The cluster analysis depicts a dendrogram that shows how genotypes are distant. Genetic distant groups, grouped based on cluster analysis in tetraploid wheat explain the differences between genotypes (Hailu et al., 2006). Cluster analysis is also useful for selecting parents to use in modeling the breeding program (Souza & Sorrells, 1991). Narouei et al. (2006) determined the genetic distance in local wheat varieties on the basis of several morphological traits. Talebi et al.

(2010) studied 24 durum wheat genotypes to determine their genetic distance, more important traits related to yield, and using cluster analysis identified three clusters. Hashjin et al. (2014) studied 116 genotypes from several different regions in order to establish the genetic distance between them.

The aim of this research was to study phenotypic correlations and identify the most important traits to improve durum wheat productivity. Establishing the genetic distance between parents and their hybrids by cluster analysis for complete and effective use of the results of diallel crosses.

## MATERIALS AND METHODS

The research was conducted in the experimental field of the Field Crops Institute - Chirpan. The standard local technology for growing durum wheat adopted at the institute was used. The soil type is Eutric Vertisols (by FAO), characterized by medium organic matter (1.5-2.4%), with slightly acid to neutral soil reaction. The experiments were sown in the optimal period for durum wheat in Bulgaria on October 20-30. The genotypes heading time was on May 8-16. The plants were taken (harvested) on July 7-10 in full maturity. Meteorological conditions during the three-year period of the study were characterized by higher temperatures than the multi-annual norm. The first two harvest years of 2014 and 2015 were favourable in terms of soil moisture and rainfall higher than the average for many years. The third harvest year was characterized as the hottest and at the same time with 20% less precipitation. The genotypes were tested under field conditions by the randomized block design in three replications in the period 2014-2016. Each parent and F<sub>1</sub> was hand sown in two rows; each row was two-meter long; spaces between rows were twenty cm and five cm between plants. Modern durum wheat varieties were used as parents: Victoria (11), Deni (22), Superdur (33), Progres (44), Predel (55). The included varieties were crossed in a diallel design without reciprocal ones and their ten F<sub>1</sub> hybrid combinations were obtained: Victoria x Deni (12), Victoria x Superdur (13), Victoria x Progres (14), Victoria x Predel (15), Deni x Superdur (23), Deni x Progres (24), Deni x

Predel (25), Superdur x Progres (34), Superdur x Predel (35), Progres x Predel (45). The inclusion of F1 in the present study is due to the fact that the F1 generation is heterozygous but absolutely phenotypic homogeneous. It contains the genes of both parents and with the replications of the cross will always have the same genotype.

Nine quantitative traits were studied. Seven are related to productivity and two are related to grain quality. The following traits were studied: plant height, productivity tillering, spike length, spikelets number per spike, grains number per spike, grains weight per spike, thousand-grain weight, grain protein content, and wet gluten content. Productivity tillering by counting them (pcs.). Spike length by measurement of spike length in centimeters. Spikelets number per spike by counting (pcs.). Grains number per spike by counting (pcs.). Grains weight per spike by weighing the grains of the main spike in grams (g). Thousand-grain weight was calculated by the formula (grains weight per spike/grains number per spike)\*1000 in grams (g). Grain protein content in percentage was estimated by measuring N according to the Kjeldahl method. The following formula was used: Protein, % = N (% DM) x 5.7 to convert the N content to protein content (BDS EN ISO 20483:2014). Wet gluten content in percentage was established by the standard mechanical method for their determination with perten glutomatic system (BDS EN ISO 21415-2:2008). In full maturity, from each replication, a total of twenty plants necessary for the biometric measurements and the technological analysis were randomly selected from plots every year. The data from the three years were combined and their average value was calculated. On the results for the mean of parents and their hybrid combinations was conducted Duncan's test for multiple comparing the means at the detected significant differences ( $p < 0.05$ ) (Duncan, 1955). Correlation and variation analysis was performed by Lidanski (1988). From the multivariate methods was used hierarchical cluster analysis in order to group the genotypes by genetic similarity and distance by the method of Ward (1963), as the data was standardized to unify the scale. The data analysis software system Statistica 10 was used to calculate the data.

## RESULTS AND DISCUSSIONS

Apart from the parents, the meanings of F1 generations can also be considered as separate genotypes. They are heterozygous, but at the same time extremely homogeneous, due to the same genotype of each individual from the individual hybrid combination in this generation. Duncan's test for multiple comparing revealed significant differences between genotypes for all studied traits (Table 1). This was evidence of a significant diversity between the tested genotypes. The mean, maximum and minimum values indicate that the traits were within the normal range for durum wheat. The calculated coefficients of variation reveal that the trait of productivity tillering varied the most (CV = 10.7), followed by the trait grains weight per spike and the grains number per spike, and the trait with lowest CV was wet gluten content. The other traits occupied an intermediate position in terms of coefficients of variation. According to Alam et al. (2013) in durum wheat the grains number per spike had the highest coefficient of variation. Sabaghnia et al. (2014) reported that the traits of productivity tillering and grains number per spike were the most varied. Adhikari et al. (2018) report high coefficients of variation for the grains number per spike. A similar result for coefficients of variation for the trait of productivity tillering was reported by Tambe et al. (2013).

From a breeding point of view, correlations between the traits related to productivity are of particular importance. A correlation analysis was performed between the traits included in the study involving parents and F1 hybrids. The obtained values of correlation coefficients are presented in Table 2. From all 36 possible correlations, only 9 were statistically significant. The trait productivity tillering had only one significant positive correlation with the spike length ( $r = 0.61^*$ ). Nofouzi (2018) also reported the same correlation. Plant height had four significant positive correlation coefficients. One positive with a thousand grain weight ( $r = 0.72^{**}$ ) and three negative with spike length ( $r = -0.53^*$ ) with grain protein content ( $r = -0.87^{**}$ ) and wet gluten content ( $r = -0.63^*$ ).

Table 1. Duncan's multiple range test, mean values, standard deviation, min, max, CV and standart error of 15 genotypes for 9 quantitative traits

Genotypes	Plant height, cm	Productivity tillering, pcs	Spike length, cm	Spikelets number per spike, pcs	Grains number per spike, pcs	Grains weight per spike, g	Thousand grain weight, g	Grain protein content %	Wet gluten content %
Victoria	103.7 <sup>g</sup>	7.4 <sup>ab</sup>	6.3 <sup>a</sup>	21.3 <sup>ab</sup>	47.8 <sup>abc</sup>	2.2 <sup>ab</sup>	47.5 <sup>cd</sup>	14.3 <sup>a</sup>	29.6 <sup>a</sup>
Deni	90.3 <sup>bcd</sup>	8.3 <sup>abcd</sup>	8.5 <sup>cefg</sup>	22.9 <sup>ef</sup>	43.4 <sup>a</sup>	2.0 <sup>a</sup>	46.5 <sup>bcd</sup>	15.7 <sup>ab</sup>	32.0 <sup>bc</sup>
Superdur	79.6 <sup>a</sup>	7.7 <sup>ab</sup>	8.4 <sup>bcd</sup>	21.7 <sup>abcd</sup>	50.2 <sup>abcd</sup>	2.0 <sup>a</sup>	39.3 <sup>a</sup>	16.5 <sup>c</sup>	32.0 <sup>ab</sup>
Progres	98.5 <sup>cdef</sup>	7.7 <sup>ab</sup>	8.6 <sup>efg</sup>	21.4 <sup>abc</sup>	44.8 <sup>ab</sup>	2.2 <sup>ab</sup>	50.5 <sup>cd</sup>	15.1 <sup>ab</sup>	31.3 <sup>ab</sup>
Predel	87.8 <sup>bc</sup>	7.7 <sup>ab</sup>	8.0 <sup>bcd</sup>	22.1 <sup>bcd</sup>	53.6 <sup>cde</sup>	2.1 <sup>ab</sup>	40.3 <sup>ab</sup>	16.4 <sup>c</sup>	32.0 <sup>bc</sup>
Victoria x Deni	99.4 <sup>efg</sup>	6.9 <sup>a</sup>	7.9 <sup>bd</sup>	23.6 <sup>f</sup>	52.4 <sup>bcd</sup>	2.4 <sup>ab</sup>	47.7 <sup>cd</sup>	15.6 <sup>ab</sup>	30.9 <sup>ab</sup>
Victoria x Superdur	98.0 <sup>efg</sup>	8.1 <sup>abcd</sup>	7.9 <sup>bcd</sup>	22.4 <sup>cd</sup>	57.9 <sup>de</sup>	2.6 <sup>bc</sup>	47.4 <sup>cd</sup>	15.7 <sup>ab</sup>	32.3 <sup>bc</sup>
Victoria x Progres	102.5 <sup>fg</sup>	7.2 <sup>ab</sup>	8.1 <sup>bcd</sup>	22.7 <sup>def</sup>	52.3 <sup>bcd</sup>	2.4 <sup>abc</sup>	49.6 <sup>cd</sup>	15.2 <sup>ab</sup>	31.0 <sup>ab</sup>
Victoria x Predel	96.7 <sup>def</sup>	7.6 <sup>ab</sup>	8.0 <sup>b</sup>	22.6 <sup>def</sup>	56.8 <sup>de</sup>	2.6 <sup>c</sup>	47.0 <sup>cd</sup>	15.8 <sup>bc</sup>	31.2 <sup>ab</sup>
Deni x Superdur	93.2 <sup>cde</sup>	8.3 <sup>abcd</sup>	9.1 <sup>gk</sup>	22.9 <sup>ef</sup>	56.9 <sup>de</sup>	2.6 <sup>bc</sup>	48.0 <sup>cd</sup>	15.6 <sup>ab</sup>	29.7 <sup>a</sup>
Deni x Progres	92.7 <sup>cde</sup>	9.7 <sup>cd</sup>	9.2 <sup>k</sup>	22.3 <sup>bcd</sup>	49.8 <sup>abcd</sup>	2.5 <sup>bc</sup>	51.0 <sup>d</sup>	15.7 <sup>abc</sup>	31.4 <sup>ab</sup>
Deni x Predel	89.2 <sup>bc</sup>	9.0 <sup>bcd</sup>	9.1 <sup>gk</sup>	22.8 <sup>ef</sup>	56.1 <sup>de</sup>	2.6 <sup>bc</sup>	46.0 <sup>bcd</sup>	16.1 <sup>bc</sup>	32.3 <sup>bc</sup>
Superdur x Progres	87.6 <sup>bc</sup>	8.0 <sup>abc</sup>	8.5 <sup>cdefg</sup>	20.6 <sup>a</sup>	57.3 <sup>cde</sup>	2.6 <sup>c</sup>	46.0 <sup>bcd</sup>	16.2 <sup>bc</sup>	33.6 <sup>d</sup>
Superdur x Predel	84.8 <sup>ab</sup>	10.0 <sup>d</sup>	8.5 <sup>bcd</sup>	21.4 <sup>abc</sup>	58.2 <sup>c</sup>	2.6 <sup>bc</sup>	43.9 <sup>abc</sup>	16.1 <sup>bc</sup>	32.5 <sup>bc</sup>
Progres x Predel	87.9 <sup>bc</sup>	8.7 <sup>abcd</sup>	8.9 <sup>fgk</sup>	21.4 <sup>abc</sup>	52.2 <sup>cde</sup>	2.5 <sup>abc</sup>	47.2 <sup>cd</sup>	16.1 <sup>bc</sup>	32.7 <sup>bc</sup>
Mean	92.8	8.2	8.3	22.1	52.6	2.4	46.5	15.7	31.6
Min	79.	6.9	6.3	20.6	43.4	2.0	39.3	14.3	29.6
Max	103.7	10.0	9.2	23.6	58.2	2.6	51.0	16.5	33.6
CV	7.4	10.7	8.5	3.6	8.9	9.4	7.0	3.5	3.3
± m	1.7	0.22	0.18	0.20	1.22	0.05	0.84	0.14	0.27

Mean values ± standard deviation values (in each column), followed by the same letters (in index) are not significantly different at  $p < 0.05$  according to Duncan's multiple range test (DMRT)

Table 2. Correlation analysis between 9 quatitative traits

Traits	1	2	3	4	5	6	7	8	9
1. Plant height	1	-0.51	-0.53*	0.31	-0.18	0.19	0.72**	-0.87**	-0.63*
2. Productivity tillering		1	0.61*	-0.19	0.24	0.36	0.02	0.37	0.37
3. Spike length			1	0.13	0.15	0.26	0.10	0.59*	0.41
4. Spikelets number per spike				1	0.03	0.04	0.20	-0.05	-0.39
5. Grains number per spike					1	0.76**	-0.20	0.42	0.26
6. Grains weight per spike						1	0.44	0.03	0.10
7. Thousand grain weight							1	-0.64*	-0.34
8. Grain protein content								1	0.73**
9. Wet gluten content									1

\* -  $p < 0.05$ , \*\* -  $p < 0.01$

From these correlation coefficients, it can be seen that tall genotypes had a larger grain, a shorter spike, and lower grain quality indicators. Authors Khan et al. (2013) and Rathwa et al. (2018) also find a significant positive correlation between plant height and the thousand grains' weight. Spikelets number per spike had no statistically significant correlation with another of the studied traits.

The grains number per spike correlated significantly only with the weight of grains per spike ( $r = 0.76^{**}$ ). The correlation was positive and showed that the increase in the number of grains per spike led to an increase in the productivity of the spike. Stefanova-Dobrova & Muhova (2020) reported a significant positive correlation between the number of grains per spike and grains weight per spike. A number of

studies showed that the weight of the grains per spike was the main breeding indicator determining the productivity of the genotype. Therefore, the number of grains per spike is extremely important for breeding. These two traits had no significant correlations with the other traits in the study. This means that the number of grains per spike was extremely important for productivity. Other authors also found a positive correlation between the grains number per spike and the weight of the grains per spike in durum wheat (Popova & Neykov, 2013; Rathwa et al., 2018). They concluded that increasing these two traits would lead to a significant increase in productivity. Therefore, when leading a selection in the next segregating generations, special attention must be paid to the grains numbers per spike. The grain protein content correlated negatively with the thousand-grain weight ( $r = -0.64^*$ ). The grain wet gluten content correlated very strongly and positively with grain protein content. Authors Taneva et al. (2019), Dragov et al. (2019), Stefanova-Dobrova & Muhova (2020a) also reported a significant and positive correlation between wet gluten content and grain protein content. Hannachi et al. (2013) and Gerema (2020) reported that grains number per spike and grain yield were in a positive significant correlation. On the other hand, grains weight per spike had a positive significant correlation with yield (Sourour et al., 2018). The established correlation coefficients could be used to optimize the selection in the segregating generations. They should be taken into account, especially in the breeding program for increasing the productivity of durum wheat. The most important traits were grains number per spike and grains weight per spike and selection should be led by these traits to increase productivity.

Cluster analysis was applied to measure the genetic distance of parents and their hybrids in this diallel cross. The data of the obtained results for parents and F1 hybrids on all studied traits were used. The values by traits for each of the studied years were included. This allowed a large group of small genes controlling the stability of traits to be included in the overall assessment and construction of the dendrogram. In this way, we got an enriched result with greater benefit for the

breeding. In order to unify the scale and more accurately assess the results obtained, the data were standardized (Siahbidi et al., 2013).

The dendrogram from the hierarchical cluster analysis is presented in Figure 1. The figure shows the separation of parents and their hybrids was in three main clusters with a sufficient degree of differences between them.

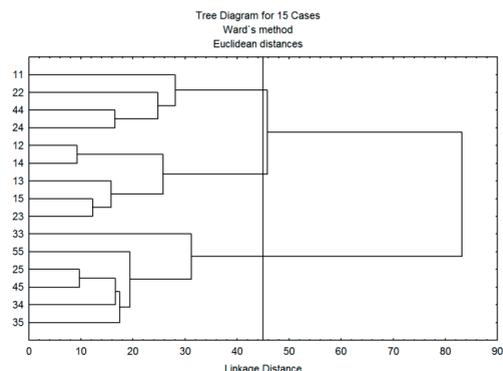


Figure 1. Dendrogram of 15 durum wheat genotypes from the diallel cross on 9 quantitative traits. Victoria (11), Deni (22), Superdur (33), Progres (44), Predel (55), Victoria x Deni (12), Victoria x Superdur (13), Victoria x Progres (14), Victoria x Predel (15), Deni x Superdur (23), Deni x Progres (24), Deni x Predel (25), Superdur x Progres (34), Superdur x Predel (35), Progres x Predel (45)

Cluster I included variety Victoria (11), variety Deni (22), variety Progres (44), and cross Deni x Progres (24). This cluster included three of the parents, as well as a cross between two of them, which indicates the presence of some genetic similarity between them. Cluster II included only crosses Victoria x Deni (12), Victoria x Progres (14), Victoria x Superdur (13), Victoria x Predel (15), and Deni x Superdur (23). This cluster included all crosses where the variety Victoria (11) participated as a parent. This indicates a strong influence of the variety Victoria (11) on the results of hybrids with it, which made them genetically closer to each other. Cluster III included variety Superdur (33), variety Predel (55) and crosses Deni x Predel (25), Progres x Predel (45), Superdur x Progres (34) and Superdur x Predel (35). Observation of this cluster shows that the variety Superdur (33) was different from the others in the cluster. This is easily explained given that the Austrian variety Superdur (33) was the only foreign variety involved in the

diallel cross. As expected, the Predel (55) variety falls into the same group as most of the hybrids in which it was a parent. Thus, a great similarity was observed between the variety Predel (55) and its F1 hybrids. A careful examination of the dendrogram shows some genetic similarity between cluster I and cluster II. On the other hand, it was observed a high degree of genetic distance between clusters I and II with cluster III. Therefore, it could be argued that the diallel cross led to the production of a relatively distant hybrid combination from their parents. In addition, some hybrids differed significantly from their parents. Diallel crossing made it possible to the creation of genetically distant hybrids in the complete combination of genetic material from a group of varieties.

Variety Predel is a standard for yield in Bulgaria. Hybrid combinations clustered with it were valuable in terms of breeding. In terms of breeding according to a complex assessment of all traits valuable were the hybrid combinations: Superdur x Predel, Superdur x Progres, Progres x Predel, Deni x Predel. They were genetically similar to the standard and in their segregating generations can be found generations that superiority it. Very valuable are the crosses showed heterosis on several traits: Deni x Predel by three traits and Superdur x Progres by two traits. These crosses can be used directly both for the production of transgression forms and for the development of hybrid breeding (Dragov, 2019). After continuing the individual selection in the hybrid combination Deni x Predel, it is possible to increase the yield and create a new durum wheat variety, as the Deni variety is genetically distant from the Predel variety. The genetic similarity and distance of the genotypes in the diallel cross from the dendrogram could be used in creating a strategy for conducting an effective breeding process in durum wheat.

The greater genetic distance between the parents was a prerequisite for heterosis manifestations in the segregated generations. Genetically closer genotypes need to be combined to achieve faster success. To achieve greater breeding progress, but in later segregating generations, it is advisable to cross genetically more distant parents. In an experiment using cluster analysis, Khodadadi et

al. (2011) and Dimitrov et al. (2021) reached the same conclusion about the breeding strategy. Many researchers used cluster analysis to study agro-morphological similarities and distances between genotypes (Awan et al., 2014; Yadav et al., 2015). Cluster analysis based on quantitative traits gave an idea of the similarity between genotypes by calculating the euclidean distance (Awan et al., 2014). This could be used to improve durum wheat by selecting a variety for hybridization from different clusters (Ghafoor et al., 2012). To use transgressive breeding in wheat, the parents should be genetically distant (Ahmad et al., 2014; Zamanianfard et al., 2015). The greater the genetic distance between the parents, the more likely it was to observe heterosis and better-segregated generations (Mehari et al., 2015). In addition, the results showed that crosses involving parents from different clusters can lead to maximum heterosis and increase genetic distance (Hailegiorgis et al., 2011; Singh & Salgotra, 2014). The result of the study was a prerequisite for optimizing the breeding program and accelerating the breeding process in durum wheat.

## CONCLUSIONS

Significant differences were found between genotypes on all studied traits. The traits grains number per spike and grains weight per spike had a high positive correlation. This relationship was of great importance in determining the right strategy for leading a selection for productivity. Diallel crossing made it possible to the creation of genetically distant hybrids in the complete combination of genetic material from a group of varieties. Parents and their hybrids fall into different clusters, indicating that hybrid combinations are genetically distant from their parents. The hybrid cross Deni x Predel is extremely valuable in terms of selection. Three clusters were observed with a significant distance between them. The genetic similarity and distance of the genotypes in the diallel cross from the dendrogram could be used in creating a strategy for conducting an effective breeding process in durum wheat. The hybrid cross Deni x Predel is extremely valuable in terms of breeding. The result of the study was a

prerequisite for optimizing the breeding program and accelerating the breeding process in durum wheat.

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## EVALUATION OF MAIZE HYBRIDS FOR GRAIN YIELD AND QUALITY TRAITS UNDER FIELD CONDITIONS FROM SOUTHWESTERN ROMANIA

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### Abstract

*This paper presents the effects of the experimental year and the hybrid (genotype) on the grain yield, protein and oil contents of Romanian commercial maize hybrids. Field trials were conducted at Agricultural Research and Development Station Șimnic located in southwestern Romania, during three successive years (2016, 2017 and 2018). The average grain yield for all hybrids tested was 7.53 t/ha. The 2018 yields were significantly higher than the 2016 and 2017 yields. The F376 hybrid achieved the highest average grain yield and oil content but the lowest protein content. The very dry year 2017 was the best year for the synthesis of protein and oil in maize grains. The average protein content for all hybrids tested was 14.5% and the average oil content was 5.1%. Oituz and F423 hybrids achieved the highest average protein content compared to other hybrids tested. The Oituz hybrid also achieved the highest average oil content (except the F376 hybrid). The combined three years of data revealed that grain yield was significantly negatively correlated with the protein and oil contents.*

**Key words:** maize, oil content, protein content, yield.

### INTRODUCTION

Agro-ecological factors are dominant in the forming process of the yield of field crops.

The year weather conditions are decisive in the yield creation process of crops and their interaction with other factors leads to regulation of particular growth phase in which the quantity and quality of the final yield are formed (Černý et al., 2011; Popović et al., 2013).

The Oltenia region located in southwestern part of Romania represents an important agricultural region, which has vast areas that can be cultivated properly only if climate restrictions are taken into account. In this region, drought and heat are common, with only two out of ten years being favorable for crops. These abiotic factors always lead to different levels of yield loss depending on its intensity and crop stage (Bonea & Urechean, 2019; Urechean & Bonea, 2017; Urechean et al., 2019; Drăghici et al., 2019; 2021). According to Bonea & Urechean (2020), the average temperatures from grain-filling period and the rainfall from sowing to anthesis period were the dominant climatic

factors that explained 94.6% and 93.3% respectively, of the inter-annual variability of maize yield.

Many researchers believe that a proper choice of cultivar, in addition to optimal cultural practices, can ensure a high yield and quality of maize grown under different agro-ecological conditions (Popovic et al., 2013; Bojović et al., 2019).

Maize (*Zea mays* L.) is one of the most important plants grown in the world from an economic point of view and it is used as a source of food, animal feed and for various industrial applications.

The oil content is an important trait of maize grains when the yield is used for animal feed because the oil has a higher calorific power than starch (Abou-Deif et al., 2012). Maize oil is also good for consumption, being recommended for cooking due to its high content of unsaturated fatty acids (Mangolin et al., 2004; Sing et al., 2014). Therefore, the promotion and cultivation of high-yielding maize hybrids with good nutritional quality and drought tolerance is a prerequisite for resolving food insecurity.

The objectives of this paper were to evaluate the effects of the experimental year and the hybrid on grain yield and the quality of Romanian maize hybrids and also to investigate the association of yield with protein and oil content.

## MATERIALS AND METHODS

Four Romanian commercial hybrids developed by NARDI Fundulea (Oituz, Iezer, F376 and F423) were used in this study.

The field experiments were conducted in three consecutive growing seasons (2016, 2017 and 2018) at the Agricultural Research and Development Station (ARDS) Simnic located in southwestern Romania, an area characterized by a temperate continental climate with sub-mediterranean influences. The trials were

designed as randomized block experiments with four maize hybrids in three replications on a reddish preluvosoil.

Standard technological practices for maize cultivation have been applied. Sowing was carried out in the last decade of April (22 and 23, respectively) in 2016 and 2018 or in the first decade of April (10) in 2017.

Harvesting was carried out in the first decade of August each year.

The grain yield per plot was adjusted to 15.5% grain moisture and was converted to tones per hectare.

The protein content and oil content of the maize grains was determined by PERTEN Inframatic 9140.

The precipitation and temperature data were collected from Weather Station Craiova (Table 1).

Table 1. Monthly average precipitation and temperature in the experimental years (ARDS Simnic, 2016, 2017 and 2018)

Year	Deviation from multiannual average ( $\pm$ )					
	April	May	June	July	August	April-August
<i>Precipitation (mm)</i>						
2016	+19.6	+16.6	+31.5	-28.8	-12.0	-12.3
2017	+10.4	+0.1	-50.5	+25.2	-40.6	-55.4
2018	-42.5	-19.9	+66.5	+52.2	-21.6	+34.7
Multiannual average	53.6	70.9	74.5	82.8	49.6	331.4
<i>Temperature (<math>^{\circ}</math>C)</i>						
2016	+2.7	-1.3	+0.5	+0.1	+0.8	
2017	-0.5	-0.3	+1.8	+0.4	+2.7	
2018	+4.0	+1.6	+0.2	-1.2	-1.4	
Multiannual average	12.1	17.6	21.4	23.8	22.3	

The year 2016 was considered moderately favorable for maize crop, the deficit being small (-12.3 mm) compared to the multiannual average. In this year April, May and June were wet with frequent rainfall but July and August were dry.

The year 2017 was characterized as an unfavorable year with severe drought stress in June and August, the deficit reaching -55.4 mm compared to the multiannual average. During these periods, the heat was more intense. April and May were optimal for plants, June was extremely dry, July was wet and August was very dry.

In 2018, April, May and August were dry, while June and July were very wet, the year

being considered moderately favorable for maize crop.

Effects of experimental year and hybrid (genotype) were evaluated by analyses of variance (two-factor ANOVA) with interaction. Significant differences between averages were reported using the Least Significant Difference (LSD) at the  $p \leq 0.05$  level. Relative dependence was defined by correlation analysis and obtained coefficients were tested for significance level of 0.05% and 0.01% (Săulescu & Săulescu, 1967). All analyses were carried out using Microsoft Office Excel program.

## RESULTS AND DISCUSSIONS

### Grain yield

Grain yield is one of the complex characters controlled by several interacting genotypic and environmental factors. Few yield components are less complex, highly inherited and less influenced by the environmental changes (Kashiani & Saleh, 2010).

According to Heidari et al. (2019), a considerable range of variations for the tested traits provide a good opportunity for selection to identify superior genotypes and to use them as a genetic source for breeding purposes versus yield improvement and the introduction of commercial varieties, especially in conditions of drought stress.

For grain yield, ANOVA results showed significant differences between years and hybrids ( $P \leq 0.01$ ) (Table 2).

Several researchers have reported that environment fluctuations have a high impact on maize yield (Haş et al., 2010; Barutcular et al., 2016; Bonea, 2016).

The maize yield varied considerably during the research period, depending on the amount and distribution of precipitation during the growing season (Table 3).

On average, in 2018 there was a significantly higher yield (8.90 t/ha) compared to 2017 (5.07 t/ha) and 2016 (8.61 t/ha). The highest yield in 2018 was achieved by the hybrid F376 (10.31 t/ha), and in 2016 was achieved by the hybrids F423 (9.48 t/ha) and F376 (9.28 t/ha). In the very dry year 2017, the best yields were given by the hybrids F376 (5.61 t/ha) and F423 (5.36 t/ha), while the hybrid Oituz had the lowest yield of 4.32 t/ha (Table 3).

Table 2. ANOVA for grain yield, protein and oil content

Source of variation	df	SS	MS	F
<i>Grain yield (t/ha)</i>				
Hybrid	3	15.64	5.21	113.80**
Year	2	109.46	54.73	1194.33**
Interaction	6	7.83	1.30	28.48**
Error	24	1.10	0.05	
<i>Protein content (%)</i>				
Hybrid	3	3.63	1.21	12.0**
Year	2	18.73	9.37	92.90**
Interaction	6	6.16	1.03	10.19**
Error	24	2.42	0.10	
<i>Oil content (%)</i>				
Hybrid	3	1.13	0.37	7.39**
Year	2	1.23	0.62	12.14**
Interaction	6	0.84	0.14	2.77*
Error	24	1.22	0.05	

\*and \*\* = significant at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively

The average yield for tested hybrids was 7.53 t/ha. The highest average yield in the research period 2016-2018 was achieved by the hybrid F376 (8.40 t/ha) followed by the hybrid F423 (7.70 t/ha), and the lowest average yield was achieved by the hybrid Oituz (6.56 t/ha).

The interaction between the analyzed factors (experimental year x hybrid) had a significant effect on grain yield ( $P \leq 0.01$ ) (Table 2).

In 2017 and 2018, the F376 hybrid had a significantly higher yield compared to all tested hybrids ( $P \leq 0.05$ ), and in 2016 the Oituz and F376 hybrids had significantly higher yields compared to all tested hybrids.

### Protein content

The quality of the maize yield is a complex trait that is formed during ontogenesis representing

the phenotypic expression of genotype x environment interaction (Bonea, 2016). ANOVA results for protein content showed significant differences between years and

hybrids ( $P \leq 0.01$ ) (Table 2). On average, the protein content was significantly higher in the very dry year 2017 (15.5%) compared to 2016 (14.1%) and 2018 (13.5%) (Table 3).

Table 3. The average grain yield, protein and oil content under field conditions from ARDS Şimnic (2016, 2017 and 2018)

Traits	Hybrid (A)	Year (B)			
		2016	2017	2018	Average per hybrid
Grain yield (t/ha)	Oituz	6.89	4.32	8.46	6.56
	Iezer	8.79	4.98	8.58	7.45
	F376	9.28	5.61	10.31	8.40
	F423	9.48	5.36	8.26	7.70
	Average per year	8.61	5.07	8.90	7.53
Protein content (%)	Oituz	15.0	15.8	13.5	14.8
	Iezer	13.2	15.6	13.7	14.2
	F376	13.6	15.3	13.4	14.1
	F423	14.7	15.1	13.5	14.8
	Average per year	14.1	15.5	13.8	14.5
Oil content (%)	Oituz	5.5	5.4	4.7	5.2
	Iezer	4.9	5.1	4.8	4.9
	F376	5.3	5.5	5.4	5.4
	F423	5.0	5.4	4.7	5.0
	Average per year	5.2	5.4	4.9	5.1

Indicator	LSD test	A	B	A x B
Grain yield	5%	0.13	0.15	0.25
Protein content	5%	0.19	0.22	0.38
Oil content	5%	0.13	0.15	0.27

Many researchers have noted that high air temperature increased protein content (Halford et al., 2015; Mayer et al., 2016).

According to Prasanna et al. (2001), the protein content in maize ranges between 6-14% depending on genotype and environment. In this study, the average value of protein content for the researching period (2016-2018) was 14.5%. The highest average protein content was achieved by the hybrids F423 and Oituz (14.8%), and the lowest content by Iezer (14.2%) and F376 (14.1%). These results are in agreement with those obtained by Bonea (2016).

The year x hybrid interaction also had a significant effect on protein content ( $P \leq 0.01$ ) (Table 3). In 2016, the hybrid Iezer had significantly lower protein content compared to all hybrids tested but in 2017 this hybrid had a significantly higher yield compared with the tested hybrids (except the Oituz hybrid). This difference may be due to the low temperature during grain-filling stage, which may be

detrimental to protein synthesis (Ahmed & Fayyazul, 2015).

Our results are consistent with the results of several researchers who stated that the protein content is characteristic of the genotype but strongly influenced by the environment (Mikhaylenko et al., 2000; Haş et al., 2010; Popovic et al., 2013),

A different range of variation (8.22 and 13.94%) was observed by Abou-Deif et al. (2012), and Oliveira et al. (2006) found that the averages of total protein content in dent and flint populations were 9.67% and 10.51%, respectively, and the average of their hybrid combinations was 11.86%.

#### Oil content

The results of ANOVA have indicated that the oil content differed significantly between years and hybrids ( $P \leq 0.01$ ) (Table 2).

On average, a significantly higher oil content (5.4%) was recorded in 2017 compared to 2016 (5.2%) and 2018 (4.9%) (Table 3).

The highest average oil content in the research period was achieved by hybrid F376 (5.4%) followed by Oituz (5.2%), and the lowest oil content by the hybrid Iezer (4.9%).

Interaction between year x hybrid had a statistically significant effect on the oil content ( $P \leq 0.05$ ). In 2018, the Oituz hybrid had a significantly lower oil content compared to F376 but in 2016 this hybrid had a significantly higher yield compared to Iezer and F423.

These results are in agreement with those obtained by Bonea (2016). Mittelmann et al. (2006) reported similar results for the average oil content in 10 maize genotypes and their hybrids, which varied from 4.22% to 4.94%. Abou-Deif et al. (2012) showed average values of 7.67% and 11.56% in inbred lines of maize, and the value for crosses were between 9.27 to 11.29%. According to Laurie et al. (2004), the kernels of a modern maize hybrid contain ~ 4% oil, 9% protein, 73% starch, and 14% other constituents (mostly fiber). Normal maize kernels contain 3-4% oil, while high oil genotypes have about 6% oil (Singh et al., 2014).

Large variations in the grain yield, protein and oil content indicated that these traits were affected by agro-ecological factors, in addition to genetic factors and management practices.

### Correlations analysis

The dependence between grain yield and protein and oil contents were determined by correlation analysis (Table 4).

The correlation coefficients between grain yield and protein content for the individual year were negative and non-significant in 2016 and 2018 ( $r = -0.486$ ;  $r = -0.538$ , respectively), but in 2017 the correlation coefficient was negative and significant ( $r = -0.687^0$ ).

The combined three years of data showed that grain yield was significantly negatively correlated with protein content ( $r = -0.838^{00}$ ;  $p \leq 0.01$ ).

On the contrary, Prakash et al. (2006) reported that grain yield was significantly positively correlated with protein content. Aliu et al. (2012) reported a negative and non-significant correlation between yield and protein content, but a positive and non-significant correlation with oil content.

Different relationships were observed between grain yield and oil content depending on the experimental year. The 2016 results indicated a negative and non-significant correlation

( $r = -0.559$ ). Opposite correlations were found in 2017 and 2018, when the dependence between grain yield and oil content was slightly positive ( $r = 0.200$ ) and significantly positive ( $r = 0.788^{**}$ ;  $p \leq 0.01$ ), respectively. These changes may be due to the total amount of precipitation in July, which favored both the yield and the oil content.

Table 4. Correlations between traits

Trait	Grain yield	Protein content	Oil content
<b>2016</b>			
Grain yield	1		
Protein content	-0.486	1	
Oil content	-0.559	0.323	1
<b>2017</b>			
Grain yield	1		
Protein content	-0.687 <sup>0</sup>	1	
Oil content	0.200	0.061	1
<b>2018</b>			
Grain yield	1		
Protein content	-0.538	1	
Oil content	0.788 <sup>**</sup>	-0.321	1
<b>Three years average</b>			
Grain yield	1		
Protein content	-0.838 <sup>00</sup>	1	
Oil content	-0.352 <sup>0</sup>	0.394 <sup>*</sup>	1

<sup>\*</sup>, <sup>0</sup> Significant at 0.05 probability level, <sup>\*\*</sup>, <sup>00</sup> Significant at 0.01 probability level

The average yield for the research period (2016-2018) was negative and significantly correlated with the oil content ( $r = -0.352^0$ ,  $p \leq 0.05$ ). Also, the protein content was positive and significantly correlated with the oil content ( $r = 0.394^*$ ,  $p \leq 0.05$ ).

Zdunic et al. (2012) observed a low relationship between yield and oil content in two testcross populations of maize. According to Singh et al. (2014), maize oil content is negatively correlated with grain yield and it is influenced by both genetic makeup and environmental conditions.

### CONCLUSIONS

It was concluded that year, hybrid, and hybrid x year interaction cause statistically significant differences in the grain yield, protein and oil contents of the maize hybrids tested.

The average yields were significantly higher in 2018 (8.90 t/ha) compared to the 2016 and 2017 yields.

The very dry year 2017 was the best year for the synthesis of protein and oil from maize grain (15.5% and 5.4%, respectively).

The F376 hybrid had the highest average grain yield and oil content but the lowest protein content. The F423 hybrid also had a significant average grain yield.

Oituz and F423 hybrids obtained the highest average protein content compared to the other hybrids tested. The Oituz hybrid also had the highest average oil content, except F376.

The combined three years of data showed that grain yield was significantly negatively correlated with protein and oil contents.

It can be recommended that growing F376 or F423 hybrid in order to obtain maximum grain yield, growing Oituz or F423 hybrid for maximum protein content, and growing F376 or Oituz hybrid for maximum oil content under the environmental conditions of southwestern Romania.

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## RESEARCHES CONCERNING THE EFFECTIVENESS OF THE MAIZE LEAF WEEVIL CONTROL (*Tanymecus dilaticollis* Gyll), IN THE COMMERCIAL FARM CONDITIONS, FROM THE SOUTH-EAST OF ROMANIA

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### Abstract

Maize leaf weevil (*Tanymecus dilaticollis* Gyll) is one of Romania's most harmful pests of maize crops. The purpose of this study is to evaluate the effectiveness of the different control methods of the maize leaf weevil in conditions of the high pest pressure from a commercial farm, located in one of the most favorable areas for this weevils species, in the southeast of Romania (Mihail Kogălniceanu, Ialomița County). This study has tested foliar treatment with acetamiprid and lambda-cyhalothrin active ingredients, seeds treatment with imidacloprid and cyantraniliprole active ingredients, and granules application with the lambda-cyhalothrin active ingredient. In the spring of 2021, at the experimental site, it has registered a high pest density (15-20 weevils/m<sup>2</sup>). As a result of the weather's unfavorable conditions for weevils activity on the ground, the pest attack was moderate when maize was in early vegetation stages. At the control (untreated) variant, most of the maize plants had leaves chaffed in the proportion of 50-75%, but the majority of the plants survived after the attack. In this study, lower weevils attack has registered in the case of the variants with seeds treated with imidacloprid and cyantraniliprole active ingredients. From all experimental variants, higher maize yield was recorded in the case of the variant with seeds treated with imidacloprid active ingredient (12501 kg/ha). This study, effectuated in conditions of high pest pressure, in commercial farm conditions, demonstrates that seed treatment with systemic insecticides adequately protects the maize plants in early vegetation stages (BBCH 10-BBCH 14) against maize leaf weevil attack.

**Key words:** maize, weevil, farm conditions, control.

### INTRODUCTION

Maize leaf weevil (*Tanymecus dilaticollis* Gyll) is a polyphagous pest; that belongs to the Curculionidae family (Coleoptera order). The literature has mentioned that these weevils attacked more than 70 host plants (Čamprag, 1963; Čamprag et al., 1969; 2006; Paulian, 1972; Popov and Bărbulescu, 2007). However, the insects from this specie have preferences for maize and sunflower plants (Paulian et al., 1979; Voinescu, 1985; Popov, 2002; Popov et al., 2005; Bărbulescu et al., 2001b; Georgescu et al., 2014; Troțuș et al., 2019). If the attack occurs when these crops are in the first vegetation stage (BBCH 10-BBCH 14), weevils can destroy young plants, and farmers

must sow again (Čamprag et al., 1969; Paulian, 1972; Bărbulescu et al., 2001b). Drought and high temperatures recorded during the first vegetation stages of the primary host plants favored weevils attack (Popov et al., 2006). Data from the literature suggest that maize monoculture; for several years have consequence increasing the maize leaf weevil's population in the following years and high yield losses as a result of this pest attack (Bărbulescu and Voinescu, 1998; Voinescu and Bărbulescu, 1998; Popov and Bărbulescu, 2007). A possible explication for this is because maize roots provide optimum conditions for larva development (Paulian, 1972; Boguleanu et al., 1980; Roșca and Istrate, 2009). As a result, maize plants provide

better conditions for the entire biological life cycle of the *T. dilaticollis*. The same authors mentioned that, from the wild plant's species, creeping thistle (*Cirsium arvense*) roots provide optimum conditions for larva development while leaves offer good conditions for the adults feeding. According to the data from the literature, in Romania, every year there were attacked by maize leaf weevil (*T. dilaticollis*) one million hectares cultivated with maize and one half million hectares cultivated with sunflower (Bărbulescu et al., 1991; 1993; 1995; Bărbulescu, 2001; Popov, 2002; 2003; Popov et al., 2005; 2007a; Popov and Bărbulescu, 2007). Early data from the Romanian literature mentioned average maize yield losses of 34% at a pest density of 25-30 weevils/m<sup>2</sup> (Paulian, 1972). The same author noted that the economic damage threshold for this specie is five weevils/m<sup>2</sup>. More recent data from the Romanian literature report higher densities of this pest in the favorable areas in south and south-east of Romania, ranging from 15 to 80 weevils/m<sup>2</sup> (Bărbulescu et al., 1991; 1993; 1995; Bărbulescu, 2001; Popov et al., 2005; 2007a). In our previous research, conducted in conditions of a commercial farm located in an area with high pest pressure (25-30 weevils/m<sup>2</sup>), untreated maize plants were destroyed by the weevils (Georgescu et al., 2018). At the same time, Badiu et al. (2019) report a reduction of the maize density by 25-50% due to this pest attack in the south, southeast, and east of Romania. Seed coating with systemic insecticides, with rapid translocation of the active ingredients in the plants, after the emergence, was the most effective method of controlling maize leaf weevils in Romania and neighbored countries (Voinescu, 1985; Bărbulescu et al., 1993; 2001b; Vasilescu et al., 2005; Popov and Bărbulescu, 2007; Čamprag, 2007; Popov et al., 2006; 2007b; Keszthelyi et al., 2008; Trotuș et al., 2011; 2019; Georgescu et al., 2021b). According to European Commission regulations 218/783, 218/784, and 218/785, imidacloprid, clothianidin, and thiamethoxam active ingredients were banned in the European Union, both for seed and foliar treatments, starting from 2019. As a result, no insecticides remain available for maize seed treatment for controlling the *T. dilaticollis* attack in

Romania. Alternatives methods such as foliar treatments, granules application, crop rotations, or early sowing data have low effectiveness in controlling the weevil's attack on maize crop compared with seed treatment with systemic insecticides, in conditions of high pest pressure, from the favorable areas located in the south and southeast of the Romania (Paulian, 1981; Bărbulescu and Voinescu, 1998; Voinescu and Bărbulescu, 1998; Georgescu et al., 2018; 2019; 2021b; Toader et al., 2020; Toshova et al., 2021). Because of a lack of effective alternatives to controlling the maize leaf weevil, between 2014 and 2022, Romania has granted temporary authorization for maize seed treatment with neonicotinoids for controlling the *T. dilaticollis* attack in spring. Moreover, from 2013 to 2019, the whole EU has granted 206 temporary authorizations to use neonicotinoids as active ingredients for seed treatment and foliar application to field crops or sugar beet (Abnett, 2021). Meissle et al. (2010) mentioned that maize leaf weevil is a regional pest in European Union, being localized in south-east of the continent. However, because of the higher level of the maize leaf weevil population recorded in Romania, especially in the favorable areas of this pest, the main damaging area of *T. dilaticollis* adults from EU27 is in Romania. The lack of alternatives for seed treatment can harm Romanian maize growers and can have negative economic consequences for this country (Ionel, 2014). In the absence of seed treatment, the maize growers will use foliar spray with pyrethroid active ingredients, for controlling pests attack, with a negative impact on the environment (Kathage et al., 2018). The present study aimed finding of possible alternatives for maize seeds treatment with imidacloprid active ingredient, for controlling the maize leaf weevils, in high pest pressure condition, from a commercial farm located in south-east Romania.

## MATERIALS AND METHODS

The field trial were carried in 2021, at the commercial farm Sopema SRL, located at Mihail Kogălniceanu, Ialomița County, Romania (latitude: 44°42'N, longitude: 27°40', altitude: 18 m a.s.l). This location is placed in the favorable area of *T. dilaticollis* in south-east

of Romania. In this trial it has assessed seed treatment with cyantraniliprole (625 g/l) active ingredient from diamid class, foliar application with acetamiprid (20 %), from neonicotinoid class and lambda-cyhalothrin (50 g/l) active ingredient from pyrethroid class. Also, it has assessed granules application with lambda-cyhalothrin (4 g/kg) active ingredient, from pyrethroid class. These variants were compared with standard treatment of maize seeds with imidacloprid active ingredient, from neonicotinoid class, that was banned according to EU regulations, but in Romania it has granted temporary authorization for spring of 2021 (Table 1). The intensive technology presented below is specific for commercial farms from this area (south-east of Romania). Maize were sowed on 26 April, full plants emergence was on 5 May. It has used Kashmir maize hybrid (FAO 370) at a density of 78000 seeds/ha and 75 cm distance between rows. Maize was sowed after maize, for having a high pest pressure. One day before sowing it was applied liquid fertilizer UAN (32% N) on dose of 230 l commercial product/ha. In same time with sowing, it has applied micro granules fertilizer (NPK 10:40:40) in dose of 240 kg c.p./ha.

Table 1. Active ingredients used in this study

Variant no.	Commercial product name	Active ingredient	Insecticid class	Rate <sup>1</sup>	Type of application
1	control (untreated)	—	—	—	—
2	Mospilan 20 SG	acetamiprid (20 %)	Neonicotinoid	0.1 kg/ha	Foliar application <sup>2</sup>
3	Nuprid 600 FS-std.	imidacloprid (600 g/l)	Neonicotinoid	2.20 µl/grain	Seed treatment <sup>3</sup>
4	Lumiposa	cyantraniliprole (625 g/l)	Ryanoid	2.20 µl/grain	Seed treatment <sup>3</sup>
5	Ercole	lambda-cyhalothrin (4 g/kg)	Pyrethroid	12.00 kg/ha	Granules application <sup>4</sup>
6	Karate Zeon	lambda-cyhalothrin (50 g/l)	Pyrethroid	0.15 l/ha	Foliar application <sup>2</sup>

1-commercial product

2-seed treatment (before sowing, BBCH 00)

3-foliar applications (when maize was in BBCH 12-13 stage, 14.05.2021)

4-granules application at sowing (BBCH 00, 26.04.2021)

The soil from experimental site (commercial farm Sopema) is chernozem with medium texture, humus content of 1.8%, pH of 7.93, nitrogen content of 0.179%, potassium content of 29 mg/kg and phosphorus content of 208 mg/kg. For crop protection against

monocotyledonous and dicotyledonous weeds, several herbicides were applied. Nine days before maize sowing (17.04.2021) it has applied Adengo 465 SC herbicide [(isoxaflutol (225 g/l) + tiencazabon-metil 90 g/l + ciprosumfamid (safener) (150 g/l) active ingredients] in dose of 0.4 l c.p./ha. When maize crops was in BBCH 16 stage it has applied Temsa SC herbicide [mesotrione (100 g/l) active ingredient] in dose of 1.5 l c.p./ha (22.05.2021). When maize was in an advanced stage (BBCH 18) it has applied both herbicides, Cerlit Super [fluroxipir (333 g/l) active ingredient] in dose of 0.54 l c.p./ha and Samson Extra OD [nicosulfuron (60 g/l) active ingredient] in dose of 0.4 l c.p./ha (06.06.2021). For this trial the area of each experimental plot was of 2100 m<sup>2</sup>.

**Field assessments: phytotoxicity** was evaluated on whole plot, when maize plants were in four leaves stage, according EPPO PP1/135 standard (EPPO standards, 2014).

**Plants density** it has made twice, first assessment when maize plants were in BBCH 14 stage (17.05.2021) and second assessment when maize plants were in BBCH 16 stage (28.05.2021). On each variant, it has established four assessment points. At each assessment point, it has counted maize plants from 20 row meters (80 row meters/variant).

The purpose of these assessments is to see if there was a plants reduction density as result of the weevils attack.

**Attack incidence** of weevils at maize plants was assessed when maize was in four leaves stage (BBCH 14). At each variant, it has established four assessment points. At each assessment point it has evaluated 50 maize plants, from five rows (10 plants/row). Before assessment plants from each row were marked with sticks, in stair system. Each plant from the assessment points was photographed with Panasonic G9 photo camera, with Leica DG O.I.S. lens (12-60 mm, f 2.8/4), then images were analyzed at computer to see if there were bites produced by the weevils.

**Attack intensity** of weevils was assessed when maize plants were in four leaves stage (BBCH 14). Attack incidence and intensity of maize leaf weevils was assessed at the same plants. Weevils attack was rated on a scale from 1 to 9 (Figure 1), as follows:

- Note 1: plant not attacked;
- Note 2: plant with 2-3 simple bites on the leaf edge;
- Note 3: plants with bites or clips on all leaf edge;
- Note 4: plants with leaf chafed in proportion of 25 %;
- Note 5: plants with leaf chafed in proportion of 50 %;
- Note 6: plants with leaf chafed in proportion of 75 %;
- Note 7: plants with leaf chafed almost at the level of the stem;
- Note 8: plants with leaf completely chafed and beginning of the stem destroyed;
- Note 9: plants destroyed, with stem chafed close to soil level.

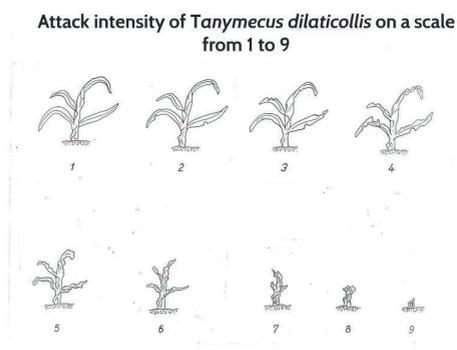


Figure 1. Attack intensity scale (Paulian, 1972, cited by Roşca and Istrate, 2009)

**Maize yield** were assessment at the harvest of the maize, from this trial, on 5 November, 2021. Each variant it has harvested separately. It has determined the humidity of the maize at harvest, hectoliter mass (Hgl) and yield.

**Meteorological data** were collected from automatic weather station of the Sopema farm, located at 1 km from experimental site. It has monitoring daily air temperature and rainfalls amount occurred in the most sensitive stage of the maize plants at weevils attack, from emergence (BBCH 00) to four leaves stage (BBCH 14).

**Statistical analysis.** The results of the field trial were presented as the absolute and mean values for maize phytotoxicity, plants density on row meter, attack incidence and weevils attack intensity, the standard deviation from the average values (SD), the coefficient of

variation (CV), replicate F and treatment F. The data were **statistical analyzed** using Student-Newman-Keuls (SNK) test for multiple comparisons (Student, 1927; Neuman, 1939; Keuls, 1952) using ARM software.

## RESULTS AND DISCUSSIONS

At the experimental site, from Sopema Farm (Mihail Kogalniceanu, Ialomita County), the spring of the year 2021 was characterized by average air temperatures lower than multiyear average in April (with a negative deviation of 2.40°C), and May (with a negative deviation of 1.47°C). The rainfall amount recorded in April was slightly over the multiyear average, with a positive deviation of 5.9 mm. Also, it was marginally lower than the multiyear average in May, with a negative deviation of 9.9 mm. It has registered high air temperatures between night and day in the first 15 days of May. Average air temperatures registered between 5 and 7 May were below 18°C, while average temperatures during the daytime, recorded in the same period, were over 25°C. After 8 May, the minimum temperature decreased, but the maximum temperature during the day was higher than 20°C. As a result, the differences between maximum and minimum temperature were higher, and the favorable period for weevils activity during the day was shorter.

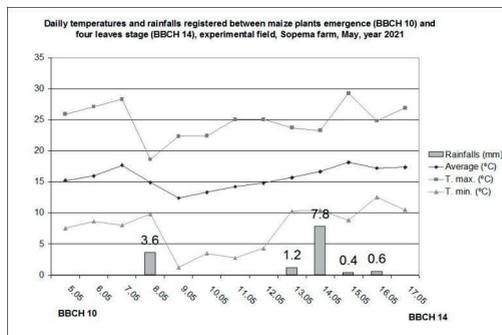


Figure 2. Daily temperatures and rainfalls recorded between maize plants emergence and four leaves stage in the spring of the year 2021 at Sopema commercial farm

According to Popov et al. (2006) higher temperatures and draught favored pest activity on the ground ant attack at maize plants. In this trial, as result of the unfavorable air temperatures recorded between 5 and 17 May,

and high air temperature differences between night and day, that sometimes exceeded 22.00°C, the weevils activity of the ground surface, when the maize plants were most sensitive to this pest attack (BBCH 10-BBCH 14), was lower. The plants were less attacked compared with previous testing years at the same location (Georgescu et al., 2018). Analyzing the data from Table 2 it has ascertained that there weren't phytotoxic effect at maize plants, as result of seed treatment before sowing; foliar application, when maize is in BBCH 11-12 stage, or granules application in same time with sowing. All type of the treatments with the active ingredients used in this trial, in conditions of the commercial farm, was safe for maize crop.

Table 2. Result of the phytotoxicity and plants density assessments

Variant no.	Active ingredient	Rate	Phytotoxicity (%) BBCH 14	Density (plants/row meter)	
				BBCH 14	BBCH 16
1	—	—	0a 0 StDev	5.05a 0.57 StDev	4.78a 1.11 StDev
2	acetamidrid (20%)	0.1 kg/ha	0a 0 StDev	5.64a 0.20 StDev	5.31a 0.28 StDev
3	imidacloprid (600 g/l)	2.20 µl/grain	0a 0 StDev	5.78a 0.06 StDev	5.35a 0.11 StDev
4	cyantranilprole (625 g/l)	2.20 µl/grain	0a 0 StDev	5.85a 0.16 StDev	5.51a 0.28 StDev
5	lambda-cyhalothrin (4 g/kg)	12.00 kg/ha	0a 0 StDev	5.65a 0.21 StDev	5.58a 0.18 StDev
6	lambda-cyhalothrin (50 g/l)	0.15 l/ha	0a 0 StDev	5.74a 0.18 StDev	5.40a 0.20 StDev
LSD (p<0.05)			0	0.444	0.736
CV			0	5.250	9.180
Replicate F			0	0.466	1.162
Replicate Prob(F)			1.000	0.710	0.357
Treatment F			0	3.833	1.362
Treatment Prob(F)			1.000	0.020	0.293

Means followed by same letter do not significantly differ (p<0.05; Student-Newman-Keuls); StDev-Standard Deviation

When maize crop was in four leaves stage (BBCH 14), plants density has a slight variation, ranged from 5.05 to 5.85 plants/row meters. Also, the coefficient of variation has low value (5.25). In this stage, plants density was normal, with normal variability among the field. At the second assessment, made when maize crop was in six leaves stage (BBCH 16) it has ascertained a slight decreasing of the plants density, comparing with previous assessment. In the same time, the coefficient of

variation, increasing. In both cases there weren't significant statistical differences between plants density registered at untreated variant, and plants density registered at treated variants (p<0.5). Also, it hasn't registered significant statistical differences between plants density at treated variants. However, lowest value of plant density it has registered in case of untreated variant (4.78 plants/row meter). Regard as weevils attack incidence, in this trial, all assessed plant was attacked by this pest (Table 3). In the previous researches, in case of maize plants, attack incidence was 100 % (Georgescu et al., 2018). This situation confirms data from the literature that maize plants is the main host for maize leaf weevil.

Table 3. Result of the assessments concerning weevils attack incidence and intensity at maize plants

Variant no.	Active ingredient	Rate	Incidence (%)	Attack intensity (1-9)
1	—	—	100a 0 StDev	6.92a 0.34 StDev
2	acetamidrid (20 %)	0.1 kg/ha	100a 0 StDev	6.91a 0.53 StDev
3	imidacloprid (600 g/l)	2.20 µl/grain	100a 0 StDev	5.74a 0.15 StDev
4	cyantranilprole (625 g/l)	2.20 µl/grain	0a 0 StDev	5.66a 0.07 StDev
5	lambda-cyhalothrin (4 g/kg)	12.00 kg/ha	100a 0 StDev	6.09a 0.90 StDev
6	lambda-cyhalothrin (50 g/l)	0.15 l/ha	100a 0 StDev	6.04a 0.91 StDev
LSD (p<0.05)			0	0.879
CV			0	9.370
Replicate F			0	1.087
Replicate Prob(F)			1.000	0.385
Treatment F			0	3.663
Treatment Prob(F)			1.000	0.023

Means followed by same letter do not significantly differ (p<0.05; Student-Newman-Keuls); StDev-Standard Deviation

In this trial, attack intensity of maize leaf weevil, on a scale from 1 to 9, ranged from 5.66 (seed treatment with cyantranilprole active ingredient) to 6.92 (untreated variant). Most of the maize plants from this trial had leaves chaffed in the proportion of 50-75 % and some plants are complete destroyed as result of the weevils attack. Coefficient of the variation of whole experience was 9.370. In the same time, at variants with seed treatment (imidacloprid and cyantranilprole active ingredients) it has registered the lowest values of the standard deviation (0.15, respectively 0.07). That means that weevils attack was uniform distributed in whole plot. At the opposite it was

variants treated with lambda-cyhalothrin active ingredient, both as foliar and granules application.

Table 4. Result of the assessments concerning maize yield from this trial (Sopema farm)

Variant no.	Active ingredient	Moisture (%)	Hgl	Yield/plot (kg)	STAS yield at 14 % moisture (kg/ha)
1	—	18.2	67.0	1312	5942.50
2	acetamiprid (20 %)	18.7	67.7	1710	7697.84
3	imidacloprid (600 g/l)	18.2	68.3	2760	12501.00
4	cyantraniliprole (625 g/l)	18.9	67.3	1944	8729.70
5	lambda-cyhalothrin (4 g/kg)	18.6	66.1	1872	8437.48
6	lambda-cyhalothrin (50 g/l)	18.5	66.1	1674	7554.32

However, there weren't significant statistical differences between variants concerning weevils attack intensity ( $p < 0.5$ ). A possible reason for this situation is because of unfavorable weather conditions for weevils, registered in early vegetation stages of the maize crop. In previous study, in case of high attack of the maize leaf weevil, untreated variant was destroyed by the weevils (Georgescu et al., 2018). Also, variants with single foliar spray, without seed treatment can be destroyed (Georgescu et al., 2021a).

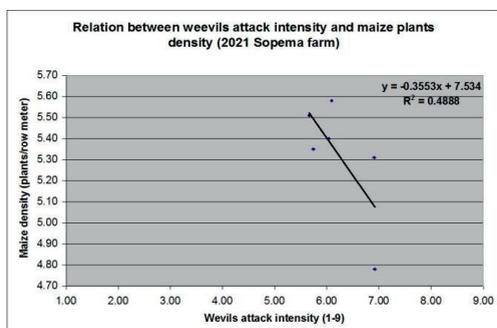


Figure 3. Relation between weevils attack intensity and maize plants density (2021 Sopema farm)

Data from table 4 reveal that in this trial, in conditions of the commercial farm, highest maize yield it has registered in case of maize seeds treated with imidacloprid active ingredient, followed by variant with seed

treatment with cyantraniliprole active ingredient. Lowest maize yield was registered in case of control (untreated variant).

In this trial, it has registered a negative correlation between maize leaf weevil attack and maize plants density (Figure 3). Data from this experience it is in accordance with previous researches. Seed treatment with systemic insecticide, with rapid translocation of the active ingredient in plants, after maize emergence, is the highest effective method for controlling of maize leaf weevil attack, when maize plants are in early vegetation stages (Bărbulescu et al., 2001b; Popov et Bărbulescu, 2007; Georgescu et al., 2018, 2021a; Trotuș et al., 2019). Further studies are necessary, in different climatic conditions and pest pressure to evaluate cyantraniliprole active ingredient effectiveness in controlling of the *T. dilaticollis* attack at maize crop.

## CONCLUSIONS

The attack of the maize leaf weevil (*Tanymecus dilaticollis* Gyll) at the maize plants, at the experimental site from Sopema farm (Mihail Kogalniceanu, Ialomița County), where it has made the assessments, was moderate, as a result of the unfavorable weather conditions from period when maize plants were in BBCH 10-BBCH 14 stage.

In the conditions of the year 2021, lowest weevils attack intensity it has registered in case of treated seeds variants.

In the conditions of the year 2021, from experimental location, it hasn't registered significant statistical differences between weevils attack at maize plants from variants with seeds treated and the rest of the variants from this experiment ( $p < 0.05$ ).

In conditions of the year 2021, from experimental location, it hasn't registered significant statistical differences between experimental variants, concerning maize plant density.

No phytotoxic effect occurred at maize plants after seed treatment, foliar or granules application of the insecticides.

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## RESULTS REGARDING THE EFFECT OF MICROGRANULATED FERTILIZERS IN HYBRID SEED MAIZE PRODUCTION

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### Abstract

*Maize is the cereal with the highest research and development budgets in the world, due to its high ecological distribution and to its vital role in human consumption and animal feeding. In the farms specialised in maize hybrid seeds production, there is a need to have increasing yields of hybrid seeds in view to be more and more efficient. But, having higher yields of hybrid seed cannot happen without the development of new fertilization strategies by farmers that multiply the seeds. Microgranulated fertilizers have been gaining an increasingly important role in these strategies due to the need to have a good development of the plants in the early growth stages. In this respect, our research aimed to study the effect of different microgranulated fertilizers on the yielding components and hybrid seed yield as well as on the overall development of the maize plants through their growth stages. The obtained results showed the following key aspects regarding the effects of microgranulated fertilizers: a better emergence of the plants in the field; a superior development of the root system and even better development where phosphorus concentrations used were higher; a better development of the leaves measurable until BBCH 18; no negative effects on nicking at pollination; increased TGW; improvement of hybrid seed yield in conditions of severe drought.*

**Key words:** maize, microgranulated fertilizers, hybrid seed yield, yielding components.

### INTRODUCTION

Increasing crop yields has been the foremost objective of research and practice in agriculture, as the XXth century was the start of a demographic boom which continues at an accelerated pace in the XXIth century, in order to be able to sustain the increasing number of people living on earth with limited arable land resources.

According to FAO data, the maize harvested area increased continuously since 1961, exceeding for the first time the area of 200 million ha in 2020 (201.98 million ha), maize ranking the second place in the world as harvested area, after wheat. This vast increases of land cultivated worldwide with maize raises the demand for quality maize seed in order to be able to attain higher yields.

The primary aim of an effective seed production programme is to supply adequate quantities of high quality seeds to farmers at the right time and place (Ibrahim, 2010). Therefore, new developments in technology that mitigates limitative factors of production are firstly adopted by farmers that multiply

seeds for big agribusiness companies with high R & D potential. This is explained by the pressure the seed companies apply on farmers, and by the incentive of the extra premium obtained by increasing yield. Hybrid maize seed multiplication is a complex process that requires a close attention to the timing of planting the parental inbred lines to ensure the coincidence of flowering time, to removal of tassels from the female rows of plants before pollen shedding and to eliminate male rows of plants after pollination, all in order to harvest high quality hybrid seeds from the female plants.

A crucial yield limitation factor is fertilization, respectively plant nutrition, which has to sustain increasingly high potential hybrids by providing enough nutrients at precise moments in time (Jeffrey et al., 2003). Classical fertilization with 1:1:1 type fertilizers or 1:1:0 at planting or before seedbed preparation has been part of hybrid seed maize production since the introduction of hybrid seed in Romania in the 1960's. Significant increases in the cost of fertilizer over the past years make it necessary to have a "minimum quantity-maximum effect"

strategy, thus making it essential to place the fertilizer granules as close to the root system as possible to minimize unused fertilizer.

In contrast to DAP and other fertilizers, which are applied as a fertilizer band at a distance of around 10 cm to the seed, microgranulated fertilizer is ideally put in the soil together with the seed, at a distance of a few centimetres (Thielicke et al., 2022). Microgranulated fertilizers appeared as a starter fertilizer option to be placed as close as possible to the grain at planting time, based on the argument that a good root development of the seedling, helped by the high phosphorus content of the microgranules, is key for a good growing of the maize plants and will therefore impact in the end the yield (Lemarchand et al., 2016).

The principle is based on the fact that as soon as imbibition of the seed starts also the solubilisation of the microgranules begin, thus the first elements of the root system will develop in a medium rich in phosphorus, nitrate and zinc helping it to develop more quickly. This type of fertilizer available on the market is usually differentiated by the content of  $P_2O_5$  and nutrients such as zinc and sulphur. The small content of Zinc in the fertilizer formulation helps especially in soils with low  $P_2O_5$  content (<15 ppm) where usually it can observe zinc deficiency (Brady & Weil, 2008) especially in monoculture conditions.

In seed production, the use of microgranulated fertilizers raises questions about whether there is any differential effect on the two parental lines used in the maize crossing process, if there is a different root development, general phenotypic development, flowering time differences, and not the least, yield differences.

The aim of this study was to evaluate all these possible effects of microgranulated fertilizer on maize inbred lines, in this case the hybrid P9889, under different fertilization situations.

## MATERIALS AND METHODS

Field experiments were conducted in 2020 and 2021 on a chernozem soil in the north-eastern part of Romania, respectively in the pedoclimatic conditions of the Moldavian Plain near the Prut River in Bivolari commune, Iasi County, on the land of the economical entity Semconsult Top SRL.

Tested variants in the field trails were based on different types of microgranulated fertilizer with different contents of  $P_2O_5$ , compared with two controls one being the classical fertilization scheme of the farmer approved by a quality control specialist of the hybrid's parent company. The field experience was based on the method of subdivided plots with 4 replications being of type 2 x 4 with the following factors (Table 1):

- Factor A - base fertilization, with 2 graduations:
  - a1 = fertilization at seedbed preparation;
  - a2 = fertilization at seedbed preparation + nitrogen application in the growth stage of the maize plants BBCH 18;
- Factor B - microgranulated fertilizer, with 4 graduations:
  - b0 = no microgranulated fertilizer;
  - b1 = Physiostart microgranulated fertilizer;
  - b2 = Greenstart microgranulated fertilizer;
  - b3 = Pannon Starter microgranulated fertilizer.

The microgranulated fertilizers used stimulates root development by providing easily assimilable phosphorus, ensuring a quick start to the crop. Nitrogen and phosphorus support strong rooting and the development of a rich and healthy foliage (Stanley & Rhoads, 1977). Microelements stimulate the metabolic and hormonal activity of plants and support generation of the necessary content of chlorophyll.

The content of nutrients in microgranules cannot replace the nutrition provided by classic fertilizers, they only augmenting the base fertilization scheme. Therefore, it was important to include base fertilization in our experiments.

In the field experiments, there was used the inbred lines of hybrid P9889, a FAO 350 hybrid with good drought tolerance, stay green effect and well suited for the climate and soil conditions found in the Moldavian plain. The planting instructions by the seed developer recommended a parity of 6:2 with 60 cm between rows, planting the Female and Male 1 first and then Male 2 after 33 GDD in order to ensure better flowering synchronisation and a longer pollen coverage time. The 6:2 ratios of female to male rows are less complicated and less costly to handle with conventional planters and harvesters (Wright, 1980).

The experimental variant had 192 m<sup>2</sup> size which resulted from 8 row plants (6 female

rows and 2 male rows) with 60 cm between rows meaning 4.8 m and 40 m length.

Table 1. The experimental factors

Experimental factors	Fertilizer products	Fertilizer application period	Fertilizer rate (kg/ha)	Nutrients rate (kg/ha)						
				N	P	K	S	Mg	Ca	Zn
a1b0	Ammonium nitrate 14:14:14+7SO+4MgO 20:20:0+0.5Zn	Autumn (preceding year)	100	33.5	0	0	0	0	0	0
		Seedbed preparation	300	42	42	42	21	12	0	0
		Seedbed preparation	150	30	30	0	0	0	0	0.75
	<i>Total - a1b0</i>				105.5	72	42	21	12	0
a1b1	a1b0 + Physiostart	Sowing	20	1.6	5.6	0	4.6	0	2.8	0.4
	<i>Total - a1b0 + Physiostart</i>				107.1	77.6	42	25.6	12	2.8
a1b2	a1b0 + Greenstart	Sowing	20	3.2	8	0	1	0.4	0	0.4
	<i>Total - a1b0 + Greenstart</i>				108.7	80	42	22	12.4	0
a1b3	a1b0 + Pannon Starter	Sowing	20	1.8	8	0	1	0	0	0.2
	<i>Total - a1b0 + Pannon Starter</i>				107.3	80	42	22	12	0
a2b0	Ammonium nitrate 14:14:14+7SO+4MgO 20:20:0+0.5Zn	Autumn (preceding year)	100	33.5	0	0	0	0	0	0
		Seedbed preparation	300	42	42	42	21	12	0	0
		Seedbed preparation	150	30	30	0	0	0	0	0.75
	Ammonium nitrate	BBCH 1.6	200	67	0	0	0	0	0	0
<i>Total - a2b0</i>				172.5	72	42	21	12	0	0.75
a2b1	a2b0 + Physiostart	Sowing	20	1.6	5.6	0	4.6	0	2.8	0.4
	<i>Total - a2b0 + Physiostart</i>				174.1	77.6	42	25.6	12	2.8
a2b2	a2b0 + Greenstart	Sowing	20	3.2	8	0	1	0.4	0	0.4
	<i>Total - a2b0 + Greenstart</i>				175.7	80	42	22	12.4	0
a2b3	a2b0 + Pannon Starter	Sowing	20	1.8	8	0	1	0	0	0.2
	<i>Total - a2b0 + Pannon Starter</i>				174.3	80	42	22	12	0

The sowing distance between seeds were of 18.3 cm, ensures thus high female crop density. The year 2020 can be characterised as being a warm and dry one, with an average temperature of 12.5°C which was the highest registered ever, and with 479.9 mm rainfall very unevenly distributed, with 1.8 mm in April and 5.8 mm in August, but with 132 mm in May and 90.4 mm in June.

The year 2021 can be characterised as being a favourable one for maize crop, with an average temperature of 10.6°C and 564.6 mm rainfall among which 390.3 mm in period April-August. Core phenotypic and yielding elements determined and studied in the field and laboratory were the following:

- Plant height - at 8-leaf stage (BBCH 18);
- The number of leaves per plant;
- Evolution of the root system up to the phase of 4 and 8 leaves (BBCH 14 and BBCH 18);
- Duration from sowing (BBCH 00) to emergence (BBCH 09) and from emergence (BBCH 09) to flowering (BBCH 51);
- The flowering time gap between male inbred and the female inbred;
- Hybrid grain yield/ha;
- Yielding components: length of the cob; number of rows/cob; number of kernels/cob; thousand grain weight (TGW).

Statistical analysis was performed using the ANOVA analysis and linear regression in order to determine the relationships between different traits. Specific tests such as Fisher's test, Tukey's test and Dunnett's were also used to compare the different variants with each other and to the 2 control variants.

## RESULTS AND DISCUSSIONS

The results revealed a significant correlation between all analysed characters and the hybrid seed yield. For each comparison the fertilized version of the microgranulated fertilizer, the products used have outperformed the control variant. Thus, there was a yield increase over the 2 years of 1188 kg compared to the control variant 1 and 300 kg when compared to the control variant 2 in the case of high P<sub>2</sub>O<sub>5</sub> microgranulated fertilisers (Table 2).

Relevant observations were made starting from the first phases of plant development, time for emergence of the plants that had microgranulated applied at sowing being smaller than for the control group by 1-3 days, according to the climatic conditions of the year (Table 3).

It can be extrapolated that the existence of available phosphorus content near the seed helped increase the speed of seedling

development thus leading to a much faster emergence.

Another important evaluation stage was the 4 leaf stage (the latest for early post-emergence herbicide application) where the development of the root system of the high phosphorus microgranulated variant has been more developed than the control variant, and the

medium phosphorus microgranulated variant, i.e. 8-9 roots vs 5-6 and vs 6 roots (Table 3).

Before cultivation, at 8 leaf-stage, it was evaluated again the development of the root system and the difference observed at the 4 leaf-stage evaluated had increased to 10-11 roots vs 6-7 and vs 8-9 roots (Table 3).

Table 2. Hybrid seed yield results

Experimental factors	Fertilizer products and their rates	Hybrid seed yield (kg/ha)			Difference to Control 1	Difference to Control 2
		2020	2021	Average		
a1b0 Control 1	Ammonium nitrate - 100 kg/ha 14:14:14+7SO+4MgO - 300 kg/ha 20:20:0+0.5Zn - 150 kg/ha	8405	8625	8515	Control	-
a2b0 Control 2	Ammonium nitrate - 100 kg/ha 14:14:14+7SO+4MgO - 300 kg/ha 20:20:0+0.5Zn - 150 kg/ha Ammonium nitrate - 200 kg/ha	9588	9818	9702.5	1188	Control
a1b1	a1b0 + Physiostart - 20 kg/ha	9075	9295	9185	670	-518
a1b2	a1b0 + Greenstart - 20 kg/ha	9750	9980	9865	1350	163
a1b3	a1b0 + Pannon Starter - 20 kg/ha	9675	9895	9785	1270	83
a2b1	a2b0 + Physiostart - 20 kg/ha	9050	9280	9165	650	-538
a2b2	a2b0 + Greenstart - 20 kg/ha	9850	10070	9960	1445	258
a2b3	a2b0 + Pannon Starter - 20 kg/ha	9875	10105	9990	1475	288

Table 3. Experimental observations

Experimental factors	Fertilizer products and their rates	Time to emergence			No of root at BBCH-14			No of root at BBCH-18			Days between Male 1 and Female flowering		
		2020	2021	Average	2020	2021	Average	2020	2021	Average	2020	2021	Average
a1b0 Control 1	Ammonium nitrate – 100 kg/ha 14:14:14+7SO+4MgO – 300 kg/ha 20:20:0+0.5Zn - 150 kg/ha	8	9	8.5	5	6	5.5	6	7	6.5	5	3	4
a2b0 Control 2	Ammonium nitrate – 100 kg/ha 14:14:14+7SO+4MgO – 300 kg/ha 20:20:0+0.5Zn - 150 kg/ha Ammonium nitrate – 200 kg/ha	8	9	8.5	5	6	5.5	6	7	6.5	4	3	3.5
a1b1	a1b0+Physiostart-20 kg/ha	8	7	7.5	6	6	6	8	9	8.5	4	3	3.5
a1b2	a1b0+Greenstart-20 kg/ha	7	6	6.5	8	9	8.5	10	11	10.5	3	2	2.5
a1b3	a1b0+Pannon Starter - 20 kg/ha	7	6	6.5	8	9	8.5	10	11	10.5	3	2	2.5
a2b1	a2b0+Physiostart-20 kg/ha	8	7	7.5	6	6	6	8	9	8.5	3	3	3
a2b2	a2b0+Greenstart-20 kg/ha	7	6	6.5	8	9	8.5	10	11	10.5	3	2	2.5
a2b3	a2b0+Pannon Starter 20 kg/ha	7	6	6.5	8	9	8.5	10	11	10.5	3	2	2.5

The number of roots are positively correlated with the final hybrid seed yield. Another key period for maize hybrid production is flowering time, which has a great impact in the final yield. The time gap between the opening of male anthers to release pollen and the emergence of female silks is an optimum of 2-3 days. In case of both types of microgranulated fertilizers, high P<sub>2</sub>O<sub>5</sub> and medium P<sub>2</sub>O<sub>5</sub>, there was no ill effects on this gap, which was one of the questions of the study as we have in the plots both male and female plants which could

react differently to fertilization. It was observed that pollen shedding was 3-5 days before silking in 2020, as drought had a protandric effect on the plants, but in 2021 pollen release started perfectly at 2-3 days before silking. The high phosphorus microgranulated fertilisers had a better effect on the gap between the opening of male anthers and the emergence of female silks, this being of 2-3 days compared to 3-5 days for control variants and 3-4 days for the medium phosphorus fertilisers (Table 3).

The value of R squared > 0.6 shows us that there is a good positive link between hybrid seed yield and the use of microgranulated fertilizers (Figure 1).

The results of the statistical analysis performed shows very strong positive correlations, for both types of microgranulated fertilizers, between yielding elements. Thus, the length of the cob, number of grains on the cob, number of rows per cob, and TGW, all these yielding elements are higher compared to the control variants. We can say that the faster start of the plants helped to have a harmonious vegetative development which helped to sustain better the

generative phase of the plants life and thus the development of productive elements.

The results of the Tukey Test (Table 5) performed, which shows if differences between variants are significant, demonstrate that the microgranulated fertilizers with a higher content of P<sub>2</sub>O<sub>5</sub> (>=40%) are providing a statistically significant yield, these being a distinct Group A in the output of Tukey testing, increase versus the 2 control variants.

Dunnett test confirms the results that all microgranulates with high phosphorus content determine statistically significant increases of yield (Table 6).

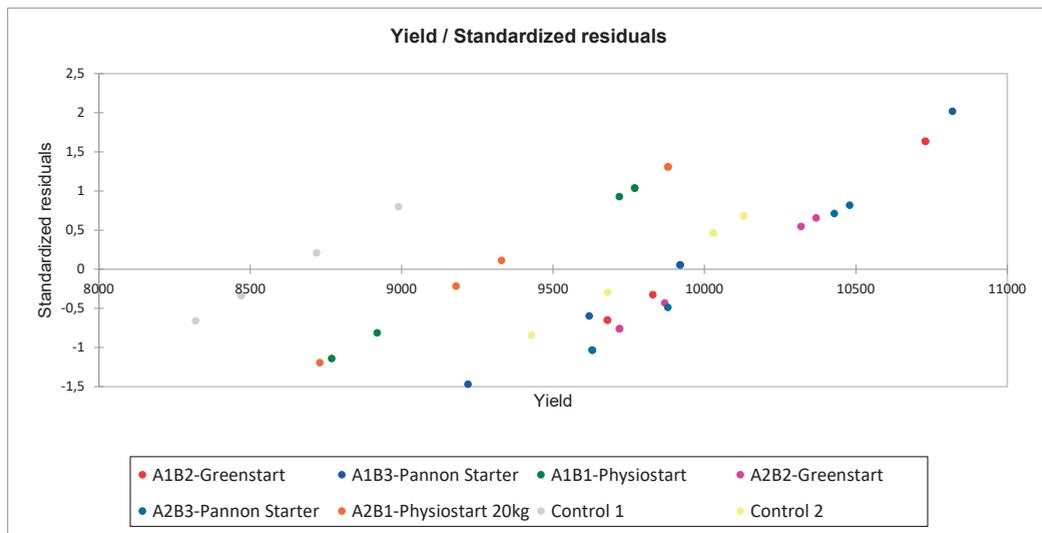


Figure 1. Yield distribution of variants

Table 4. R squared value of regression

Goodness of fit statistics (Yield):	
Observations	32
Sum of weights	32
DF	24
R <sup>2</sup>	0.599

Table 5. Tukey Test groupings

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
A2B3-Pannon Starter	10105.000	229.553	9631.226	10578.774	A	
A2B2-Greenstart	10070.000	229.553	9596.226	10543.774	A	
A1B2-Greenstart	9980.000	229.553	9506.226	10453.774	A	
A1B3-Pannon Starter 20kg	9895.000	229.553	9421.226	10368.774	A	
Control 2	9817.500	229.553	9343.726	10291.274	A	
A1B1-Physiostart 20kg	9295.000	229.553	8821.226	9768.774	A	B
A2B1-Physiostart 20kg	9280.000	229.553	8806.226	9753.774	A	B
Control 1	8625.000	229.553	8151.226	9098.774		B

Table 6. Dunnett Test significance results

Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control 1 vs A2B3-Pannon Starter	-1480.000	-4.559	2.814	913.620	0.001	Yes
Control 1 vs A2B2-Greenstart	-1445.000	-4.451	2.814	913.620	0.001	Yes
Control 1 vs A1B2-Greenstart	-1355.000	-4.174	2.814	913.620	0.002	Yes
Control 1 vs A1B3-Pannon Starter	-1270.000	-3.912	2.814	913.620	0.004	Yes
Control 1 vs Control 2	-1192.500	-3.673	2.814	913.620	0.007	Yes
Control 1 vs A1B1-Physiostart	-670.000	-2.064	2.814	913.620	0.220	No
Control 1 vs A2B1-Physiostart	-655.000	-2.018	2.814	913.620	0.238	No

The study allowed us to efficiently highlight and observe all the qualitative and quantitative components such as early root, foliar, and yielding elements development together with flowering time gap, and have a consistent direct impact, which are critical to yield development of hybrid seed maize. For each of these elements, variants fertilized with microgranulated fertilizer at sowing time have proven to perform better than the control variant, and the variants with higher P<sub>2</sub>O<sub>5</sub> content even better.

## CONCLUSIONS

The results of the performed researches show that, even though microgranulated fertilizers in seed maize production are not a key element, their use will help the overall development of the plant, the formation of the yielding elements and not the least the yield. This in the context of high economic value of the maize hybrid seeds makes the use of such fertilizers very efficient leading to higher revenues. The most effective microgranulated fertilizers are the ones with a high content of P<sub>2</sub>O<sub>5</sub> leading to a fast emergence and fast development until 8th leaf (BBCH 18). The root system develops faster and supports a very quick development of the maize plant, with a shorter time until flowering but with superior leaf area development.

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## THE EFFECT OF SOWING TIME AND FERTILIZATION ON THE WINTER WHEAT YIELD AND THE PHYSICAL CHARACTERISTICS OF ITS GRAIN QUALITY UNDER UKRAINIAN NORTHERN STEPPE CONDITIONS

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### Abstract

*The current article reports on the research results determining the effect of the sowing time and fertilization on the yield and the physical characteristics of winter wheat grain quality after bare fallow and spring barley. In this article, it has been determined that under conditions of the Ukrainian Northern Steppe, the highest yields of all the varieties are observed when sowing is performed at the middle term (22<sup>nd</sup> September). The shift in the sowing time towards the early date (7<sup>th</sup> September) or the late date (7<sup>th</sup> October) causes the drastic reduction in the yields. In comparison with the other genotypes under the study, the variety of Holubka Odeska shows the highest flexibility in terms of sowing time. It has been defined that locally applied N<sub>30</sub> fertilization permits the increase in the grain yield as much as additional 0.79-1.18 t/ha in comparison against the control group plants (without application of any fertilizers) on condition that fertilization is carried out at the end of the plant tillering stage after bare fallow at the pre-sowing application of N<sub>30</sub>P<sub>60</sub>K<sub>30</sub>; the application of the nitrogen fertilizers (N<sub>30</sub> in early spring into thawing frozen soil + N<sub>30</sub> locally) at the pre-conditioning with N<sub>60</sub>P<sub>60</sub>K<sub>30</sub> after spring barley brings the increase of 2.17-2.47 t/ha. The grain units of the winter wheats planted after the two predecessors increase, as a rule, from the early sowing time towards the later one. The durum wheat of Burshtyn variety has shown the higher values than the soft wheat varieties of Lastivka Odeska and Holubka Odeska. We also report on the positive effect of the plant fertilizing on grain glassiness development in both the durum wheat variety and the soft wheat variety, however, the favour of the durum wheat variety is higher than that of the soft wheat from the standpoint of this property.*

**Key words:** fertilization, grain quality, sowing time, variety, winter wheat, yield.

### INTRODUCTION

The winter wheat (*Triticum* L.) is a principal grain cereal grown on the territory of Ukraine. Its crops occupy 6-7 million ha and account approximately 40% of the grain cereal total areas. The winter wheat allows high yield, its grains are important raw materials for flour-and-cereals industry, bread processing, animal feed industry and other processing branches. There are several kinds of it and a plenty of varieties and forms. The principle kinds of wheat are the two: soft wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.). The grains of soft wheat are applied, as the rule, for flour production and further bread processing. The grains of durum wheat contain more proteins than those of the soft wheat, however, the bread produced from durum wheat is smaller in its volume and stales faster. Along with it, it

worth mentioning that the flour produced from the durum wheat is an indispensable raw material for pasta products (Petrychenko & Lykhochvor, 2020).

The significant importance in the technology of grain processing into flour or into cereals is attributed to physical and structural properties of the grain, in particular, the grain size (expressed in the mass of 1000 grains), grain unit (grain mass within a certain volume), endosperm consistency (glassiness) and so forth. The previous researches conducted in different climate zones of Ukraine evidence on noticeable dependence of the above-mentioned parameters on the wheat variety features and weather conditions during grain formation, grain ripening, and overmaturing (Hasanova & Konop-lova, 2011; Liubych, 2013). However, agricultural techniques of growing also have a significant effect on physical properties of wheat

grain (Zhemela & Shakalii, 2012; Cherenkov et al., 2015).

Due to the good potential for the grain yield, capability of bringing high incomes on condition of proper cultivation plus the growing demand for winter wheat on both the domestic market and the external one, this kind of wheat holds its position as a promising cereal crop in Ukraine. Among the agricultural factors to determine the harvest amount, the sowing time is extremely important, it defines the duration of autumn plant vegetation when the plant growth and development occur. The correctly determined sowing time ensures the sufficient development for root system, tillering node and over-land vegetation mass. The highest grain yield of winter wheat is obtained provided that the optimal sowing time is observed: the sowing time is to be defined with the account of soil and climate conditions, winter wheat variety specific features, agricultural techniques of plant growing, and weather conditions in the pre-sowing period (Cherenkov et al., 2008; Gandjaeva, 2019; Kryvenko, 2019).

It is defined that sowing at optimal time with meeting certain conditions ensures not only high yield but also promotes obtaining excellent physical characteristics of the wheat grains, namely increased weight of 1000 wheat grains and grain unit. Sowing at the earlier time results in worse protein characteristics and bread-baking constituents of the winter wheat grain quality (Petrychenko & Lykhochvor, 2020; Shakalii et al., 2020). Later sowing for winter wheat shows higher contents of proteins and gluten than those at optimal date since grain ripening occurs at higher temperatures and lower humidity of the soil and of the air; these shorten the period of grain formation and the yield as the resultant of it. If there is no drastic difference in the yields within the tolerable time period of sowing, the differences in wheat grain quality are insignificant (Zhemela & Musatov, 1989).

According to the researches carried out within 2009-2011 in the Ukrainian Northern Steppe, the highest winter wheat yields were obtained at sowing on 20<sup>th</sup> September both after bare fallow and after sunflower while the lowest yields were received at early date of sowing, namely 5<sup>th</sup> September. The yields of the different winter wheat varieties were somewhat lower after

sowing on the later date of 5<sup>th</sup> October as compared with the harvest of the optimal sowing date but the grain units after sunflower crop were notably higher than those with the two crops sown earlier. The crops after bare fallow gave insignificantly change in grain units dependently on sowing time (Solodushko et al., 2016).

Further, an important event to encourage both winter wheat yield increase and its grain quality is fertilization; the greater part of its positive effect is stipulated by the fact that the nutrients content gradually decreases in the soil, the nutrients are in the forms difficult for dissolving while the physiological activity of the winter wheat root system is not sufficient (Maathus & Diatloff, 2013; Usova et al., 2018; Berdnikova, 2020).

In (Hirzel et al., 2010; Lytovchenko, 2017; Litke & Gaile, 2018; Hasanova et al., 2019; Bilousova, 2019), it is suggested that nitrogen fertilizers are supreme in importance for the grain quality and the development of its physical characteristics. If the amounts of phosphorous and potassium are balanced in the soil at the beginning of vegetation, they improve the plant growth and support nitrogen compounds accumulation in the vegetative organs of the plant. At the next stage of the plant development, these compounds play a vital role in grain formation and improvement of such characteristics as the weight of 1000 grains, grain unit, glassiness, protein accumulated.

Furthermore, we also cannot ignore the data which evidence on the decrease in the weight of 1000 grains and in grain unit when the increased mineral fertilization was applied: there was greater productive plant density observed at land plots with their increased mineral fertilization forming smaller spikes and smaller grains. In particular, the experiments with winter durum wheat under irrigation in Ukrainian Southern Steppe showed that the application of nitrogen fertilizers permitted the higher yield, increase in protein content in the grains, their better glassiness, however the values of the weight of 1000 grains and grain unit showed the regular decrease at the fertilized land plots (Bazalii et al., 2011).

The analysis conducted on the scientific papers by different authors evidences that the relevant data obtained are of ambiguous character and are

controversial for certain cases. This could be explained by the facts that the researches devoted to the study how agricultural techniques of growing and weather conditions influence the winter wheat yield and its grain quality have been performed under different conditions of soil and climate zones, with different wheat varieties and the weather conditions were also not the same. This stipulates the urgent character and the necessity for the further experiments in the indicated scientific direction.

The aim of the current research is to reveal the specific effect of sowing time and mineral fertilization on the yield and grain quality of the most demanded varieties of winter soft wheat and winter durum wheat under conditions of sowing after bare fallow and after spring barley in the Ukrainian Northern Steppe.

## MATERIALS AND METHODS

The reported research has been carried out within 2017-2019 in experimental farm "Dnipro" belonging to the Institute of Grain Crops of National Academy of Agrarian Sciences of Ukraine. The experimental farm is located in the northern subzone of Ukrainian Steppe. The soil of the experimental plots of the farm is common steppe black soil, low in humus, full cross-section, medium loam. The humus content within the arable layer is close to 3.2%, general nitrogen (N) - 0.18-0.20%, mobile phosphorus ( $P_2O_5$ ) is within 90-120 mg/kg, exchangeable potassium ( $K_2O$ ) is within 70-120 mg/kg (according to Chirikov). The maximal value of the nitrification capability of the experimental farm black soil is within the arable layer and makes 17-20 mg/kg soil.

Soil solution reaction of the black soil humus horizon is close to the neutral (pH of aqueous suspension is 6.75). The ground water depth is within 8-12 m, therefore soil moisturizing for the experiments was performed only by the atmospheric precipitations. The climate of the zone is moderate continental with insufficient and unstable moisturising.

The wheat sowing was carried out with seeder CH-16 as continuous row sowing, the rate of seeding was 5 000 000 viable seeds/ha, depth of seed wrapping was 5-6 cm. The plots were located in systematical order. The area of a simple plot was 30 m<sup>2</sup>, the research repetition - three times.

The varieties of the winter soft wheat of Holubka Odeska and Lastivka Odeska and winter durum wheat of Burshtyn were sown on 7<sup>th</sup> September, 22<sup>nd</sup> September and 7<sup>th</sup> October after bare fallow and spring barley.

According to the description of the originator (Plant Breeding and Genetics Institute, NAAS of Ukraine), Lastivka Odeska variety is long stemmed wheat of intensive type, possesses increased positive response to the improvement in agricultural conditions, and along with the mentioned, it is rather tolerable to disadvantageous agricultural conditions and growing after unpaired predecessors. Holubka Odeska variety is middle stemmed wheat, it is also of intensive type, shows a good response to the fertilization, is tolerable to poor or moderate soil fertility, and perfectly adapted to the dry conditions of growing. In connection to the prolonged vernalisation (54-56 days), this wheat variety adapts better to the early sowing time. Burshtyn variety refers to the species of hordeiformes, it allows high yield, belongs to short-stemmed type, undergoes the middle vernalisation (22-25 days).

The fertilization modes applied in the reported experiment were as follows: 1 - without application of any fertilizers (control lot); 2 - pre-sowing application of the fertilizers (after bare fallow -  $N_{30}P_{60}K_{30}$  while after spring barley -  $N_{60}P_{60}K_{30}$ ); 3 - the fertilizing system ( $N_{30}$  was applied locally at the end of the plant tillering stage on the background of the pre-sowing mineral fertilization after bare fallow while  $N_{30}$  was introduced into thawing frozen soil +  $N_{30}$  locally after spring barley). All the observations were performed according to the widely accepted recommendations (Vovkodav, 2001). The account of the yield was carried out by continuous mowing, grain threshing from all the area under observation, from each plot at the stage of ripe grain and further the grain yield was weighed. Combine Sampo-500 was employed for this. On the harvesting day, such parameters as the humidity of grain and impurities were defined. The data obtained were processed into standard grain humidity value (14%) and 100% purity.

The parameters of the grain quality were determined by the methods prescribed in the valid Ukrainian national standard on wheat (DSTU 3768-2019).

## RESULTS AND DISCUSSIONS

The weather conditions in the years under study for the current article were, in general, advantageous for winter wheat. However, it should be noted that the years were different in the amounts of precipitation, temperature regimes and considerable distortions were observed for these factors from average multi-year recordings. Thus, the air temperature in 2016/17 vegetation year was +0.5°C higher than the average temperature having been recorded for many previous years while, in 2017/18 it was +2.6°C higher than the average value, in 2018/19 the temperature recording was +1.9°C higher than the average one.

The precipitation amounts during the years of the study were also higher than the normally recorded ones. In 2016/17, the amount of precipitations was 10.3 mm higher than the average multi-year values, 2018/19 vegetation year obtained 8.2 mm more of precipitations than on average, 2017/18 was described as the vegetation year of high humidity as the amount of precipitation exceeded the conventional values as much as 123.2 mm.

The fact that the research on the winter wheat was conducted under different weather conditions allows better understanding of its productivity potential.

Thus, the grain yields of various winter wheat varieties at all the research variants both after bare fallow and after spring barley were the highest in 2019 as compared with the results of 2017 and 2018.

The above-mentioned result was attributed to the better humidity for the winter wheat during the spring months (March, April and May); this stipulated the increase in the productive spikes number per the unit of the area and had a positive effect on the yield. Therefore, the richest harvest was developed under conditions of 2019 in spite of hot weather influence during the stage of grain filling, in June sickly grain development was noticed. The weight of 1000 grains was somewhat lower than in the previous years. The highest grain weight from the spike was grown in 2017, this positively influenced the winter wheat yield indexes of the year.

In 2019, the yields of the winter wheat after bare fallow showed differences per the wheat variety, sowing time and fertilization system, the range

was from 4.72 to 7.36 t/ha; after spring barley – from 2.49 to 6.20 t/ha. In other years, the yield indexes were lower.

According to the data obtained, the yields of the winter wheat varieties in 2017-2019 were higher on average from the sowings after bare fallow than those after spring barley. The increased yields of Lastivka Odeska and Burshtyn were obtained from the variants with sowing on 22<sup>nd</sup> September. Depending on the fertilizers, the yield indexes varied after bare fallow from 6.4 to 7.10 t/ha and 5.22-6.40 t/ha per winter wheat varieties, while after spring barley – from 3.39 t/ha to 5.75 t/ha and 3.11-5.53 t/ha. The sowings conducted early (7<sup>th</sup> September) or at late time (7<sup>th</sup> October) caused 0.70-1.06 t/ha decrease in the yield of Lastivka Odeska after bare fallow and 0.12-0.42 t/ha decrease after unpaired predecessor; Burshtyn variety also reacted with the decrease as much as 0.35-0.91 t/ha and 0.35-0.80 t/ha, respectively (Figures 1, 2).

The yield of Holubka Odeska was less dependent on sowing time but its sowing at the optimal date gave higher results of 6.18-7.16 t/ha after bare fallow and 3.82-6.05 t/ha - after spring barley. When sowing at early but allowable time, the yields of this variety were also rather high: they totaled 6.14-7.04 t/h and 3.74-5.91 t/ha depending of the predecessor.

Further, it has been established that the application of the mineral fertilisers had a considerable effect on yield creation in winter wheat, especially after stubble predecessor.

Compared to the control lots, the increases in average yields of experimental winter wheat grain for the period of three years after bare fallow varied from 0.51 t/ha to 0.81 t/ha (depending on the wheat variety and sowing time) at the mode of pre-sowing application of complex fertilizer (N<sub>30</sub>P<sub>60</sub>K<sub>30</sub>) while the increases from 0.79 to 1.18 t/ha were observed at the fertilization system, in which pre-sowing complex fertilization was followed by nitrogen fertilization N<sub>30</sub> performed locally at the end of winter wheat tillering stage.

After spring barley, we found large increases of the winter wheat yields to the application of mineral fertilizers than those after bare fallow, that was conditioned by the difference in the nutrients contents left after these predecessors different in their agronomic conditions.

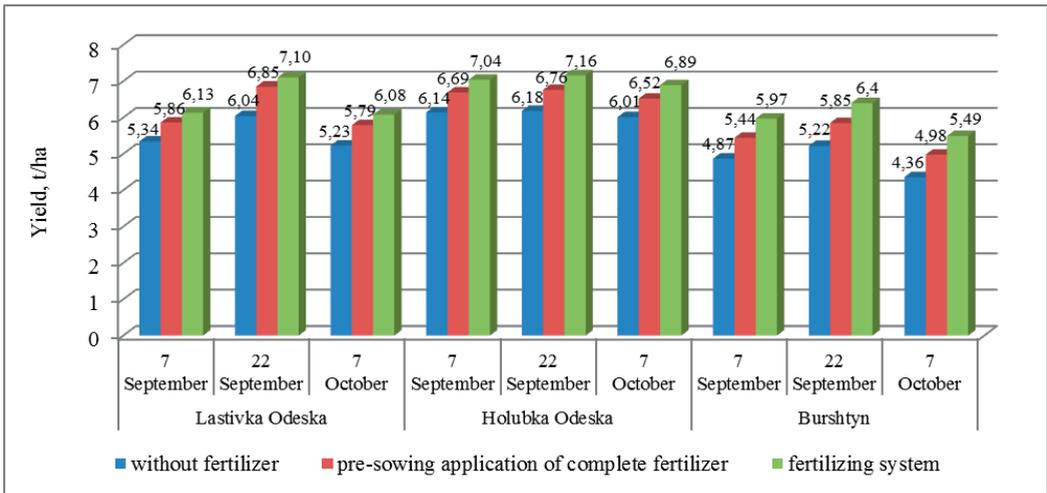


Figure 1. The effect of sowing time and fertilization on the winter wheat yield after bare fallow, t/ha, 2017-2019

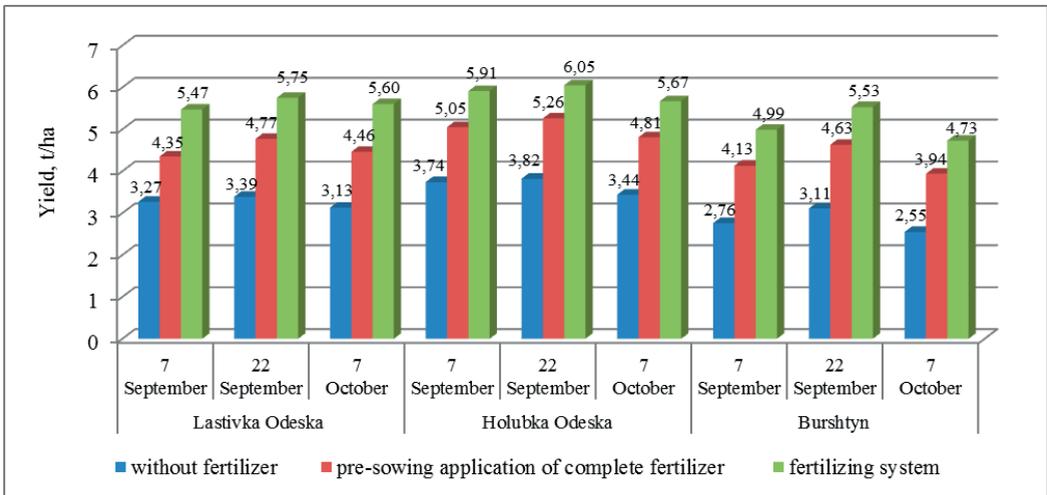


Figure 2. The effect of sowing time and fertilization on the winter wheat yield after spring barley, t/ha, 2017-2019

More-over, the content of that complete fertilizer which was applied in pre-sowing after unpaired predecessor was richer in nitrogen. The higher doses of this element were also used for spring fertilization of the winter wheat.

Thus, after spring barley, the increases in the winter wheat yields were 1.08-1.52 t/ha on condition of pre-sowing fertilization with complex fertilizer of  $N_{60}P_{60}K_{30}$ , the difference in the indexes depended on the winter wheat variety and sowing time. The results of the yield increases were obtained as compared against the control lot. These indexes were changing within the range of 2.17-2.47 t/ha at the fertilization system in which nitrogen fertilization ( $N_{30}$ ) was

conducted in thawing frozen soil on the background of pre-sowing fertilization with complex fertilizer plus local application of  $N_{30}$  at the end of plant tillering stage.

The analysis on the data obtained would not be complete without mentioning that within the years of the researches the highest grain yields were obtained from winter soft wheat of Holubka Odeska, while the lowest ones from winter durum wheat of Burshtyn after the both predecessors.

Further, it worth noting that the existing Ukrainian standard on wheat imposes the limits on such parameters as grain unit and glassiness (DSTU 3768-2019). Thus, the food grain

requirement is to possess grain units of not less than 775 g/l for soft wheat grains of the first class, 750 g/l - for the second class, 730 - for the third one. The gain of the fourth class, mainly utilised as coarse grains, is not restricted in this parameter. Glassiness value demands are to be equal or more than 50% for soft wheat of the first class, 40% for the second class, the other classes are not prescribed to undergo the limitations in this parameter.

For durum wheat grains, classified into five classes by the set of its parameters, grain unit values for the first and the second classes are to be not less than 750 g/l, for the third - 730 g/l, for the fourth - 710 g/l, no limitation in grain unit for the fifth class. Considering the certain application purpose for the durum wheat, the parameter of grain glassiness is one of the most significant parameters to attribute the wheat to the class. Therefore, for the durum wheat, the glassiness values are as follows: not less than 70% for the first class, 60% - for the second class, 50% - for the third class, 40% - for the fourth class, only the fifth class is free from glassiness limitations.

Note, that all the varieties of the winter wheat under the research had the highest values of both the weight of 1000 grains and the grain unit in the humid year of 2018. We have determined that these parameters respectively were 40.5-50.9 g and 803-845 g/l after bare fallow, while 42.0-49.2 g and 803-831 g/l after spring barley with no fertilizers applied but with the dependence on the wheat variety and the sowing time. The lowest values of the weight of 1000 grains (33.9-43.3 g after bare fallow and 32-42.3 g after stubble predecessor) and grain unit (750-818 and 759-806 g/l with respect to the predecessor) were received in 2019.

The correlation analysis of the data obtained has pointed to the close positive connection between the parameters of the weight of 1000 grains and the grain unit in winter wheat. Thus, the correlation coefficient ( $r$ ) after bare fallow was varying in the research years within the range of 0.74-0.79, while after spring barley it was 0.91-0.95. Further, the closest interconnection of the two quality parameters was revealed in 2019.

Furthermore, it was found that during the three years of the research, the physical parameters of

winter durum wheat Burshtyn were on average higher than those of winter soft varieties. Thus, after bare fallow and with the dependence on sowing time and fertilization, Burshtyn's weight of 1000 grains was within 39.3-45.9 g, its grain unit - 796-826 g/l, its glassiness - 76.5-97.7%. The variety of Lastivka Odeska showed the values of these parameters within 39.4-42.4 g, 776-803 g/l and 66.6-80.9%, respectively; Holubka Odeska - 38.7-42.1 g, 781-806 g/l and 61.5-78.8%, respectively. After the stubble predecessor, the difference in the grain unit and in the development of its grain glassiness was less manifested, however, the durum wheat appeared to be more advantageous per the parameter of 1000 grain weight than the soft wheat (Tables 1, 2).

The grain unit parameter of all the winter wheat varieties under the study both after bare fallow and after spring barley showed the increase in most cases from the early sowing time (7<sup>th</sup> September) towards the later one (22<sup>nd</sup> September and 7<sup>th</sup> October). As for the weight of 1000 grains, such regularity became a characteristic feature for winter durum wheat of Burshtyn while winter soft wheat varieties expressed less dependence of this parameter on sowing time. The higher values of glassiness at sowing on the optimal date or on tolerably later date after bare fallow were registered with all the wheat varieties, however after the stubble predecessor such a result was obtained only with Lastivka Odeska.

The research results have determined that such parameters as the weight of 1000 grains and the grain unit are insignificantly dependent on the fertilization variation but the exception is the cases when Burshtyn's 1000 grains had lower weight with the application of the fertilization system to include  $N_{30}$  local fertilization at the end of wheat tillering stage on the background of pre-sowing introduction of complete  $N_{30}P_{60}K_{30}$  fertilizer.

However, such fertilization after bare fallow allows winter durum wheat to develop the best glassiness of 96.7-97.7%. It is also worth noting that this fertilization produces positive effect on grain glassiness also after spring barley in Burshtyn.

Table 1. The effect of sowing time and fertilization on the winter wheat physical characteristics of its grain quality after bare fallow, 2017-2019

Variety	Sowing time	Fertilizer option		
		without fertilizer (control lot)	pre-sowing application of complete fertilizer*	fertilizing system**
1000 grain weight, g				
Lastivka Odeska	7 September	39.7	40.9	40.8
	22 September	39.4	39.6	39.4
	7 October	39.9	41.0	42.4
Holubka Odeska	7 September	38.8	40.0	39.2
	22 September	39.3	42.1	38.8
	7 October	38.7	39.5	40.6
Burshtyn	7 September	45.0	43.4	41.5
	22 September	40.9	44.0	39.3
	7 October	44.8	45.9	43.6
Grain unit, g/l				
Lastivka Odeska	7 September	777	776	779
	22 September	787	787	794
	7 October	800	800	803
Holubka Odeska	7 September	784	789	782
	22 September	791	800	786
	7 October	796	806	781
Burshtyn	7 September	804	796	812
	22 September	808	807	813
	7 October	826	821	815
Grain glassiness, %				
Lastivka Odeska	7 September	68.9	66.6	68.7
	22 September	76.7	79.9	80.3
	7 October	75.9	74.9	80.9
Holubka Odeska	7 September	61.5	62.9	70.0
	22 September	77.7	77.5	74.7
	7 October	73.9	70.4	78.8
Burshtyn	7 September	76.5	79.1	96.7
	22 September	92.5	92.9	97.7
	7 October	93.7	92.6	97.4

Notes: \*Pre-sowing application of complete fertilizer -  $N_{30}P_{60}K_{30}$ ; \*\* $N_{30}P_{60}K_{30} + N_{30}$  locally.

Thus, on the background with no fertilizers application, the values of glassiness were 69.4-81.5% (depending to the sowing time), the range of 79.0-84.0% was reached with pre-sowing fertilization while the enhance in glassiness up to 84.5-96.6% was attained at the fertilization system when  $N_{30}$  was applied for fertilization into thawing frozen soil +  $N_{30}$  local introduction on the background of  $N_{60}P_{60}K_{30}$ .

In general, the most cases (75%) evidence that the grain glassiness values of winter wheat varieties were better after the both predecessors under study provided that pre-sowing fertilization followed by the describe fertilization system, the comparison was made against the variants with no fertilizers employed.

## CONCLUSIONS

Eventually, winter soft wheat varieties of Lastivka Odeska and Holubka Odeska as well as winter durum Burshtyn under conditions of the Ukrainian Northern Steppe are significantly influenced by sowing time and fertilization mode in terms of their yields and physical parameters of the grain quality.

The maximal parameter values of grain wheat after bare fallow and after spring barley are registered when the middle data of sowing was observed (22<sup>nd</sup> September). Sowing time shift both to the earlier data (7<sup>th</sup> September) or to the later data (7<sup>th</sup> October) causes the considerable decrease in the yield value.

In comparison with the other genotypes, Holubka Odeska variety shows the highest flexibility for the sowing time. The highest yields of the winter wheat within the years of the

study are obtained when we applied the fertilization system in which pre-sowing fertilization is followed by complex nitrogen fertilizers.

Table 2. The effect of sowing time and fertilization on the winter wheat physical characteristics of its grain quality after spring barley, 2017-2019

Variety	Sowing time	Fertilizer option (faktor C)		
		without fertilizer (control lot)	pre-sowing application of complete fertilizer*	fertilizing system**
1000 grain weight, g				
Lastivka Odeska	7 September	40.9	40.9	41.7
	22 September	39.2	41.2	40.1
	7 October	40.9	40.2	40.9
Holubka Odeska	7 September	37.7	40.1	38.5
	22 September	41.7	40.1	40.4
	7 October	41.8	41.5	42.6
Burshtyn	7 September	40.6	40.2	41.3
	22 September	43.3	42.1	44.1
	7 October	45.8	48.6	46.3
Grain unit, g/l				
Lastivka Odeska	7 September	788	790	784
	22 September	789	789	791
	7 October	804	795	799
Holubka Odeska	7 September	778	779	779
	22 September	798	794	789
	7 October	800	798	802
Burshtyn	7 September	772	780	782
	22 September	801	805	806
	7 October	806	834	828
Grain glassiness, %				
Lastivka Odeska	7 September	72.0	74.2	72.9
	22 September	75.8	78.7	79.2
	7 October	84.6	82.5	79.9
Holubka Odeska	7 September	70.3	80.0	73.4
	22 September	72.7	86.9	80.4
	7 October	70.9	73.1	79.4
Burshtyn	7 September	81.5	84.9	84.5
	22 September	69.4	80.6	87.9
	7 October	69.7	79.0	96.6

Notes: \*Pre-sowing application of complete fertilizer -  $N_{60}P_{60}K_{30}$ ; \*\* $N_{60}P_{60}K_{30} + N_{30}$  on frozen-thawed soil +  $N_{30}$  locally.

The increases in the harvest after bare fallow on the background of  $N_{30}P_{60}K_{30}$  vary between the range of 0.51-0.81 t/ha as dependent on the sowing time; additional fertilization of  $N_{30}$  introduced locally at the end of the wheat tillering stage permits the yield increases as much as within 0.79-1.18 t/ha and specifically for Lastivka Odeska - 6.08-7.10 t/ha, for Holubka Odeska - 6.89-7.16 t/ha, for Burshtyn - 5.49-6.40 t/ha (all the mentioned increases in the grain yield are compared with the control lot where fertilization was not applied).

Winter wheat growing after spring barley and pre-sowing fertilization with  $N_{60}P_{60}K_{30}$  enables

the yield increase within 1.08-1.52 t/ha (as compared with the control lot) and shows the dependence on the wheat variety and sowing time; additional nitrogen fertilization ( $N_{30}$  in the early spring into thawing frozen soil +  $N_{30}$  locally) allows the increase of 2.17-2.47 t/ha. Thus, the variety of Lastivka Odeska at the application of the fertilization system gives the increase of 5.47-5.75 t/ha, Holubka Odeska - up to 5.67-6.05 t/ha, while Burshtyn - up to 4.73-5.53 t/ha.

The reported research has determined that grain unit values of winter wheats, as a rule, increase from the early sowing time towards the late time

after the both predecessors under analysis. The variety of winter durum wheat (Burshtyn) has higher grain unit values than the winter soft wheat varieties (Lastivka Odeska and Holubka Odeska), this parameter is varying from 796 to 826 g/l after bare fallow while after the unpaired predecessors, the varying is within the range of 772-834 g/l (depending on sowing time and fertilization). The research has established the positive effect of fertilization on the development of winter wheat grain glassiness. The significantly notable advantage in this parameter has been shown by the durum wheat over the soft wheat.

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## THE EFFECT OF THE CARBOXYL FATTY ACIDS AS A BIOLOGICAL CONTROL PRODUCT AGAINST *Brassicogethes aeneus* F. ON CANOLA

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### Abstract

The pollen beetle *Brassicogethes aeneus* F. (Coleoptera: Nitidulidae) is a major pest of oilseed rape and shows increasing resistance to commonly used insecticides. The aim of the study was to determine the effective dose of potassium salts of carboxyl fatty acids against the pest and to compare their efficacy with two pyrethroid products commonly used in conventional agriculture. The experiment was conducted on the field in the region of Plovdiv, Bulgaria in 2021. Two applications were made in an interval of 7 days between them. The treatment list contained untreated control, potassium salts of carboxyl fatty acids (in two doses: 2.5 l/ha and 5 l/ha), deltamethrin 0.05 l/ha, and tau-fluvalinate 0.2 l/ha. The results obtained show that the application of potassium salts of carboxyl fatty acids in the dose of 5 l/ha significantly reduces the number of the pollen beetle *Brassicogethes aeneus* F. and even slightly increases the oilseed rape yield. This substance could be successfully used as a plant protection product against the pollen beetle on oilseed rape as an alternative to the applied pyrethroid products.

**Key words:** *Brassicogethes aeneus*, canola, carboxyl fatty acids, pest control, pyrethroid.

### INTRODUCTION

The pollen beetle *Brassicogethes aeneus* (Fabricius, 1775) (formerly *Meligethes aeneus*) (Coleoptera: Nitidulidae) is one of the most important insect pests in winter oilseed rape (OSR; *Brassica napus* L.) in Europe (Juhel et al., 2017). To produce a high quality yield the agronomists apply a significant amount of pesticides which results in a resistance of the pests against some of the active substances, for example, pollen beetles in Europe became generally resistant to pyrethroids (Hansen, 2003; Heimbach, 2013).

Pyrethroids are mainly used to control insect pests of agriculture, horticulture, forestry and household (Bhatt et al., 2019). Products with that type of active substances are considered relatively safe but their extensive use makes them harmful for humans and animals (Kuivila et al., 2012; Burns and Pastoor, 2018; Bordoni et al., 2019). Many researchers declare their detrimental effects on non-target species including marine fish and aquatic insects (Burns and Pastoor, 2018; Lu et al., 2019). There are also evidences that deltamethrin induces inflammation, nephro- and hepatotoxicity and influences the activity of

antioxidant enzymes in tissues (Chrustek et al., 2018).

Alternative integrated pest management strategies for oilseed rape have been actively sought in the last decade, such as repellents (Mauchline et al., 2017), traps (Cook et al., 2004), or resistant cultivars (Herve et al., 2014).

Fatty acids are carboxylic acids with a long aliphatic (carbon chain) tail. They are characterized by the length of the carbon chain (the number of carbon atoms typically ranges from 4 to 24) and the number and position of double bonds (Sims et al., 2014). Toxicity of fatty acids and their salts (soaps) was initially documented almost hundred years ago using soft-bodied insects, such as aphids (Siegler and Popenoe, 1925; Tattersfield and Gimmingham, 1927; Dills and Menusan, 1935; Puritch, 1975; Parry and Rose, 1983). The authors observed that the toxicity of saturated fatty acids increased as the chain length increased, peaking at C10-C12, decreasing at C14-16, and again increasing in both the saturated and unsaturated C18 molecules. Fatty acids and their derivative soaps are mainly toxic to soft-bodied insect species, although there is considerable evidence that their range of activity covers a much

broader taxonomic spectrum (Sims et al., 2014). Commercial soaps and detergents are also toxic to other hard-bodied insects, such as beetles (van der Meulen and van Leeuwen 1929), Hemiptera (Fulton, 1930), crickets (Abbasi et al., 1984), and ants (Chen et al., 2010). There is not enough information about the action of the purified saturated carboxylic fatty acids against larger insects. Schull (1936) suggested that the fumigant effects of saturated C1-C5 fatty acids can produce significant biological effects on insect tissues. According to Mullens et al. (2009) mixtures of C8, C9 and C10 were highly repellent to houseflies and horn flies at or below 1 mg/cm<sup>2</sup> formulation. In their opinion, the low toxicity and reasonable activity and persistence of these carboxylic acids make them good candidates for development as protective materials against pest flies in livestock settings. Individual longer-chain-length fatty acids were tested, and C11 repelled houseflies for up to 5-8 days, while C12 lasted 2 days. Haritos & Dojchinov (2003) studied the effect of formic acid vapors on the respiration and suggested that they are exceedingly toxic to the rice weevil, *Sitophilus oryzae* (L.), by inhibition of cytochrome c oxidase. Most of the authors investigated the properties of short and middle-chain fatty acids. The data about the pesticidal action of the long-chain fatty acids especially on large insects are scarce. This motivated us to investigate the effects of carboxyl fatty acids on pollen beetle severity.

## MATERIALS AND METHODS

### Plant material, used substances, and experimental design

The experiment was set up in the field in the region of Plovdiv in the period October 2020 July 2021. The oil seed rape cv. DK Implement CL was planted on 02 October 2020 on a depth of 2 cm and plant density of 50 plants per m<sup>2</sup>. The experimental design included 5 variants: 1 - untreated control; 2 - potassium salts of carboxyl fatty acids 2.5 l/ha; 3 - potassium salt of carboxyl fatty acids 5.0 l/ha; 4 - deltamethrin 0.05 l/ha (Product Decis 100 EK), and 5 - tau-fluvalinate 0.2 l/ha (Product Mavrik 2 F). Every variant was set up in four replications with a plot size of 30 m<sup>2</sup> each.

The fatty acids are applied as a mixture of potassium salts of middle and long-chain carboxyl fatty acids. The applications with the potassium salts of the carboxyl fatty acids were made twice during the vegetation. The pyrethroid products were applied only once (on the day of the first treatment with potassium salts of carboxyl fatty acids). For the applications a backpack sprayer with compressed air was used. The application volume was 300l/ha, operating pressure - 2.3 bar using flat-fan nozzle type. The first spray was performed on 2 April 2021 - BBCH - 55 (majority). The air temperature during the treatment was 12.5°C, cloud cover - 30% and wind velocity - 0.8 mps. The second treatment was done during the BBCH phase 61 (majority), 7 days after the first one (9 April 2021). The air temperature was 17.4°C, cloud cover - 0%, and wind velocity - 1 mps. During the vegetation one herbicidal application was made on October 26 2020 with Cleranda in a dose 2 l/ha and one fungicidal application with Propulse in dose 1 l/ha on April 9 2021.

### Analyses of the pollen beetle (*Brassicogethes aeneus* F.) number

For the analyses of the pest number and severity, fifty branches from an experimental plot were observed and the data are presented as means. The number of the living pollen beetle was counted 5 times (1 - on the day of the first treatment, in the morning before spray; 2 - two days after the first treatment; 3 - on the day of the second treatment; 4 - four days after the second treatment, and 5 - eight days after the second treatment).

### Statistical analysis

The data were presented as mean ± SD of 4 replicates. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis using Duncan's comparative method, with the validity of the differences determined at a 95% significance level. The different letters (a, b, c, d) after the average show statistically significant differences between the analyzed variants.

## RESULTS AND DISCUSSIONS

The pollen beetle population was observed several times and the number of the living individuals was recorded. The results about the number of the observed pollen beetles are presented on Figures 1-5. On Figure 1 there is

information about the number of the pests on the day of the first treatment with carboxyl fatty acids and insecticidal substance (0 DAT 1). It is seen that there are no significant differences in the number of the pests between the tested variants at that time.

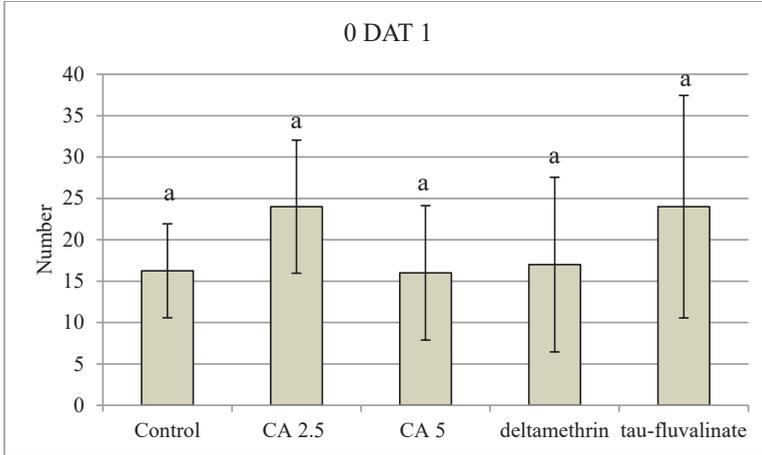


Figure 1. Number of the living pollen beetle on the day of the first treatment (0 DAT 1)

On the other hand, 2 days after the first treatment (2 DAT No 1) there are significant differences between the tested variants. The two products containing potassium salts of carboxyl fatty acids demonstrated a very good pesticide/repelling effect. The CA 2.5 treatment showed a reduction of the beetles by 22.9%

compared to the control. As we expected the reduction was even bigger after the application of CA 5 (by 80% compared to the control). The effects of deltamethrin and tau-fluvalinate were similar - a reduction of the adults' number by 81.4 and 82.9% respectively (Figure 2).

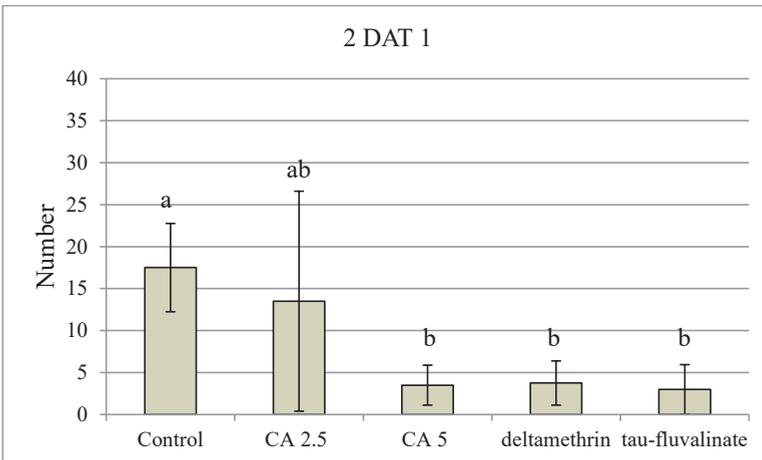


Figure 2. Number of the living pollen beetle 2 days after the first treatment (2 DAT 1)

Seven days after the first treatment a second treatment only with potassium salts of carboxyl fatty acids was performed. On the same day (0 DAT 2) another observation for the number of the pests was made and the differences between

the variants were more pronounced. The beetles in variant CA 2.5 were reduced by 59.5% in comparison to the control. On the test plots of the other treatments no living beetles were found (Figure 3).

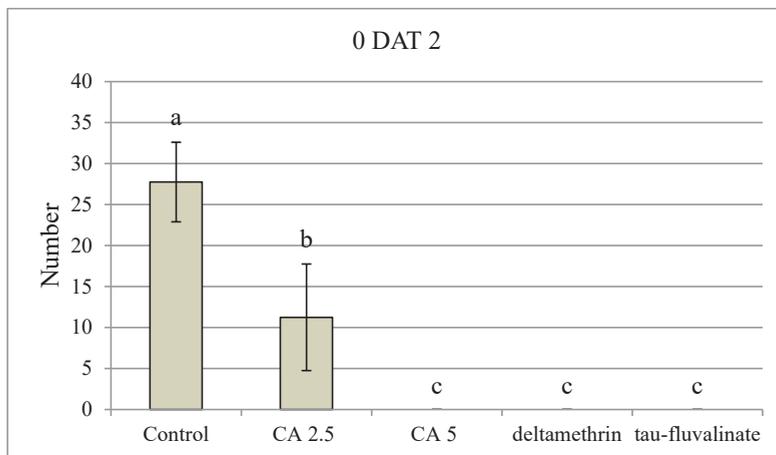


Figure 3. Number of the living pollen beetles at the day of the second treatment (0 DAT 2)

Four days after the second treatment with carboxyl fatty acids the number of the beetles in all the tested variants was very low in comparison to the untreated control. In the CA 2.5 treatment, the reduction was by 87.8% compared to the control. In the other carboxyl

acid treatment (dose 5 l/ha), there were no living beetles observed. On the plots treated with deltamethrin there were 97.4% less pests than in the control. On the test plots where tau-fluvalinate was applied, no living beetles were found (Figure 4).

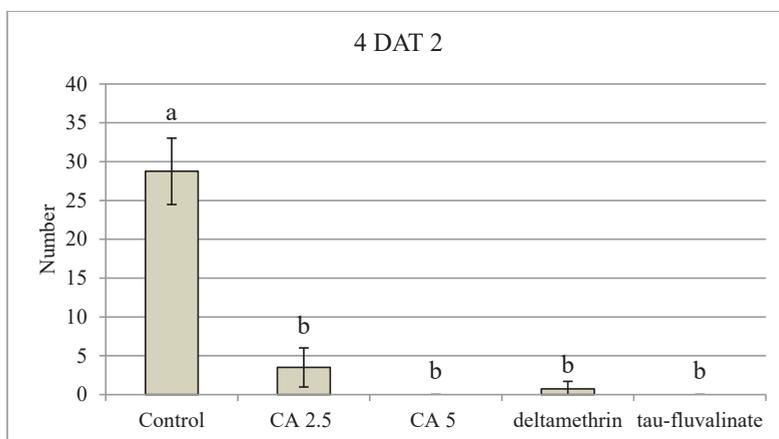


Figure 4. Number of the living pollen beetle 4 days after the second treatment (4 DAT 2)

The data about the number of the pest eight days after the second treatment with carboxyl fatty acids are presented on Figure 5. On the test plots which were treated with carboxyl

fatty acids in the dose of 2.5 l/ha the decrease of the number of the living beetles was by 34.1% in comparison to the untreated plots. There where the dose of 5 l/ha was applied, no

living beetles were found. The deltamethrin treatment reduced the pest population by 97.7%

and the spray with tau-fluvalinate - by 86.2% respectively.

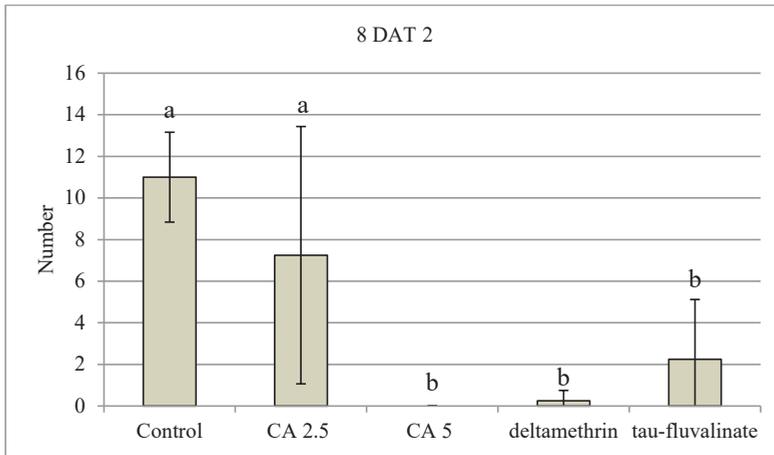


Figure 5. Number of the living pollen beetle 8 days after the second treatment (8 DAT 2)

The data about the calculated pest severity is presented on Figure 6. It is obvious that the pest severity of the control plots increased 7 days after the first treatment and is relatively high during the next 4 days. After that, the value of that parameter was extremely reduced

and 15 days after the first application it was lower than at the first day of observation. On the test plots which were treated with potassium salts of fatty acids in dose of 5 l/ha no beetles were observed from day 7 on till the end of the experiment.

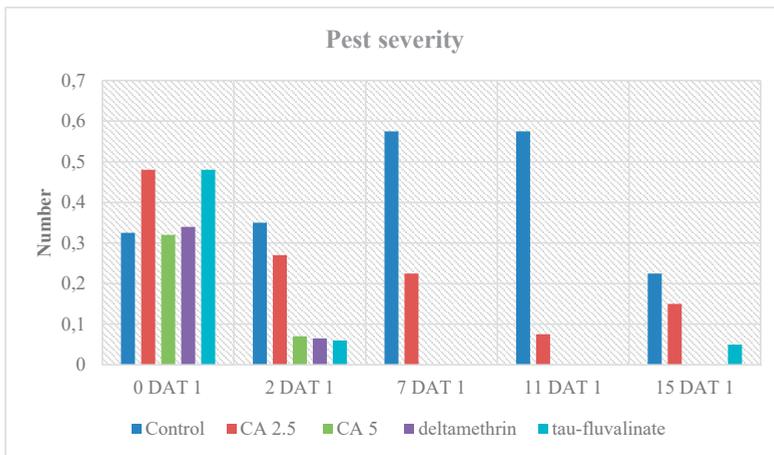


Figure 6. Pest severity (number of living beetles per branch)

The use of carboxylic acid in the dose of 2.5 l, also led to a decrease of the insect number but not to a total lack. The insecticidal treatments extremely reduced the pest population from day 2 on. Till the final count no living beetle was observed on the plot treated with deltamethrine but some beetles were found on the plot treated

with tau-fluvalinate on day 15. The toxicity effects of fatty acids and their salts (soaps) was documented decades ago using soft-bodied insects, such as aphids (Sieglar and Popenoe 1925). Later many other researchers speculated about the connection between the length of the acids and their detrimental or repelling effects

(Sims et al., 2014). They examined the effect of different length chain fatty acids (C14-C20) and reported that the repellent properties of middle length acids (C14) was highly reduced and their toxicity effect was insignificant.

On the other hand, there are several reports about the insecticidal and repellent properties of carboxyl fatty acids with shorter chains. For example, Krzyzowski et al. (2020) used short chain carboxyl fatty acids to investigate their effect on one of the most common pests of stored legumes - the cowpea weevil, *Callosobruchus maculatus*. The authors used undiluted fatty acids including formic, acetic, propionic, butyric, and valeric acid and the results obtained show that these substances demonstrate very highly repellent properties. In our experiment, we used a mixture of middle and long-chain fatty acids in the form of potassium salts and the results show that two applications of these substances could provide very good protection of the treated plots. The application of 5 l potassium salts of fatty acids

reduced the pest severity for 15 days. This effect was similar to the effect of one single application of a pyrethroid product.

According to Dheeraj et al. (2013) and Mohamad et al. (2013), potassium salts of fatty acids could be effectively used as synthetic chemicals against snap beans pests and their application led to an increase of the yield quality and quantity. In the current experiment, the yield was also measured and there was a slight increase in all of the tested variant in comparison to the control although it was not statistically significant (Figure 7).

The crop productivity was almost unaffected after the application of 2.5 l/ha potassium salts of carboxyl fatty acids but the rate of 5 l/ha led to an increase by about 4% compared to the untreated control.

The enhancement of the yield was by 6% and by 4% on the plots witch were treated with deltamethrin and tau-fluvalinate respectively (Figure 7.).

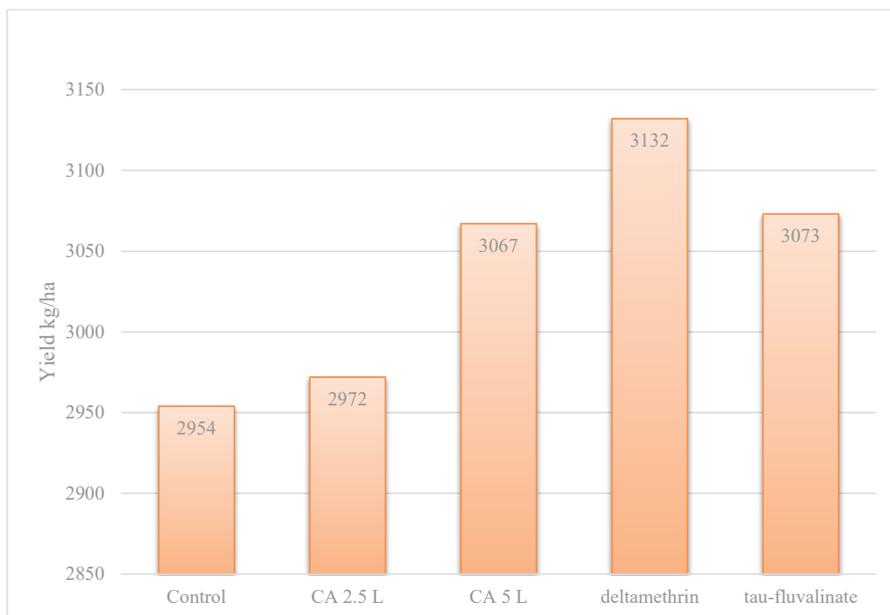


Figure 7. Oilseed rape yield kg/ha

## CONCLUSIONS

The current experiment aimed to investigate the effect of the potassium salts of carboxyl fatty acids against the pollen beetle *Brassicogethes*

*aeneus* F. on oilseed rape. The results obtained showed that the application of products containing salts of carboxyl acids resulted in a significant reduction of the pollen beetle adults' number. Both of the tested doses (2.5 l/ha and 5

l/ha) decreased the pest number, but the effect was more pronounced after the application of the higher test dose of 5 l/ha. Fifteen days after the first treatment no living insects were observed on the test plots sprayed with carboxyl acids in the dose of 5 l/ha. The application of the lower dose of 2.5 l/ha could not provide long-lasting protection against the pollen beetle *Brassicogethes aeneus* F. Furthermore the use of the tested substances did not affect the yield quality or quantity negatively but even slightly increased it when 5 l/ha potassium salts of fatty acids were applied. After the analyses performed, we could suggest that the potassium salts of middle and long-chain fatty acids are a potential health-friendly tool for managing the control of *Brassicogethes aeneus* F. In order to minimize the application of pyrethroid insecticides and according to the results obtained, we could recommend the application of potassium salts of middle and long-chain fatty acids in the rate of 5 l/ha as a promising substance for pollen beetle control in oilseed rape.

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## RESEARCH ON THE EFFECT OF APPLYING THE SECUIENI METHOD TO THREE VARIETIES OF MONOECIOUS HEMP, IN TERMS OF PRODUCTION (SEED, STEMS, FIBER), IN THE PEDOCLIMATIC CONDITIONS OF A.R.D.S. SECUIENI

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### Abstract

*In this paper we present the results regarding the evolution of monoecious hemp crop on the yield of seeds, stems and fiber, by following the Secuieni Method under the pedoclimatic conditions in the Agricultural Research and Development Station Secuieni Neamț (A.R.D.S. Secuieni, Neamț). The experience takes place in the experimental field of the unit, and it is a multifactorial experience, of the type 3 x 2 x 3, in three repetitions: A factor - variety (Denise, Diana, Dacia), B factor - distance between rows (25 cm; 50 cm), C factor - „Secuieni metho” (uncut, one cut, two cuts). On average, during the three years of experimentation, the above factors greatly influenced the seed yield obtained, which varied widely, from 806 kg • ha<sup>-1</sup> (Denise x 50 cm x uncut) to 1117 kg • ha<sup>-1</sup> (Dacia x 50 cm x two cuttings). Regarding the yield of stems, this also varied quite a lot, from 9219 kg • ha<sup>-1</sup> (Denise x 50 cm x two cuttings) up to 12634 kg • ha<sup>-1</sup> (Dacia x 50 cm x uncut).*

**Key words:** yield, method, monoecious hemp, variety.

### INTRODUCTION

Industrial hemp disappeared almost completely after World War II and, despite a ban by the United Nations, Canada, China and the European Union are cultivating it again. In 2018, industrial hemp recorded record cultivation figures in Europe (48605 ha), Canada and China, registering a total of 150000 ha (EIHA Conference, 2020). Incidentally, cultivation has also begun in the US (EIHA Conference, 2019).

Hemp is considered a plant of increasing importance for Europe (Ranalli, 2004) and is used for the extraction of fiber, oil and as a medicinal herb (Sandru et al., 1996).

The hemp fiber is the most resistant vegetable fiber and as such, in the past, was the most valuable raw material of the textile industry worldwide (Forgo, 1957).

Hemp is an extraordinary crop, with enormous social and economic value, since it can be used to produce food, textiles, clothing, biodegradable plastics, paper, paint, biofuel, and animal feed, as well as lighting oil. Various parts of the hemp plant represent a valuable source of

food and ingredients for nutritional supplements (Pellegrino et al., 2021).

Industrial hemp (*Cannabis sativa* L.), is one of the most important traditional natural fiber crops, had almost been forgotten for the last several decades. Nowadays, industrial hemp has many agro-industrial applications, such as agriculture, textile, papermaking, construction, bio-fuel (Xinlin et al., 2021).

The technological properties of fibers in terms of strength (tensile, torsional, friction, rot), extensibility (elastic and plastic), spinning capacity and high length (Sandru, 1996) determine its use in a wide range of areas such as the manufacture of quality paper, braids and fabrics, fine fabrics, plastic castings (Small and Marcus, 2002), fiber-reinforced cement (Zhijian et al., 2004), thermal insulation.

Small in 2015 says that *Cannabis sativa* has been employed for thousands of years, primarily as a source of a stem fiber (both the plant and the fiber termed “hemp”) and a resinous intoxicant (the plant and its drug preparations commonly termed “marijuana”).

Struik et al. (2000), shows that the fiber hemp may yield up to 25 t above ground dry matter per

hectare (20 t stem dry matter ha<sup>-1</sup>) which may contain as much as 12 t ha<sup>-1</sup> cellulose, depending on environmental conditions and agronomy. Its performance is affected by the onset of flowering and seed development.

Cannabinoids represent the most studied group of compounds, mainly due to their wide range of pharmaceutical effects in humans, including psychotropic activities. The therapeutic and commercial interests of some terpenes and phenolic compounds, and, in particular, stilbenoids and lignans, are also highlighted in view of the most recent literature data (Andre et al., 2016).

Kasula et al. (2021) said that the nutrient composition of hemp products provides evidence that these potentially serve as valuable livestock feed ingredients and may enhance human health.

Hemp products available on the market may be used in meat processing as valuable sources of nutrients such as n3 fatty acids, proteins and minerals. They could be used to create functional meat products. The aim of this work was to compare the quality of pork loaves produced with the addition of hemp seeds (5%), de-hulled hemp seeds (5%), hemp flour (5%), and hemp protein (5%). The technological value and the consumers' acceptance were also evaluated (Zajac et al., 2019).

Cannabidiol (CBD) oil are low tetrahydrocannabinol product derived from *Cannabis sativa* that have become very popular over the past few years. Patients report relief for a variety of conditions, particularly pain, without the intoxicating adverse effects of medical marijuana (Harrison et al., 2019).

During the last decade, the popularity of hemp products has been rising rapidly. Products containing cannabidiol (CBD) are of predominant interest. Traditional hemp products are frequently enriched by CBD due to their potential therapeutic effects. Cannabidiol occurs naturally in hemp juice together with other biologically active substances, such as terpenes, flavonoids, and stilbenoids (Tremlova et al., 2021).

The byproducts of industrial hemp (*Cannabis sativa* L.), including inflorescences, represent an exploitable material to produce niche products for the pharmaceutical, nutraceutical, cosmetic and pesticide industry (Fiorini et al., 2019).

Alexa et al. (2012) are aiming with a study to determine the nutritional value of hemp seeds expressed by oil content and metal concentration (Ca, Mg, K, Fe, Mn, Zn and Cd), for five approved Romanian monoecious and dioecious hemp.

Currently, the cultivation of hemp varieties with multifunctional capacity, both for seed production and for obtaining stems and fiber, has opened new challenges in many research sectors such as breeding (Baldini et al., 2018).

## MATERIALS AND METHODS

During 2018-2021 period, a multifactorial experiment was set up at Agricultural Research and Development Station Secuieni (A.R.D.S. Secuieni), the purpose of which was to establish the yields capacity of stems, fiber and monoecious hemp seeds, by applying the "Secuieni method" at different distances between rows. The experiment was of the type 3 x 2 x 3, in three repetitions, where A Factor - varieties - a1 - Denise, a2 - Diana, a3 - Dacia, B Factor - distances between rows - b1 - 25 cm and b2 - 50 cm, and C Factor - "Secuieni method" - c1 - uncut, c2 - one cut, c3 - two cuts (Figure 1).



Figure 1. Applied Secuieni method and shoot formation (source: original photo)

The Secuieni method was applied when the plants go into the phase of intense growth and have 5-6 floors with opposite leaves. (Figure. 1). Following the pruning applied from the insertion of the leaves, 2-6 lateral shoots develop, which

remain in culture in this form, but the height of the plants will not exceed 3 m. After the first pruning, when the shoots have developed enough, the second pruning above the first cut, at 15-20 cm (Găucă et al., 1990).

The experience was placed on a typical cambic faeoziom (chernozem) soil, with medium texture, characterized as being well supplied with phosphorus ( $P_2O_5$  - 39 ppm) and mobile potassium ( $K_2O$  - 161 ppm), moderately supplied with nitrogen, the soil nitrogen index being 2.1, weakly acidic, with pH values (in aqueous suspension) of 6.29 and poorly fertile, with a humus content of 2.3% (Leonte et al., 2021).

The experiment was done on subdivided plots, and the applied technology was the one specific to the conditions in the Center of Moldavia. The content of hemp fiber extracted from the stem was determined in the laboratory, and specific biometric measurements were made during the vegetation period. The experimental production data were processed by statistical-mathematical methods specific to the multifactorial experiments, and the interpretation of the results was performed by analyzing the variations (Ceapoiu, 1968; Jitäreanu, 1999).

## RESULTS AND DISCUSSIONS

During the vegetation period, a series of observations were made about the number of branches on the plant following the application of the "Secuieni method" and how the length and diameter of the stem was influenced.

Of the three varieties studied, the largest size of 2.9 m, with a diameter of 10.7 mm was recorded at the interaction of the Dacia variety x 25 cm x uncut, but by applying two cuttings its size was reduced to 2.0 m, with a stem diameter of 4.3 mm (Table 1).

By applying the "Secuieni method", the plants developed branches, which varied within fairly large limits, from 1.5 branches/plant (Diana x 25 cm x two cuttings) and up to 3.3 branches/plant (Denise x 25 cm x two cuttings) (Table 1).

Correlating the plant height with the stem diameter (average), it is observed that it is a direct link, the correlation coefficients ( $r$ ) were statistically assured and interpreted as very significant (Figure 2).

The yield of the stems varies quite a bit from one year to the next, so the lowest yield of 7537

$kg \cdot ha^{-1}$  was obtained in 2021, at the interaction of the Denise variety x 25 cm x two cuttings, and the highest yield was obtained in 2019, of 15680  $kg \cdot ha^{-1}$ , at the Dacia x 50 cm x uncut interaction.

Table 1. Biometric measurements on monoecious hemp culture after applying the "Secuieni method", (*Cannabis sativa* L.), year average

Variety	Row distance (cm)	Applied work	Ramif. /plant	Height (m)	Stem Diam. (mm)
1	2	3	6	7	8
Denise	25	NR	-	2.7	10.7
		R1	1.8	2.1	6.2
		R2	3.3	1.8	4.5
	50	NR	-	2.7	10.1
		R1	1.6	2.2	6.9
		R2	3.0	1.7	4.1
Diana	25	NR	-	2.6	8.5
		R1	1.5	2.2	5.6
		R2	3.0	1.8	3.8
	50	NR	-	2.7	8.4
		R1	1.6	2.4	6.1
		R2	2.5	2.0	4.2
Dacia	25	NR	-	2.9	7.4
		R1	1.5	2.6	6.1
		R2	2.7	2.0	4.3
	50	NR	-	2.9	8.6
		R1	1.7	2.6	6.5
		R2	2.4	2.1	4.8

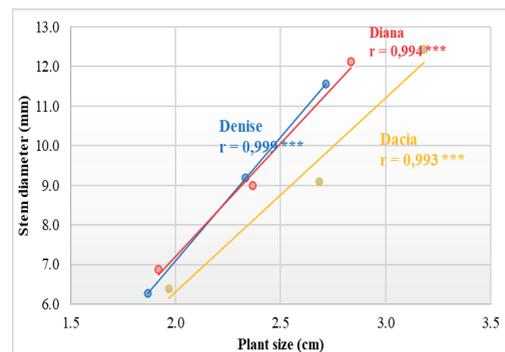


Figure 2. Correlation between plant size and stem diameter

On average, over the three years studied, the studied factors highly influenced the yield of strains obtained, which ranged from 9219  $kg \cdot ha^{-1}$  to the Denise interaction x 50 cm x two cuttings, up to 12634  $kg \cdot ha^{-1}$  (Dacia x 50 cm x uncut). Yield increases were also obtained in five interactions, two being very significant (Dacia x 25, respectively 50 cm x uncut), two being distinctly significant (Denise x 25 cm x uncut, Diana x 50 cm x uncut) and one significant (Diana x 25 cm x uncut) (Figure 3).

Seed yield varied quite a bit year-on-year, so the lowest yield was obtained with the Denise x 50 cm x uncut interaction of 668 kg•ha<sup>-1</sup> (2021), and the highest yield was obtained at the Dacia interaction x 25 cm x two cuttings, of 1386 kg•ha<sup>-1</sup> (2019). In the three years studied, the factors influenced the seed yield obtained, which ranged quite a lot, from a maximum of 1117 kg•ha<sup>-1</sup>, at Dacia x 50 cm x two cuttings to

806 kg•ha<sup>-1</sup> in the uncut version sown at a distance of 50 cm with the Denise variety. Distinctly significant yield increases were obtained, compared to the control (average experiment) in some variants in which two cuttings were applied, namely Denise x 25 cm and Dacia x 25, 50 cm, and significant at the interaction Denise x 50 cm (Figure 4).

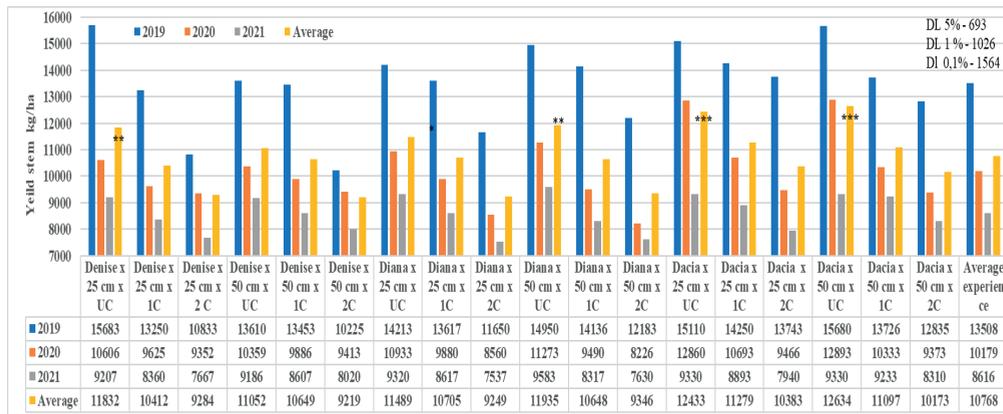


Figure 3. Stem yield obtained at monoecious hemp

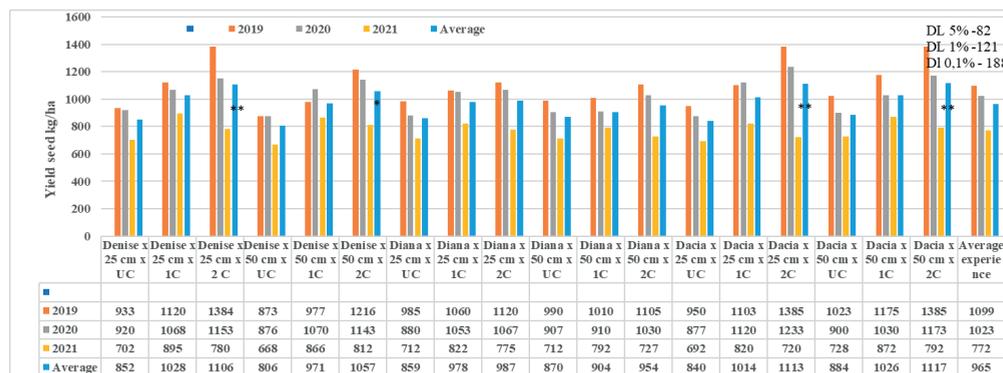


Figure 4. Seed yield obtained at monoecious hemp

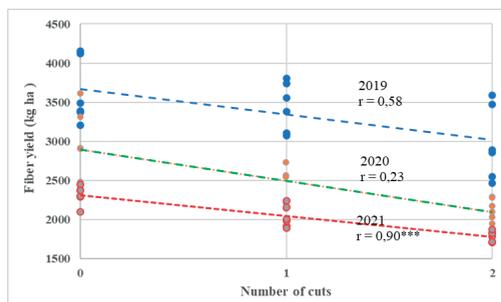


Figure 5. Correlation between number of cuts and fiber yield

Correlating the number of cuttings applied with the yield of hemp fiber obtained, it can be observed that in 2021 it was directly influenced, statistically assured and interpreted as very significant, and in the other two years of experimentation they had no significance (Figure 5). The percentage of hemp fiber obtained in the three years of experimentation varied from 21.6% in 2020 (Denise x 50 cm x one cut) to 27.5% in 2019 (Dacia x 25 cm x uncut). In the three years studied, the factors influenced the fiber percentage obtained, which ranged quite a

lot, from a minim of 22.6%, at the interaction Denise x 50 cm x one cut to 27.0% at the uncut

version sown at a distance between rows of 25 cm, with the Dacia variety (Figure 6).

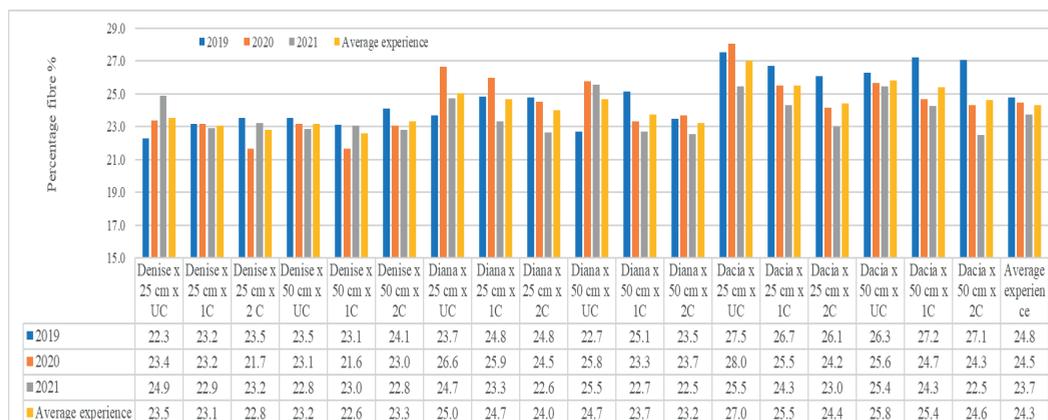


Figure 6. Fiber content extracted from monoecious hemp stalks

## CONCLUSIONS

By applying the "Secuieni method" to the hemp crop, for the three varieties studied, this had a negative effect on the stem yield and on the fiber percentage, but positively influencing, instead, the seed yield.

By reducing the size but also the diameter of the stem, the seed can be harvested more easily by the fact that the ivy of the combine reaches the inflorescence of the plant.

In the three years of study, the highest yield was recorded during year 2019, the research carried out showed superiority of the Dacia x 50 cm x uncut interaction, of 15680 kg·ha<sup>-1</sup>, the increases observed being very significant for the stem crops, and also at the level of the same year, the seed yield were significantly better for the same variety, Dacia, but at a distance between rows of 25 cm and by applying two cuttings (1386 kg·ha<sup>-1</sup>).

The combination of the three factors studied, in the three years, generated the highest stem yield in the case of the Dacia x 50 cm x uncut variant (12634 kg·ha<sup>-1</sup>), as well as the highest seed yields, for the established using the Dacia x 50 cm (1115 kg·ha<sup>-1</sup>), to which two cuttings were applied.

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## EFFECTIVENESS TESTING OF DIFFERENT ORGANIC FERTILIZERS ON CROP YIELDS UNDER CLIMATIC CONDITIONS OF ARGES COUNTY

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### Abstract

*Application of organic fertilizers has proven a lot of benefits which converge to better yield parameters and food of high quality. The objectives of our study were to research the efficiency of three fertilizers accepted for organic agriculture (CODAMIX - F1, ECOAMINOALGA - F2 and ECOAMINOALGA PLUS - F3) on four different field crops, to evaluate their effects on yield parameters and to compare the results with those obtained in a previous experimental year. The study was developed in Albota, Argeș County during 2020-2021 and the field crops subjected to this investigation were winter wheat (Trivale variety), sunflower (PG4 hybrid), maize (T.332 hybrid), soybean (Raluca TD variety). The experimental scheme was composed from four plots and four variants (control, F1, F2, F3) for each crop. Organic fertilizers (F1, F2, F3) were applied for all field crops as two foliar treatments during vegetation period. The obtained results evidenced that application of organic fertilizers increased yield parameters and the efficiency of treatments is following the order F3>F2>F1. The results obtained during 2020-2021 are in good agreement with those reported for 2019-2020.*

**Key words:** crop, foliar application, organic fertilizer, yield components.

### INTRODUCTION

Finding new fertilizers and formulations destined for organic agriculture has gained a lot of interest lately because are environmentally friendly, may contribute to higher yields and improve soil characteristics. Furthermore, organic production regulations allow the use of specific inputs that has to comply strict standards, the accepted products being regulated by Commission Regulation (EC) No 889/2008 and by Regulation (EC) No 2003/2003 of the European Parliament and of the Council. For instance, protein hydrolysates of plant and animal origin and different amino acids fertilizers tested on field (Mihalache et al., 2014; Mihalache & Stanescu, 2017; Nicu et al., 2021) or on horticultural crops (Colla et al., 2017; Roupheal et al., 2021; Soteriou et al., 2021) has proven their efficiency. Other study (Moosavi et al., 2013) evidenced positive effects of a formulation based on free aminoacids on maize grain yield.

Panayotova (2019) tested the efficiency of organic seaweed bioproduct Bioalpha (Alga 300) on seven sunflower hybrids grown under organic farming conditions. Among all tested hybrids, DKF2120 achieved higher yields and was most adaptable to be use in organic conditions.

Furthermore, the effectiveness of various combinations of organic manures (green manure - GM, farm yard manure - FYM, poultry litter - PL, press mud - PM, sewage sludge - SS) at a rate of 10 t/ha on a spring wheat cultivar evidenced that variant consisting from GM+PL+SS treatment produced maximum yield that was 137% higher than control variant with no fertilization (Hammad et al., 2011). Moreover, use of two green manure crops (red and white clover) on organically grown maize hybrids under climatic conditions from Greece has proven the efficiency on grain yield and protein content (Kanas et al., 2020). Also, a meta-analysis using 133 maize studies developed worldwide evidenced that rational

use of organic and mineral fertilizers improved maize yield, reduced N and C losses and increased soil organic carbon sequestration (Wei et al., 2020).

Alam et al. (2018) investigated the impact of crop rotations using green manure, green manure incorporation timing and application of pelletized dehydrated poultry manure on organic wheat production. The results demonstrated that leguminous green manure and organic inputs significantly enhanced wheat biomass, yields and protein content.

In the context of our previous researches concerning effects of fertilization on quality and yield parameters for different crops (Mihalache et al., 2015; Mot et al., 2017; Madjar et al., 2018; Ilie et al., 2018; Ilie et al., 2018; Madjar et al., 2018; Madjar et al., 2019; Mihalache et al., 2020; Ilie et al., 2020; Madjar

et al., 2021), we have developed an experiment with the aim to investigate the effects of organic inputs (CODAMIX - F1, ECOAMINOALGA - F2 and ECOAMINOALGA PLUS - F3) on different field crops (winter wheat, sunflower, maize, soybean) and to compare their effects on yield performances.

## MATERIALS AND METHODS

### *Experiment location*

The experimental study was developed during 2020-2021 in Albota, Argeş County, where the dominant soil type is albic luvisols (Mihalache et al., 2015). Albota is located in the south part of Argeş County, at 10 km far from Piteşti (Figure 1).

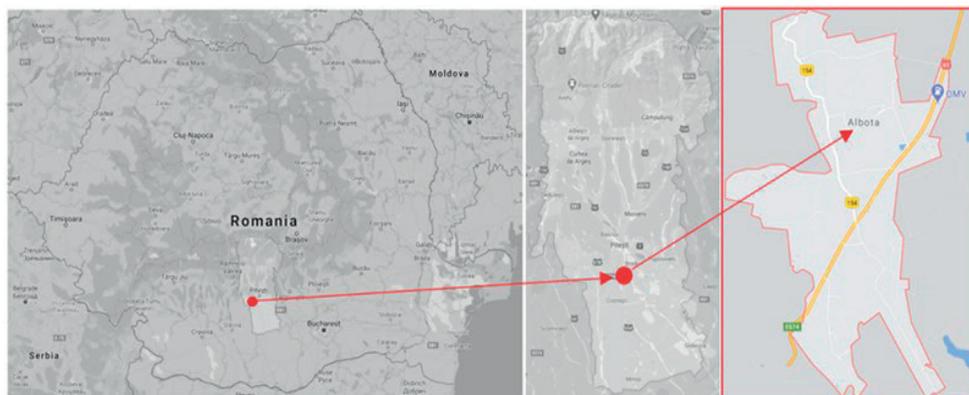


Figure 1. Position of Albota on geographical map

### *Experiment description*

To achieve the proposed objectives, it was chosen for testing the following species: winter

wheat, sunflower, maize and soybean. The details of the experiment are presented in Table 1.

Table 1. Experiment characterization

Field crop	Winter wheat	Sunflower	Maize	Soybean
Variety/hybrid	Trivale	PG 4	T.332	Raluca TD
Sowing date	22.10.2020	05.05.2021	11.05.2021	28.04.2021
Harvest date	08.07.2021	16.09.2021	24.09.2021	01.10.2021
Lot surface*	500 m <sup>2</sup>	500 m <sup>2</sup>	500 m <sup>2</sup>	500 m <sup>2</sup>

\*4 variants/lot and 3 repetitions/variant

### *Soil agrochemical characterization*

The experiment was developed on albic luvisol with clay texture. Soil reaction (pH = 5.27) is moderately acidic, humus content is considered as medium level (2.21%). Mobile phosphorus (P<sub>AL</sub> = 35 mg/kg) and mobile potassium

(K<sub>AL</sub> = 84 mg/kg) are associated with medium content in soil, meanwhile inorganic sulphur level is 21 mg/kg and corresponds to high content. Microelements' contents are depicted in Table 2.

### Characterization of used fertilizers

To fulfil the objectives, for this study was chosen three inputs accepted for organic agriculture: CODAMIX (coded F1),

ECOAMINOALGA (coded F2) and ECOAMINOALGA PLUS (coded F3). The inputs' full chemical characterizations are presented in Table 3 and Table 4.

Table 2. Soil microelements' content

Microelement	Total form, mg/kg	Mobile form, mg/kg
Co	7	-
Cu	15	2.6
Mn	603	58
Ni	23	-
Zn	50	1.6

Table 3. CODAMIX (F1) chemical characterization

Guaranteed analysis	%w/w	%w/v
Iron (Fe) complexed and water soluble	4.00	5.12
Manganese (Mn) complexed and water soluble	2.00	2.56
Zinc (Zn) complexed and water soluble	0.50	0.64
Copper (Cu) complexed and water soluble	0.12	0.15
Boron (B) water soluble	0.30	0.38
Molybdenum (Mo) water soluble	0.08	0.10
Complexing agent: lignosulfonates		

Table 4. ECOAMINOALGA (F2) and ECOAMINOALGA PLUS (F3) chemical characterizations

Parameter	F2	F3
	Content, %	
Organic nitrogen	3	3
Organic matter	46	45
Phosphorus (P <sub>2</sub> O <sub>5</sub> )	-	0.2
Potassium (K <sub>2</sub> O)	6	7.7
Iron (Fe)	-	0.03
Manganese (Mn)	-	0.01
Zinc (Zn)	-	0.005
Copper (Cu)	-	0.06
Boron (B)	-	0.01

### Fertilization scheme

In Table 5 are presented treatments for each experimental crop. The foliar treatments with F1, F2 and F3 inputs were applied twice for

each experimental crop during vegetation, using 2.5L solution 0.5%/ha/treatment. Total applied volume was 150 L.

Table 5. Fertilization scheme

Experimental crop	Winter wheat	Sunflower	Maize	Soybean
Preceding crop	Maize	Wheat	Sunflower	Wheat
Basal fertilization*	300 kg/ha	300 kg/ha	300 kg/ha	300 kg/ha
First treatment application (phenophase) <sup>#</sup>	28.04.2021 (boot)	23.05.2021 (4-5 leaves)	27.05.2021 (5 leaves)	22.05.2021 (2 <sup>nd</sup> trifoliate leaf)
Second treatment application (phenophase) <sup>#</sup>	16.05.2021 (heading)	04.06.2021 (7 leaves)	08.06.2021 (7 leaves)	07.06.2021 (4 <sup>th</sup> trifoliate leaf)

\*Basal fertilization complex NPK 20:20:0. <sup>#</sup>Foliar fertilization with F1, F2, F3

## RESULTS AND DISCUSSIONS

### 1. The efficiency of foliar fertilization with CODAMIX, ECOAMINOALGA and ECOAMINOALGA PLUS on winter wheat yield parameters

Foliar application of organic fertilizers produced positive effects and, excepting TKW after F1, all yield parameters increased (Table 6). The best results were obtained after F3 treatment in comparison with control. Consequently, yield parameters (total biomass,

spikes biomass, seeds biomass, TKW) after F3 increased with 73.64%, 69.27%, 79.97% and 13.42% respectively, in comparison with control (Figure 2).

The efficiency of treatments on yield parameters is following the order F3>F2>F1. The proper climatic conditions, during experiment influenced positively the results.

The superiority of F2 as against F1 on winter wheat yield parameters was already reported in a previous paper (Madjar et al., 2021).

Table 6. The efficiency of foliar fertilization on winter wheat yield parameters

Experimental variant (dose; number of treatments)	Total biomass, kg/ha	Spikes biomass, kg/ha	Seeds biomass, kg/ha	TKW, g
<b>Control</b>	7333	3840	2167	35.0
<b>F1</b> (2.5 L/ha; 2)	8867	4307	2367	34.7
<b>F2</b> (2.5 L/ha; 2)	12200	6157	3733	38.0
<b>F3</b> (2.5 L/ha; 2)	12733	6500	3900	39.7*
DL 5% =	6100	3610	2177	4.6
DL 1 % =	10310	5460	3299	7.0
DL 0.1% =	16570	8780	5303	11.3

F1 = CODAMIX; F2 = ECOAMINOALGA; F3 = ECOAMINOALGA PLUS; \*significant difference; \*\*distinct significant difference \*\*\*very significant difference.

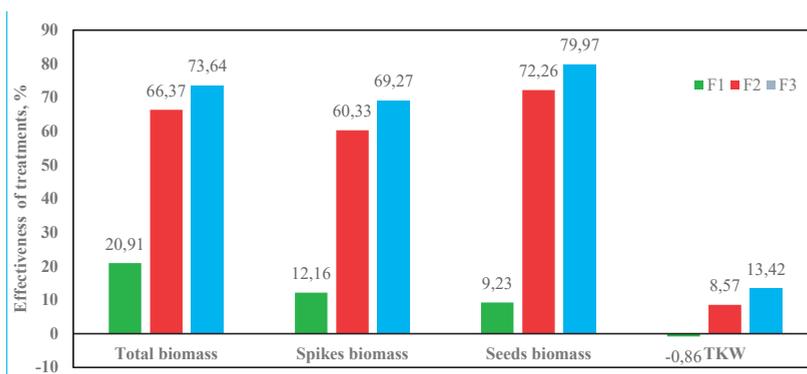


Figure 2. The effectiveness of treatments on yield parameters (%) for winter wheat in comparison with control variant

### 2. The efficiency of foliar fertilization with CODAMIX, ECOAMINOALGA and ECOAMINOALGA PLUS on sunflower yield parameters

Applied treatments on sunflower crop evidenced the same trend for yield parameters, as in the case of winter wheat. Excepting calatidium biomass which remained constant after F1 treatment in comparison with control variant, all the other registered values presented increases (Table 7). It has been proven that treatment with F3 produced the highest increases of yield parameters in comparison

with control, as if follows: 23.37%, 30.12%, 11.40% and 17.64%, respectively (Figure 3).

The efficiency of F1 and F2 treatments in comparison with control were reported previously (Madjar et al., 2021) and in that case it was found better values after F1 in comparison with F2 for calatidium biomass, seeds biomass and TKW. Literature data provide information regarding influence of different foliar treatments on sunflower crop. For example, Akuaku et al. (2020) reported that foliar application of organic inputs produced increase of protein content and seed yield.

Table 7. The efficiency of foliar fertilization on **sunflower** yield parameters

Experimental variant (dose; number of treatments)	Total biomass, kg/ha	Calatidium biomass, kg/ha	Seeds biomass, kg/ha	TKW, g
<b>Control</b>	9267	5533	2780	61.2
<b>F1</b> (2.5 L/ha; 2)	10667	5533	2890	63.0
<b>F2</b> (2.5 L/ha; 2)	10900	7167	3093	65.3
<b>F3</b> (2.5 L/ha; 2)	11433*	7200	3097	72.0**
DL 5% =	1961	2199	431	5.1
DL 1 % =	2513	3359	653	9.8
DL 0.1% =	4069	5215	1049	13.2

F1=CODAMIX; F2=ECOAMINOALGA; F3=ECOAMINOALGA PLUS; \*significant difference; \*\*distinct significant difference \*\*\*very significant difference.

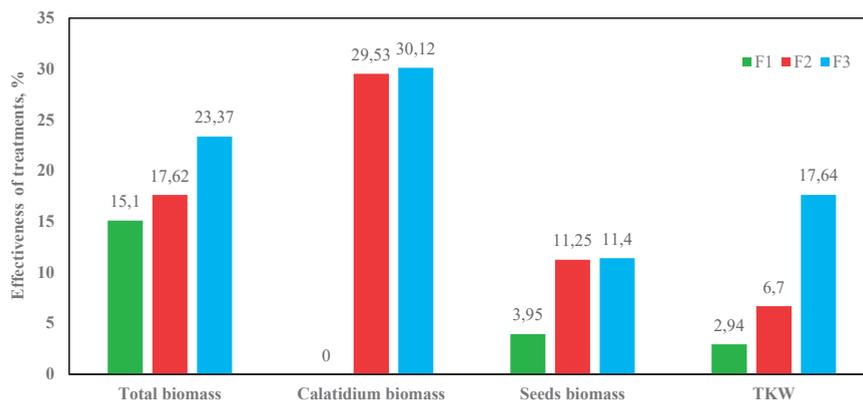


Figure 3. The effectiveness of treatments on yield parameters (%) for **sunflower** in comparison with control variant

Other study (Mihalache & Stanescu, 2017) evidenced that foliar fertilizers based on protein hydrolysates presented a favorable influence on accumulation of biomass and increasing the number of seeds/capitulum.

### 3. The efficiency of foliar fertilization with CODAMIX, ECOAMINOALGA and ECOAMINOALGA PLUS on maize yield parameters

Treatments with organic fertilizers presented positive effects on yield parameters for maize crop in comparison with control variant,

excepting TKW value after F1 treatment (Table 8). The highest values were registered after F3 in comparison with control variant, the increases being of 31.56%, 27.57%, 24.64% and 11.49%, respectively (Figure 4).

Values for total biomass, cobs biomass and seeds biomass in the case of maize hybrid used in the experiment (T.332) were higher than those obtained in previous experimental year for other maize hybrid (F 376) in similar conditions and using the same fertilizers (F1 and F2) (Madjar et al., 2021).

Table 8. The efficiency of foliar fertilization on **maize** yield parameters

Experimental variant (dose; number of treatments)	Total biomass, kg/ha	Cobs biomass, kg/ha	Seeds biomass, kg/ha	TKW, g
<b>Control</b>	18440	8920	7060	287
<b>F1</b> (2.5 L/ha; 2)	21640	10520	8100	282
<b>F2</b> (2.5 L/ha; 2)	22200	10980	8200	290
<b>F3</b> (2.5 L/ha; 2)	24260	11380	8800	320
DL 5% =	6860	3981	3376	95
DL 1 % =	10382	6029	5112	144
DL 0.1% =	16688	9685	8213	232

F1=CODAMIX; F2=ECOAMINOALGA; F3=ECOAMINOALGA PLUS; \*significant difference; \*\*distinct significant difference \*\*\*very significant difference.

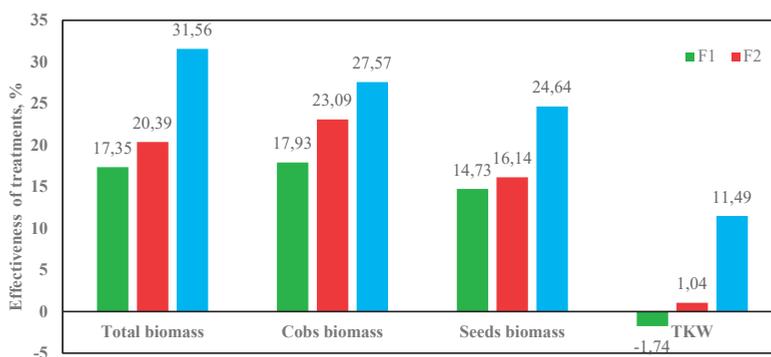


Figure 4. The effectiveness of treatments on yield parameters (%) for **maize** in comparison with control variant

#### 4. The efficiency of foliar fertilization with CODAMIX, ECOAMINOALGA and ECOAMINOALGA PLUS on soybean yield parameters

Even if all yield parameters for soybean crop are higher after treatments with organic fertilizers, in comparison with control variant, the differences between them are small (Table 9).

The superiority of treatment F3 is confirmed by recorded values of yield parameters that increased with 7.84%, 4.49%, 11.75% and 20.4% respectively, in comparison with control variant (Figure 5). The efficiency of organic inputs F1 and F2 on yield parameters for soybean crop has been previously reported (Madjar et al., 2021).

Table 9. The efficiency of foliar fertilization on **soybean** yield parameters

Experimental variant (dose; number of treatments)	Total biomass, kg/ha	Siliques biomass, kg/ha	Seeds biomass, kg/ha	TKW, g
Control	5022	2605	1744	98
F1 (2.5L/ha; 2)	5334**	2688	1918*	110*
F2 (2.5L/ha; 2)	5380***	2695	1933**	111*
F3 (2.5L/ha; 2)	5416***	2722	1949**	118**
DL 5% =	155	120	126	10.2
DL 1 % =	229	178	186	15.1
DL 0.1% =	355	275	289	23.4

F1=CODAMIX; F2=ECOAMINOALGA; F3=ECOAMINOALGA PLUS; \*significant difference; \*\*distinct significant difference \*\*\*very significant difference.

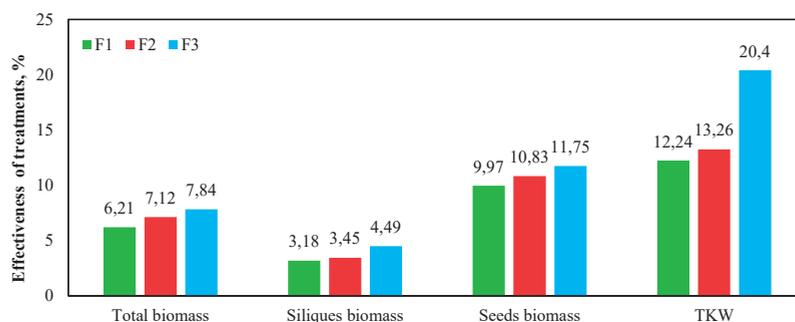


Figure 5. The effectiveness of treatments on yield parameters (%) for **soybean** in comparison with control variant

## CONCLUSIONS

Evaluation of the efficiency of three selected inputs accepted for organic agriculture (CODAMIX, ECOAMINOALGA,

ECOAMINOALGA PLUS) on different field crops (wheat, sunflower, maize, and soybean) evidenced positive effects on yield parameters in comparison with control variant. For all investigated crops it has been evidenced that F3

treatment provided the best results, followed by F2 and F1. This experiment confirmed and extended our previous results regarding the efficiency of organic fertilizers on field crops.

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## EFFECTS OF THE BIOSTIMULANTS APPLICATION AT CASTOR BEAN CROP

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### Abstract

*Castor bean (Ricinus communis L.) is an oil plant of great importance worldwide. It has a very low share in the structure of agricultural crops in Romania, but with a tendency to grow, due to multiple economic advantages.*

*In the context of climate change new approach to cultivation technology is needed. Improving the effects of stressors is the benefit of using biostimulants. Under this circumstances, the aim of this paper is to present the results of the researchers performed in South Romania with respect to the application of biostimulants at castor bean crop.*

*In the years 2019, 2020, and 2021, at Agricultural Research and Development Station Teleorman (ARDS Teleorman) located in South Romania, a multifactorial field experiment of type 2 x 2 x 2 was set up, having the following factors: Factor A - biostimulant product: a1 = product containing auxins (Kelpak); a2 = product containing amino acids (Amer Micro); Factor B - application phase: b1 = application at 4-5 leaves; b2 = application before the appearance of the main raceme; Factor C - application rate: c1 = 1 l/ha; c2 = 2 l/ha.*

*The application of the studied biostimulants determined significant increases in seed yield at castor bean. The obtained results in the three experimental years show that the application of the biostimulants should be done before the appearance of the main raceme and in the rate of 2 l/ha. The effects determined by Amer Micro biostimulant (based on amino acids) was more consistent than those of Kelpak biostimulant (based on auxins).*

**Key words:** castor bean, biostimulants, auxins, amino acids, seed yield.

### INTRODUCTION

Castor bean (*Ricinus communis* L.) is an oil plant of great importance, which provides oil used as raw material in many industries, as well as used for medicinal and cosmetic purposes, as lubricating oil and biofuel (Ion, 2021).

As raw material, the castor bean oil is used in industries such as those of textiles, leather, machine building and aeronautics, paints, varnishes and emulsions etc. (Sturzu et al., 2014).

Castor bean has a tremendous future potential as an industrial oilseed crop because of its high seed oil content (more than 48%), unique fatty acid composition (90% ricinoleic acid content), and ability to be grown under drought and saline conditions (Severino et al., 2012).

Castor bean plants are able to withstand harsh weather conditions, such as drought, and can be cultivated on marginal lands which are not favourable for cultivation of food crops (Hammad et al., 2019).

In Romania, castor bean is a niche crop, whose share in the structure of agricultural crops is very low, but increasing, due to its advantages: cultivation involves relatively low costs and ensures good yields, with a high oil content; after crop harvest, the land remains with no weeds and the soil is aerated and structured; the plants put very well into value the natural fertility of the soil and the residual effect of fertilizers applied to the preceding crop; the crop capitalizes very well on rainfall and groundwater intake.

In the context of climate change and the emergence of new varieties of castor bean, a new approach to cultivation technology is needed to target the latest concepts and agricultural inputs. A cultivation practice that could be used for improving the performance of castor bean crops is the application of biostimulant products (Santos et al., 2014).

Biostimulants are products capable of acting on the metabolic and enzymatic processes of plants, improving crop productivity and yield quality.

It also helps plants cope with abiotic stress, especially in the early stages of plant development (Xu and Geelen, 2018).

Improving the effects of abiotic stress is the most commonly mentioned benefit of using biostimulants, as 60-70% of production losses in agriculture are estimated to be attributed to abiotic strains (Rouphael et al., 2017; Yakhin et al., 2017).

Biostimulants have positive effects on plant growth, stress reduction and disease prevention, so their use contributes to increasing plant production, yield and quality (Xu and Geelen, 2018). Studies have shown that foliar application of algae extract leads to a major root development in a variety of species, including corn (Jeannin et al., 1991), tomatoes (Crouch and Van Staden, 1992), strawberries (Alam et al., 2013), and autumn rape (Jannin et al., 2013). Lateral root growths, total root volume, and root length have been observed and attributed to the presence of phytohormones such as auxins and cytokinins in seaweed (Khan et al., 2011a; 2011b).

The chlorophyll content of the leaves increased after the application of seaweed extract in a number of studies. This increase has been associated with a reduction in chlorophyll degradation and delayed senescence, rather than a net increase in the rate of photosynthesis (Jannin et al., 2013).

An innovative technology with promising application potential is the use of hydrolysed proteins (PHs). PHs are "mixtures of polypeptides, oligopeptides, and amino acids that are made from protein sources using partial hydrolysis" (Schaafsma, 2009). They are available as liquid extracts or in soluble powder and in granular form and can be incorporated laterally near the root or applied as foliar sprays (Colla et al., 2015). PHs are produced mainly by chemical hydrolysis (acid and alkaline hydrolysis), thermal and enzymatic hydrolysis of a wide range of both animal waste and plant biomass (Halpern et al., 2015).

Foliar and root applications have been shown to improve the absorption and efficient use of macro- and micronutrients (Halpern et al., 2015). Improved nutrient uptake performance in PHs-treated plants has been largely associated with changes in root architecture (density, length and number of lateral roots), as

well as an increase in nutrient availability in the soil solution resulting from nutrient complexation by peptides and amino acids. improved microbial activity (Colla et al., 2015; Du Jardin, 2015).

With the trend towards of using less the chemical fertilizers, biostimulants derived from by-products are expected to be used more frequently if the recovery chain is well established, but the mode of action has to be further investigated.

This paper is aimed to present the results of the researchers performed in South Romania with respect to the application of biostimulants at castor bean crop.

## MATERIALS AND METHODS

The researches were carried out at the Agricultural Research and Development Station Teleorman (ARDS Teleorman) located in South Romania (Teleorman county). These were performed under rainfed conditions on a soil of cambic chernozem type, with a loam-clay texture on the depth of the ploughed layer (0-25 cm). From the point of view of physico-chemical properties, the soil is characterized by a content of 45% clay, 3.1% humus, 0.166% total nitrogen content, 40-60 ppm phosphorus mobile, 250 ppm mobile potassium, and by a weakly acid soil reaction (pH = 6.1-6.5).

In the years 2019, 2020 and 2021 a multifactorial field experiment of type 2 x 2 x 2 was set up having the following factors:

- Factor A - biostimulant product, with 2 graduations: a1 = product containing auxins (Kelpak); a2 = product containing amino acids (Amer Micro);
- Factor B - application phase, with 2 graduations: b1 = application at 4-5 leaves; b2 = application before the appearance of the main raceme;
- Factor C - application rate, with 2 graduations: c1 = 1 l/ha; c2 = 2 l/ha.

The field experiments were based on the method of subdivided plots with 3 replications. The surface of the experimental plot was 14 m<sup>2</sup> (L = 5 m, l = 2.8 m).

The technological parameters were the following: the plant density was of 60,000 plants/ha; the distance between rows was 70 cm; the number of plants/plot was 84,

and the number of plants/row was 21; the distance between plants/row was 23.8 (cm); the sown variety was Rivlas, which is a mid-late variety created at ARDS Teleorman, Romania; the sowing was performed in the last decade of April.

The biostimulants used in field experiment were Amer Micro and Kelpak.

Kelpak is a pure concentrated biostimulant of seaweed (*Ecklonia maxima*), a very fast-growing algae due to phytohormones (auxins and cytokinins). It contains the optimal ratio of auxins/cytokinins (11 mg equivalent of biological auxins - 0.03 mg equivalent of cytokinins). The application of the Kelpak biostimulant causes the rapid growth of the root tips, which implicitly leads to an increase in the cytokinin content; the development of the root system makes the plants more resistant to certain stressors (drought, waterlogging, nutrient deficiencies, high salt content in the soil, infestation with nematodes and soil pathogens); the better supply of nutrients, correlated with a high level of cytokines in plants, determines the growth and optimal development of crops, and in the end higher yields and better quality are obtained.

Amer Micro has a high content of amino acids (43.8%), which have the role of stimulating root growth and increase tolerance of crops to extreme temperatures, salinity and even drought. Composition: total nitrogen 7.5%, organic carbon 23%, cobalt 0.001%, iron 0.2%, organic nitrogen 7%, zinc 0.01%, free amino

acids 16%, boron 0.05%, manganese 0.1%, molybdenum 0.005%, total amino acids 43.8%. During the vegetation period, phenological observations and biometric determinations specific to castor bean culture were performed. The seeds were harvested and peeled by hand on each sample.

The calculation and interpretation of the results was made based on the analysis of the variance of the experiments placed in the subdivided plots.

**Climatic data.** In terms of temperature, during the years of experimentation, castor bean plants benefited during the whole vegetation period of temperatures that satisfied their specific thermal needs (Table 1).

In the pre-sowing months, the average monthly temperatures exceeded the multiannual average of the area in all years of experimentation. April 2021, when the sowing was done, was the coldest in the 3 years of experimentation (9.5°C) compared to the multiannual average of the area (11.9°C). During the period of castor vegetation, the average monthly temperatures exceeded the multiannual average of the area by + 5.3°C on average over the three years of experimentation.

In terms of water, in all the three years of experimentation, in the first part of the vegetation period, respectively in May and June, the precipitations were quantitatively higher than the multiannual average, while in July and August there was registered a significant to severe water deficit (Table 1).

Table 1. Evolution of average monthly temperatures and rainfall at ARDS Teleorman in the experimental years

Average monthly temperature							
Month	II	III	IV	V	VI	VII	VIII
Year 2019	3.5	9.3	11.4	17.0	22.7	23.1	25.2
Year 2020	4.9	8.0	11.9	16.6	21.1	24.7	25.4
Year 2021	3.1	4.7	9.5	17.3	21.0	26.3	25.5
<b>Multiannual average</b>	<b>-0.5</b>	<b>4.6</b>	<b>11.9</b>	<b>16.8</b>	<b>20.6</b>	<b>22.6</b>	<b>22.4</b>
Rainfall							
Month	I	III	IV	V	VI	VII	VIII
Year 2019	27.0	26.0	69.8	109.2	171.6	34.8	25.8
Year 2020	68.5	74.0	20.0	79.0	84.0	2.8	0.0
Year 2021	10.5	98.0	36.0	83.0	99.0	1.0	36.0
<b>Multiannual average</b>	<b>30.9</b>	<b>35.0</b>	<b>41.8</b>	<b>61.2</b>	<b>72.6</b>	<b>60.9</b>	<b>46.8</b>

## RESULTS AND DISCUSSIONS

The application of the biostimulants together with pesticides creates the possibility of the simultaneous execution of several technological links in a single passage of aggregates in the field, thus contributing to the rationalization of energy consumption. In the choice of the application phase of the biostimulants these considerations were taken into account. After the emergence of the main raceme, weeds are no longer a major problem for the castor bean crop, because they no longer have favourable growing conditions (water, nutrients, and especially light), the ground being covered by the castor bean plant canopy. Therefore, after this phenological phase, there is no need to control the weed through technological measures. These ideas were taken into consideration in the establishment of the application phase of the biostimulant products. Also, it was taken into account that the application of the biostimulants could cause an abundant vegetative growth to the detriment of yield, but also a prolongation of the vegetation period, which required a careful observation of the growth and development phenophases.

**Plant phenology.** Differences in the date of emergence of the main raceme, flowering date, and maturity date were not significant by applying biostimulants compared to the untreated control variant. The number of days from sowing to maturity was on average on the studied variants of 129 days in 2019, 144 days in 2020 and 140 in 2021. Due to climate change, the average monthly temperatures in recent years exceeded in all months of the vegetation period the multiannual average values. As a consequence, the vegetation period of castor bean plants is no longer restricted by the first autumn frosts, this being the reason that early Romanian varieties were created. So, in the present, this aspect is no longer a problem for the castor bean crop in South Romania.

**Broken plants** is a phenomenon that can cause significant yield losses at castor bean due to the specific architecture of plant. Studies have shown that the use of biostimulants stimulate the development of the root system, which gives the plants a better resistance to breakage.

In order to determine the influence of the biostimulants on the percentage of broken plants, biometric determinations were performed in this respect, in two moments, respectively at the end of the period of formation of the productivity elements and at the physiological maturity of the main raceme. The data are presented as average values of the two determinations (Table 2).

In average over the years of experimentation and for the variants treated with biostimulants, the percentage of broken plants registered the lowest value (3.0%) in 2021, while in 2020 this was higher (3.7%), and in 2019 it was the highest (9.4%), this year being the most humid (Table 2). In 2019, in the first decade of June, when the yielding elements begin to form and the plants are vigorous in their architecture, 114 mm of precipitation fell. The rains were associated with strong storms that broke the castor bean plants, due to the fistulous stem and the fact that the plant's cells were turgid. As the stems were also burdened by racemes, they were prone to breaking. Even after the extreme weather events (storm) of the first decade of June, it was observed that in all experimental variants with application of biostimulants there were fewer broken plants compared to the untreated control.

On average per biostimulant product, the lowest percentage of broken plants had been registered for the variants with Amer Micro biostimulant (based on amino acids). The variants in which the biostimulant product was applied before the appearance of the main raceme had a lower percentage of broken plants compared to the variant of application in the phase of 4-5 leaves. As concerning the application rate, for both biostimulants, the lower percentage of broken plants was registered for the variants with 2 l/ha.

**Plant height**, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 159.4 cm in the more humid year 2019, while this was of 125.8 cm in 2021 and of 113.0 cm in 2020 (Table 2).

The height of the castor bean plant did not show significant differences in the case of application of the two biostimulant products. However, an increase of the plant height is observed for both products in the case of the

application before the appearance of the main raceme and at the application rate of 2 l/ha. On average per biostimulant product, the plant

height registered higher values in the case of application of Kelpak biostimulant (based on auxins).

Table 2. Influence of biostimulants on the percentage of broken plants and the plant height at castor bean

No.	Biostimulant	Application phase	Rate (l/ha)	Percentage of broken plants (%)				Plant height (cm)			
				Year			Average	Year			Average
				2019	2020	2021		2019	2020	2021	
1	<i>Control</i>	<i>Untreated</i>	-	15.7	6.2	6.5	9.5	158	118	126	134.0
2	Kelpak	4- 5 leaves	1	12.5	4.2	3.7	6.8	154	108	129	130.3
3			9.3	3.4	3.1	5.3	159	116	127	134.0	
4		Before appearance of main raceme	1	10.3	5.0	4.2	6.5	165	111	122	132.7
5			2	6.8	4.2	2.9	4.6	168	122	129	139.7
<i>Average for Kelpak</i>				9.7	4.2	3.5	5.8	161.5	114.3	126.8	134.2
6	Amer Micro	4- 5 leaves	1	13.7	5.0	4.1	7.6	147	116	121	128.0
7			2	10.1	2.5	2.1	4.9	155	111	125	130.3
8		Before appearance of main raceme	1	6.2	3.4	3.0	4.2	164	111	126	133.7
9			2	5.9	1.7	1.2	2.9	163	109	127	133.0
<i>Average for Amer Micro</i>				9.0	3.2	2.6	4.9	157.3	111.8	124.8	131.3
<i>Average for variants with biostimulants</i>				9.4	3.7	3.0	5.4	159.4	113.0	125.8	132.7

**Main raceme length**, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 44.1 cm in the more humid year 2019, while this was of 33.3 cm in 2021 and of 32.1 cm in 2020 (Table 3).

The length of the main raceme registered increased average values for both biostimulants compared to untreated variant.

An increase of the length of the main raceme is observed for both biostimulants in the case of the application before the appearance of the main raceme and at the application rate of 2 l/ha. On average per biostimulant product, the main raceme length registered higher values in the case of application of Amer Micro biostimulant (based on amino acids), with 37.7 cm the length of the main raceme, compared to the application of Kelpak biostimulant (based on auxins), with 35.3 cm the length of the main raceme.

**Thousand Grain Weight (TGW)**, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 303.6 g in the more humid year 2019, while this was of 229.0 g in 2021 and of 219.7 g in 2020 (Table 3).

The TGW registered increased average values for both biostimulant products compared to untreated variant.

In the case of Amer Micro biostimulant, the higher average value was obtained when it was applied in the rate of 2 l/ha in the phase of 4-5 leaves and in the rate of 1 l/ha before the appearance of the main raceme. In the case of Kelpak biostimulant it was in the contrary, respectively the higher average value was obtained when it was applied in the rate of 1 l/ha in the phase of 4-5 leaves and in the rate of 2 l/ha before the appearance of the main raceme.

**Number of capsules on the main raceme**, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 78.1 in the more humid year 2019, while this was of 51.0 in 2020 and of 48.4 in 2021 (Table 4).

The number of capsules on the main raceme registered increased average values for both biostimulant products compared to untreated variant.

The highest values of the number of capsules on the main raceme were registered in the case of application of biostimulant products before the appearance of the main raceme and at the application rate of 2 l/ha. Also, the highest values were registered in the case of application of Amer Micro biostimulant (based on amino acids), with 63.1 capsules on the main raceme in average, while the application of the Kelpak

biostimulant (based on auxins) determined an average value of 55.3 capsules on the main raceme.

**Seed weight on the main raceme**, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 81.6 g in the more humid year 2019, while this was of 25.5 g in 2020 and of 24.2 g in 2021 (Table 4).

The seed weight on the main raceme registered increased average values for both biostimulant products compared to untreated variant.

The highest values of the seed weight on the main raceme were registered in the case of application of biostimulant products before the appearance of the main raceme and at the application rate of 2 l/ha. Also, the highest values were registered in the case of application of Amer Micro biostimulant (based on amino acids), with 45.0 g in average, while the application of the Kelpak biostimulant (based on auxins) determined an average value of 42.5 g for the seed weight on the main raceme.

Table 3. Influence of biostimulants on the length of the main raceme and TGW at castor bean

No.	Biostimulant	Application phase	Rate (l/ha)	Main raceme length (cm)				TGW - Thousand Grain weight (g)			
				Year			Average	Year			Average
				2019	2020	2021		2019	2020	2021	
1	<b>Control</b>	<b>Untreated</b>	-	<b>47</b>	<b>26</b>	<b>24</b>	<b>32.3</b>	<b>305</b>	<b>210</b>	<b>215</b>	<b>243.3</b>
2	Kelpak	4-5 leaves	1	41	29	27	32.3	302	218	228	249.3
3			2	45	30	29	34.7	303	211	231	248.3
4		Before appearance of main raceme	1	42	31	32	35.0	300	208	230	246.0
5			2	47	35	36	39.3	308	228	235	257.0
<b>Average for Kelpak</b>				<b>43.8</b>	<b>31.3</b>	<b>31.0</b>	<b>35.3</b>	<b>303.3</b>	<b>216.2</b>	<b>231.0</b>	<b>250.2</b>
6	Amer Micro	4-5 leaves	1	38	32	34	34.7	304	214	220	246.0
7			2	43	33	36	37.3	306	240	229	258.3
8		Before appearance of main raceme	1	49	32	35	38.7	306	228	222	252.0
9			2	48	35	37	40.0	300	210	237	249.0
<b>Average for Amer Micro</b>				<b>44.5</b>	<b>32.9</b>	<b>35.5</b>	<b>37.7</b>	<b>304.0</b>	<b>223.0</b>	<b>227.0</b>	<b>251.3</b>
<b>Average for variants with biostimulants</b>				<b>44.1</b>	<b>32.1</b>	<b>33.3</b>	<b>36.5</b>	<b>303.6</b>	<b>219.7</b>	<b>229.0</b>	<b>250.7</b>

Table 4. Influence of biostimulants on the number of capsules and the seed weight on the main raceme at castor bean

No.	Biostimulant	Application phase	Rate (l/ha)	No of capsule on main raceme				Seed weight on main raceme (g)			
				Year			Average	Year			Average
				2019	2020	2021		2019	2020	2021	
1	<b>Control</b>	<b>Untreated</b>	-	<b>70</b>	<b>40</b>	<b>38</b>	<b>49.3</b>	<b>79.0</b>	<b>20.0</b>	<b>19.7</b>	<b>39.6</b>
2	Kelpak	4-5 leaves	1	70	41	40	50.3	79.2	21.4	20.3	40.3
3			2	77	43	47	55.7	84.2	22.5	21.9	42.9
4		Before appearance of main raceme	1	74	48	45	55.7	74.4	24.4	23.8	40.9
5			2	80	50	48	59.3	84.4	28.0	25.3	45.9
<b>Average for Kelpak</b>				<b>75.3</b>	<b>45.6</b>	<b>45.0</b>	<b>55.3</b>	<b>80.6</b>	<b>24.1</b>	<b>22.8</b>	<b>42.5</b>
6	Amer Micro	4-5 leaves	1	75	50	49	58.0	82.6	22.8	21.7	42.4
7			2	84	60	52	65.3	81.9	25.9	24.9	44.2
8		Before appearance of main raceme	1	75	50	50	58.3	88.1	28.0	27.5	47.9
9			2	90	66	56	70.7	77.8	30.8	28.3	45.6
<b>Average for Amer Micro</b>				<b>81.0</b>	<b>56.4</b>	<b>51.8</b>	<b>63.1</b>	<b>82.6</b>	<b>26.9</b>	<b>25.6</b>	<b>45.0</b>
<b>Average for variants with biostimulants</b>				<b>78.1</b>	<b>51.0</b>	<b>48.4</b>	<b>59.2</b>	<b>81.6</b>	<b>25.5</b>	<b>24.2</b>	<b>43.8</b>

**Seed yield** of any cultivated plant is the result of the interaction of all factors that participate in one way or another in the formation of the harvest. The yield level is in relation to the degree to which each factor and all together come close to the optimal values required by plant biology. This global condition is rare in the natural environment, but it can be improved by combining different agronomic practices: soil preparation, sowing period, plant density, crop rotation, choice of variety or hybrid, fertilization, weed, disease and pest control, equipment used, and the ability to manage vegetation factors so that the “site supply” is as close as possible to the biology of the cultivated plants (Pop et al., 2013).

Seed yield, in average over the years of experimentation and for the variants treated with biostimulants, registered the highest value of 2633.5 kg/ha in the more humid year 2019, while this was of 1422.5 kg/ha in 2020 and of 1307.9 kg/ha in 2021 (Table 5). Compared to the seed yield obtained in 2019, on average for

the study years, there is a difference of -1211 kg/ha in 2020 and of -1325.6 kg/ha in 2021. This difference is due to the climatic conditions of 2020 and 2021, when the drought was installed during the formation of the yielding elements.

The seed yield registered increased values for both biostimulant products compared to untreated variant in all experimental years and in all application phases and rates.

The highest values of the seed yield were registered in the case of application of biostimulant products before the appearance of the main raceme and at the application rate of 2 l/ha. Also, the highest values of the seed yield were registered in the case of application of Amer Micro biostimulant (based on amino acids), with 1855.3 kg/ha in average, while the application of the Kelpak biostimulant (based on auxins) determined an average value of 1720.6 kg/ha. Amer Micro biostimulant determined an average seed yield increase of 134.7 kg/ha compared to Kelpak biostimulant.

Table 5. Seed yields obtained by application of biostimulants on castor bean

No	Biostimulant	Application phase	Rate (l/ha)	Seed yield (kg/ha)			
				Year			Average
				2019	2020	2021	
1	<i>Control</i>	<i>Untreated</i>	-	<b>2289</b>	<b>1187</b>	<b>935</b>	<b>1470.3</b>
2	Kelpak	4-5 leaves	1	2341	1281	1120	1580.7
3			2	2511	1319	1210	1680.0
4		Before appearance of main raceme	1	2594	1339	1278	1737.0
5			2	2833	1441	1380	1884.7
<i>Average for Kelpak</i>				<b>2569.8</b>	<b>1345.0</b>	<b>1247.0</b>	<b>1720.6</b>
6	Amer Micro	4-5 leaves	1	2409	1367	1271	1682.3
7			2	2838	1461	1387	1895.3
8		Before appearance of main raceme	1	2465	1501	1345	1770.3
9			2	3077	1670	1472	2073.0
<i>Average for Amer Micro</i>				<b>2697.3</b>	<b>1499.9</b>	<b>1368.8</b>	<b>1855.3</b>
<i>Average for variants with biostimulants</i>				<b>2633.5</b>	<b>1422.5</b>	<b>1307.9</b>	<b>1787.9</b>

In order to determine the influence of each factor, but also their interaction on the seed yield of castor bean, the analysis of variance for the multifactorial experiment with 2 biostimulant products x 2 application phases x 2 application rates was performed for each year of study (Table 6). Analysing the variance table for the seed yield of the multifactorial experience of type 2 x 2 x 2, one observes the very significant influence of factors A

(biostimulant product), B (application phase) and C (application rates) in the years of experimentation 2019 and 2020, and the distinctly significant influence of these factors in 2021. In 2019, the interactions between experimental factors were very significant, and the triple interaction A x B x C significant, but in 2020 and 2021, due to less favourable climatic conditions for castor bean crop interactions between factors were insignificant.

Table 6. Analysis of variance (ANOVA) for seed yield on the multifactorial experiment with biostimulants (2 biostimulant products × 2 application phases × 2 application rates)

Source of Variation	Sum of Squares (SS)			DF*	Mean Squares (MS)			F-test		
	2019	2020	2021		2019	2020	2021	2019	2020	2021
Biostimulant	97282.7	169680.2	89182.0	1	97282.7	169680.2	89182.0	106.6***	77.7***	30.6**
Application phase	282968.2	466488.2	89182.0	1	282968.2	466488.2	89182.0	545.0***	204.9***	11.2**
Biostimulant x Application phase	29540.2	3408.2	10710.4	1	29540.2	3408.2	10710.4	56.9***	1.5 <sup>NS</sup>	1.3 <sup>NS</sup>
Application rate	462592.7	153920.2	71177.0	1	462592.7	153920.2	71177.0	218.6***	44.3***	15.9**
Biostimulant x Application rate	353322.7	1504.2	975.4	1	353322.7	1504.2	975.4	167.0***	0.4 <sup>NS</sup>	0.2 <sup>NS</sup>
Application phase x Application rate	131128.2	6080.2	198.4	1	131128.2	6080.2	198.4	62.0***	1.8 <sup>NS</sup>	0.04 <sup>NS</sup>
Biostimulant x Application phase x Application rate	19153.5	11008.2	0.04	1	19153.5	11008.2	0.0	9.1*	3.2 <sup>NS</sup>	0 <sup>NS</sup>

\*DF - Degrees of Freedom

Comparing the average seed yields per biostimulant product, one notices that in 2019 the Amer Micro biostimulant determined a distinctly significant increase in seed yield of 127.5 kg/ha, compared to the seed yield obtained in the case of Kelpak biostimulant (Table 7). Significant increase of the seed yield of 154.9 kg/ha and of 121.8 kg/ha was also obtained in 2020 and respectively in 2021 when applying the Amer Micro biostimulant, compared to the seed yield obtained in the case of Kelpak biostimulant.

Comparing the average seed yields per application phase of the two biostimulants, one notices that in 2019 and 2020 the application before the appearance of the main raceme determined very significant increase in seed yield of 217.5 kg/ha and respectively of 130.75 kg/ha, compared to the seed yield obtained in

the case of application in the phase of 4-5 leaves. But in 2021, despite the fact that the seed yield registered an increase by applying the biostimulant products before the appearance of the main raceme compared to the application in the phase of 4-5 leaves, the yield increase of 121.75 kg/ha was not significant.

Comparing the average seed yields per application rate of the two biostimulant products, one notices that in 2019 application of 2 l/ha determined a very significant increase in seed yield of 362.5 kg/ha, compared to the seed yield obtained in the case of application of 1 l/ha. Distinct significant increase of the seed yield of 100.8 kg/ha and of 108.8 kg/ha was also obtained in 2020 and respectively in 2021 in the case of application of 2 l/ha compared to the application of 1 l/ha.

Table 7. Limit differences for the influence of experimental factors on castor bean seed yield

LSD (kg/ha)	For biostimulant			For application phase			For application rate		
	2019	2020	2021	2019	2020	2021	2019	2020	2021
5%	52.9	83.5	94.7	25.9	54.2	202.3	43.4	55.6	63.0
1%	122.2	170.8	218.7	42.8	89.6	334.8	63.2	80.9	91.6
0.1%	389.0	470.7	695.8	80.2	167.7	626.6	94.8	121.3	137.4

In 2019, an increase of 287.5 kg/ha was obtained when the Kelpak biostimulant was applied before the appearance of the main raceme, and an increase of 147.5 kg/ha when the Amer Micro biostimulant was applied before the appearance of the main raceme, compared to application in the phase of 4-5 leaves. Also compared to application in the

phase of 4-5 leaves, in 2020, the Kelpak biostimulant applied before the appearance of the main raceme determined an increase of 90 kg/ha, and the Amer Micro biostimulant an increase of 171.5 kg/ha, while in 2021 the application of the Kelpak biostimulant applied before the appearance of the main raceme determined an increase of 164 kg/ha, and the

Amer Micro biostimulant an increase of 79.5 kg/ha.

Analysing the seed yields obtained when applying the same biostimulant product but in different application rates, it is observed that in 2019, the increase of the Amer Micro biostimulant application rate from 1 l/ha to 2 l/ha brings a very significant yield increase of 520.5 kg/ha, while in the year 2020 and 2021 the increase was distinctly significant, with 131.5 kg/ha and respectively 121.5 kg/ha. Increasing the application rate of Kelpak biostimulant from 1 l/ha to 2 l/ha brings a significant increase in seed yield only in 2019, with 204.5 kg/ha.

Analysing the seed yields obtained at the same application phase of Kelpak biostimulant, but at different application rates, it was found that at the application phase of 4-5 leaves, increasing the application rate from 1 l/ha to 2 l/ha brings a significant increase in seed yield only in 2019

(170 kg/ha). At the application stage before the appearance of the main raceme, increasing the application rate from 1 l/ha to 2 l/ha brings a very significant increase in seed yield in 2019 (239 kg/ha) and significantly increase in 2020 and 2021 (102 kg/ha in both years of experimentation).

Analysing the seed yields obtained at the same application phase of the Amer Micro biostimulant, but at different application rates, it was found that at the application phase of 4-5 leaves, increasing the application rate from 1 l/ha to 2 l/ha brings a significant increase in seed yield in 2019 (429 kg/ha) and 2021 (116 kg/ha). At the application stage before the appearance of the main raceme, increasing the application rate from 1 l/ha to 2 l/ha brings a significant increase in seed yield in all years of experimentation (612 kg/ha in 2019, 169 kg/ha in 2020, and 127 kg/ha in 2021).

Table 8. Limit differences for all combinations of experimental factors on castor bean seed yield

LSD (kg/ha)	Interactions											
	a1b2-a1b1			a2b1-a1b1			a1c2-a1c1			b1c2-b1c1		
	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021
5%	36.6	76.6	143.1	57.9	83.5	126.2	61.4	78.6	89.1	61.4	78.6	89.1
1%	60.6	126.7	236.7	153.3	170.8	238.6	89.3	114.4	129.5	89.3	114.4	129.5
0.1%	113.4	237.1	443.1	359.2	470.7	584.7	134.0	171.6	194.3	134.0	171.6	194.3
LSD (kg/ha)	Interactions											
	b2c2-b1c2			a2c2-a1c2			a1b1c2-a1b1c1			a1b2c1-a1b1c1		
	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021
5%	59.1	92.9	105.7	108.2	132.9	151.2	32.6	32.6	72.5	83.6	83.6	125.1
1%	65.8	183.7	209.0	120.0	147.3	167.7	47.5	47.5	105.5	93.0	93.0	139.2
0.1%	103.6	482.3	548.9	293.1	359.9	409.6	71.2	71.2	158.2	146.6	146.6	219.4

## CONCLUSIONS

The application of the studied biostimulants determined significant increases in seed yield at castor bean.

The studied biostimulants did not extend the growing period of the castor bean plants.

The application of the studied biostimulants before the appearance of the main raceme and in the rate of 2 l/ha reduces the percentage of broken plants. The Amer Micro biostimulant (based on amino acids) determined the lowest percentage of broken plants and a less effect on the plant height.

Also, the application of the studied biostimulants before the appearance of the main raceme and in the rate of 2 l/ha determined an increase of the length of the main raceme, of the number of capsules on the main raceme, of the seed weight on the main raceme, and finally of the seed yield.

The obtained results in the three experimental years show that the application of the studied biostimulants should be done before the appearance of the main raceme and in the rate of 2 l/ha.

The effects determined by Amer Micro biostimulant (based on amino acids) was more

consistent than those of Kelpak biostimulant (based on auxins).

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## RESEARCH REGARDING THE ACHIEVEMENT OF THE QUALITATIVE PARAMETERS OF WORK ON SEEDLING PLANTING MACHINES

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### Abstract

*This paper presents some research on the achievement of qualitative parameters of work by seedling planting machines, especially the position of the seedling from the vertical, the degree of plant damage and planting mistakes, defining parameters for evaluating the planting work. The mechanized planting work is done, in overwhelming proportion, with semi-automatic machines or with automatic machines to a lesser extent. Depending on the constructive and functional solutions and the planting conditions (type and condition of the soil, working speed, and skill of the operator) different degrees of fulfillment of the qualitative working parameters mentioned above are obtained. It turned out that the solution with a planting machine with rotary bucket distributors meets a higher percentage of qualitative working parameters, especially the vertical position at planting and the degree of damage. These types are also found in two constructive variants, namely: with articulated bucket dispenser, which places the seedling in a gutter, opened by a coulter, and with non-articulated bucket dispenser, which places the seedling directly in the soil.*

**Key words:** *planted seedlings, quality parameters, efficiency.*

### INTRODUCTION

The experience gained in agriculture and other economic sectors with an advanced degree of mechanization, shows that mechanization is a form of activity that ensures the reduction of labor, increases the quality of work, increases productivity, all leading to lower production costs. Seedling planting machines are designed for mechanizing the work of installing crops through seedlings in well-prepared soil, loose and free of weeds.

When mechanized planting, the intervals between rows as well as the distances between plants per row, must comply with the cultivation scheme prescribed by the working technology. The rows planted should be straight and parallel to allow mechanization of subsequent work.

The planting machine must fix the plant in an upright position as recommended in the technical prescriptions, be covered with soil, without being damaged by planting equipment. In general, a planting equipment should perform the following three functions: open the gutter in the soil; place the seedling upright in the gutter or directly in the soil; close the gutter and compact the soil around the seedling without damaging it. Mechanized planting of

vegetable seedlings is widespread worldwide with a wide variety of seedling planting machines and the offer of machines in the field of vegetable planting is particularly rich (Vlahidis.V., 2018; Yonetani, 1999).

Also, the literature is very rich in articles on the topic addressed in this thesis (Han, 2018; Mitrache, 2019; Zhipeng, 2020) and other sites in the bibliography, these being just some of the multiple references.

The planting operation is one of the most important works in seedling cultivation technology, the correctness of this operation influencing the subsequent maintenance and harvesting of the crop, all having a final impact on the size of production per unit area (Nabu, 1992; Vlahidis, 2018).

Depending on how the seedling is placed in the planting machine and put it into the gutter, seedling planting machines can be classified into:

- machines without a planting apparatus, in which the seedling is placed directly in the gutter by the operator;
- machines with the manual taking over and introduction of the seedling in the planting apparatus followed by the mechanical introduction of the seedling in the gutter, these being generically called, semi-automatic machines;

- machines pick-up, automatic introduction of the seedling into the planting apparatus and mechanical introduction of the seedling into the gutter, these being generically called automatic machines.

The working performance of vegetable seedling machines depends on the frequency of alimentation the seed taking over system (for automatic machines), the frequency of the seedling unit, the distance between seedlings per row, the distance between rows and the optimum operating speed (to minimize damage and planting mistakes), work capacity, type of crop and other operating parameters.

Most researchers and manufacturers reported data on planting frequency and working speed and less on machine performance. Central Institute of Agricultural Engineering - CIAE, India, 2004 reported a working speed of 0.9 km h<sup>-1</sup> and a working capacity of 0.1 ha h<sup>-1</sup>, for planting tomatoes, at a distance between rows of 60 cm and 45 cm between plants per row, using a semi-automatic planting machine pulled by the tractor, in two rows, with planting system with pocket type organs.

The field performance of a two-row semi-automatic planting machine with pocket-type planting system, reported by the University of Agriculture in Punjab - PAU, India in 2004, is shown in Table 1 (Prasanna, 2014). The adequate operating speed to obtain a minimum of planting errors proved to be from 0.9 to 1.1 km h<sup>-1</sup> for various crops. With the increase of the working speed, the percentage of mistakes also increases, thus being necessary two workers who servind one row, in order to keep

the percentage of mistakes within acceptable limits. The planting machine, models 1500, FWD from Holland Transplater Co. 1500, and 1600, and Mechanical Transplanter models 1000, 1000B-3, 1000 2, 1980, 2000 and 22C, provided two operators for a single row (Prasanna, 2014).

Marr (Marr, 1994) stated that the planting machine must work at a speed that allows the operator to place the seedling correctly in the planting organ and also to observe the operation of the machine in general.

That is why a semi-automatic planting machine with a rotary bucket distributor, (in which the seedling is placed by simple hand release), allows a higher working speed than one equipped with a pocket-type planting organ at which the placement of the seedling is more difficult.

Tamil Nadu Agricultural University-TNAU, Coimbatore India, 2004, reported a working capacity of 0.14 ha h<sup>-1</sup> for planting tomatoes, cauliflower, hot peppers and eggplants, using a semi-automatic planting machine with rotary bucket distributor, on three rows, at an average working speed of 1.4 km h<sup>-1</sup> (Abhijit, 2018)

## MATERIALS AND METHODS

The Bureau of Indian Standard (BIS) has not yet formulated test codes for performance testing of vegetable planters. However, based on the available literature, the limiting values for performance of vegetable planters can be classified as very good, good, satisfactory and inadequate, Table 2 (Abhijit, 2018).

Table 1. Field performance of two-row semi-automatic transplanted developed by PAU (2004)

Parameter	Vegetable			
	Tomato	Chile Pepper	Cabbage	Cauliflower
Row spacing, cm	67	67	67	67
Plant spacing, cm	25-30	50-54	25-30	25-30
Working speed, km h <sup>-1</sup>	0.90	1.10	0.95	0.90
Working capacity, ha h <sup>-1</sup>	0.082	0.090	0.092	0.084
Plant mortality, %	5.0-7.0	5.0-7.0	1.0	7.0
Percentage missed plantings, %	3.0-8.0			
Labor requirement per row	One person			

Table 2. Performance criteria for mechanical vegetable planters

Classification	Field efficiency, (%)	Planting efficiency (%)	Miss planting, (%)	Multiple planting (%)
Very good	>75	>90	<5	<5
Good	65-75	80-90	5-10	5-10
Satisfactory	55-65	70-80	10-15	10-15
Inadequate	<55	<70	>15	>15

In Romania, several agro-technical and technological recommendations have been identified that must be met and implemented by the technical equipment for planting seedlings, as follows:

- rows planted to be straight, deviations from permitted row axis are max. 5% within  $\pm 20$  mm;
- the row spacing is adjustable, preferably continuous, starting at min. 300 mm, to allow maintenance and harvesting operations to be mechanized;
- the distance between the plants in turn can be adjusted, either continuously or in steps of 50 mm, within 100-1200 mm;
- the planting depth can be achieved within 30-150 mm;
- the position of the planting seedlings is as close as possible to the vertical;
- the percentage of seedlings improperly planted (inclined more than  $30^\circ$  to the vertical, ground covered, left on the ground or damaged) is less than 5% (Popescu, 2006).

The field experiments, for determining the qualitative working parameters, were carried out with a semi-automatic seedling plant in a row, symbolized MPA (INMA, 2018) in the

unit with the 45 hp tractor, New Holland TCE50 and were carried out in the experimental polygon of INMA Bucharest.

The MPA machine, Figure 1, consists of the following main assemblies: frame (1), planting device (2), type with vertical rotary distributor and buckets, transmission (3), rear support wheels (4), compaction wheels (5), rack or crate support (6), trace marker (7).

The experiments were performed in two functional variants, as follows:

- with the machine that places the seedling in a gutter made by a coulter, followed by its covering with earth, by two fins and the additional fixing it of two compaction wheels, named Variant I;
- with a machine that places the seedling directly in the soil, followed by an additional fixing it of two compaction wheels, named Variant II.

Constructively, the Variant II missing the coulter, a component part of the planting device.

The experiments were performed with the planting device equipped with three and four buckets, thus achieving two distances between plants in a row, of 620 mm and 460 mm.



Figure 1. Machine for planting vegetable seedlings and medicinal plants in a row, MPA symbol (INMA, 2018)

## RESULTS AND DISCUSSIONS

The vertical position of the plant after planting, for the two distances between plants tested in turn, 620 mm and 460 mm, for the two tested variants, is presented in Tables 3- 4, in which:

$v_1$  - working speed, km/h;

$\alpha_i$  - position of the plant in relation to the vertical, degrees;

$\alpha_m$  - average position of the plant relative to the vertical, degrees.

Aspects during the experiments are shown in Figure 2.

Table 3. Vertical position of the plant after planting, Variant I

Nr. crt.	The distance between plants on row, 620 mm - 3 buckets			The distance between plants on row, 460 mm - 4 buckets		
	$v_1$ km/h	$\alpha_i$ degrees	$\alpha_m$ degrees	$v_1$ km/h	$\alpha_i$ degrees	$\alpha_m$ degrees
1	1.1	1.2	1.4	1.2	1.4	1.48
		1.8			1.8	
		1.3			1.7	
		1.5			1.3	
		1.2			1.2	
2	1.54	1.5	1.88	1.59	1.9	2.48
		1.8			2.8	
		2.1			2.6	
		2.3			3.3	
		1.7			2.7	
3	2.25	2.2	2.2	2.35	3.2	3.16
		2.4			3.4	
		1.9			2.9	
		2.3			3.0	
		2.2			3.3	
4	2.95	3.2	2.96	2.86	3.7	3.34
		2.6			3.6	
		2.8			2.8	
		3.0			3.3	
		3.2			3.3	

Table 4. Vertical position of the plant after planting, Variant II

Nr. crt.	The distance between plants on row, 620 mm - 3 buckets			The distance between plants on row, 460 mm - 4 buckets		
	$v_1$ km/h	$\alpha_i$ grade	$\alpha_m$ grade	$v_1$ km/h	$\alpha_i$ grade	$\alpha_m$ grade
1	1.18	3.5	4.92	1.11	4.5	4.90
		4.6			4.3	
		4.8			4.4	
		5.4			5.3	
		6.3			6.0	
2	1.55	5.5	5.8	1.52	4.5	5.76
		6.3			5.8	
		4.8			5.8	
		6.3			6.4	
		6.1			6.3	
3	2.15	12	15.4	2.25	21	22.8
		14			23	
		14			23	
		17			22	
		20			25	
4	2.88	25	30.5	2.86	27	31.8
		32			28	
		28			32	
		25			35	
		25			32	



Figure 2. MPA: a- seedling planting machine during experiments; b- planted seedling

The comparative diagram of the variation of the plant position with the speed of work is presented in Figure 3.

The assessment of planting faults (covered with soil, left on the soil) and of damaged plants is made as a percentage, and its value must be less than 5%, according to the requirements (Popescu, 2006).

The results determined in experiments in order to assess the faults and the degree of damage at planting, for the two tested variants, are presented in Tables 5-6, in which:  $g_i$  - mistake or identified damaged plant, %.

The comparative diagram of the variation of faults and damaged plants with working speed is presented in Figure 4.

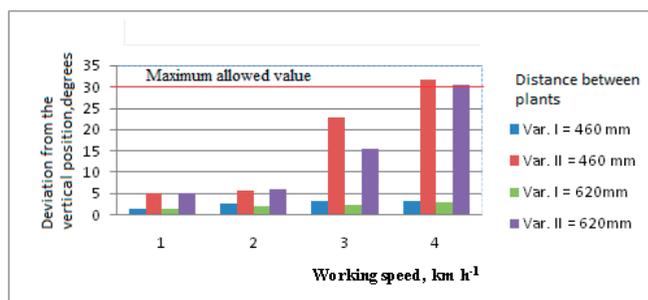


Figure 3. Comparative variation of plant position with working speed

Table 5. Mistakes and damaged plants, Variant I

Number of seedlings planted	The distance between plants on row, 620 mm - 3 buckets			The distance between plants on row, 460 mm - 4 buckets		
	$v_1$ km/h	Mistakes and damaged plants, $g_i$		$v_1$ km/h	Mistakes and damaged plants, $g_i$	
		buc.	%		buc.	%
30	1.1	0	0	1.2	0	0
30	1.54	0	0	1.59	0	0
30	2.25	1	3.33	2.35	1	3.33
30	2.95	2	6.67	2.86	3	10

Table 6. Mistakes and damaged plants, Variant II

Number of seedlings planted	The distance between plants on row, 620 mm - 3 buckets			The distance between plants on row, 460 mm - 4 buckets		
	$v_1$ km/h	Mistakes and damaged plants, $g_i$		$v_1$ km/h	Mistakes and damaged plants, $g_i$	
		buc.	%		buc.	%
30	1.18	0	0	1.11	0	0
30	1.55	1	3.33	1.52	1	3.33
30	2.15	1	3.33	2.25	3	10
30	2.88	3	10	2.86	4	13.33

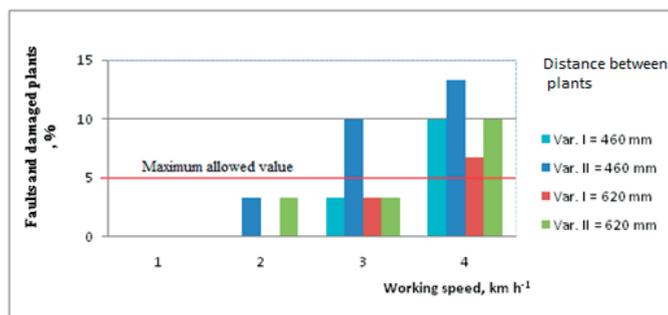


Figure 4. Comparative variation of faults and damaged plants with working speed

## CONCLUSIONS

1. The planting position of the seedling is a qualitative index of work for the planting work, respectively the deviation from the vertical position of the plant, which according to the agro-technical requirements, must not exceed 30°.
2. From the performed determinations it resulted that the inclination angle is less than 30° for working speeds lower than 2.15-2.25 km/h, according to Tables 3-4, there was a tendency to increase the inclination of the plant to the vertical with increasing working speed, Figure 3. However, the angle of inclination is much higher for the planting Variant II Tab. 4, at speeds of over 2.25 km/h, approaching the maximum limit accepted by planting technologies.
3. It is therefore recommended that when using the Variant II, the working speed does not exceed 3 km/h, regardless of the distance between plants per row or the number of buckets used.
4. Regarding the mistakes and damage of the plants, registered in the planting process, it can be stated that they tend to increase with the increase of the working speed in the gutter.
5. From the analysis of Table 5 it is found that up to working speeds of approx. 1.6 km/h in Variant I, no mistakes or injuries were registered and at a speed of approx. 2.35 km/h, they have determined values of 3.33%, lower than the accepted ones of 5%, while at speeds of approx. 3 km/h the values of faults and injuries exceed the acceptable limit, the determined values being between 6.67-10%.
6. In the case of planting Variant II, the percentage of determined faults and injuries is

higher than in Variant I, the percentage being between 10-13.33% for working speeds of approx. 3 km/h, which means more than double the accepted values.

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## INFLUENCE OF SEED AGE ON QUALITY, GERMINATION AND SEED HEALTH IN SOYBEANS

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### Abstract

*Due to its multiple uses: in human and animal nutrition but being also successfully used in rotations with the main cereal crops, soybean is one of the most valuable crops in the world. Seed germination can be influenced by a number of factors: genetic, technological, age of storage but also by the presence of pathogens inside or outside the seeds. Seeds infected with pathogens may have low storage capacity, low germination and weight, and low quality of oil and food. There are a number of pathogens that attack soybean seeds and can greatly affect the quality of the seeds. The most common pathogens are: Phomopsis sp., Cercospora kikuchii, Cercospora sojina, Colletotrichum sp., Pernospora manshurica but also a number of pathogens and secondary saprophytes, including Alternaria, Fusarium, Cladosporium and Penicillium (Telenko, 2018). The paper aimed to present the effects of seed age on soybean germination, fungal load and chemical composition. Seeds of Onix soybean variety, from 4 years (2017-2020) were stored in laboratory conditions and used in the experiment. Germination, chemical composition and also sanitary quality were significantly influenced by storage.*

**Key words:** fertilizers, yield, soybean, seed composition.

### INTRODUCTION

Soybeans are desired on the marketplace as a valuable source of protein used as feed, with food applications and oil which is broadly incorporated into food, feed, and some industrial applications (Clemente and Cahoon, 2009).

The importance of soybean crop is also related to the property of this plant to fix nitrogen in the atmosphere and to use it with high efficiency in the assimilation process, without requiring material efforts from growers. Biological fixation of atmospheric nitrogen has the great advantage that it is a natural and non-polluting process. The symbiosis between soybean plants and *Rhizobium japonicum* bacteria provides over 50% of the nitrogen needed for plant growth and development, after harvesting in the soil remaining between 80-140 kg N/ha (Haş, 2006). Legumes, for both grain and fiber, play an important role in human nutrition and animal feed due to their high protein intake.

Soybean is considered the "golden plant" of mankind and provides over 60% of the world's protein needs and due to its high oil content

(over 20%) is considered an oleo-protein plant, providing over 25% of world oil needs. Soybeans are in the first place among oil plants and is ranked 4th in the world in the cultivated area.

Globally, many developing countries are struggling with an acute shortage of protein, the expansion of soybean crops being a future solution to eradicate malnutrition due to this deficit. Soybean seed germination and plant sensitivity in the early stages of vegetation are the main problems of this crop. To meet the requirements of quality for sowing, soybean seed must have a physical purity of over 98% and germination over 80%.

Minimum temperature for seed germination is 7°C, with an optimal temperature between 12-14°C. According to Arif et al. (2013) soybean seed must absorb 50 percent of its weight in moisture to germinate.

Seed germination is a highly complex physiological and biochemical phenomenon in which a number of biological factors acting on the embryo are involved.

Seed age and storage conditions are important factors affecting soybean germination, chemical

composition and also seeds sanitary quality with negative consequences on grain yield (Kandil et al., 2013; Milošević et al., 2004). Several diseases, including *Phomopsis* spp., *Cercospora kikuchii*, *Cercospora sojina*, *Colletotrichum* spp., *Pernospora manshurica*, *Alternaria*, *Fusarium*, *Cladosporium* and *Penicillium* are known to affect soybean crop.

The deterioration of soybeans during storage causes a decrease in the supply of high-quality seeds, being the main limiting factor of the production (Susilawati et al., 2019).

## MATERIALS AND METHODS

Onix, an early soybean variety was used in the laboratory experiment that was conducted at the Research and Development Station for Agriculture Turda (RDSA Turda) and at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (UASVM Cluj-Napoca). Germination and seed composition were measured both for fresh seeds and at 1-year-storage interval to 3 years. Soybean seeds were harvested in 4 different years: 2017, 2018, 2019 and 2020 and stored under ambient conditions until 2020. For standard germination test 100 seeds with 4 replications for 8 days were evaluated by using classical method between paper. Germination was performed by counting the germinated seeds and appreciating the germs.

The germinated seeds were counted twice. The first count was made to determine the germination energy, and the second at the end of the germination period, to determine the final germination. The counting was done at each separate repetition, starting with the first, by removing the normally germinated seeds with tweezers. Using the formula purposed by Krishnasamy and Seshu (1990) germination percentage was calculated (Germination (%) = number of seeds germinated/number of seeds tested x 100).

Soybean unground samples were scanned using NIR spectroscopy (Tango, Bruker Optik GmbH, Ettingen, Germany) to quickly determine soybean seed composition: dry matter, protein content, oil content, stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3).

To determine the health of the seeds, the presence of pathogens on the surface of the grains or under the tegument was also analysed. During the research, the seeds were monitored daily in order to observe and identify certain pathogens that may develop simultaneously with their germination. The identification of pathogens was performed by macroscopic examination of the symptoms on the seeds (exudate, mycelium, spores) and microscopically by obtaining preparations from the affected parts in order to be included in the genera from which they come.

### Data analysis

The experimental data was prepared and processed in Polifact for ANOVA test and the mean comparison was done by Excel (Microsoft, USA).

## RESULTS AND DISCUSSIONS

The results from this study reveals that germination is very significant influenced by seed age (Table 1). Fresh seeds had the highest germination (93.5%) with small variation in the first and in the second year of storage. Onix soybean variety germination capacity decreased by 5.5%, 6% and 41.75% when seeds were stored for 1 year, 2 years and 3 years, respectively (Table 2).

Many researchers have also reported that germination, chemical composition and also sanitary quality are significantly influenced by storage (Carvalho et al., 2014; Conceição et al., 2016).

Table 1. ANOVA test for seed germination

Cause of variability	SP	DF	s <sup>2</sup>	F
S (Storage)	4401.68	3	1467.2	151.673***
R (Replication)	3.6875	3	1.2291	
SxR	87.0625	9	9.6736	
Error S	87.0625	9	9.6736	
Total	4492.43	15		

Table 2. Effect of storage on seed germination

Storage period		Germination (%)	Difference (%)	Statistical signification
Fresh seeds	93.8	0		Mt.
1 year	88.0	-5.5		0
2 years	87.5	-6		0
3 years	51.75	-41.75		000

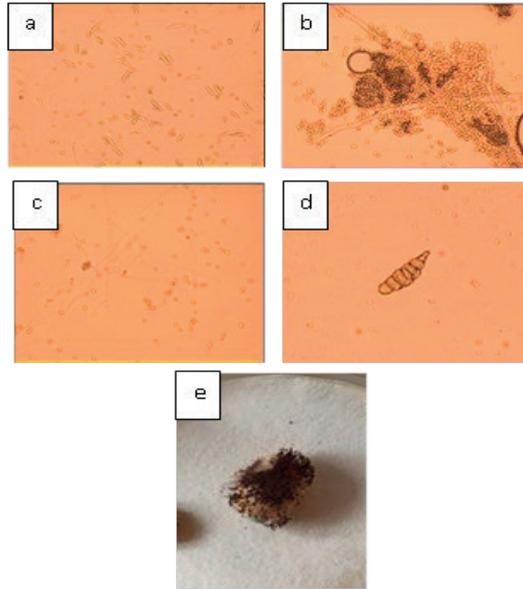


Figure 1. Microorganisms associated to the seeds with 3 years of storage (a. *Fusarium* sp.; b. *Aspergillus*; c. *Penicillium* sp.; d. *Alternaria*; e. *Rhizopus*)

In our research, seed age affected the abundance of *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria* and *Rhizopus* species, all being identified when seeds were stored 3 years in the laboratory conditions (Figure 1).

If there was a small infection percentage associated to the seeds with 1 or 2 years of storage, after 3 years seed age caused the highest affection leading to a significant decrease in germination. According to Lezcano (2015), also

for other legumes, the infection of the seeds increased with the passing of the storage time.

Experimental data presented in Figure 2 highlights that seed size (TKW) was not affected by storage time. It varied between 128 g for the seeds harvested in 2018 and 183 g, this value being obtained for the 2019 harvest.

The germination capacity of soybeans is influenced not only by storage but also by sanitary quality of the seeds.

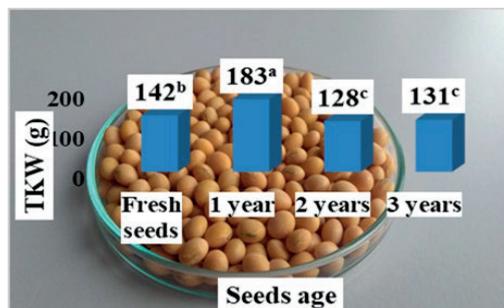


Figure 2. The influence of seed age on TKW

According to Duncan's test with respect to chemical composition of the seeds, after 3 years of storage maximum values for dry matter [%], fat content [%], stearic, oleic and linoleic acid content [%] were obtained. While no significant changes occurred for the fat content, protein content varied from 36.7% to 38.67% but it wasn't influenced by the seed age. Many researchers have reported that storage affected soybean seed composition (Table 3).

Table 4 reveals the estimates of the correlation coefficients evaluated for the studied parameters. Statistically, a positive close correlation between germination and moisture ( $r$

$= 0.97$ ) can be observed which comply with the reported literature. Also the significant correlations identified between seed size and protein content is very important to support breeding line selection in the soybean breeding program. Similar results were obtained by Maestri et al (1998). A strong negative statistical proven correlation was established ( $r = -0.97$ ) between the fat content and linolenic acid content. The well documented inverse relationship between protein and oil content is shown by the negative value of the correlation coefficients obtained for these quality parameters (Filho et al., 2001).

Table 3. The influence of seed age on quality parameters

No	Parameters	Seed age			
		Fresh seeds	1 year	2 years	3 years
1.	Dry matter [%]	93.30 <sup>b</sup>	93.5 <sup>b</sup>	93.5 <sup>b</sup>	93.8 <sup>a</sup>
2.	Protein [g/100 gDM]	37.23 <sup>bc</sup>	38.67 <sup>a</sup>	36.7 <sup>c</sup>	37.4 <sup>b</sup>
3.	Fat [g/100 g DM]	21.20 <sup>b</sup>	21.17 <sup>b</sup>	22.00 <sup>a</sup>	22.03 <sup>a</sup>
4.	Stearic acid [%]	5.00 <sup>a</sup>	5.30 <sup>a</sup>	5.33 <sup>a</sup>	5.07 <sup>a</sup>
5.	Oleic acid [%]	25.93 <sup>a</sup>	24.80 <sup>b</sup>	25.07 <sup>b</sup>	25.67 <sup>a</sup>
6.	Linoleic acid [%]	51.87 <sup>b</sup>	53.83 <sup>a</sup>	53.73 <sup>ab</sup>	54.10 <sup>a</sup>
7.	Linolenic acid [%]	8.60 <sup>a</sup>	8.27 <sup>a</sup>	7.40 <sup>b</sup>	7.30 <sup>b</sup>

Table 4. Correlation coefficients of studied parameters

	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Fat	Moisture	Protein	TKW	Germination
Stearic acid	1	-0.23	0.30	-0.68	0.74	-0.08	-0.75	-0.64	0.02
Oleic acid	-0.23	1	-0.62	0.15	0.04	-0.01	-0.46	-0.57	-0.26
Linoleic acid	0.30	-0.62	1	-0.76	0.59	-0.77	0.23	0.06	-0.59
Linolenic acid	-0.68	0.15	-0.76	1	-0.97 <sup>0</sup>	0.78	0.44	0.60	0.69
Fat	0.74	0.04	0.59	-0.97 <sup>0</sup>	1	-0.70	-0.63	-0.77	-0.65
Moisture	-0.08	-0.01	-0.77	0.78	-0.70	1	-0.02	0.28	0.97*
Protein	-0.75	-0.46	0.23	0.44	-0.63	-0.02	1	0.94*	0.05
TKW	-0.64	-0.57	0.06	0.60	-0.77	0.28	0.94	1	0.38
Germination	0.02	-0.26	-0.59	0.69	-0.65	0.97*	0.05	0.38	1

## CONCLUSIONS

The results clearly showed that seed storage for one or two year period does not reduce seed germination while a high decrease in final germination percentage was obtained from seeds stored for three years.

The seeds stored three years germinated but microorganisms such as *Fusarium*, *Aspergillus*, and *Rhizopus* also developed. On the seeds from 2018-2020, the incidence of microorganisms was lower, being present, in small percentages

*Fusarium*, *Aspergillus* and *Penicillium*. As the storage time of soybean seeds increases, germination decreases, increasing the risk of installing certain pathogens.

The seed size was not influenced by the storage period, this quantitative character being dependent on the environmental conditions of the year in which the seeds were harvested. Similar results were obtained for the chemical composition of the seeds, the fat and protein content of the grains not being influenced by the age of the seeds.

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## GRAIN YIELDS OF TRITICALE VARIETIES GROWN UNDER BIOLOGICAL AND CONVENTIONAL AGRICULTURE

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### Abstract

*Triticale is mainly used as a source of feed and the tendency is to include in people's diet. Information concerning comparison of yields under biological and conventional agriculture is needed. The aim of the research is to evaluate the productivity of triticale varieties depending on the fertilization, under biological and conventional agriculture. Two by two-factorial trials were conducted during the period 2014-2017. The experiments were performed after predecessor sunflower on Pelic Vertisols. Three varieties of triticale were tested, certified organic fertilizer from red Californian worm on biological field and mineral fertilizers containing nitrogen and phosphorus on conventional field were applied. The results showed an increase between 31.3%-52.4% after organic fertilization and between 28.6%-55.4% after mineral fertilization. Concerning variety an increase between 15.8%-23.0% under biological system was established. Under conventional system, 83.0% lower compared to control and 6.9% more was established. A specific reaction of the varieties to the agricultural system was found. The Respect variety achieved the highest productivity under biological system and the Boomerang variety under conventional system. The average grain yield under biological system was 32.6% lower. The effects of year, farming system, variety and fertilization on grain yields were confirmed.*

**Key words:** biological farming, conventional farming, fertilization, grain yield, triticale.

### INTRODUCTION

After its artificial creation, triticale is confirmed as an important cereal crop. According to FAOSTAT data (2018), in Europe the largest production was reported in Poland, Germany and France, and the registered areas in the world were 3.81million ha, which is 1.5 more than in 2000-around 2.49 million ha.

The main agro-technical approach in the conventional agriculture is the application of nitrogen mineral fertilization. Nitrogen fertilizers have a rapid effect and guarantee high yields. The discovery of the Haber-Bosch process in the early 19th century led to production of comparative cheap nitrogen fertilizer (Erismann et al., 2008; Follett et al., 2010) and the use of mineral fertilizers has become a regular worldwide strategy to eliminate nitrogen deficiency and increase yields (Piepho et al., 2017). Nitrogen fertilization leads to an increase in soil nutrients and improved crop growth conditions (Hu et al., 2020), but on the other hand over time the stock of soil organic matter may be exhausted (Šimon et al., 2018). The increase of the

intensity of agricultural practices in recent decades caused environmental problems such as water pollution, soil degradation and biodiversity loss (Kalnina et al., 2013). In response to these negative trends alternative agricultural practices have developed, whose beginning was placed before the beginning of the new century. Professor King - the author of the book "Farmers of the 40th Century", called for a "global movement" in agricultural reform, and the detailed report presented for previous organic farming of China, Korea and Japan, was adopted as a validation of the principles of organic farming (Paull, 2011). The organic farming is an alternative farming system based mainly on crop rotations, the use of legumes, intensive soil treatments and the application of licensed organic preparations. In this regard, the use of vermicompost is widely used. In agricultural practice the vermicompost gives remarkable benefits regarding soil aggregation, plant nutrition and the development of beneficial microorganisms against phytopathogens (Pereira et al., 2014). One of the first researchers of the relationship between soil fertility and earthworms, subsequently having a direct connection with biological

farming was Charles Darwin (Darwin, 1881). Earthworms consume different soil substrates and release nutrients (Whalen and Janzen, 2002). The resulting product "is one of the richest nutrient organic fertilizer and shows a positive effect on plant growth and development" (Bhat et al., 2018).

Currently the biological farming has become one of the most dynamic agricultural sectors in European Union (Mikulioniene and Balezentiene, 2009). From 2010 to 2017, the certified area under biological farming, occupied by field crops in Bulgaria has increased by 59.1% and in Europe by 81.5% (FAOSTAT, 2018). Demand for high quality agricultural products has grown worldwide, especially for those produced under biological farming conditions (Oleynikova et al., 2020). Farmers, who practice intensive production, perceive the transformation into biological as difficult, risky and uncertain in terms of production results (Orlando et al., 2020). Many studies have confirmed that yields from biologically grown crops are lower. According to De Ponti et al. (2012) biological yield from individual crops are on average 80% from conventional, and the difference in yield has increased with an increase in conventional yield. Study by Brückler et al. (2018) has showed that the difference in yields between biological and conventional system varies between different types of crops, regions and in some locations crop yields are close to conventional. The cited data shows that the topic of yield comparison between biological and conventional system is not exhausted and is current. In Bulgaria no field research was conducted to compare triticale yield grown under biological and conventional system, given the use of triticale mainly as a source of feed, and the data is important for future production decisions in livestock farms.

The aim of the research is to evaluate the productivity of triticale varieties depending on the fertilization, under biological and conventional agriculture.

## MATERIALS AND METHODS

Two by two-factorial trials were conducted during the period 2014-2017 at the Institute of Field Crops, Bulgaria on certified biological

and conventional field. The experiments were performed after predecessor sunflower in four replications on Pelic Vertisols. Three Bulgarian triticale varieties, i.e. - Colorit (Standard for Bulgaria), Boomerang and Respect, were tested in both agricultural systems. The grain yield (GY) was evaluated. The sowing on both fields was carried out on 13 November 2014, 3 November 2015 and 20 October 2016 with a density of 550 seeds per m<sup>2</sup>.

Certified organic fertilizer Lumbrical from red Californian worm (0, 1,400.0 and 1,750.0 kg/ha) was applied on the biological field. The commercial product contains: organic substances 45-60%; humic-acids up to 14%; fulvic-acids 7%; ammonium nitrogen (NH<sub>4</sub>-N)-33.0 ppm; nitric nitrogen (NO<sub>3</sub>-N)-30.5 ppm; P<sub>2</sub>O<sub>5</sub>-1410 ppm; K<sub>2</sub>O-1910 ppm. The fertilizer was applied in autumn, at the same time with the main tillage. No disease and pest control was applied. Mechanical treatments were applied to reduce weeds. The experimental plot was 18 m<sup>2</sup>.

Different rates of nitrogen fertilizer (ammonium nitrate)-0, 60, 120 and 180 kg/ha were tested. Phosphorus fertilizer (ternary superphosphate) at a rate of 60 kg/ha was applied to options N<sub>60</sub>, N<sub>120</sub> and N<sub>180</sub> and was incorporated in autumn with the main tillage before sowing. Nitrogen fertilizer was applied in early spring in the tillering phase. Herbicide combination of pinoxaden and iodosulfuron-methyl - sodium with amidosulfuron was applied to control the weeds. The experimental plot was 12 m<sup>2</sup>.

A two-factor date analyze was performed using ANOVA. Significant differences in the mean values were determined using the LSD test at significance level 5.0, 1.0 and 0.1%.

## RESULTS AND DISCUSSIONS

The data presented in Figure 1 shows that the sum of temperature during the growing seasons 2014-2015 and 2015-2016 were higher by 248.3°C and 515.5°C, respectively over the average for the period 1928-2013. In the first vegetation period the precipitations were 285.0 mm more compared to climatic average. During October (135.4 mm), December (142.3 mm) and March (134.9 mm) the amount of precipitation was higher than multi-annual average-37.5 mm, 54.0 mm and 37.0 mm,

respectively. The amount of precipitation in 2015-2016 period (400.0 mm) was about

climatic average (428.5 mm). In the third year the registered precipitations were 51.6 mm less.

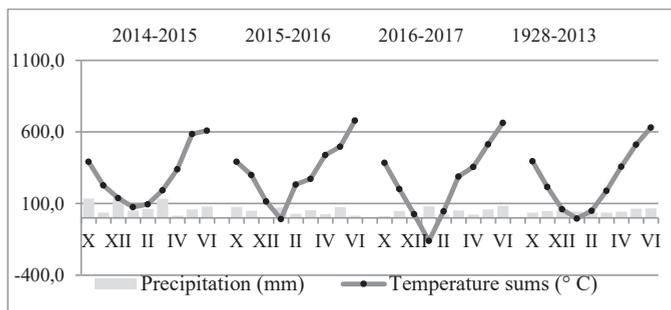


Figure 1. Monthly temperature sum and rainfall for 2014-2017 in the 1928-2013 reference periods

The results of the average triticale grain yields data presented in Table 1, illustrate considerable differences in individual years within systems of agriculture. Compared to the grain yield obtained under biological field in 2015 harvest year, for all three years under conventional agriculture and under biological field in 2016 the values were significant. This data shows that even if the weather conditions were the same, the variation in the average yield of both systems could be due of biotic and

abiotic factors within the two agroecosystems. Under conventional system in 2017 highest average number of spikes (385.5 per m<sup>2</sup>) was obtained compared to 2015 (369.9 per m<sup>2</sup>) and 2016 (312.1 per m<sup>2</sup>) (Stefanova-Dobrevna, 2019). Similarly, the yield obtained in 2016 under biological system can be explained by the highest average number of spikes (509.6 per m<sup>2</sup>), compared to 2015 (481.2 per m<sup>2</sup>) and 2017 (496.3 per m<sup>2</sup>) (Muhova, 2018).

Table 1. Influence of year and system of agriculture on triticale grain yield for the period 2015-2017

Agriculture system	GY (kg/ha)	% Control
2015		
Biological	1,168.1	100.0
Conventional	3,883.5***	332.5
2016		
Biological	1,784.6***	152.8
Conventional	3,645.7***	312.1
2017		
Biological	1,080.3 <sup>ns</sup>	92.5
Conventional	4,828.3***	413.3
LSD		
5.0%	335.7	28.7
1.0%	446.0	38.2
0.1%	579.1	49.6

\*\*\* significance at  $p=0.1\%$ ; <sup>ns</sup> no significance.

According to Table 2, the average yield under conventional farming was 206.4% higher compared to biological farming and statistically significant. This data corresponds to threefold increase. The data obtained by Kronberga et al. (2013) for triticale showed a yield under conventional farming two time higher compared to biological farming and Oljača et

al. (2010) reported 7.8% higher average yield for rye. The data from the present study showed that the average yield under biological system is 32.6% lower compared to conventional. This data confirms the results obtained by Torikov et al. (2020). The authors reported 33.7% and 32.9% lower grain yield under biological system compared to conventional for two

triticale cultivars. An analyses made by Brückler et al. (2018) showed a 68.4% lower triticale yield under biological system compared to the conventional one. The difference in average yield between the agricultural systems, can be explained by the applied agricultural techniques and the

incoming nitrogen levels. The analyses for evaluation of cereal varieties under conventional and biological management strategies showed that there are differences in entry levels under conventional and biological systems (Le Champion et al., 2020).

Table 2. Influence of agricultural system on triticale grain yield for the period 2015-2017

Agricultural system	GY (kg/ha)	% Control
Agricultural system		
Biological	1,344.3	100.0
Conventional	4,119.2***	306.4
LSD		
5.0 %	193.8	14.4
1.0 %	257.5	19.2
0.1 %	334.4	24.9

\*\*\*significance at  $p=0.1\%$ .

Table 3. Effect of the year and agricultural system on triticale grain yield for the period 2014-2017

Source of variation	df	SS	$\eta$ (%)	MS	F	P value
Options	5	1.515223E+08	93.11	3.030446E+07***	178.62	0.00000
A	2	2213824	1.36	1106912**	6.52	0.00296
B	1	1.385957E+08	85.17	1.385957E+08***	816.9	0.00000
A×B	2	1.071283E+07	6.58	53565416***	31.57	0.00000
Error	66	1.119693E+07	6.88	16964950.4		

A - year; B - agricultural system; \*\*, \*\*\*significance at  $p = 1.0\%$  and  $p = 0.1\%$ , respectively.

The results presented in Table 3 show the significance of the year, the agricultural system and their complex influence, which is obvious from Table 1 and Table 2. The greatest effect on triticale grain yields had the agricultural system - 85.17% of the total variation, followed by the interaction of factors (6.58%) and the year conditions (1.36%).

The results of the analysis on yield of the varieties showed a different increase in yield compared to Colorit variety (Table 4). On a three year average, the yield varied between 15.8% and 23.0% within the varieties compared to the control under the biological system. On average, the highest average productivity (1,464.4 kg/ha) was observed at the Respect variety. Under conventional system the yield varied from 83.6% lower to the control option and 6.9% more to the control. The highest productivity was registered at the Boomerang variety (4,586.5 kg/ha). It is obvious that the studied varieties show different productivity under the two systems of agriculture.

This can be established from the results presented on Table 5, where is visibly that in both systems of agriculture the influence of the variety is significant - under biological system is 6.74% of the total variation and under conventional - 17.59%. Torikov et al. (2018) have reported similar results. In their study two varieties of triticale showed different productivity under conventional and biological farming system. The study made by Kronberga et al. (2013) for the evaluation of triticale productivity under biological and conventional systems showed that some genotypes are more productive under biological system, but their yield under conventional is below average yields. In the present study, the Respect variety showed the highest average productivity under biological system (1,464.4 kg/ha), but the yield under conventional system (3,560.4 kg/ha) was lower compared to the average for the period 2014-2017 (4,145.3 kg/ha) (Table 4).

Table 4. Influence of variety on triticale grain yield under biological and conventional system for the period 2014-2017

Variety	Biological system (kg/ha)	% Control	Conventional system (kg/ha)	% Control
Colorit	1,190.6	100.0	4,289.0	100.0
Boomerang	1,379.0*	115.8	4,586.5*	106.9
Respect	1,464.4**	123.0	3,560.4 <sup>ns</sup>	83.0
Average	1,344.6		4,145.3	
LSD				
5.0%	175.0	14.7	277.8	6.5
1.0%	231.6	19.5	367.1	8.6
0.1%	299.0	25.1	472.7	11.0

\*, \*\*significance at  $p = 5.0\%$  and  $p = 1.0\%$ , respectively; <sup>ns</sup> no significance.

Table 5. Effect of variety and fertilization on triticale grain yield for the period 2015-2017

Source of variation	df	SS	$\eta$ (%)	MS	F	P value
Biological system						
Options	8	7116096	33.93	889512***	6.36	0.00001
C	2	1413568	6.74	706784**	5.05	0.00830
D	2	5537136	26.40	2768568***	19.78	0.00000
C×D	4	165392	0.79	41348 <sup>ns</sup>	0.30	0.88020
Error	99	1.385462E+07	66.07	139945.7		
Conventional system						
Options	11	8.962329+07	58.92	8147573***	17.21	0.00000
C	2	2.675943E+07	17.59	1.337971E+07***	28.27	0.00000
D	3	5.942963E+07	39.07	1.980988E+07***	41.85	0.00000
C×D	6	3434240	2.26	572373.3 <sup>ns</sup>	1.21	0.30489
Error	132	6.248423E+07	41.08	473365.4		

C - variety; D - fertilization; \*, \*\*, \*\*\*significance at  $p = 1.0\%$  and  $p = 0.1\%$ , respectively; <sup>ns</sup> no significance.

Table 6 presents the results for the influence of organic and mineral fertilization on triticale grain yield. The average results showed a 31.3% and 52.4% higher yields, respectively when 1,400.0 and 1,750.0 kg/ha organic fertilizers were applied. The highest grain yield obtained when Lumbrical 1,750.0 kg/ha was applied can be explained by larger amount of imported macronutrients. A similar trend was observed under the influence of nitrogen fertilization on triticale yield under conventional system. The yield obtained when fertilizing with N<sub>60</sub>, N<sub>120</sub> and N<sub>180</sub> was 28.6%, 40.5% and 55.4% higher than the non-fertilizing option. Under both systems of agriculture, the maximum increase in yield was similar-52.4% under biological system and 55.4% under conventional system more than control option, but the difference in kg/ha was an advantage for the conventional system. This data suggests that in both agricultural systems,

fertilization plays a crucial role for yield formation.

The results in Table 7 show the influence of organic fertilization with rate of 1,400.0 and 1,700.0 kg/ha Lumbrical on grain yield among all triticale varieties. Thus, at Colorit variety the grain yields were 27.5% and 51.3% higher than the control, at Boomerang, 51.4% and 69.7% higher and at Respect variety, 60.4% and 88.8% respectively. The largest increase in yields compared to the control was observed at Respect variety. A similar trend concerning fertilization was observed in the conventional system. Boomerang variety registered the highest productivity when applying N<sub>60</sub>, N<sub>120</sub> and N<sub>180</sub>, the increase in yields varying from 41.3% to 72.2% compared to the control. At Colorit variety, the increase in grain yields varied from 30.3% to 60.7% and at Respect, from 8.2% to 26.1%.

Table 6. Influence of fertilization on triticale grain yield under biological and conventional system for the period 2015-2017

Fertilization	GY (kg/ha)	% Control	LSD		
			%		% Control
Organic fertilization					
0	1,051.1	100.0	5.0	175.0	16.6
1,400.0	1,380.6***	131.3	1.0	231.6	22.0
1,750.0	1,602.3***	152.4	0.1	299.0	28.4
Mineral fertilization					
N <sub>0</sub>	3,161.6	100.0	5.0	320.8	10.2
N <sub>60</sub> P <sub>60</sub>	4,064.8***	128.6	1.0	423.8	13.4
N <sub>120</sub> P <sub>60</sub>	4,442.6***	140.5	0.1	545.8	17.3
N <sub>180</sub> P <sub>60</sub>	4,912.3***	155.4			

\*\*\*significance at  $p = 0.1\%$ .

Table 7. Influence of fertilization and variety on triticale grain yield under biological and conventional system for the period 2015-2017

Variety	Biological system			Conventional system		
	Organic fertilization (kg/ha)	GY (kg/ha)	% Control	Mineral fertilization (kg/ha)	GY (kg/ha)	% Control
Colorit	0	942.8	100.0	N <sub>0</sub>	3,211.0	100.0
	1,400.0	1,202.0 <sup>ns</sup>	127.5	N <sub>60</sub> P <sub>60</sub>	4,184.8***	130.3
	1,750.0	1,426.9**	151.3	N <sub>120</sub> P <sub>60</sub>	4,599.5***	143.2
				N <sub>180</sub> P <sub>60</sub>	5,160.8***	160.7
Boomerang	0	1,109.9 <sup>ns</sup>	117.7	N <sub>0</sub>	3,376.5	105.2
	1,400.0	1,427.5***	151.4	N <sub>60</sub> P <sub>60</sub>	4,536.5***	141.3
	1,750.0	1,599.5***	169.7	N <sub>120</sub> P <sub>60</sub>	4,904.5***	152.7
				N <sub>180</sub> P <sub>60</sub>	5,528.6***	172.2
Respect	0	1,100.6 <sup>ns</sup>	116.7	N <sub>0</sub>	2,897.3 <sup>ns</sup>	90.2
	1,400.0	1,512.3***	160.4	N <sub>60</sub> P <sub>60</sub>	3,473.0 <sup>ns</sup>	108.2
	1,750.0	1,780.4***	188.8	N <sub>120</sub> P <sub>60</sub>	3,823.8*	119.1
				N <sub>180</sub> P <sub>60</sub>	4,047.4**	126.1
LSD						
5.0%	303.0		32.1	555.6		17.3
1.0%	401.1		42.9	734.1		22.9
0.1%	518.0		54.9	945.4		29.4

\*, \*\*, \*\*\*significance at  $p = 5.0\%$ ,  $p = 1.0\%$  and  $p = 0.1\%$ , respectively; <sup>ns</sup>no significance.

Based on the data presented it can be summarized that under both systems of agriculture triticale varieties show a specific reaction according to the tested doses of organic and mineral fertilizer and subsequently had different productivity. The Respect and Boomerang varieties show high responsiveness to the applied fertilization, respectively under biological and conventional system. The Respect variety manifests high ecological plasticity according to the conditions of the biological system. The results showed that the effect of the fertilization under biological and conventional systems was respectively 26.40% and 39.07% of the total variation and statistically significant (Table 5). The complex influence of factors was found to be low and with no significant effect under both tested

systems (0.79% and 2.26% of the total variation).

## CONCLUSIONS

Triticale grain yield was influenced by the characteristic conditions of the years and the agricultural system. So the conditions of the years had low effect, and the agricultural system had a significant, large impact on triticale grain yields. The average grain yield under biological system was 32.6% lower compared to conventional. For both systems of agriculture a crucial role for the yield formation had fertilization and less degree the variety. The Respect variety showed the highest productivity under condition of biological system when applying Lumbrical organic

fertilizer at rate of 1,750.0 kg/ha and under conventional system the Boomerang variety when fertilized with N<sub>180</sub>.

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## THE QUALITY OF MIXTURES OF PERENNIAL GRASSES AND LEGUMES EXPLOITED IN HAY REGIME UNDER CENTRAL OF MOLDOVA CONDITIONS

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### Abstract

*The study was conducted during 2017-2019, within ARDS Secuieni and aimed to analyse the influence of fertilization and species mixture on fodder quality, as well as the determination of fodder content in crude protein (CP), NDF (neutral detergent fiber), ADF (acid detergent fiber) and calculation of relative forage quality (RFQ). Experimental factors were represented by fertilization (factor A), with four graduations: a1-N0P0; a2-N40P40; a3-N80P40; a4-N80+40P40, and by the mixture of perennial grasses and perennial legumes (factor B), with five graduations: b1 - 20% perennial grasses + 80% legumes; b2 - 65% perennial grasses + 35% legumes; b3 - 70% perennial grasses + 30% legumes; b4 - 70% perennial grasses + 30% legumes; b5 - 80% perennial grasses + 20% legumes. The results showed that the lowest content in crude protein, of 15.24 g/100 g d. s. was achieved in the variant fertilized with N40P40 and the highest value of CP (15.94 g/100 g d. s.) was obtained in the non-fertilized variant (control). The applied fertilizers influenced the crude protein content of each of the studied mixtures, the differences obtained being statistically assured.*

**Key words:** fertilization, perennial grasses, perennial legumes, hay.

### INTRODUCTION

When making the mixtures, the biological properties of the species will be taken into account, depending on the use and the duration of the existence of the temporary grasslands. Thus, when used for hayfields, tall species are used, with a close growth rate, high speed of growth and high growth energy, resistance to soil compaction and higher vivacity (Belesky, 2002; Sanderson, 2005; Vintu et al., 2010).

The introduction of aggressive species into mixtures along with those with reduced competition capacity leads to the elimination of the latter over time. Competitiveness (competition capacity) is a species trait, but it is greatly influenced by environmental conditions and the manner of exploitation (Leconte et al., 1991; Skinner et al., 2006; Lazaridou, 2008).

Temporary grasslands established with a mixture of perennial grasses and perennial legumes, in a balanced ratio, give the obtained fodder an optimal quality and content of mineral elements, which then have positive effects on the animals. The fodder obtained

from these grasslands is well consumed both in the green state and in the form of hay (Golinski, 2008; Thumm, 2008; Tomic et al., 2011).

Temporary grasslands play a remarkable role in improving soil properties, thus its structure will be more stable, the content of mineral and organic substances increases, microbiological activity develops, water and air regime improves, and subsequent crops give large yields with reduced quantities of fertilizers (Vintu et al., 1996; Samuil et al., 1999, 2018; Carlsson et al., 2008; Tomić et al., 2011).

It is known that doses of nitrogen fertilizers are administered in smaller quantities to mixtures of perennial grasses and legumes, compared to grasses grown in monoculture, because this element is provided biologically by symbiotic bacteria, but fertilization also plays an important role in obtaining large yields in these mixtures (Rotar et al., 1994; 1995; Simtea et al., 1988; Cardaşol et al., 2001; Rotar et al., 2002; Cardaşol et al., 2003; Motca et al., 2008). Because grasslands are constituted of species with different nutrient requirements, some react

strongly, others react more weakly to improving the dietary regime. These particularities determine the differentiation of the fertilization according to the vegetation period, the pedoclimatic conditions, the type of fertilizer and the way of using the grasslands.

The application in a timely manner and in appropriate combinations can significantly improve both fodder production and fodder quality (Aguilar et al., 2012; Al-Juhaimi et al., 2014).

The aim of this study was to analyse the influence of fertilization and mixture of species on fodder quality, by determining the crude protein content (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF).

## MATERIALS AND METHODS

The placement of the experiments was carried out in the experimental field of the ARDS Secuieni, Neamț County, in the spring of 2017 and is of the stationary type. The soil on which the experiments were conducted is of the typical cambic chernozem type, characterized as well supplied with active humus (2.33%), very well supplied with phosphorus (189 mg/kg), potassium (304 mg/kg) and poorly supplied in nitrogen (9.4 mg/kg N-NO<sub>3</sub>), being slightly acidic with a pH (in aqueous suspension) of 5.83. Given the soil content in macro elements, it was aimed to set up a 4 x 5 type bifactorial experiment, placed based on the method of subdivided plots, in four repetitions (Jitareanu G., 1998).

Factor A is represented by nitrogen fertilization (on a phosphorus background), with four graduations: a<sub>1</sub> - N<sub>0</sub>P<sub>0</sub>; a<sub>2</sub> - N<sub>40</sub>P<sub>40</sub>; a<sub>3</sub> - N<sub>80</sub>P<sub>40</sub>; a<sub>4</sub> - N<sub>80+40</sub>P<sub>40</sub>, and factor B, a mixture of perennial grasses and legumes, with five graduations: b<sub>1</sub> - 20% grasses + 80% legumes (20% *Dactylis glomerata* L. + 80% *Medicago sativa* L.) - Mt; b<sub>2</sub> - 65% grasses + 35% legumes (30% *Bromus inermis* Leyss + 35% *Dactylis glomerata* L. + 35% *Onobrychis viciifolia* Scop.); b<sub>3</sub> - 70% grasses + 30% legumes (30% *Dactylis glomerata* L. + 40% *Lolium perenne* L. + 20% *Medicago sativa* L. + 10% *Lotus corniculatus* L.); b<sub>4</sub> - 70% grasses + 30% legumes (30% *Festuca arundinacea* Schreb. + 20% *Dactylis glomerata* L. + 20% *Festuca pratensis* Huds. + 20% *Medicago*

*sativa* L. + 10% *Trifolium pratense* L.); b<sub>5</sub> - 80% grasses + 20% legumes (45% *Festuca pratensis* Huds. + 35% *Festuca arundinacea* Schreb. + 20% *Trifolium pratense* L.).

The harvestable area of the experimental plot was 8 m<sup>2</sup> and the plot surface area was 10 m<sup>2</sup>. In the year of establishing the experiment, three uniformity mows were performed.

Following the research conducted at ARDS Secuieni, three production cycles were carried out, both in 2018 and in 2019, in the phenophase of grasses sprouting and legumes efflorescence, at an interval of 53 days for the first cycle, 42 days for the second cycle and 52 days for the third cycle.

Background fertilization was done with phosphate fertilizers administered in the fall, and nitrogen-based fertilizers were administered in early spring, at the start of vegetation, except for the N80 + 40 dose, the difference of which was administered after the first mowing. Harvesting was done mechanically, using the "Bertolini" mower, at a height of 4-5 centimeters from the ground. The dry matter was determined by treating the samples at 105°C for 3 hours.

The determination of the nitrogen content was performed using the Kjeldahl method, and the Van Soest method was used to determine the NDF and ADF content. The crude protein was determined by converting total nitrogen to CP by multiplying by a factor of 6.25. RFQ (Relative Forage Quality) calculation was performed using Equation 1 (Ward R. et al, 2008; Linn J.G., et al 2012).

$$RFQ = \frac{(4,898 + 89,796x(1,085 + 0,0124xADF))x^{\frac{120}{NDF}}}{1,23}$$

Equation 1

The results were interpreted statistically by analysing the variance and calculating the boundary differences. The correlations between the quantity of nitrogen applied and the fodder content in BP, NDF, ADF and RDQ for each of the studied mixtures were calculated.

The meteorological conditions recorded during the experimenting period (2017-2019) were variable, the deviation from the multiannual average of the temperatures (8.9°C) was between 0.6°C (2017) and 1.4°C (2018), and compared to the multiannual average, the period was characterized as normal in 2017 and warm in 2018 and 2019 (Figure 1).

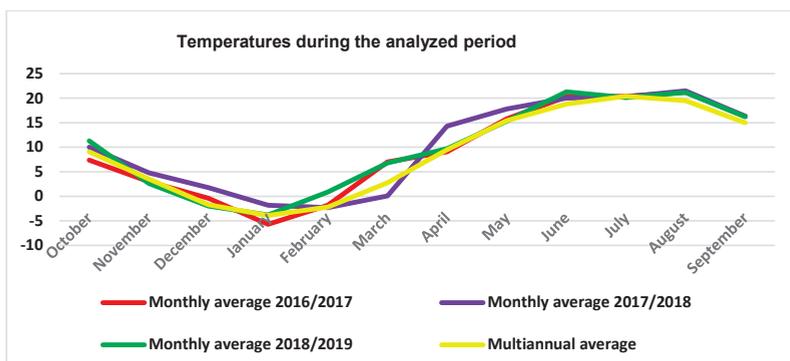


Figure 1. Temperatures recorded at the ARDS Secuieni Weather Station

From the rainfall point of view, the studied years were classified as rainy agricultural years (2016-2017), normal years (2017-2018) and

less dry agricultural years (2018-2019), compared to the multiannual average which is 544.3 mm (Figure 2).

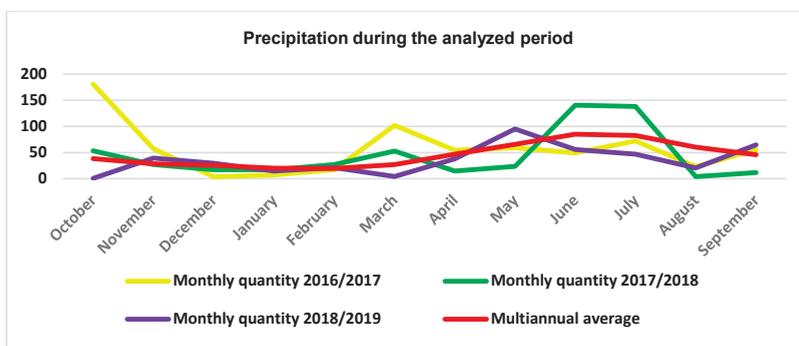


Figure 2. Precipitation recorded at the ARDS Secuieni Weather Station

## RESULTS AND DISCUSSIONS

The yield is positively influenced by the fertilization with mineral fertilizers, and the results obtained depended both on the doses of nitrogen fertilizers applied and on the mixtures studied. The manner of exploitation of the temporary grasslands proved to be an important factor in the level of yields, thus in the years of experimentation, three mows (harvests) were performed for the mixtures used as meadow, and the exploitation period was 143 days.

On average, over the two years, at the first harvest, the lowest yield of 5.73 t/ha d.s. was obtained in the non-fertilized variant, sown with the mixture of *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%. Comparing the yield obtained with that of the control variant (*Dactylis glomerata* 20% + *Medicago sativa* 80%), the difference in yield was negative and very significant.

In the studied variants, the results showed that the highest d.s. yield of 11.15 t/ha d.s. was obtained for the *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% variant, fertilized with N<sub>80</sub>P<sub>40</sub>. Compared to the control variant, it achieved very significant production increases (Table 1). At the second harvest, the highest yield obtained (12.04 t/ha d.s.) was for the fertilization with N<sub>80+40</sub>P<sub>40</sub> to the mixture of *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%, the difference compared to the control being positive and very significant.

In all the other variants analysed it is observed that the differences from the control were positive and very significant. At the third harvest, yields decreased by half compared to the second harvest, due to the deficit in precipitation, the highest yield of 5.42 t/ha d.s. being achieved for the *Bromus inermis* 30% +

*Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% fertilized with N<sub>80</sub>P<sub>40</sub>, and the lowest, for the *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20% mixture, without fertilization.

Analysing the influence of the interaction between mixtures of perennial grasses and legumes and fertilization, in the second year of production, it was observed that the highest yields of 28.01 t/ha d.s. were obtained in the case of fertilization with N<sub>80</sub>P<sub>40</sub>, in the mixture

of *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%.

Compared to the control plot (18.04 t/ha d.s.), the differences of all variants studied were positive and very significant, the exception being represented by the mixture of *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10% without fertilization that is not statistically assured (Table 1).

Table 1. The influence of the interaction between the mixture and fertilization on d.s. average production during 2018-2019, to use as hay

Variant		Yield (t/ha d.s.)				
		I harvest	II harvest	III harvest	TOTAL	
a <sub>1</sub> - N <sub>0</sub> P <sub>0</sub> (mt)	b <sub>1</sub> - D.g.20%+M.s.80%(mt)	7.50 <sup>Mt</sup>	6.42 <sup>Mt</sup>	4.12 <sup>Mt</sup>	18.04 <sup>Mt</sup>	
	b <sub>2</sub> - B.i.30%+D.g.35%+O.v.35%	7.56	9.19***	4.32	21.07***	
	b <sub>3</sub> - D.g.30%+L.p.40%+M.s.20%+L.c.10%	6.96 <sup>o</sup>	7.03***	4.60***	18.59	
	b <sub>4</sub> - F.a.30%+D.g.20%+F.p.20%+M.s.20%+T.p.10%	7.04 <sup>oo</sup>	9.90***	3.76 <sup>ooo</sup>	20.70***	
	b <sub>5</sub> - F.p.45%+F.a.35%+T.p.20%	5.73 <sup>ooo</sup>	10.00***	3.07 <sup>ooo</sup>	18.80*	
a <sub>2</sub> - N <sub>40</sub> P <sub>40</sub>	b <sub>1</sub> - D.g.20%+M.s.80%(mt)	8.47***	7.21***	4.69***	20.37***	
	b <sub>2</sub> - B.i.30%+D.g.35%+O.v.35%	9.45***	9.98***	4.56***	23.99***	
	b <sub>3</sub> - D.g.30%+L.p.40%+M.s.20%+L.c.10%	8.15**	7.39***	4.54***	20.08***	
	b <sub>4</sub> - F.a.30%+D.g.20%+F.p.20%+M.s.20%+T.p.10%	7.79	10.51***	4.34*	22.64***	
	b <sub>5</sub> - F.p.45%+F.a.35%+T.p.20%	6.80 <sup>oo</sup>	10.70***	3.70 <sup>ooo</sup>	21.20***	
a <sub>3</sub> - N <sub>80</sub> P <sub>40</sub>	b <sub>1</sub> - D.g.20%+M.s.80%(mt)	10.04***	8.12***	5.24***	23.40***	
	b <sub>2</sub> - B.i.30%+D.g.35%+O.v.35%	11.15***	11.44***	5.42***	28.01***	
	b <sub>3</sub> - D.g.30%+L.p.40%+M.s.20%+L.c.10%	9.45***	8.22***	5.30***	22.97***	
	b <sub>4</sub> - F.a.30%+D.g.20%+F.p.20%+M.s.20%+T.p.10%	9.48***	11.61***	5.39***	26.48***	
	b <sub>5</sub> - F.p.45%+F.a.35%+T.p.20%	8.59***	11.90***	5.21***	25.70***	
a <sub>4</sub> - N <sub>80+40</sub> P <sub>40</sub>	b <sub>1</sub> - D.g.20%+M.s.80%(mt)	9.42***	7.83***	5.15***	22.40***	
	b <sub>2</sub> - B.i.30%+D.g.35%+O.v.35%	10.72***	11.75***	5.00***	27.47***	
	b <sub>3</sub> - D.g.30%+L.p.40%+M.s.20%+L.c.10%	9.38***	8.32***	5.04***	22.74***	
	b <sub>4</sub> - F.a.30%+D.g.20%+F.p.20%+M.s.20%+T.p.10%	8.75***	11.57***	4.81***	25.13***	
	b <sub>5</sub> - F.p.45%+F.a.35%+T.p.20%	7.47	12.04***	4.40**	23.91***	
DL		5%	0.43	0.31	0.21	0.65
		1%	0.57	0.42	0.28	0.87
		0.1%	0.75	0.55	0.36	1.15

The quality of the fodder obtained from the temporary grasslands was influenced by the doses of fertilizers applied, by the proportion of participation of the species in the sowing norm, but also by the climatic conditions during the exploitation period.

In 2018, the lowest content in crude protein (CP) of 15.24 g/100 d.s. was obtained for the variant fertilized with N<sub>40</sub>P<sub>40</sub>, and highest CP content of the fodder, of 15.94 g/100 d.s., was obtained for the variant without fertilization (control). Compared to the control variant, all the differences obtained for the three fertilized variants were statistically assured (Table 2).

Analysing the influence of fertilization on the fodder content in NDF (neutral detergent fiber), it is observed that the variant fertilized with N<sub>80+40</sub>P<sub>40</sub> obtained the highest fodder content in NDF, 51.40 g/100 g d.s., and the lowest, of 44.73 g/100 g d.s., was registered in the variant without fertilization. With the exception of the variant fertilized with N<sub>40</sub>P<sub>40</sub>, all differences from the control obtained were distinctly significant and very significant. Fertilization has significantly and distinctly significantly influenced the content of the fodder in ADF (acid detergent fiber), having positive differences between the non-fertilized variant

(control) and the other fertilized variants. From the results obtained in the first year of exploitation, it is observed that the fertilization also influenced the fodder content in RFQ (relative forage quality). The highest forage value, 155, was recorded in the non-fertilized variant belonging to quality class 0 (excellent) (Hancock DW, 2011), and the lowest value, 128, was recorded in the variant fertilized with N<sub>80+40</sub>P<sub>40</sub> quality class 1 (good) (Table 2).

Table 2. The influence of the fertilization on fodder quality, in 2018, to use as hay

Variant	Quality parameters				
	CP (g/100 g d.s.)	NDF (g/100 g d.s.)	ADF (g/100 g d.s.)	RFQ	
a <sub>1</sub> - N <sub>0</sub> P <sub>0</sub> (mt)	15.94 <sup>Mt</sup>	44.73 <sup>Mt</sup>	28.38 <sup>Mt</sup>	155 <sup>Mt</sup>	
a <sub>2</sub> - N <sub>40</sub> P <sub>40</sub>	15.24 <sup>o</sup>	47.37	29.76	143 <sup>o</sup>	
a <sub>3</sub> - N <sub>80</sub> P <sub>40</sub>	15.35 <sup>o</sup>	49.64 <sup>**</sup>	31.20 <sup>*</sup>	134 <sup>oo</sup>	
a <sub>4</sub> - N <sub>80+40</sub> P <sub>40</sub>	15.44 <sup>o</sup>	51.40 <sup>***</sup>	31.72 <sup>**</sup>	128 <sup>ooo</sup>	
DL	5%	0.49	2.89	2.20	11
	1%	0.71	4.16	3.17	15
	0.1%	1.04	6.12	4.66	23

Analysing the crude protein content of the fodder in 2019, it was found that it was between 15.40 g/100 g d.s. in the variant fertilized with N<sub>80</sub>P<sub>40</sub> and 15.11 g/100 g d.s. in the variant fertilized with N<sub>40</sub>P<sub>40</sub>. Compared to the control variant, the three fertilized variants were not statistically assured (Table 3).

From the results obtained it is observed that the fertilization influenced the fodder content in NDF, having values between 48.53 g/100 g d.s. in the variant fertilized with N<sub>40</sub>P<sub>40</sub> and 50.97 g/100 g d.s. in the variant fertilized with N<sub>80</sub>P<sub>40</sub>. All differences obtained from the control were positive, significant and distinctly significant.

Analysing the influence of fertilization on the quality of the fodder in the ADF, the differences obtained were between 1.85-3.35 g/ 100 g d.s. For the variant fertilized with N<sub>80</sub>P<sub>40</sub> was obtained the highest content in ADF (31.45 g/100 g d.s.) and the smallest, of 28.10 g/100 g d.s., was registered in the non-fertilized variant. Compared to the control variant, the differences obtained in the variants fertilized with N<sub>80</sub>P<sub>40</sub> and N<sub>80+40</sub>P<sub>40</sub> were positive, significant and distinctly significant.

The biggest difference compared to the control variant, in terms of the influence of fertilization on the relative forage quality (RFQ) is observed in the variant fertilized with N<sub>80</sub>P<sub>40</sub>, (24 units). The highest forage value, 153 units,

was recorded in the non-fertilized variant belonging to quality class 0 (excellent) (after Hancock DW, 2011), and the lowest value, of 129 units, was recorded in the variant fertilized with N<sub>80</sub>P<sub>40</sub> belonging to quality class 1 (good). All differences obtained in the fertilized variants were statistically assured (Table 3).

Table 3. The influence of the fertilization on fodder quality, in 2019, to use as hay

Variant	Quality parameters				
	CP (g/100 g d.s.)	NDF (g/100 g d.s.)	ADF (g/100 g d.s.)	RFQ	
a <sub>1</sub> - N <sub>0</sub> P <sub>0</sub> (mt)	15.25 <sup>Mt</sup>	45.30 <sup>Mt</sup>	28.10 <sup>Mt</sup>	153 <sup>Mt</sup>	
a <sub>2</sub> - N <sub>40</sub> P <sub>40</sub>	15.11	48.53 <sup>*</sup>	29.95	139 <sup>o</sup>	
a <sub>3</sub> - N <sub>80</sub> P <sub>40</sub>	15.40	50.97 <sup>**</sup>	31.45 <sup>**</sup>	129 <sup>ooo</sup>	
a <sub>4</sub> - N <sub>80+40</sub> P <sub>40</sub>	15.24	50.76 <sup>**</sup>	31.18 <sup>*</sup>	130 <sup>oo</sup>	
DL	5%	0.57	3.03	2.31	11
	1%	0.82	4.35	3.33	16
	0.1%	1.21	6.40	4.89	24

Compared to 2018, in 2019 the fodder content in crude protein was lower due to the high percentage of grasses obtained in these variants, and the NDF, ADF and RFQ values are very close.

The influence of the mixture on the quality of the fodder was also monitored, thus in 2018 the crude protein content of the fodder decreased from 16.55 g/100 g d.s. for the mixture *Dactylis glomerata* 20% + *Medicago sativa* 80% (control) to 13.64 g/100 g d.s. for the mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%. Compared to the control variant (*Dactylis glomerata* 20% + *Medicago sativa* 80%), *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% and *Dactylis glomerata* 20% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus*, 10% mixtures were statistically assured (Table 4).

The NDF content of the fodder for the mixtures studied was between 45.1 g/100 g d.s. for *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10% mixture and 54.14 g/100 g d.s. for *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% mixture.

Compared to the control variant sown with the *Dactylis glomerata* 20% + *Medicago sativa* 80% mixture, for the variant sown with the *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% mixture, the difference obtained was distinctly significant. For mixtures sown with *Dactylis glomerata*

30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10% and *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%, the differences obtained were negative, distinctly significant. Analysing the fodder ADF content for the studied mixtures, is observed that for *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10%, *Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20% + *Medicago sativa* 20% + *Trifolium pratense* 10% and *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%, the differences obtained from the control variant (*Dactylis glomerata* 20% + *Medicago sativa* 80%) were negative, distinctly significant and very significant. For the variant sown with the *Bromus inermis* 30% + *Dactylis*

*glomerata* 35% + *Onobrychis viciifolia* 35% mixture, there were no statistically assured differences from the control variant.

In the case of the analysis of the influence of the mixture on the relative forage quality (RFQ), it is observed that between the mixture in the control variant and the other studied mixtures, the differences were assured statistically. In the variant sown with the mixture *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%, the highest value of RFQ of 154 units was obtained, the fodder belonging in the quality class 0 (excellent), and the lowest, of 119 units, was recorded in the variant sown with the mixture *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%, the feed belonging to quality class 1 (good) (Table 4).

Table 4. The influence of the used mixture on the fodder quality in 2018, to use as hay

Variant		Quality parameters			
		CP (g/100 g d.s.)	NDF (g/100 g d.s.)	ADF (g/100 g d.s.)	RFQ
b <sub>1</sub> - D.g.20% +M.s.80%(mt)		16.55 <sup>Mt</sup>	49.19 <sup>Mt</sup>	32.86 <sup>Mt</sup>	131 <sup>Mt</sup>
b <sub>2</sub> - B.i.30%+D.g.35%+O.v.35%		13.64 <sup>oo</sup>	54.14 <sup>**</sup>	33.11	119 <sup>o</sup>
b <sub>3</sub> - D.g.30%+L.p.40%+M.s.20%+L.c.10%		14.47 <sup>o</sup>	45.10 <sup>oo</sup>	28.87 <sup>oo</sup>	152 <sup>**</sup>
b <sub>4</sub> - F.a.30%+D.g.20%+F.p.20%+M.s.20%+T.p.10%		16.51	47.07	29.41 <sup>oo</sup>	145 <sup>*</sup>
b <sub>5</sub> - F.p.45%+F.a.35%+T.p.20%		16.28	45.93 <sup>oo</sup>	27.07 <sup>ooo</sup>	154 <sup>***</sup>
DL	5%	1.62	3.00	2.18	10
	1%	2.45	4.55	3.31	14
	0.1%	3.93	7.31	5.31	23

In the conditions of 2019, the crude protein content of fodder decreased from 16.29 g/100 g d.s. for the *Dactylis glomerata* 20% + *Medicago sativa* 80% mixture (control) to 13.44 g / 100 g d.s. for *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% mixture. The difference between the control variant and the variant for which the lowest CP fodder content was obtained was of 2.85 g/100 g d.s., being negative, distinctly significant (Table 5).

Both in 2018 and 2019, the lowest fodder content of crude protein of 13.64 g / 100 g d.s. respectively 13.44 g / 100 g d.s. was achieved for the variant sown with the *Bromus inermis* 30% + *Dactylis glomerata* 20% + *Onobrychis viciifolia* 35% mixture.

Analysing the fodder NDF content, it is observed that the difference between the control variant (*Dactylis glomerata* 20% + *Medicago sativa* 80%) and the *Bromus inermis*

30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% mixture for which the highest fodder NDF content was obtained was of 4.39 g / 100 g d.s.

Compared to 2018, this mixture maintained the same significance compared to the control variant, the differences were positive distinctly significant.

From the analysis of the mixture influence on ADF fodder content, it is found that for the variant sown with *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20% was obtained the lowest content of ADF in the fodder, of 27.19 g / 100 g d.s., and the highest was recorded for the variant sown with the *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% mixture, of 32.82 g / 100 g d.s. The differences between the control variant - *Dactylis glomerata* 20% + *Medicago sativa* 80% and the variants sown with *Dactylis glomerata* 30% + *Lolium*

perenne 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10%, *Festuca arundinacea* 30% + *Dactylis glomerata* 20% + *Festuca pratensis* 20% + *Medicago sativa* 20% + *Trifolium pratense* 10% and *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20% were negative, significant and distinctly significant, with the exception of the variant sown with the *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% variant, where the difference did not have statistical significance.

Analysing the influence of the mixture on the relative forage quality (RFQ), it is observed

that the differences obtained were statistically assured. The difference between the control variant (*Dactylis glomerata* 20% + *Medicago sativa* 80%) and the variant in which the lowest value of the feed was obtained in RFQ (*Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35%), was 11 units. Compared to the control mixture, the other mixtures studied showed significant and distinctly significant differences in both years of experimentation (Table 5).

In 2018, the studied factors had a differentiated influence on the fodder content in crude protein, in NDF, ADF and RFQ.

Table 5. The influence of the used mixture on the fodder quality in 2019, to use as hay

Variant	Quality parameters				
	CP (g/ 100 g d.s.)	NDF (g/ 100 g d.s.)	ADF (g/ 100 g d.s.)	RFQ	
b <sub>1</sub> - D.g.20%+M.s.80%(mt)	16.29 <sup>Mt</sup>	49.24 <sup>Mt</sup>	32.57 <sup>Mt</sup>	131 <sup>Mt</sup>	
b <sub>2</sub> - B.i.30%+D.g.35%+O.v.35%	13.44 <sup>oo</sup>	53.63 <sup>**</sup>	32.82	120 <sup>o</sup>	
b <sub>3</sub> - D.g.30%+L.p.40%+M.s.20%+L.c.10%	14.62	46.82	29.21 <sup>o</sup>	146 <sup>**</sup>	
b <sub>4</sub> - F.a.30%+D.g.20%+F.p.20%+M.s.20%+T.p.10%	16.02	47.77	29.06 <sup>o</sup>	143 <sup>*</sup>	
b <sub>5</sub> - F.p.45%+F.a.35%+T.p.20%	15.90	46.99	27.19 <sup>oo</sup>	150 <sup>**</sup>	
DL	5%	1.82	2.79	2.38	10
	1%	2.76	4.22	3.60	15
	0.1%	4.44	6.78	5.79	24

Analysing the influence of the quantity of nitrogen on the crude protein content of each of the mixtures studied, in 2018, at harvest I, it is found that there are positive correlations statistically assured as significant. The exception was the mixture of *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%, where there is a negative correlation between the quantity of nitrogen and the crude protein content, the correlation coefficient was not statistically assured (Figure 3).

In 2019, at harvest I, it is found that between the quantity of nitrogen applied and the crude protein content, in most of the studied mixtures, there are statistically positive assured correlations being significant. For the mixtures *Dactylis glomerata* 20% + *Medicago sativa* 80% and *Dactylis glomerata* 30% + *Lolium perenne* 40% + *Medicago sativa* 20% + *Lotus corniculatus* 10% the correlation coefficients were not statistically assured (Figure 4).

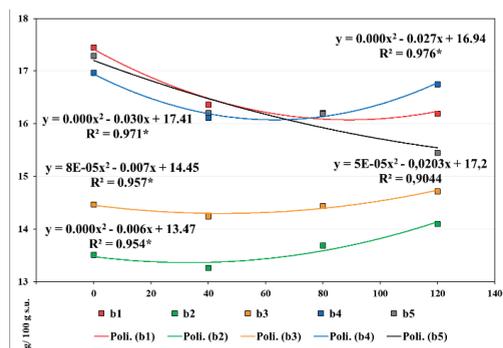


Figure 3. The correlation between the quantity of applied nitrogen and the CP content, at each of the studied mixtures, at the first harvest, in 2018, to use as hay

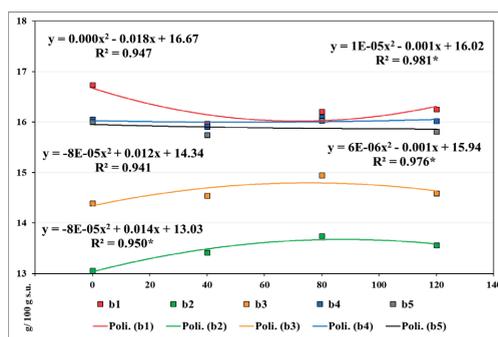


Figure 4. The correlation between the quantity of applied nitrogen and the CP content, at each of the studied mixtures, at the harvest scythe, in 2019, to use as hay

Both in 2018 and in 2019, at harvest I, it is found that there are positive correlations between the quantity of nitrogen applied and the NDF content, the correlation coefficients being statistically assured and interpreted as significant and distinctly significant (Figures 5 and 6).

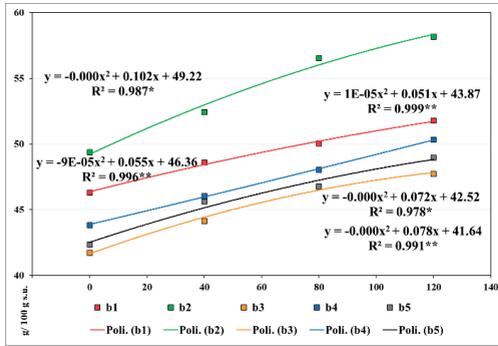


Figure 5. The correlation between the harvest of applied nitrogen and the NDF content, at each of the studied mixtures, at the first harvest, in 2018, to use as hay

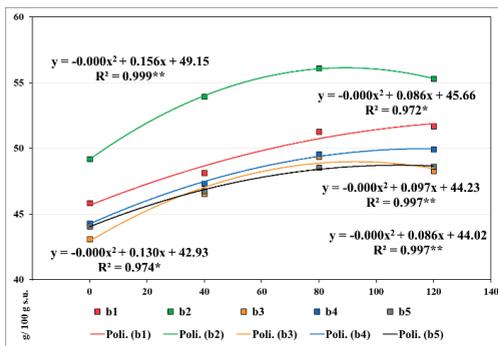


Figure 6. The correlation between the quantity of applied nitrogen and the NDF content, at each of the studied mixtures, at the first harvest, in 2019, to use as hay

Between the quantity of nitrogen applied and the ADF content, in each of the studied mixtures, in the two years of experimentation there is a direct correlation, the coefficients were statistically assured and interpreted as significant and distinctly significant (Figures 7 and 8).

The correlation between the quantity of nitrogen applied and the RFQ was analysed, in each of the mixtures studied, in 2018, at harvest I, it is found that there are negative correlations, and the correlation coefficients were not statistically assured (Figure 9).

In the second year of exploitation, 2019, it was found that between the quantity of applied nitrogen and RFQ for the studied mixtures, there are statistically significant, significant and distinctly significant negative correlations (Figure 10).

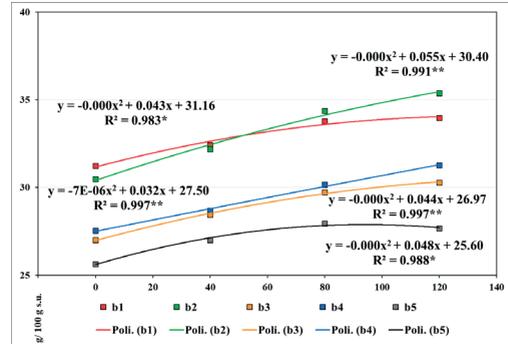


Figure 7. The correlation between the quantity of applied nitrogen and the ADF content, at each of the studied mixtures, at the harvest scythe, in 2018, to use as hay

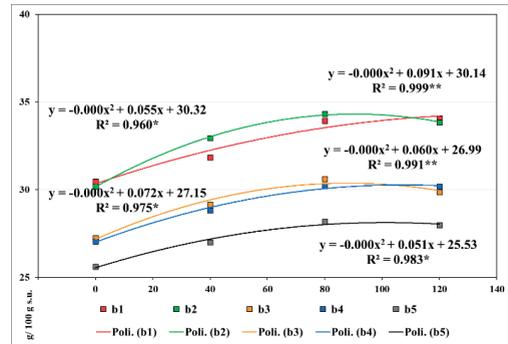


Figure 8. The correlation between the quantity of applied nitrogen and the ADF content, at each of the studied mixtures, at the first harvest, in 2019, to use as hay

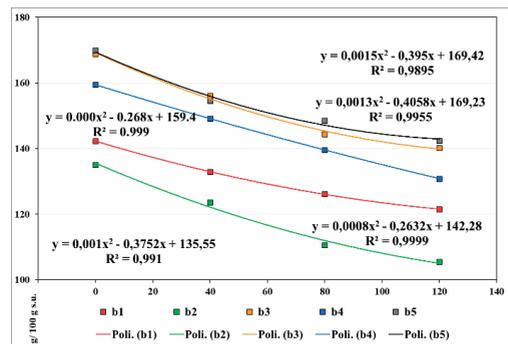


Figure 9. The correlation between the quantity of applied nitrogen and the RFQ content, at each of the studied mixtures, at the first harvest, in 2018, to use as hay

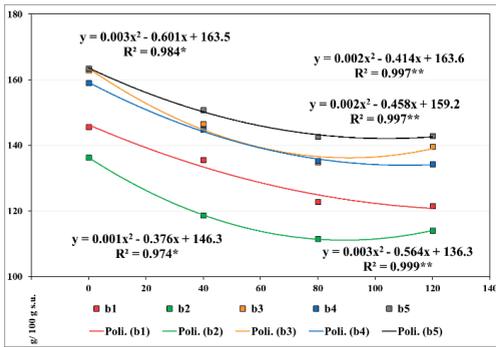


Figure 10. The correlation between the quantity of applied nitrogen and the RFQ content, at each of the studied mixtures, at the first harvest, in 2019, to use as hay

## CONCLUSIONS

During the years of experimentation, for the mixtures used as hay, three harvest were performed, and the exploitation period was 143 days.

The interaction between the mixtures of perennial grasses and legumes and fertilization, in 2018, shows that the mixture with *Bromus inermis* 30% + *Dactylis glomerata* 35% + *Onobrychis viciifolia* 35% recorded the highest yields, with a maximum of 28.01 t/ha d.s. when being fertilized with N<sub>80</sub>P<sub>40</sub>.

The quality of the fodder obtained from the temporary grasslands was influenced by the doses of fertilizers applied, by the proportion of participation of the species in the sowing norm, but also by the climatic conditions during the exploitation period.

In 2018, as well as in 2019, the best relative forage quality RFQ was registered in the mixture *Festuca pratensis* 45% + *Festuca arundinacea* 35% + *Trifolium pratense* 20%, of 170 units, respectively 163 units, belonging to the quality class 0 (after Hancock DW, 2011).

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## EFFECTS OF AGROTECHNICAL MEASURES ON WEED DYNAMICS AND WATER BALANCE IN SOIL FOR DIFFERENT CROPS

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### Abstract

*In conditions of the modernization of agriculture, knowledge about the weeds can influence the production are required. The researches were performed during the 2018-2021, in the experimental field of NARDI Fundulea and aimed to study the influence of agrotechnical practices on the weeds and water balance in soil. The paper presents the results obtained in long term experiences with fertilizers, soil tillages and crop rotations. The crops showed an infestation with monocotyledonous and dicotyledonous weeds, represented as follows: weed wheat crop 27%, weed maize crop 80%. Weeding sources and the number of weeds are higher in the soils where the manure is administered, it is not herbicided and for the seed in uncultivated land. The dynamics of the soil moisture reserve was correlated with the precipitation regime and the water consumption of the plants. The reporting values for determining the soil moisture were as follows: Field capacity - 4,391 m.c./ha; Withering coefficient - 2,132 m.c./ha. Monthly determinations of soil moisture revealed atypical values of humidity at a depth of 0-75 cm, finding a variable amount of water.*

**Key words:** wheat, maize, weed dynamics, water balance, soil.

### INTRODUCTION

Romania has agricultural areas facing increasingly severe drought, with insufficient water resources for crops and the restoration of groundwater reserves, which threatens food security, economic development and local quality of life.

Water stress has a significant effect on water consumption and corn and wheat yields. A positive linear relationship between water production and use has been established in many studies (Fatih et al., 2008; Istanbuluoglu et al., 2002; Bouazzama et al., 2012).

Soil water conservation is directly related to all the phenomena of penetration, circulation, retention, use and loss. By agrotechnical methods can be directly or indirectly influenced, one or more components of the water regime, so as to bring it as close as possible to the requirements of plants for water. Soil water dynamics is influenced by climatic conditions, applied technological links and the consumption of the crop plant (Popescu and Bucur, 1999; Sin and Ionita, 1997). The study of weeding of agricultural crops is a means of

knowing the structure of the plant flora, in order to establish measures to control weeds and prevent the increase in the number of weeds that may appear on agricultural land. The causes of weeding, its seasonal, annual and multiannual dynamics are a matter of interest in agriculture.

The sources of weeding of agricultural crops are given by the existing weed seed reserve in the arable soil layer, by the uncultivated areas, by the heads of the unsown plots and by the unconditioned seed used for sowing the agricultural crops. Thus, the total elimination of weeds from agricultural lands is impossible and, for ecological reasons, it is not desirable. Because of this, "thresholds" of damage have been established, up to which the number of weeds in a species does not cause damage that would justify the control (Zanin et al., 1994; Ionescu, 2010).

The reduced competition of crop plants in the fight against weeds, especially in the first phenophases of growth (Wilson, 1988), requires specific research on the causes of weeds, weed species and the possibilities to control them (Berca, 2008; Maxwell et al.,

2007; Partal et al., 2017; Serban and Maturaru, 2019).

Climate change can have various effects on agricultural production, depending on the type of soil and the technological measures applied in agricultural crops (Moss et al., 2010; Paraschivu et al., 2017).

## MATERIALS AND METHODS

The tests were performed between 2019 and 2021, on a chernozem cambic soil specific for southern Romania, in a long-term experience at NARDI Fundulea.

Regarding the physical characteristics of the soil, the humus content is higher in the first 15 cm due to the former bedding and gradually decreases to depth. The soil consists of several horizons:

- Ap+Aph - 0-30 cm, clay-clay-dust with 36.5% clay and permeability 492, pH 5.9.

- Am - 30-45 cm, clay-clay with 37.3% clay, compacted, DA 1.41g/cm<sup>3</sup>, pH 5.9.

- A/B (45-62 cm), Bv1 (62-80 cm), Bv2 (82-112 cm), Cnk1 (149-170 cm), Cnk2 (170-200 cm).

Depending on the agricultural year, the water supply of the soil is favorable for field crops, groundwater at 10-12 meters.

Experimental factors included A- crop rotation with 4 graduations: monoculture, rotation of 2, rotation of 3 and rotation of 4 years; B - soil work with 3 graduates: no-tillage, disk tillage and autumn plowing; C - fertilization with 3 graduates: unfertilized, N<sub>90</sub>P<sub>75</sub>, manure 20 t/ha applied at 4 years.

The size of the plot was 56.0 m<sup>2</sup> (4 rows x 20 m long x 70 cm distance between rows).

Regarding the weed dynamics, counts and determinations were performed in different vegetation phases of the crop plants.

Regarding the dynamics of the soil moisture reserve, it was correlated with the precipitation regime and with the water consumption of the plants. During the vegetation, from sowing to harvesting, soil samples were collected at a depth of 1.25 m to determine the state of humidity. The values taken into account when determining the soil moisture were the following: Field capacity - 4391 m.c./ha; Withering coefficient - 2132 m.c./ha; Minimum ceiling - 3264 m.c./ha.

The principle of the method of determining the soil moisture: the soil is dried at a temperature of 105°C to a constant weight and then weighed. The difference in weight obtained before and after drying, represents the humidity that is expressed as a percentage (%).

Materials used: termoadjustable drying oven; analytical balance; weighing ampoules; dryer.

The experimental data obtained were presented in the form of tables and graphs.

In order to reduce the weeding of crops and the best possible conservation of water in the soil, it is necessary to apply differentiated agrotechnical practices throughout the vegetation period in correlation with technological inputs.

## RESULTS AND DISCUSSIONS

### Climatic aspects

In 2019, the precipitations recorded the lowest values, of 6.2 mm in September, compared to the multiannual average of 48.5 mm and in August by 12.6 mm compared to the multiannual average of 49.7 mm. The greatest amount of precipitation occurred in July, with 87.4 mm, about 16.3 mm above the multiannual average. Regarding the thermal regime, in the period June-September, the recorded values show that the average monthly temperatures were higher than the multiannual average, in June by 2.8°C above the multiannual average.

The year 2020 was an extremely dry one, with an accentuated water deficit and high temperatures, compared to the multiannual average. The months with the lowest rainfall were 5.4 mm compared to 49.7 mm on average and July with 34.2 mm compared to 71.1 mm on average. The deficit of precipitation influenced the weeding of crops and the dynamics of water in the soil. The average temperatures recorded in the agricultural year 2020 were 13.5°C, compared to the multiannual average of 10.9°C, with an increase of 2.6°C.

The 2021 was a normal year in terms of water levels, but with an uneven distribution of rainfall, especially in July, August and September. Temperatures recorded an annual average of 12.1°C and a difference of 1.2°C from the multiannual average. The rainfall here averages 584.3 mm. In 2019, the amount of

precipitation was 42.9 mm lower than the multiannual average, in 2020 by 161.1 mm and in 2021 by 31.1 mm. The climatic data

obtained were corroborated with the elements followed during the vegetation period of the crops (Table 1).

Table 1. The meteorological parameters in the experimental period (Fundulea, 2019-2021)

Years/Months	Jan	Febr	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	Total/ Average	
Precipitations (mm)	2019	53.8	21.4	22.4	51.4	124	74.6	87.4	12.6	6.2	38.2	33.2	16.2	541.4
	2020	2.0	16.6	29.8	14.0	57.8	68.4	34.2	5.4	68.6	24.0	20.0	77.6	423.2
	2021	77.0	16.2	59.0	31.0	57.6	135	21.2	24.2	4.0	56.4	33.8	37.6	553.2
<b>50 years average</b>	<b>35.1</b>	<b>32.0</b>	<b>37.4</b>	<b>45.1</b>	<b>62.5</b>	<b>74.9</b>	<b>71.1</b>	<b>49.7</b>	<b>48.5</b>	<b>42.3</b>	<b>42.0</b>	<b>43.7</b>	<b>584.3</b>	
Temperatures (°C)	2019	-1.1	3.8	9.3	11.2	17.2	23.6	22.9	24.7	19.3	12.0	11.0	4.0	13.2
	2020	0.9	5.2	8.3	12.4	16.8	21.8	25.1	25.5	20.8	12.8	6.2	4.0	13.5
	2021	1.6	3.2	5.1	9.7	17.2	21.1	25.3	24.2	17.3	10.2	7.7	2.6	12.1
<b>50 years average</b>	<b>-2.4</b>	<b>-0.4</b>	<b>4.9</b>	<b>11.3</b>	<b>17.0</b>	<b>20.8</b>	<b>22.7</b>	<b>22.3</b>	<b>17.3</b>	<b>11.3</b>	<b>5.4</b>	<b>0.1</b>	<b>10.9</b>	

### Weed dynamics

The maize crop was infested with weeds in a proportion of approximately 90%, represented as follows: annual and perennial monocotyledons - 46%; dicotyledonous - 42%; other insignificant weeds - 2% (Figure 1).

Of the total of 46% of the annual and perennial monocotyledonous weeds, the following species are part of the component:

*Setaria* spp. - 25%;

*Echinochloa crus-galli* - 12%;

*Shorghum halepense* - 9%;

Of the total of 42% of the dicotyledonous weeds, the following weeds are part:

*Amaranthus retroflexus* - 19%;

*Chenopodium album* - 15%;

*Xanthium strumarium* - 8%.

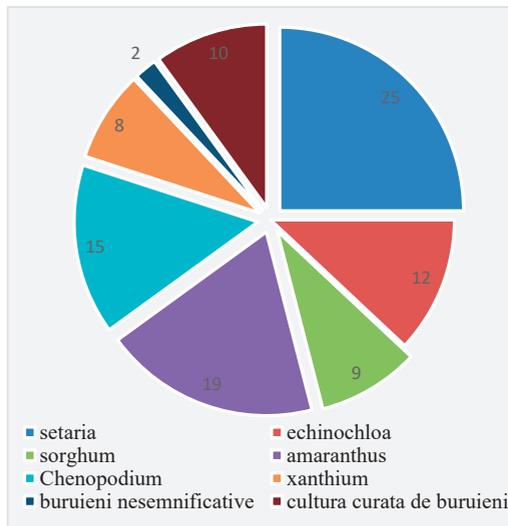


Figure 1. The presence of weeds in maize crop - expressed as a percentage in 2020

For maize, one of the advantages of combining crop rotation and tillage is to control most weed species and significantly reduce the supply of weed seeds in the soil.

In 2020, the associated variant A4B3 - 4 year rotation + autumn plowing led to the appearance of a number of 52 weeds/m<sup>2</sup> for the non-fertilized and fertilized version with N<sub>90</sub>P<sub>75</sub> and for 53 weeds/m<sup>2</sup> for the version with manure application 20 t/ha (Table 2).

The application of the basic works of the soil with the disc achieves a more efficient control of the weeds in comparison with the no-tillage one, regardless of the technological combinations made, but less efficient compared to the variant of the autumn plowing of the soil. Thus, the association with fertilization with manure 20 t/ha, led to the appearance of a number of weeds between 56-118 weeds/m<sup>2</sup> depending on the crop rotation applied. The lowest number of weeds was recorded in the A4B2C3 variant with 56 weeds/m<sup>2</sup>.

Crop rotation has fluctuated depending on the technological combination applied. Thus, the monoculture variant achieved the lowest weed control rate, with 170 weeds/m<sup>2</sup> in the variant associated with no-tillage and unfertilized.

The 3-year crop rotation registered the lowest number of weeds when associated with the basic work of plowing through autumn plowing and the application of manure 20 t/ha, with 77 weeds/m<sup>2</sup>.

The appearance of such a large number of weeds in the variants where fertilization with manure was applied 20 t/ha, regardless of the associations with crop rotation or soil work, is due to the very high content of viable seeds present in the manure.

Thus, in the experience was recorded a number between 118-162 weeds/m<sup>2</sup> for the monoculture variant, between 78-100 weeds/m<sup>2</sup>

for the 2-year rotation variant, between 77 - 102 weeds/m<sup>2</sup> for the 3-year rotation variant and between 53-63 4-year rotation weeds.

Table 2. The number of weeds depending on the technological combination applied to the maize crop - 2020

Variants		C1		C2		C3	
		Weeds*	%	Weeds*	%	Weeds *	%
A1	B1	170	100	170	100	162	100
	B2	127	74.7	127	74.1	118	73
	B3	146	85.9	146	85.2	130	80
A2	B1	80	100.0	80	100	100	100
	B2	65	81.2	65	73.0	78	78
	B3	75	93.7	75	85.7	86	86
A3	B1	110	100	108	100	102	100
	B2	80	72.7	80	74.0	88	86
	B3	110	100	72	66.6	77	75
A4	B1	59	100	59	100	63	100
	B2	54	91.5	54	84.8	56	88
	B3	52	88.1	52	93.5	53	84

\*number of weeds/m<sup>2</sup> - average

In the agricultural year 2021, for maize cultivation, the number of weeds/m<sup>2</sup> registered significant variations depending on the applied technological variant. Thus, the highest number of weeds/m<sup>3</sup> was registered in the technological variant A1B1C1 - monoculture + no-tillage + unfertilized with 181 weeds/m<sup>2</sup>. The technological variant with the lowest number of weeds was the associated A4B3C2 - 4 years rotation + autumn plowing + N<sub>90</sub>P<sub>75</sub> fertilization, with 40 weeds/m<sup>2</sup> (Table 3). Manure application 20 t/ha recorded the lowest number of weeds in association with 4 years

crop rotation + autumn plowing, with 65 weeds/m<sup>2</sup>, followed by association of 4 years rotation + disc, with 67 weeds/m<sup>2</sup>. The application of fertilization has registered notable variations depending on the technological associations. Thus, compared to manure fertilization 20 t/ha, the variant fertilized with N<sub>90</sub>P<sub>75</sub> registered a smaller number of weeds, regardless of the technological variant or the grading of factors such as crop rotation and tillage, with values between 44-126 weeds/m<sup>2</sup> as an average of the associated variants.

Table 3. The number of weeds depending on the technological combination applied to the maize crop - 2021

Variant		C1		C2		C3	
		Weeds*	%	Weeds*	%	Weeds *	%
A1	B1	181	100	150	100	170	100
	B2	135	75	110	73	135	79
	B3	150	83	120	80	125	73
A2	B1	90	100	70	100	110	100
	B2	77	85	50	71	92	84
	B3	85	94	58	83	85	77
A3	B1	110	100	90	101	109	100
	B2	109	99	70	78	95	87
	B3	90	82	70	78	85	78
A4	B1	70	100	48	100	73	100
	B2	65	93	45	93	67	91
	B3	62	88	40	83	65	89

\*number of weeds/m<sup>2</sup> - average

The sunflower crop showed an infestation with weed species in the proportion of about 80%, and these were represented as follows: annual and perennial monocotyledonous weeds - 36%;

dicotyledonous weeds - 42%; other insignificant weeds - 2%. Of the total percentage of 36% of annual and perennial monocotyledonous weeds, the most

representative are the following weeds: *Setaria spp.* - 13%; *Echinochloa crus-galli* - 8%; *Shorghum halepense* - 15%, and of the total of 42% of dicotyledonous weeds the following representative weeds are part: *Amaranthus retroflexus* - 19%; *Cirsium arvense* - 15%; *Xanthium strumarium* - 8% (Figure 2).

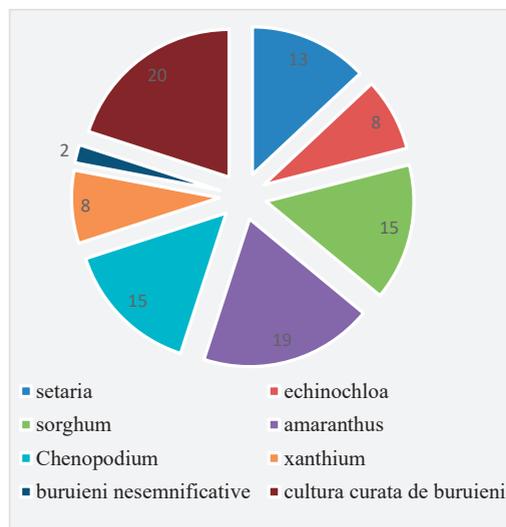


Figure 2. The presence of weeds in sunflower crop - expressed as a percentage - 2020

The weeds that recorded the largest presence in the culture were: *Shorghum halepense* and *Amaranthus retroflexus*.

In the sunflower crop, in 2019, the association of the 4-year rotation + tillage by autumn plowing led to the appearance of a number of 61 weeds/m<sup>2</sup> for the non-fertilized version, for 31 weeds/m<sup>2</sup> for the version fertilized with N<sub>90</sub>P<sub>75</sub> and for 40 weeds/m<sup>2</sup> for the fertilized version with manure 20 t/ha. The application of the basic works of the soil with the disc achieves a more efficient control of the weeds in comparison with no-tillage one, regardless of the technological combinations made, but less efficient compared to the variant of the autumn plowing of the soil. Thus, the association with manure fertilization led to a number of 41 weeds/m<sup>2</sup> at 4-year rotation and 52 weeds/m<sup>2</sup> at 2-year rotation (Table 4).

The uncultivated variant registers a high number of weed species and implicitly a large number of weeds of each species, compared to the other tillage variants. Thus, associated with non-fertilization and monoculture, a number of 150 weeds/m<sup>2</sup> was registered, as an average of the experiments. In the variant associated with N<sub>90</sub>P<sub>75</sub> fertilization, the number of weeds dropped to 125/m<sup>2</sup>.

Table 4. The number of weeds depending on the technological combination applied to sunflower crop - 2019

Variant	C1		C2		C3		
	Weeds*	%	Weeds*	%	Weeds*	%	
A1	B1	150	100	125	100	155	100
	B2	120	80.0	101	80.8	118	76.0
	B3	102	68.0	88	70.4	105	68.0
A2	B1	70	100	52	100	61	100
	B2	61	87.1	43	82.7	52	85.0
	B3	52	74.3	35	67.3	44	72.0
A3	B1	100	100	74	100	80	100
	B2	91	91.0	66	89.2	73	91.0
	B3	70	70.0	56	75.6	59	73.0
A4	B1	66	100	40	100	56	100
	B2	61	92.4	38	95.0	41	73.0
	B3	58	63.7	31	77.5	40	71.0

\*number of weeds/m<sup>2</sup> – average

In 2020, in the sunflower crop there were variations in the number of weeds depending on the graduations of the experimental factors, of 68.0-102.0%. Thus, the lowest number of weeds was registered in the variant associated with the 4-years rotation + autumn plowing + fertilization with N<sub>90</sub>P<sub>75</sub>, with 42 weeds/m<sup>2</sup>, followed by the variant with 2-years rotation +

autumn plowing + fertilization with N<sub>90</sub>P<sub>75</sub>, with 46 weeds/m<sup>2</sup> (Table 5). The no-tillage variant registered values of the number of weeds very high, regardless of the association with other factors, between 135-165 weeds/m<sup>2</sup>. The application of manure 20 t/ha led to the appearance of a number of 51 weeds/m<sup>2</sup> at the association with the rotation of 4 years +

autumn plowing and of 59 weeds/m<sup>2</sup> at the variant with the rotation of 2 years + autumn plowing. The variation of weeds is high due to

the low competition capacity of the crop plants and due to the monocotyledons which have a high capacity for twinning and growth.

Table 5. The number of weeds depending on the technological combination applied to the sunflower crop - 2020

Varianta		C1		C2		C3	
		Weeds*	%	Weeds*	%	Weeds*	%
A1	B1	165	100	135	100	142	100
	B2	117	71.0	115	85.2	115	81.0
	B3	117	71.0	100	74.0	96	68.0
A2	B1	80	100	63	100	71	100
	B2	71	88.7	54	85.7	62	87.0
	B3	62	77.5	46	73.0	59	83.0
A3	B1	109	100	85	100	89	100
	B2	102	93.5	88	103	80	89.0
	B3	80	73.4	68	80.0	73	82.0
A4	B1	76	100	51	100	78	100
	B2	70	92.1	49	96.1	60	76.0
	B3	68	89.5	42	82.4	51	70.0

\* number of weeds / m<sup>2</sup> – average

### Water balance in soil

The soil samples taken in order to determine the soil moisture highlight the climatic character of the agricultural year and establish the influence on field crops.

Soil moisture indicates the reserve with respect to the wilting coefficient, the deficit with respect to the field capacity and the water reserve with respect to the minimum ceiling.

Soil water, between the withering coefficient (CW) and the water capacity of the soil in the field (FC), is the water accessible to plants. The field capacity is the water content of the soil at the maximum saturation point and the cessation of the downward movement of the water; the

wilting coefficient (wilting point) is the minimum humidity that the plant needs in order not to wither.

The humidity determinations performed as an average in 2019, showed normal values of humidity in the case of all variants, on the depth of 0-75 cm, finding a satisfactory amount of water, which is made available to the plants. These humidity values are lower in the 75-125 cm layer, but remain constant. The soil samples taken show us a reserve compared to the wilting coefficient of 1367.2 m<sup>3</sup>/ha, a deficit compared to the field capacity of 891.8 m<sup>3</sup>/ha and a water reserve compared to the minimum ceiling of 235.2 m<sup>3</sup>/ha (Table 6).

Table 6. Determination of soil moisture in spring crops - average in 2019

Depth (cm)	Bulk density (g/cm <sup>3</sup> )	Momentary water reserve		Coefficient withering CW (m <sup>3</sup> /ha)	Field capacity FC (m <sup>3</sup> /ha)	Reserve by CW (m <sup>3</sup> /ha)	Deficit from FC (m <sup>3</sup> /ha)	Minimum ceiling MC (m <sup>3</sup> /ha)	Reserve by MC (m <sup>3</sup> /ha)
		%	m <sup>3</sup> /ha						
0 - 25	1.33	21	698.3	405	884	293.3	-185.8	645	53.3
25 -50	1.38	20.9	721.1	464	929	257.1	-208.0	697	24.0
50 -75	1.43	20.5	732.9	469	928	263.9	-195.1	699	33.9
75 -100	1.36	20.4	693.6	426	837	267.6	-143.4	632	61.6
100 -125	1.32	19.8	653.4	368	813	285.4	-159.6	591	62.4
TOTAL	-	-	<b>3499.2</b>	2132	4391	<b>1367.2</b>	<b>-891.8</b>	3264	<b>235.2</b>

Humidity determinations calculated as an average of 2020, showed low values of humidity in all variants, on the whole depth of 0-125 cm.

The soil samples taken do not show a reserve of the wilting coefficient of 965.2 m<sup>3</sup>/ha, a deficit

compared to the field capacity of -1293.8 m<sup>3</sup>/ha and a negative water reserve compared to the minimum ceiling of -166.8 m<sup>3</sup>/ha. The water reserve compared to the minimum ceiling registered negative values and made the vegetation of the plants difficult in March,

April, August, September and October. The deficit in field capacity registered negative values in each month of 2020, thus showing the non-uniformity of the quantities of water

accumulated in the soil. The determination of soil moisture highlighted the unfavorable nature of the agricultural year 2020 for field crops (Table 7).

Table 7. Determination of soil moisture in spring crops - average in 2020

Depth (cm)	Bulk density (g/cm <sup>3</sup> )	Momentary water reserve		Coefficient withering CW (m <sup>3</sup> /ha)	Field capacity FC (m <sup>3</sup> /ha)	Reserve by CW (m <sup>3</sup> /ha)	Deficit from FC (m <sup>3</sup> /ha)	Minimum ceiling MC (m <sup>3</sup> /ha)	Reserve by MC (m <sup>3</sup> /ha)
		%	m <sup>3</sup> /ha						
0 - 25	1.33	16.2	538.7	405	884	133.7	-345.4	645	-106.4
25 -50	1.38	22.0	759.0	464	929	295.0	-170.0	697	62.0
50 -75	1.43	19.4	693.6	469	928	224.6	-234.5	699	-5.5
75 -100	1.36	17.0	578.0	426	837	152.0	-259.0	632	-54.0
100 -125	1.32	16.0	528.0	368	813	160.0	-285.0	591	-63.0
<b>TOTAL</b>	-	-	<b>3097.2</b>	2132	4391	<b>965.2</b>	<b>-1293.8</b>	3264	<b>-166.8</b>

The humidity determinations performed on average in 2021, showed satisfactory values of humidity in all variants, on a depth of 0-75 cm, finding a larger amount of water, which is made available to plants. These humidity values are lower in the 75-125 cm layer, but

remain constant. The soil samples taken show a reserve with respect to the wilting coefficient of 1389.1 m<sup>3</sup>/ha, a deficit with respect to the field capacity of 869.9 m<sup>3</sup>/ha and a water reserve with respect to the total minimum ceiling of 257.1 m<sup>3</sup>/ha (Table 8).

Table 8. Determination of soil moisture in spring crops - average in 2021

Depth (cm)	Bulk density (g/cm <sup>3</sup> )	Momentary water reserve		Coefficient withering CW (m <sup>3</sup> /ha)	Field capacity FC (m <sup>3</sup> /ha)	Reserve by CW (m <sup>3</sup> /ha)	Deficit from FC (m <sup>3</sup> /ha)	Minimum ceiling MC (m <sup>3</sup> /ha)	Reserve by MC (m <sup>3</sup> /ha)
		%	m <sup>3</sup> /ha						
0 - 25	1.33	19.9	661.7	405	884	256.7	-222.3	645	16.7
25 -50	1.38	20.8	717.6	464	929	253.6	-211.4	697	20.6
50 -75	1.43	21.2	757.9	469	928	288.9	-170.1	699	58.9
75 -100	1.36	21.0	714.0	426	837	288.0	-123.0	632	82.0
100 -125	1.32	20.3	669.9	368	813	301.9	-143.1	591	78.9
<b>TOTAL</b>	-	-	<b>3521.1</b>	2132	4391	<b>1389.1</b>	<b>-869.9</b>	3264	<b>257.1</b>

The determinations regarding the soil moisture, carried out on an annual average, showed different values depending on the studied variants, on the depth of 0-75 cm, finding a higher amount of water, which is made available to the plants, and in the layer 75-125 cm, the values are lower but remain constant. From the analysis of the soil moisture values it was found that the water reserves from the spring are a decisive indicator in the adaptation of the agrotechnical measures to the annual particularities of the climatic conditions.

## CONCLUSIONS

The reduction of the total weeding up to the limit of excluding the competition for the crop plants can be achieved by observing the 3-4

years crop rotation associated with the execution of the basic soil works by autumn plowing and fertilization with N<sub>90</sub>P<sub>75</sub> or the application of manure 20 t/ha to 4 years. Crop rotation contributes to a decrease in the number of weeds/m<sup>2</sup> by up to 25-30%.

For the efficient conservation of the water from the soil, it is necessary to apply differentiated agrotechnical practices throughout the vegetation period in correlation with the technological contributions. Among the agrotechnical methods of water conservation in the soil we mention: the practice of crop rotation for 3-4 years, the execution of soil tillages in the optimal working interval, avoiding the mobilization of the soil at greater depths than necessary, both for basic tillages and preparation of germination bed.

The dynamics of water in the soil is directly proportional to the amount of precipitation and the vegetation phase of the crop plant, which specifies the level of consumption of the plant. The basic tillages of the soil by autumn plowing will be carried out by alternation, at 3-4 years with the disk tillage, taking into account the advantages it brings to the soil, and last but not least to the productivity of crops in the two systems of tillages.

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## THE IMPACT OF DIFFERENT TILLAGE SYSTEMS ON THE QUALITATIVE INDICATORS OF SOWING WORKS AND WHEAT CULTURAL DEVELOPERS

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### Abstract

Soil tillage methods have complex effects on the physical, chemical and biological properties of the soil with the influence on the crop. In the paper it was proposed to study the effect that agricultural works for the preparation of the germination bed have on the work of sowing wheat or the degree of sunrise. The studies started in the southwestern part of Olt County, on an area of 4.5 hectares divided into plots of 0.5 hectares. Germination bed preparation works were carried out on each plot as follows: conventional works for autumn sowing, land covered with mowed vegetable waste (TAV), reduced work using heavy disc harrows on land covered with plant debris (LDG), work with harrow with vertical rotors. (LGR). After the execution of the works for each plot, the soil moisture, the degree of crushing, the degree of compaction, the uniformity of the incorporation depth of the seeds were determined. The study will be continued by tracking the crop throughout the growing season, including crop production.

**Key words:** soil tillage methods, TAV, LDG, LGR.

### INTRODUCTION

Soil tillage methods have complex effects on the physical, chemical and biological properties of the soil. Due to changes in the physical and chemical properties of the soil through tillage methods, the biological properties of the soil can also change. These changes are indirect results of tillage. The modified physical and chemical properties of the soil through soil tillage methods affect parameters directly related to soil microbial activities such as organic matter, soil moisture, temperature and ventilation, as well as the degree of interaction between soil minerals and organic matter. As a result of these effects, significant differences can be observed in the population of soil microbial activities (Wardle, 1995; Lavelle, 2000; Kladviko, 2001; Sagar et al., 2001).

In this study, the effects of three different tillage systems on the degree of plant emergence were investigated.

### MATERIALS AND METHODS

Land area of 4.5 hectares  
CLASS CELTIS 446 RC Tractor  
Plug PP 3-30 + star harrow type GS-1,2

V3 type heavy disc harrow  
GRC type 3 rotary harrow  
SUP seed drills 29  
Wheat GLOSA variety with MMB = 43 g  
Humidometer type Delta-T Devices Hh2  
Moisture Meter  
Penetrometer type Fieldscout SC900  
Three granulometric sieves with a diameter of  
25 mm orifices; 50 mm and 100 mm  
respectively, a frame with an area of 1 m<sup>2</sup>  
presented below:



### Field of study

This study was carried out on a soil in the southern part of Olt County on an area of 4.5 hectares which is an agricultural company. According to the soil taxonomy classification system, the soils are chernozem zonal, whose properties are presented in table no.1 Soils are simple and almost simple, inclined 0 + 1% and deeply profiled, located on very old alluvial subsoil.

### Climate conditions

The land surface, which is in the temperate-transitional continental climate zone, has a mild winter with frosty days and nights, along with climate change, slightly humid. Free from rain and snow a dry and hot summer climate.

According to the average climate data of 30 years, the average annual temperature is 19.1°C. The field work was carried out on 1.5 hectares sub-plots on which different methods of tillage were applied. Three tillage methods were established and each tillage method was replicated three times, yielding 18 subplots. Each plot was 12 m wide and 40 m long, covering an area of 480 m.

### Soil tillage methods

Three different tillage methods were applied in this study. These methods were conventional soil tillage with residues (TAV), reduced heavy tillage (LDG) tillage, low tillage with rotary rotor harrow (LDR). These methods have been replicated three times. Details of the tillage operations applied for Glosa winter wheat are given in Table 2.

Prior to the determination of the qualitative work and energy indices, the soil parameters and the conditions under which the experiments were carried out were determined, respectively the resistance of the soil to penetration, the degree of crushing and the soil moisture.

Table 1 shows the soil moisture values at 3 test points, at a depth of 30 cm, maximum working depth.

To determine the degree of compaction of the soil, the resistance to penetration was determined using a penetrometer provided with

a rod with a penetration cone of 1 cm<sup>2</sup> and 60° the top angle to which the rod was attached to the cone by screwing and mounting them to the "data logger".

The measurements were performed to a depth of 30 cm, at an average humidity of 12.36%.

The distribution of the forces of resistance to the penetration of the cone in the soil layers, in kPa measured in 5 test points, is presented in Table 2.

Table 1. Measured soil moisture values

Measuring depth, cm	Humidity value, %
10	12.50
20	12.80
30	11.80
<b>Average</b>	<b>12.36</b>

Table 2. Determined values for penetration resistance

Measuring depth, cm	No. test/Resistance to penetration, kPa					
	1	2	3	4	5	Mediated
10	75	95	125	115	105	103
20.0	210	350	455	350	312	335
30.0	512	650	680	775	665	656

Soil resistance to penetration is classified according to the I.C.P.A. methodology, middle class (260-500) kPa.

The SUP 29 seed drill has been set for 270 kg/hectare, respectively position C-18 in the Northon box, and the distance between rows of 12.5 cm. At the sowing test, the grains were counted for 1 m<sup>2</sup> of sowing, the result being 558-560 seeds/m<sup>2</sup>.

## RESULTS AND DISCUSSIONS

### Degree of soil shredding

To determine the degree of crushing of the soil, three sieves with a diameter of 25 mm were used; 50 mm and 100 mm, respectively, a frame with an area of 1 m<sup>2</sup>, and a food dynamometer for weighing.

By size fractions, for each plot on which the experiments were performed it is presented in Table 3.

Table 3 Degree of soil shredding

Degree of crushing	Agricultural work	Value, %		
		Fractions below 25 mm	Fractions between 25-50 mm	Fractions over 50 mm
Subplot no. 1	<ul style="list-style-type: none"> <li>• Chopping vegetable waste</li> <li>• Plowing 28 cm + harrowing</li> <li>• Sowing wheat to a depth of 4 cm</li> </ul>	71	17	12
Subplot no. 2	<ul style="list-style-type: none"> <li>• Chopping vegetable waste</li> <li>• Disc with heavy disc harrow 16-18 cm (2 times)</li> <li>• Sowing wheat to a depth of 4 cm</li> </ul>	76	15	9
Subplot no. 3	<ul style="list-style-type: none"> <li>• Chopping vegetable waste</li> <li>• Processed soil with a rotary harrow 16-18 cm</li> <li>• Sowing wheat to a depth of 4 cm</li> </ul>	82	16	2

### The establishment of culture

The SUP 29 sowing machine was regained for the amount of 270 kg/hectare, respectively position C-18 in the Northon box, and the distance between rows of 12.5 cm.

At the sowing test, the seeds were counted for 1 m<sup>2</sup> of sowing, the result being 558-560 seeds/m<sup>2</sup>.

### Land preparation before sowing



Figure 1. Disc with heavy disc harrow 16-18 cm (2 times)



Figure 2. Chopping vegetable waste Plowing 28 cm + harrowing



Figure 3. Processed soil with a rotary harrow 16-18 cm

### Determination of the degree of emergence

It was done by counting the plants grown on an area of 1 m<sup>2</sup>.



Figure 4. Sunrise after 10 days



Figure 5. Sunrise after 10 days



Figure 6. Sunrise after 10 days

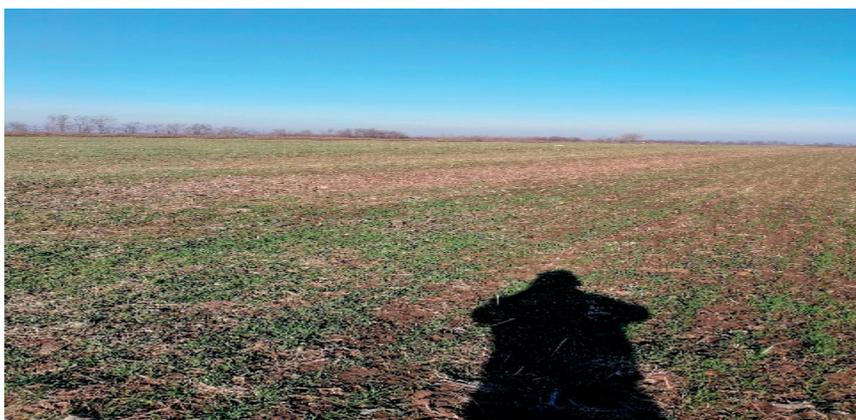


Figure 7. Sunrise after 10 days General view for the surface of 4.5 ha

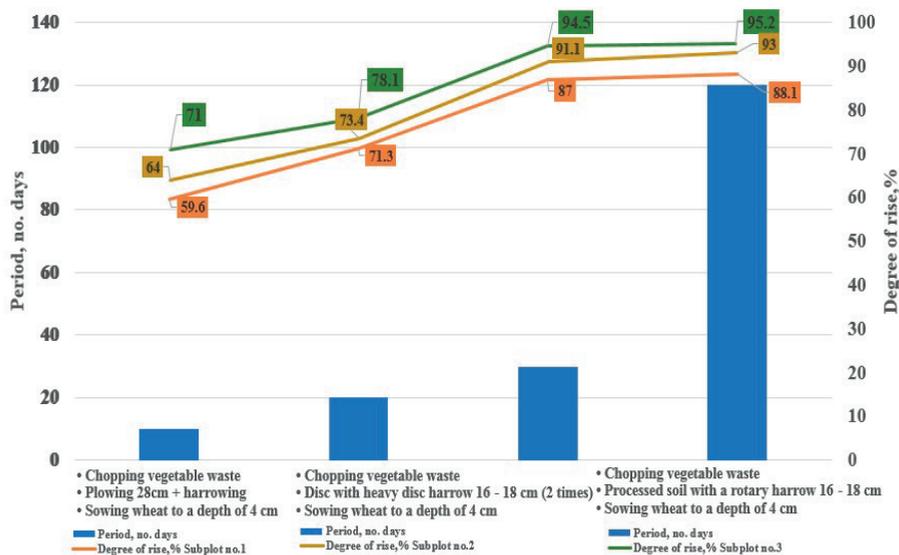


Figure 8. Degree of emergence after 10, 20 days, 30 days, 120 days depending on the land preparation works

Table 4. Statistical-mathematical analysis of the degree of seed germination

No	Groups	Frequencies	Relative frequencies	Cumulative absolute frequencies	Cumulative relative frequencies
1	59-66	2	0.17	2	0.17
2	67-74	3	0.25	5	0.42
3	75-82	1	0.08	6	0.50
4	83-90	2	0.17	8	0.67
5	91-98	4	0.33	12	1.00
	Total	12			

From the statistical-mathematical analysis by grouping the statistical data by intervals, we can interpret that only 33% of the sown grains emerged between 91-98% representing the highest percentage.

We can also observe that after analyzing the absolute cumulative frequencies, 8% of the grains manage to achieve an emergence of up to 82%, representing 50% of the total grains according to the cumulative relative frequencies, and 25% of these grains emerge in a percentage between 67-74% (Table 4).

A percentage of 17% rose in a percentage of 59-66% and 83-90%, respectively.

The degree of emergence is thus influenced by the agricultural works performed for the preparation of the germination bed

## CONCLUSIONS

In this study, the effects of three different tillage methods on germination and wheat germination. Every effect the soil is subjected to has positive or negative reactions. Soil is a living, dynamic system whose physical, chemical, and biological properties are constantly interacting with each other, and changes in any properties affect other properties. By tillage, the chemical and biological characteristics of the soil are also affected due to the physical manipulation to which the soil has been subjected.

The results of the study showed that most of the physical properties of the soil are adversely affected by conventional tillage methods to

which three tillage operations apply. There were differences between tillage methods and these differences proved to be significant.

In general, the best results related to the degree of germination of wheat crop were obtained with rotary rotor harrow (LDR). (LDG), led to a decrease in the degree of emergence and even a uniformity of emergence

From similar studies, the effects of tillage methods may differ depending on climatic, regional and environmental factors. These factors must be taken into account before applying the tillage methods. Otherwise germination and emergence are affected. It can be considered that in the year in which the study was carried out the decrease in the degree of emergence was also influenced by climatic conditions and lack of humidity and low temperatures.

It is recommended that the method of preparing the germination bed with a rotary harrow (LDR) can be used for tillage.

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## EVALUATION OF THE BIOMASS QUALITY OF WHITE SWEETCLOVER, *Melilotus albus*, AND PROSPECTS OF ITS USE IN MOLDOVA

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### Abstract

Legume plants play a major role in promoting sustainable agriculture, at global, regional and national levels. We investigated the biomass quality of the local ecotype of white sweetclover, *Melilotus albus* L., grown in an experimental field of the National Botanical Garden (Institute), Chişinău. That the green mass cut in the flowering period contained 27.9% dry matter. The dry matter of whole plants contained 132 g/kg CP, 82 g/kg ash, 381 g/kg CF, 386 g/kg ADF, 567 g/kg NDF, 64 g/kg ADL, 322 g/kg Cel, 181 g/kg HC, 86 g/kg TSS, with 58.8% DMD, RFV = 97, 11.66 MJ/kg DE, 9.57 MJ/kg ME, 5.59 MJ/kg NEI. The fermented fodder from *Melilotus alba* contained 127 g/kg CP, 414 g/kg CF, 99 g/kg ash, 407 g/kg ADF, 581 g/kg NDF, 58 g/kg ADL, 62 g/kg TSS, 348 g/kg Cel, 174g/kg HC, with nutritive and energy values: 57.2 % DMD, RFV = 92, 11.38 MJ/kg DE, 9.34 MJ/kg ME and 5.30 MJ/kg NEI. The biochemical methane potential of white sweetclover green mass substrate reached 267 L/kg organic matter and white sweetclover silage substrate - 278 L/kg organic matter. The local ecotype of white sweetclover can be used for the restoration of degraded lands, as a component of the mix of grasses and legumes for the creation of temporary grasslands. The harvested biomass can be used as alternative fodder for farm animals or as substrates in biogas generators for the production of renewable energy.

**Key words:** biochemical composition, biochemical methane potential, forage quality, green mass, *Melilotus albus*, silage.

### INTRODUCTION

Population growth, economic development and awareness about healthy nutrition increase the demand for animal products day by day and require more efficient use of limited production resources such as water and soil. Selecting the right forages and suitable management practices are the most effective strategies for reasonable forage production under limited natural resources and under the conditions of climate change. The increasing need for animal feed and the insufficiency of production resources have caused a high interest in plants that can produce high yields under different climate and soil conditions and can be cultivated without great expense. *Fabaceae* plants play a major role in promoting sustainable agriculture, at global, regional and national levels. Legumes have a symbiotic relationship with atmospheric nitrogen fixing bacteria that live in root nodules and that make legumes independent of nitrogen fertilization. As a consequence, legume fodders commonly

have higher concentrations of protein than other grasses. In this context, neglected and underutilized legume plants have gained a lot of attention recently.

*Melilotus* is a genus in the family *Fabaceae*, and its representatives occur commonly in grasslands or as weeds in fields. *The Plant List* includes 205 scientific plant names of species rank for the genus *Melilotus*, 22 of them are accepted species names, distributed in the Mediterranean countries, Asia and North America, Central and Eastern Europe. There are 6 species of the genus *Melilotus* in Romania and 4 species in the Republic of Moldova. The most known and common species are white sweet clover *Melilotus albus* Medik. and yellow sweet clover *Melilotus officinalis* L. (Stoian, 1987; Negru, 2007).

White sweetclover or honey clover *Melilotus albus* Medik. (syn. *Melilotus alba* L., *Melilotus leucanthus* W.D.J. Koch ex DC.) is annual or biennial herb, develops erect or ascending stems, robust, 30-180 cm tall, greenish-yellow, pubescent in the upper part. The leaves are

bright green, trifoliolate. The basal leaves have obovate leaflets, cuneate at the base and obtuse at the tip, the margins are slightly and irregularly serrated, the leaflets of the upper leaves are narrow, elongated-lanceolate, with slightly toothed margins, with scattered hairs on the adaxial side, the middle leaflet petiolate, the stipules are narrow, entire and slightly toothed at the base, subulate at the tip. The white flowers are arranged in a loose raceme, 5-8 cm long. It blooms from June to September. The fruit is a glabrous pod, obovate, rostrate and reticulated, blackish, 3-4 mm long, brown, indehiscent, with 1-2 yellowish, oval seeds that are 2.0-2.5 mm long and 1.5 mm wide. The weight of 1000 seeds is 2.0-2.5 g. The tap root is strongly branched, grows into the soil to a depth of 2.0 m. It forms a symbiotic relationship with the bacteria *Rhizobium meliloti*. The non-scarified seeds need high amounts of moisture in the germination bed, but the amount of water absorbed by the scarified seeds, for germination, decreases by 30-40%. The seeds germinate at a soil temperature of 2-4°C; in spring, the seedlings can withstand, temperatures as low as 3-6°C. In winter, the plants can withstand temperatures of -30°C, under the snow cover. In summer, it is more drought tolerant than alfalfa and sainfoin. White sweetclover cannot tolerate shade, thus, in mixed culture the foliage is sparse and the shoots are lignified. This species has relatively low requirements to the soil, due to the development of a strong root system, the high ability to solubilize phosphorus and other elements from combinations which are hard to dissolve for other plants. Strong and deep penetrating taproots make it a suitable candidate as a nurse crop in derelict land compacted soils. White sweetclover prefers neutral or moderately alkaline soils. It can grow on slightly sandy, solonchak, loamy, calcareous, stony and unstructured soils. It has shown some tolerance to saline soils with a salt concentration of 0.4-0.6%. Acidic and excessively moist soils should be avoided (Medvedev & Smetannikova, 1981; Guerrero-Rodríguez, 2006; Gucker, 2009; Anderson, 2013; Annaeva et al., 2020; Țiței & Roșca, 2021). *Melilotus albus* contains condensed tannins. The low level of tannins (2-3%) in

ruminant feeding has a beneficial effect as it reduces protein degradation in the rumen while the high level of tannins negatively affects protein digestion, microbial and enzyme activities. On the other hand, approximately 21-25% of anthropogenic CH<sub>4</sub> release worldwide is produced in the animal digestive system. In the rumen, this tannin protects proteins from degradation and ruminants excrete less urinary nitrogen (Kumar & Singh, 1984). White sweetclover is a promising honey plant, with a potential productivity of 200-500 kg/ha (Popa & Cîrnu, 1960; Glukhov, 1974). An important area of the foreign research is a breeding program with a biennial ecotype of *Melilotus albus* to lengthen the duration of flowering and increasing the plant leaf coverage, to optimize the nutritional value of green mass by reducing the content of lignin and coumarin (Kosolapov et al., 2021). Due to the intensive growth of biomass during the growing season *Melilotus albus* has the prospect of being used for renewable energy production (Popp et al., 2015; Hunady et al., 2020; Kintl et al., 2020; 2021). In recent years, the interest in white sweetclover herba product in the official pharmacopoeia has been increasing, the antimicrobial, antibiofilm, and antioxidant potentials is being studied (Stefanovic et al., 2015).

The main objectives of this study were to evaluate the quality of green mass and silage from white sweetclover, *Melilotus albus* and the prospects of its use as fodder for farm animals or as substrates for renewable energy production

## MATERIALS AND METHODS

The local biennial ecotype of white sweetclover - *Melilotus albus*, which was cultivated in the experimental plot of the National Botanical Garden (Institute) Chișinău, N 46°58'25.7" latitude and E 28°52'57.8" longitude, served as subject of the research, and the traditional fodder crops - common sainfoin, *Onobrychis viciifolia* Scop. and Sudan grass, *Sorghum sudanense* (Piper) Stapf were used as control variants.

The experimental design was a randomized complete block design with four replications, and

the experimental plots measured 50 m<sup>2</sup>. The *Melilotus albus* seeds were sown in late March, at a depth of 2.0 cm at a distance between rows of 15 cm, the sowing density was 120 scarified seeds/m<sup>2</sup>. The plant growth, development and productivity were assessed according to methodical indications. The white sweetclover and Sudan grass green mass samples were collected in full flowering period, while the common sainfoin in budding - flowering stage. The leaf/stem ratio was determined by separating leaves and flowers from the stem, weighing them separately and establishing the ratios for these quantities. For this purpose, samples of 1.0 kg harvested plants were taken. The sweetclover and Sudan grass silages were prepared from harvested green mass, but common sainfoin haylage was produced from wilted green mass, cut into small pieces and compressed in glass containers. The containers were stored for 45 days, and after that, they were opened and the organoleptic assessment and the determination of the organic acids composition of the persevered forage were done in accordance with the Moldavian standard SM 108. The fresh mass and fermented fodder samples were dehydrated in an oven with forced ventilation at a temperature of 60°C. At the end of the fixation, the biological material was finely ground in a laboratory ball mill. The quality of the biomass was evaluated by analyzing such indices as: crude protein (CP), crude fibre (CF), crude ash (CA), total soluble sugars (TSS), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), digestible dry matter (DDM), digestible organic matter (DOM) which have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research and Development Institute for Grassland Braşov, Romania. The concentration of hemicellulose (HC) and cellulose (Cel), the digestible energy (DE), the metabolizable energy (ME), the net energy for lactation (NEI) and the 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 biogas potential (Y<sub>b</sub>) and the methane potential (Y<sub>m</sub>) were calculated according to Dandikas et al., 2014.

## RESULTS AND DISCUSSIONS

Analyzing the results of the assessment of agrobiological peculiarities, it can be noted that in the first year of vegetation, the growth and development rates of *Melilotus albus* were low in comparison with *Onobrychis viciifolia*, it developed the rosette and 1-3 erect shoots about 57-78 cm long at the end of the growing season. In the second year, the growth and development of the local ecotype of white sweetclover plants began when the average temperature was above 5°C, in the first days of April. It was established that the revival of *Melilotus albus* plants from dormant buds was uniform, characterized by faster grow and development rates. In full flowering period, at the time when the green mass was harvested, the erect shoots of *Melilotus albus* reached 129.7 cm, the yield was 43.1 t/ha green mass or 12.58 t/ha dry matter, containing 70.2% leaves and flowers in the harvested mass (Table 1). The productivity of control crops, *Onobrychis viciifolia* plants reached 42.3 t/ha green mass or 10.1 t/ha matter with 53.7% leaves and 46.3% stems, but the yield of *Sorghum sudanense* was 27.9 t/ha or 7.2 t/ha dry matter with 51.6 % leaves and panicles.

The morphological structure of the whole plant has a significant impact on the nutrient content of the green mass. Analysing the results of the biochemical composition of dry matter, Table 2, we would like to mention that *Melilotus albus* fodder was characterised by reduced concentration of crude proteins as compared with *Onobrychis viciifolia* but higher content as compared with Sudan grass. The level of crude cellulose, acid detergent fibre, neutral detergent fibre, cellulose and hemicellulose in *Melilotus albus* fodder was low as compared with Sudan grass and higher as compared with *Onobrychis viciifolia*. The concentrations of acid detergent lignin in white sweetclover fodder were also high as compared with the control species, which negatively affected the digestibility of the organic matter. The dry matter in white sweetclover fodder contained a low amount of minerals and soluble sugars as compared with traditional fodder crops. The energy concentrations were optimal in white sweetclover fodder, but reduced as compared with sainfoin fodder.

Table 1. Some agrobiological peculiarities and the structure of the green mass of the studied crops

Plant species	Plant height, cm	Stem, g		Leaf + flower, g		Yield, t/ha	
		fresh mass	dry matter	fresh mass	dry matter	fresh mass	dry matter
<i>Melilotus albus</i>	129.7	10.2	3.0	30.6	8.4	43.1	12.0
<i>Onobrychis vicifolia</i> , first cut	99.2	10.1	2.5	12.5	2.9	42.3	10.1
<i>Sorghum sudanense</i> , first cut	212.0	33.1	3.0	12.5	3.2	27.9	7.2

Table 2. The biochemical composition and the fodder value of the green mass of the studied crops

Indices	<i>Melilotus albus</i>	<i>Onobrychis vicifolia</i>	<i>Sorghum sudanense</i>
Crude protein, g/kg DM	132	177	85
Crude fibre, g/kg DM	381	293	392
Minerals, g/kg DM	81	96	95
Acid detergent fibre, g/kg DM	386	309	413
Neutral detergent fibre, g/kg DM	567	447	656
Acid detergent lignin, g/kg DM	64	49	41
Total soluble sugars, g/kg DM	86	114	138
Cellulose, g/kg DM	322	260	372
Hemicellulose, g/kg DM	181	138	243
Digestible dry matter, g/kg DM	525	669	517
Digestible organic matter, g/kg DM	449	615	506
Relative feed value	97	135	80
Digestible energy, MJ/ kg	11.66	12.73	10.39
Metabolizable energy, MJ/ kg	9.57	10.45	8.52
Net energy for lactation, MJ/ kg	5.59	6.48	5.28

Different results regarding the productivity, biochemical composition and the nutritive value of the green mass from *Melilotus* species are given in the specialized literature. According to Medvedev & Smetannikova (1981), the green mass productivity of white sweetclover *Melilotus albus* was 42.3-50.0 t/ha, 21-23% dry matter, 16-22% CP, 2-4% EE, 24-34% CF, 30-45% NFE, 7-9% ash. Stoian (1987) found that, under the conditions of the Danube Delta, *Melilotus alba* yielded 32 t/ha fresh mass or 6.4 t/ha dry matter. Kshnikatkina et al. (2005) reported that the dry matter from *Melilotus officinalis* forage harvested in the flowering stage contained 8.19% CP, 2.62% EE, 23.47% CF, 50.62% NFE, 7-9% ash, 1.40%Ca, 0.8% P. Heuzé & Tran (2015) revealed that *Melilotus indicus* fresh aerial part contained 22.8% DM, 18.0% CP, 2.0% EE, 30.8% CF, 51.2% NDF, 36.3 % ADF, 9.2% lignin, 9.2% WSC, 11.5% ash, 21.5 g/kg Ca and 3.7 g/kg P, 70.3% ODM, 18.1 MJ/kg GE, 12.2 MJ/kg DE, 9.6 MJ/kg ME. Bozhanska et al., (2016), mentioned that the chemical composition and energy nutritional value of *Melilotus albus* was: 11.76% CP, 3.16% EE, 32.59% CF, 33.60% NDF, 24.15% ADF; 3.05% ADL, 9.45% HC, 21.10% Cel, 5.28%

ash, 1.84% Ca, 0.25% P, 17.11 MJ/kg GE, 7.48 MJ/kg ME, 0.69 feed unit of milk and 0.63 feed unit of growth. Makarov & Andrusova (2016) compared the quality of several *Melilotus* species and mentioned that *Melilotus albus* contained 17.8% CP, 1.9% EE, 22% CF, 48% NFE, 10.6% ash., 1.73% Ca, 0.26% P, 113 g/kg DP, 0.76 feed unit/kg, but *Melilotus suaveolens* contained 20.7-22.1% CP, 2.1-2.4% EE, 15.2-16.8% CF, 49.9-50.5% NFE, 9.8-10.5% ash., 1.87-2.09% Ca, 0.20-0.22% P, 155-166 g/kg DP, 0.82-0.86 feed unit/kg DM. Dashkevich et al. (2018) found that the concentrations of nutrients and energy in the dry matter of the tested ecotypes of *Melilotus albus* were 13.33-19.56% CP, 1.90-2.26% EE, 14.60-23.94% CF, 46.19-56.80% NFE, 7.43-10.12% minerals, 0.46-0.90% coumarin and 9.81-10.83 MJ/kg ME, in *Melilotus officinalis* plants 16.20-20.73% CP, 2.02-2.28% EE, 13.18-18.66% CF, 49.57-57.80% NFE, 7.73-11.09% minerals, 0.28-1.13% coumarin and 10.57-11.29 MJ/kg ME, but in the *Melilotus wolgicus* plants: 16.93-21.17% CP, 2.00-2.29% EE, 15.64-20.52% CF, 48.90-54.41% NFE, 8.10-10.14% minerals, 0.79-1.13% coumarin and 10.40-10.95 MJ/kg ME, respectively. Ateş & Seren (2020) remarked that the *Melilotus caeruleus*

forage harvested in different growth stages contained 17.98-20.67% CP, 39.09-42.33% NDF, 28.24-30.83% ADF. Annaeva et al. (2020) found that, in saline areas of Uzbekistan, the productivity of *Melilotus albus* cv. Kibray ranged from 31.6 to 38.0 t/ha green mass at the first cut and 22.6-28.1 t/ha green mass at the second cut, but *Medicago sativa* cv. Tashkent yielded 17.5-22.5 t/ha and 17.4-20.9 t/ha respectively. Avetisyan et al. (2020) revealed that under the forest-steppe conditions of the Krasnoyarsk territory, Russia the productivity of *Melilotus albus* was 49.5 t/ha green mass, 11.3 t/ha dry matter, 122.0 GJ/ha metabolizable energy, 0.15 nutritive units/kg green mass and 35 g digestible protein /kg green mass, but *Sorghum sudanense* yielded 43.2 t/ha green mass, 9.9 t/ha dry matter, 97.0 GJ/ha metabolizable energy, 0.21 nutritive units/kg green mass and 23 g digestible protein /kg green mass. Hunady et al. (2021), evaluating of the biomass quality of 14 wild leguminous plant species collected in the budding stage, reported that *Melilotus alba* dry matter contained 87.23% OM, 16.68% CP, 4.43% EE, 19.89% CF, 35.16% NDF, 27.82% ADF; *Onobrychis viciifolia* - 92.18% OM, 12.47% CP, 2.77% EE, 26.08% CF, 46.97% NDF, 38.48% ADF; *Medicago sativa* - 94.98% OM, 15.33% CP, 2.75% EE, 25.30% CF, 47.83% NDF, 38.27% ADF. Kosolapov et al. (2021) reported that the tested *Melilotus albus* varieties were characterized, in first year of life, by a growing season of 106-110 days, 78.0-88.1 cm plant height, 14.48-16.39 t/ha green mass with 52.0-58.6% leaf coverage, 2.88-23.51 t/ha dry matter with 18.4-20.2% CP and 22.10-24.20% CF, but in the second year of life - growing season of 61-71 days, 145-182 cm plant height, 29.67-38.54 t/ha green mass with 53.9-66.9 % leaf coverage, 6.71-9.74 t/ha dry matter with 19.10-23.0% CP and 22.10-25.20% CF.

The ensiling process has substantial effects on the nutritive value of the prepared feed and animal performance. During the sensorial assessment, it was found that, in terms of colour, the silage from white sweetclover had olive stems with dark green leaves and smell specific to pickled apples, the haylages prepared from *Onobrychis arenaria* consists of yellowish-green leaves and yellow-green

stems; it has a pleasant smell of pickled vegetables; while the silage made from Sudan grass was homogeneous, green-yellow, with pleasant smell, specific to pickled vegetables. The texture of the plants stored as silage and haylage was preserved well, without mold and mucus. The results regarding the quality of the prepared fermented fodder are shown in Table 3. It has been determined that the pH values of the fermented fodder depended on the species, thus, *Melilotus albus* silage had higher pH than *Sorghum sudanense*, but lower than *Onobrychis viciifolia* haylage. The content of organic acids in the fermented fodder from the studied leguminous crops did not vary essentially, but was lower than in *Sorghum sudanense*. Most organic acids were in fixed form, butyric acid was detected only in Sudan grass silage. According to the Moldavian standard SM 108, the ration of acetic acid and lactic acid of the studied fermented fodder corresponds to the 1<sup>st</sup> class quality. It was found that during the process of ensiling, the concentrations of crude protein and acid detergent lignin decreased, but the level of minerals, neutral detergent fibre increased in comparison with the green mass. In white sweetclover silage, the amount of crude protein was high as compared with Sudan grass silage, but lower as compared with sainfoin haylage. The energy concentrations in the prepared silage were at the same level, but reduced compared with sainfoin haylage.

Several studies have evaluated the potential of white sweetclover *Melilotus albus* as silage. Medvedev & Smetannikova (1981) mentioned that white sweetclover silage contained 23.1% DM, including 3.4% CP, 1.6% EE, 9.4% CF, 7.2% NFE, 1.6% ash, 2.2 g/kg Ca, 0.64 g/kg P and 84 mg/kg carotene. Popp et al. (2015) reported that sweet clover silage was characterized by 22.2% DM, pH 4.5, lactic acid 0.1 g/kg fresh mass, acetic acid 1.3 g/kg fresh mass, butyric acid 0.32 g/kg fresh mass, 8.6% CP, 0.9% EE, 38.0% NFE, 41.0 % Cel, 31.0% HC and 15.0% lignin. Kintl et al. (2020) remarked that the quality of silage from pure white sweetclover was 316.4 g/kg DM, 96.75% OM, 11.62% CP, 3.83%EE, 4.51% starch, 28.16% CF, 31.62% ADF, 48.67% NDF, 14.32% ADL, 17.51 mg/kg coumarin, the maize silage contained 333.8 g/kg DM, 96.04%

OM, 8.13% CP, 4.26% EE, 20.66% starch, 16.73% CF, 20.35% ADF, 42.14% NDF, 6.16% ADL, 1.84 mg/kg coumarin, but silages from different weight shares of these two crops 321.0-341.3 g/kg DM, 94.89-96.69% OM, 8.10-

11.34% CP, 2.59-4.03% EE, 11.13-4.03% starch, 17.59-25.49% CF, 29.49-38.84% ADF, 37.21-57.10% NDF, 3.90-16.33% ADL, 4.35-14.25 mg/kg coumarin, respectively.

Table 3. The biochemical composition and the nutritive value of the fermented fodder from studied crops

Indices	<i>Melilotus albus</i> silage	<i>Onobrychis viciifolia</i> haylage	<i>Sorghum sudanense</i> silage
pH index	4.42	4.68	3.82
Organic acids, g/kg DM	24.0	23.4	30.0
Free acetic acid, g/kg DM	0.3	1.1	2.3
Free butyric acid, g/kg DM	0	0	0.0
Free lactic acid, g/kg DM	2.3	4.4	10.2
Fixed acetic acid, g/kg DM	3.1	2.2	2.1
Fixed butyric acid, g/kg DM	0	0	0.2
Fixed lactic acid, g/kg DM	18.3	15.7	15.2
Total acetic acid, g/kg DM	3.4	3.3	4.4
Total butyric acid, g/kg DM	0	0	0.2
Total lactic acid, g/kg DM	20.6	20.1	28.4
Acetic acid, % of organic acids	14.20	14.10	14.67
Butyric acid, % of organic acids	0	0	0.66
Lactic acid, % of organic acids	85.8	85.90	84.67
Crude protein, g/kg DM	127	142	57
Crude fibre, g/kg DM	414	312	392
Minerals, g/kg DM	99	118	109
Acid detergent fibre, g/kg DM	407	317	402
Neutral detergent fibre, g/kg DM	581	470	652
Acid detergent lignin, g/kg DM	58	40	39
Total soluble sugars, g/kg DM	62	135	108
Cellulose, g/kg DM	349	277	363
Hemicellulose, g/kg DM	174	153	250
Digestible dry matter, g/kg DM	509	653	57.5
Digestible organic matter, g/kg DM	416	582	53.8
Relative feed value	92	127	82
Digestible energy, MJ/ kg	11.38	12.63	11.43
Metabolizable energy, MJ/ kg	9.34	10.37	9.38
Net energy for lactation, MJ/ kg	5.30	6.38	5.54

Table 4. Biochemical composition and biomethane production potential of substrates from studied species

Indices	<i>Melilotus albus</i>		<i>Onobrychis viciifolia</i>		<i>Sorghum sudanense</i>	
	fresh mass	silage	fresh mass	haylage	fresh mass	silage
Minerals, g/kg DM	81.0	99.0	96.0	118.0	95.0	109
Nitrogen, g/kg DM	21.1	20.3	28.3	22.7	13.6	9.1
Carbon, g/kg DM	510.6	500.6	502.2	490.0	502.8	495
Ratio carbon/nitrogen	24.2	24.6	17.7	21.6	37.0	54.3
Hemicellulose, g/kg DM	181.0	174.0	138.0	153.0	243.0	250.0
Acid detergent lignin, g/kg DM	64.0	58.0	49.0	40.0	41.0	39.0
Bio gas potential, L/kg VS	518	543	569	608	627	636
Biomethane potential, L/kg VS	267	278	291	311	321	326

Replacing fossil fuels with renewable energy alternatives has become a major global issue of the XXI century and a key to sustainable development. Biogas plants for anaerobic digestion utilize different substrates organic materials like residual waste from livestock, food production, effluents from industry or sludge from wastewater treatment plants,

energy crops. However, in order to consolidate the role of biogas production via anaerobic digestion as a renewable energy production technology, it is important to ensure the availability of sustainable biomass sources. Installations do not only produce energy but also digestate and fugate are believed to be good fertilizers, which are rich in plant

available nutrients such as nitrogen, phosphate and potash, and could serve as a replacement for fossil based mineral fertilizers. The results regarding the quality of substrates and their biochemical methane potential are illustrated in Table 4. We found that the investigated substrates from *Melilotus albus*, according to the carbon nitrogen ratio, which constituted 24, met the established standards, but the high concentrations of acid detergent lignin negatively influenced the activity of bacteria and decomposition processes, as compared with the other investigated substrates. The biochemical biogas and biomethane potential of white sweetclover green mass substrate reached 518 L/kg and 267 L/kg organic matter, but white sweetclover silage substrate - 543 L/kg and 278 L/kg organic matter, respectively, being lower as compared with the potential of the substrates of Sudan grass and sainfoin.

According to Popp et al. (2015) the cumulative methane yield of substrate codigestion of sweet clover silage and cow manure was almost equal: 266 L/kg VS for the 2.5% coumarin content substrate and 259 L/kg VS for the 5.0% coumarin substrate, respectively, and 265 L/kg VS for the coumarin-free control substrate. Kintl et al. (2020) found that the average methane yield, after 21 days of the experiment, of ensiled sweetclover was 0.161 m<sup>3</sup>/kgVS, of maize silage - 0.271 m<sup>3</sup>/kg VS and of silage mixture of these two crops - 0.199-264 m<sup>3</sup>/kg VS. Hunady et al. (2021) calculated the theoretical methane yield and revealed that the values of biomass from *Galega orientalis*, *Lathyrus pratensis*, *Trigonella foenum-graecum* and *Melilotus alba* ranged from 0.161 to 0.172 m<sup>3</sup>/kg VS, the methane yield of biomass from *Onobrychis viciifolia*, *Astragalus cicer*, *Dorycnium germanicum*, *Vicia sylvatica* ranged from 0.141 to 0.160 m<sup>3</sup>/kg VS and the absolutely lowest value – 0.12-0.14 m<sup>3</sup>/kg VS – was calculated for *Medicago sativa*.

## CONCLUSIONS

The productivity of the local ecotype of white sweetclover in the first year reached 1.74 kg/m<sup>2</sup> green mass or 0.4 kg/m<sup>2</sup> dry matter, but in the second year 4.31 kg/m<sup>2</sup> green mass or 1.2 kg/m<sup>2</sup> dry matter. The dry matter of harvested mass, in the second year, contained 132 g/kg CP, 82

g/kg ash, 381 g/kg CF, 386 g/kg ADF, 567 g/kg NDF, 64 g/kg ADL, 322 g/kg Cel, 181 g/kg HC, 86 g/kg TSS, with 58.8 % DMD, RFV= 97, 11.66 MJ/kg DE, 9.57 MJ/kg ME, 5.59 MJ/kg NEL.

The silage prepared from *Melilotus albus* is characterized by pH = 4.72, 3.4 g/kg acetic acid, 20.6 g/kg lactic acid, contained 127 g/kg CP, 414 g/kg CF, 99 g/kg ash, 407 g/kg ADF, 581 g/kg NDF, 58 g/kg ADL, 62 g/kg TSS, 348 g/kg Cel, 174g/kg HC, with nutritive and energy values: 57.2 % DMD, RFV= 92, 11.38 MJ/kg DE, 9.34 MJ/kg ME and 5.30 MJ/kg NEL.

The biochemical methane potential of white sweetclover green mass substrate reaches 267 L/kg organic matter and white sweetclover silage substrate - 278 L/kg organic matter.

The local ecotype of white sweetclover can be used for the restoration of degraded lands, as a component of the mix of grasses and legumes for the creation of temporary grasslands. The harvested biomass can be used as alternative fodder for farm animals or as substrates in biogas generators for the production of renewable energy.

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## EVALUATION OF COTTON GENE POOL SAMPLES IN DIFFERENT YEARS OF HEAT SUPPLY IN THE CONDITIONS OF THE SOUTHERN STEPPE OF UKRAINE

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### *Abstract*

*The article presents the results of studying of the gene pool of cotton in different years of heat supply, with and without irrigation. As a result of many years of research from the collection of the gene pool, more than 30 sources of economic and valuable traits have been identified, which are characterized by high adaptability to environmental factors, excellent indicators of product quality. On the basis of the studied material, two working signs and a training cotton collection were formed. Valuable samples of cotton were isolated from the gene pool on the basis of economically valuable features. These included drought resistance, fiber color, a combination of signs of precocity and partial pubescence of seeds, high attachment of the first sympodial branch and large capsule, long fiber, ultra-early maturity and high productivity. Also, medium-fiber high-yielding very early varieties of cotton - 'Dniprovskiy 5' and 'Pidozerskiy 4' were created.*

**Key words:** cotton collection, climate change, precocity, yield, ripening period.

### **INTRODUCTION**

In recent years, the climate has become drier, there are observed global changes on the planet (Adamenko, 2014; Barabash et al., 2004). Climate change in the conditions of the Southern Steppe of Ukraine is no exception (Vozhegova & Kovalenko, 2013; Vozhegova, 2014a). According to the Kherson Agrometeorological Station, significant climate changes have also taken place in the south of Ukraine over a 132-year period, as evidenced by the hydrothermal coefficient of 0.6-0.7 (Gusev et al., 2005; Melnichuk & Adamenko, 2011). The period with average daily air temperatures of 10°C and above, which suggests active growth and development of plants, lasts 183-189 days. The sum of positive air temperatures above 10°C during this period varies from 3285°C in the north to 3415°C in the center of the region. In some years, these figures ranged from 2850 to 3685°C.

In general, increasing the aridity of the climate in the southern region contributes to the probability of increasing arid years to 60-75% (Kovalenko, 2012), which allows to refer it to the zone of risky agriculture with unstable agro-ecological conditions, and in some years –

with extreme (Netis, 2004). Researchers claim that over the past 50 years, the average annual air temperature has risen by 1.8°C, winters have become warmer and shorter, while the autumn period - on the contrary, became longer (Adamenko, 2014; Vozhegova, 2014a). The duration of the vegetation period of agricultural crops in the Kherson region lasts 229-237 days: it starts on March 20-25 and ends on November 9-14.

Over the last five years, in 2011-2015, the amount of precipitation decreased compared to the period from 1972 to 2010, on average, from 457 to 381 mm or 76 mm. In this area there is a shortage of water supply to plants, so an important role is given to irrigation (Balyuk, 2009). Analysis of data on climate change in the Kherson region shows that the water and heat regimes of this zone for most crops have deteriorated over the past five years. The reasons for these changes may be global world processes, the cyclical nature of solar activity, anthropogenic factors of human economic activity and their combined action.

If for the majority of crops grown in the southern region of the country, the limiting factor is water, for cotton the limiting factor is

heat supply, to which it has high requirements. Scientists have found that the most risky for harvesting raw material, before the onset of frost, in this area are the years with the coincidence of cool temperatures and frequent precipitation in the period of August - September (Borovik et al., 2011). Therefore, the increase of air temperature, increase the amount of dry days, decrease of rainfall - cotton perceives all of it quite positively. The onset of the main phases of its development depends largely on the weather conditions of the year.

## MATERIALS AND METHODS

The main task in conducting research is to obtain reliable comparative assessments of samples on the main economic and valuable features and biological properties provided by the criteria in collection nurseries. The subjects of research were samples of cotton from collection nursery. The research was conducted in non-irrigated and irrigated conditions in the fields of the selection department of the Institute of Irrigated Agriculture. Morphological description, classification by economic and biological properties was carried out according to the "Wide unified classifier" - reference book of the genus *Gossypium hirsutum* L. (Vozhegova et al., 2014). Statistical processing of the obtained data was performed according to the method by Vozhegova (Vozhegova, 2014b).

To characterize the weather conditions were used data from the Kherson Agrometeorological Station, located near the experimental field. Weather conditions in the years of research were typical for the zone of the southern region of Ukraine, which contributed to the objective assessment of the introduced material, the selection of the best samples on the basis of economic and valuable features. The soil of the experimental field is dark brown, hard loamy, residually weakly saline with humus content in the arable layer of 2.15-2.3%. Soil density is 1.2-1.3 g/cm<sup>3</sup>, wilting moisture is 7.8-9.8%, and the lowest moisture content of 0.7 m layer is 20.5-22.4%. Groundwater lies deeper than 15 m. Cotton growing techniques were typical for the Southern Steppe of Ukraine.

## RESULTS AND DISCUSSIONS

Evaluation of 282 samples of cotton gene pool was carried out during 1993 and 1999-2012. Meteorological indicators for the test years, according to the sum of effective temperatures above 10°C, were as follows: the most favorable were 1999, 2002 and 2005, 2011, 2012, close to them - 1998, 2001, 2008, 2009, 2010; medium - 1996, 2000, 2003, 2006, 2007 and very cold and wet - 1993 and 2004. Too cold 1993 (the sum of effective temperatures above 10°C was 1397°C) accelerated the differential brutal culling of collection material according to their maturity groups (Borovik, 2009). Practically, the ripening phase before frost was recorded in only 30% of cotton varieties of Bulgarian selection.

1999 was characterized by favorable temperature conditions for most varieties of different maturity groups. During the growing season, the sum of positive temperatures above 10°C reached its maximum - 1735°C (norm 1492°C). Ripening of most varieties and even late ripening came almost simultaneously with the standard - 23-27.09. The opening of the capsules, even in mid-late varieties, occurred in the 2nd - 3rd decades of September, which is 3 weeks earlier than the optimal time. 2006 and 2007 were characterized by similar weather conditions.

The length of the growing season in varieties of different maturity groups in 2000 was differentiated and ranged from 110 to 144 days. In 2001, the maturity of most samples, even medium-late ones, was 14 to 28 days earlier. Before the frosts it was obtained the 90-98% of raw materials. Despite the fact that in 2003 the sum of effective temperatures above 10°C was 1614.1°C (norm 1475.2°C), there was a delay in the onset of the phase of full ripeness of cotton due to damage of silver Y (*Autographa gamma*) and cotton bollworm (*Helicoverpa armigera*). Scoops caused great damage to the culture by damaging almost all the first buds at the 2nd - 5th sympodial branching and thus contributed to mass decline. As a result, the onset of mass flowering and ripening in 2003 was delayed for 20-30 days, regardless of the maturity group of the variety. In fact, before the frost, which was observed on October 25, ripening of raw material took place only in single plants of precocious forms.

The onset of phases of growth and development and their duration is determined by the biological characteristics of the variety and agro-climatic conditions of the growing area. In years with a large amount of precipitation, the duration of the development phases is prolonged, and in drought is reduced (Kruchinina, 2014). It is established that the years with the coincidence of cool temperatures and frequent precipitation during the period of formation - ripening of boxes are the most risky for obtaining the harvest of raw materials before the onset of frost in the south of Ukraine, and 2004 can be considered as an example. In the extreme conditions of 2004, the

result of effective temperatures above 10°C was 1424°C (norm 1546°C). The maximum air temperature rose to 38.0°C (August 3). There were 39 days of drought during the summer, and 23 days were normal. Precipitation for the season was 104.5 mm (79.2% of normal), most of which was observed in the second (28.6 mm) and third (64.4 mm) decades of June, as well as in the third decade of July (19, 4 mm). Weather with a large amount of precipitation led to low yield – only 1.4 t/ha. Capsules of these plants were formed at the 4th - 9th sympodial branching. Before the onset of frost 0.62 t/ha of raw material was harvested (Figure 1).

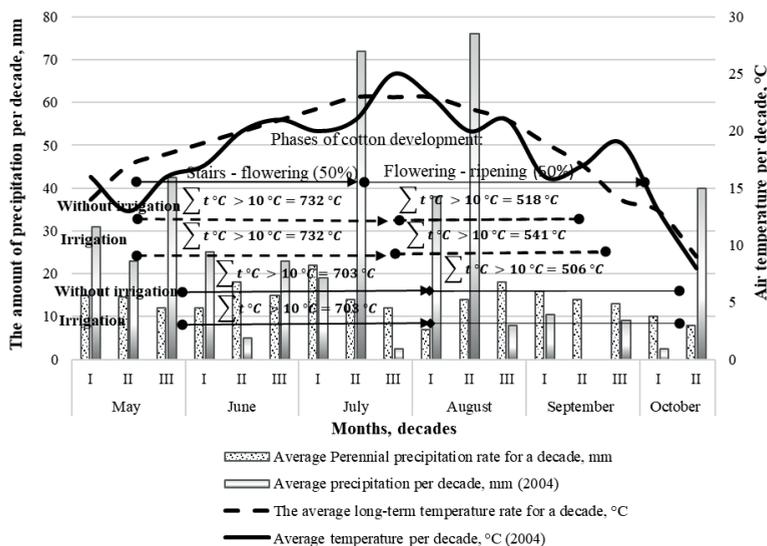


Figure 1. The course of the main indicators of weather and phases of development of cotton in 2004

The maximum potential of cotton appeared in 2002 and 2005-2012, when, practically, only pre-frost yield of raw material was obtained - 70-90% of the total, and the maturation of varieties of different

maturity groups accelerated: their growing season was 105-115 days. Groups of cotton ripeness, formed on average over the years of research, are presented in Table 1.

Table 1. Classification of cotton collection samples by maturity groups depending on the sum of effective temperatures above 10°C (average for 1993, 1999-2012\*)

Maturity group	The sum of effective temperatures from sowing to			The number of plants that have entered the ripening phase, %
	stairs	flowering	maturation in 50% of plants	
Ultra-early (110-115 days)	146*	1398/1399	1532/1534	3.7/2.1
Precocious (116-130 days)	146	1416/1417	1560/1562	13.4/7.9
Medium ripe (131-140 days)	146	1433/1434	1597/1598	30.5/29.7
Late ripening (141-150 days)	146	1476/1478	1735/1737	22.2/25.2
Very late ripening (> 150 days)	146	1490/1491	1742 and more	30.2/35.1

\*With the exception of 2004, when a very small yield of pre-frost raw material was obtained;  
 \*The numerator presents the results of research in non-irrigated conditions, the denominator - with irrigation;  
 \*Results of research in the conditions of irrigation are presented for 1993-2002.

During studying the collection of the cotton gene pool both on non-irrigated and irrigated lands, it was found that the formation of different groups of maturity of samples largely depends on the weather conditions in the south of Ukraine. During the years of research in non-irrigated conditions, in fact, ultra-early forms were observed in 3.7%, and in irrigated - 2.1% (Borovik & Stepanov, 2014). Watering delayed the process of maturing cotton. The ripening phase of late-maturing specimens under irrigation was also somewhat prolonged with the change of the maturity group in the direction of increase. Significant rainfall (68.4

mm) in the first decade of May 2009, as well as warm weather in the second and third, contributed to the friendly germination of cotton, which appeared on 29.05 (long-term average – 19.05). In comparison, late seedlings were obtained because of late sowing dates. The further course of positive temperatures and the distribution of precipitation during the growing season accelerated the development cycle of varieties of all maturity groups. Flowering took place in most varieties in long-term calendar terms - July 28-29. An important factor determining the suitability of the variety for mechanized harvesting is the height of the bookmark of the first sympodial branching. In 2009, this value was in the range of 7.8-12.3 cm.

Table 2. The best samples of cotton in the collection nursery by economic-valuable features (average for 2011-2012)

National Catalogue Number	Sample name	Country of origin	Duration of the growing season, days	Plant height / attachment of the 1st sympodial branching, cm	Resistance to the most common diseases, score		Weight of the 1 capsule, g	Productivity of the 1 plant, piece	Fiber length, mm
					<i>Verticillium dahliae</i> (verticillium wilt)	<i>Xantomonas mahaveceanum</i> (homozyg)			
1	2	3	4	5	6	7	8	9	10
UF0800001	Dniprovskiy 5	UKR	105	58.1/14.1	9	9	5.2	6.6	30.4
UF0800220	500y	UKR	104	58.3/12.8	9	9	5.4	6.8	28.9
UF0800212	1135/94	UZB	104	64.9/14.2	9	9	4.8	7.9	30.7
UF0800031	Pidozerskiy 4	UKR	103	63.6/18.1	9	9	5.9	10.2	32.4
UF0800238	Sort 534	RUS	104	60.6/14.2	9	9	5.4	6.4	31.2
UF0800050	2362	BGR	107	59.8/14.2	9	9	5.5	6.4	29.6
UF0800281	Trakiya	BGR	106	60.7/15.1	9	9	5.7	9.4	29.4
UF0800123	N267	BGR	108	62.4/14.6	9	9	5.2	6.6	28.5
UF0800062	144F	UZB	106	56.2/12.4	9	9	5.4	10.1	28.4
UF0800159	Acala 90	USA	108	64.6/16.2	9	9	5.5	6.2	31.5
UF0800019	Chyrpan 603	BGR	109	74.6/15.3	9	9	5.1	7.1	29.6
UF0800185	Interlinear hybrid №64 irradiated	BGR	108	66.9/15.1	9	9	5.4	7.6	30.2
UF0800282	Joloten 14	KGZ	135	86.1/15.6	9	9	6.5	9.7	31.9
LSD <sub>05</sub>								5.4	

In 2010, the height of attachment of the first fruit branch of all Bulgarian varieties was not lower than 13.1 cm. In warm and hot years, a larger number of capsules was formed on plants under irrigation conditions, especially in 2011 and 2012. The course of weather conditions had a positive effect on the development of cotton. At the sum of effective temperatures more than 1600°C (norm 1491.8°C), at the time of harvest, samples of all maturity groups reached their maturity. The exception was

UF0800282 Joloten 14, the maturation period of which was 135 days (Table 2). Samples with such a maturation period (131-140 days) cannot be grown in the southern region of Ukraine, as with the sum of effective temperatures less than 1598°C there is a risk of obtaining the maximum yield of open capsules for the period of collection of raw material. As a result of many years of research, breeders of the Institute have selected from the collection of more than 30 sources of economic and valuable

features, which are characterized by high adaptability to environmental factors, excellent indicators of product quality. In 2010, a working feature collection of cotton was formed in the amount of 40 samples of basic economic and valuable features and received a Certificate of registration of the collection of plant gene pool in Ukraine (№ 107, 22.12.10) (Borovik et al., 2010). In 2014 a working collection of cotton was formed on the basis of valuable economic and main features of differences in irrigation conditions (Certificate №164, 10.12.2014). A training collection was also created (Certificate №179, 11.12.2014). Valuable specimens were selected and registered from the collection on the basis of economically valuable features: cotton sample UF0800031 Pidozerskyi 4 - a source of drought resistance (Certificate № 736, 22.12.10); UF0800265 Rude - a source of colored fiber (Certificate № 737, 22.12.10); UKR006 0850 L 191/13 - source of combination of signs of precocity and partial pubescence of seeds (Certificate № 1227, 22.10.2014); UKR006 0851 L 165/13 - source of combination of very high attachment of the first sympodial branch and large capsule (Certificate № 1228, 22.10.2014); UF0800029 417u - a source of ultra-early maturity and high productivity (Certificate № 1229, 22.10.2014); UKR006 0852 Dohvoloknystyi - a source of long fiber (Certificate № 1226, 22.10.2014).

The formed basic collection of cotton is a component of the European Integrated System of Gene Banks (AEGIS): Agreement dated by March 26, 2012 between the Institute of Irrigated Agriculture of the National Academy of Agrarian Sciences of Ukraine in the person of Director R. Vozhegova and the National Coordinator [AEGIS] of the ECPGR for Ukraine, Ryabchun V. – the head of the National Center for Plant Genetic Resources of Ukraine of the Institute of Plant Breeding named after V. Yuriev. For introduction into production, the Institute of Irrigated Agriculture NAAS recommends the best medium-fiber varieties created by breeders: very early-maturing (95-105 days) cotton varieties: Dniprovskyi 5 with a potential yield of 2.0-2.5 t/ha for non-irrigated and irrigated conditions and variety Pidozerskyi 4 with a potential yield of 2.5-3.0 t/ha, which is best

sown on irrigated lands of the arid climate of southern Ukraine.

## CONCLUSIONS

Climate change on the planet and, accordingly, in the conditions of the Southern Steppe of Ukraine in the direction of warming has had a positive effect on the maturity of cotton. As a result of research of 282 samples of cotton gene pool during 1993, 1999-2012 in different heat supply years both on non-irrigated and irrigated lands, it was found that the formation of different maturity groups of samples largely depends on weather conditions in southern Ukraine. During the years of research in non-irrigated conditions, in fact, ultra-early forms were observed in 3.7%, and in irrigated in 2.1%.

As a result of many years of research from the collection of the gene pool, more than 30 sources of economic and valuable traits have been identified, which are characterized by high adaptability to environmental factors, excellent indicators of product quality. Based on the studied material, two working collections and a training collection for cotton were formed. Also from the gene pool on economically valuable features were selected and registered valuable samples of cotton, sources of: drought resistance (UF0800031 Pidozerskyi 4), colored fiber (UF0800265 Rude), a combination of signs of precocity and partial pubescence of seeds (UKR006 0850 L), combination the first sympodial branch and large capsule (UKR006 0851 L 165/13); ultra-early and high productivity (UF0800029 417y); long fiber (UKR006 0852 Dohvoloknystyi).

It was created medium-fiber high-yielding very early-maturing varieties of cotton: Dniprovskyi 5 with a potential yield of 2.0-2.5 t/ha for non-irrigated and irrigated conditions and Pidozerskyi 4 with a potential yield of 2.5-3.0 t/ha, which is best sown on non-irrigated lands of the arid climate of southern Ukraine.

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## INFLUENCE OF FERTILIZATION WITH LIQUID ORGANIC FERTILIZER ON THE PRODUCTIVITY AND BOTANICAL COMPOSITION OF NATURAL *Agropyron repens* GRASSLAND

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### Abstract

The aim of this study was to establish the influence of organic leaf fertilization on the productivity and botanical composition of natural grassland *Agropyron repens* type in the region of South-Central Bulgaria (305 m altitude). The experiment was carried out with leaf organic fertilizer Naturamin Plus, during the period 2018-2019. The trial was designed by the block method in 4 repetitions and 3 doses of fertilizer were tested: 1,500; 2,500 and 3,500 ml.ha<sup>-1</sup>. Results obtained for the yield were statistically processed by ANOVA. The use of the Naturamin Plus in natural grassland *Agropyron repens* type has been found to have a positive effect on productivity regardless of weather conditions over the years. Average for the period of investigation, more green and dry mass were obtained by treatment with a dose of 2,500 ml.ha<sup>-1</sup> - respectively 31.6% and 30.7% more compared to the control. In grassland with predominant species *Agropyron repens* (L.) P. Beauv., the largest share is occupied by perennial cereal grasses, while legumes have a small share. Fertilization with liquid organic fertilizer Naturamin Plus increases the participation of perennial cereal and legume grasses and reduces that of weeds.

**Key words:** botanical composition, fertilization, natural grassland, productivity.

### INTRODUCTION

Nowadays, natural grasslands are accepted not only as an enormous natural resource, which allows for natural and low cost raising of ruminant animals but they also have valuable ecologic functions: protect the soil from water and wind erosion, protect groundwater from contamination, decrease the effect of greenhouse gasses by absorbing part of CO<sub>2</sub> in the process of photosynthesis, conserve the biodiversity (Călina & Călina, 2015; Kirilov & Mihovski, 2014; Pavlov, 2005; Pavlov & Mihovsky, 2007; Štýbnarová et al., 2012; Trofimov et al., 2014). For that reason, in the attempts to stabilize the natural grassland yields, contemporary science develops new approaches which include the use of treatments identical to natural, in order to improve the provision of plants with nutrients and their resistance to stress influences in the environment. Fertilization is one of the main agro-technical measures, contributing to the increase of productivity and nutritional value of forage obtained from grasslands. (Avarvarei & Chelariu, 2011; Kharkevich et al., 2015; Kozhouharov & Lingorski, 2011; Păcurar et al., 2012; Štýbnarová et al., 2012).

An important factor, influencing the process of optimization of the grasslands fertilization, is the type of fertilizer and the application method. On the most widely distributed natural grasslands in Central Balkan mountains - a natural meadow (*Chrysopogon gryllus* L. Trin. type) and a natural pasture (*Nardus stricta* L. type) Iliev et al. (2020) tested foliar fertilization with the organic humic fertilizer Biostim (BG). It was established that this method of fertilization was more effective, compared to variable mineral fertilization and had a positive effect on the bioproductive characteristics of both grassland types. The application of foliar fertilizers and biostimulators was an effective method to correct the shortage of nutrients and had a positive effect on the resistance and adaptive mechanisms of plants to outside influences, the grassland density, plant height, productiveness and nutritional value of the obtained forage (Ivanova & Zavarukhina, 2015; Xu & Huang, 2010).

In order to decrease the negative impact on the environment Chourkova (2011; 2013), Churkova & Bozhanska (2016), Bozhanska et al. (2017a; 2017b), Petrova, (2017), Pigareva & Zhugdurov (2013), Wolski et al. (2019) used

liquid biofertilizers to optimize the feed rate of grass species. It was established the effect of application of different treatments, doses and types of biofertilizers and growth regulators in the production of birdsfoot trefoil (*Lotus corniculatus* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), sainfoin (*Onobrychis viciifolia* Scop.), perennial cereal grasses and legume grass mixtures, natural grasslands. The obtained results determined biofertilizers and growth regulators as an alternative for reducing mineral fertilization and obtaining ecologically clean forage production, and according to the authors, its effectiveness is influenced by the biological characteristics of the grass species and the weather conditions.

The aim of the present study was to determine the influence of the liquid organic fertilizer Naturamin Plus (Daymsa, ES) on the productivity, botanical composition, and agronomic efficiency of fertilization in natural grassland *Agropyron repens* (L.) P. Beauv. type in the ecological conditions of South-Central Bulgaria.

## MATERIALS AND METHODS

The study was conducted in two vegetation seasons in the period 2018–2019 on natural grassland *Agropyron repens* (L.) P. Beauv. type, located in the area of village Yagoda, Stara Zagora region, 305 m altitude. The trial was designed by the block method in 4 repetitions and the size of the experimental plot was 10 m<sup>2</sup>. The soils in the area are Gleic Chromic Luvisols, with low humus content (3.42-3.93%), low acidity, low available nitrogen (31.3-38.1 mg.kg<sup>-1</sup> soil) and phosphorus content (3.1-4.3 mg.100 g<sup>-1</sup> soil) but with high available potassium content (42-44 mg.100 g<sup>-1</sup> soil).

Tested was the influence of the combined fertilizer Naturamin Plus, which has the following composition: total 400 g.l<sup>-1</sup> amino acids, free amino acids - 200 g.l<sup>-1</sup>, Nitrogen (N) - 75 g.l<sup>-1</sup>, Iron (Fe) - 12 g.l<sup>-1</sup>, Manganese (Mn) - 7.5 g.l<sup>-1</sup>; Boron (B) - 1.3 g.l<sup>-1</sup>, Copper (Cu) - 1.2 g.l<sup>-1</sup>, Molybdenum (Mo) - 0.5 g.l<sup>-1</sup>, Zinc (Zn) - 2.5 g.l<sup>-1</sup>. The preparation is certified according to European Council Regulation (EC) No 834/2007 for use in organic production. The following doses were used: 1,500, 2,500 and 3,500 ml.ha<sup>-1</sup>.

Fertilization with the liquid organic fertilizer Naturamin Plus was made a single time during grassland vegetation in each of the trial years at the time of growing (height 10-15 cm) of the grassland. A small sprayer pump with fine dispersion nozzle with 300 l.ha<sup>-1</sup> spraying solution was used for spraying. The experimental plots were harvested via mowing in the spike growth phase of the dominant grass species *Agropyron repens* (L.) P. Beauv. During the years of the experiment the plots were mowed twice.

The following indicators were evaluated: green mass and dry matter yield; botanical composition of the grassland (%) was determined via weight analysis of fresh grass samples taken immediately before mowing; the share of the main botanic groups (perennial cereal grasses, legume grasses and weeds); agronomic efficiency of fertilization (AE), partial fertilization productivity (PFP).

Agronomic efficiency of fertilization was calculated the following way: ratio of the difference between yield with fertilization (YF) and yield without fertilization (YF<sub>0</sub>) divided by the fertilization rate (F).

Formula for agronomic efficiency of fertilization:  $(AE) = (YF - YF_0)/F$ ;

Partial fertilization productivity (PFP) was calculated the following way: ratio of the yield (Y) to the applied fertilizer (F).

Formula for partial fertilization productivity:  $(PFP) = Y/F$ .

Data processing was performed by a two-way dispersion analysis (Lidanski, 1988), using MS Excel software - 2010.

The results for the yield were statistically processed with ANOVA LSD test for statistical significance of the differences, standard deviation and coefficient of variation (Lidanski, 1988), using MS Excel software - 2010.

## RESULTS AND DISCUSSIONS

Weather conditions during the period of the study are shown on Table 1. Water is one of the main factors determining the growth and development of grassland. Regarding rainfall, the years of the study are characterized as moderately favorable. The multiannual (1936-2017) average rainfall during the vegetation period (April-September) was 299 mm. In

2018, the quantity of rainfall during active grassland vegetation was with 10.5 mm above normal, and in 2019 with 14.7 mm above normal. This had a favorable influence on the complex biochemical processes related with the growth and development of the plants and helped for the formation of two undergrowth. The average of annual air temperature calculated for the multiannual period (1936-2017) was 12.5°C. Due to the warm winter and relatively small altitude, spring arrives

comparatively early in the region. Already in the first ten days of March (4-7 March) the temperatures permanently stay above 5°C, and as a result, the active vegetation of the grassland begins. The air temperature during the active vegetation (April-September) on average for the multiannual period (1936-2017) was 19.4°C. The temperature during vegetation in the trial period (2018-2019) was with 1.0-1.5°C higher than the multiannual average in both years of the study.

Table 1. Climate conditions of South-Central Bulgaria

Years	Months													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I-XII	IV-IX
Total rainfall, mm														
2018	19.0	115.0	89.9	2.9	99.3	85.2	75.2	27.0	19.9	40.8	30.8	21.6	626.6	309.5
2019	39.9	15.3	5.3	57.9	63.5	108.7	52.0	11.0	20.6	14.2	11.7	4.0	404.1	313.4
1936-2017	41.7	35.8	37.2	45.1	62.8	61.7	51.2	43.5	34.7	42.7	48.9	47.8	553.1	299.0
Average temperature, °C														
2018	2.4	3.6	6.7	15.7	19.1	21.8	23.7	25.1	19.7	14.4	7.8	2.0	13.5	20.9
2019	2.3	4.5	8.9	11.6	17.4	23.4	23.9	25.2	20.6	14.2	11.7	4.0	14.0	20.4
1936-2017	1.0	2.8	6.6	12.0	17.1	21.2	23.7	23.3	19.0	13.0	7.4	2.7	12.5	19.4

Productivity is the most important and synthesizing indicator for the application of a particular technology. During the study, the yield of green mass, from natural grassland *Agropyron repens* (L.) P. Beauv. type, varied

based on the treatment with the liquid organic fertilizer Naturamin Plus. As shown on Table 2, for the two years period the annual average yield varied from 11,643.8 to 15,706.3 kg.ha<sup>-1</sup>.

Table 2. Green mass yield by the years and average for the period 2018–2019 (kg.ha<sup>-1</sup>)

Variant	Dose ml.ha <sup>-1</sup>	2018		2019		Average	
		kg.ha <sup>-1</sup>	%	kg.ha <sup>-1</sup>	%	kg.ha <sup>-1</sup>	%
Control (untreated)		10,191.7	100.0	13,812.5	100.0	12,002.1	100.0
Naturamin Plus	1,500	11,275.0a	110.6	16,487.5*ab	119.4	13,881.3a	115.7
Naturamin Plus	2,500	13,608.3**b	133.5	17,975.0**b	130.1	15,791.7**b	131.6
Naturamin Plus	3,500	11,500.0*a	112.8	14,550.0a	105.3	13,025.0a	108.5
Average		11,643.8	114.2	15,706.3	113.7	13,675.0	113.9
LSD.P< 0.05		1,301.7	11.2	2,464.7	17.8	1,883.2	15.7
LSD.P< 0.01		1,870.3	16.1	3,541.2	25.6	2,705.7	22.5
LSD.P< 0.001		2,751.3	23.6	5,209.4	37.7	3,980.4	33.2
SD						2,809.03	
CV						20.54	
SE						496.57	
Min						9,850	
Max						21,500	

\*Different letters indicate statistically significant differences among variants at P < 0.05

\*, \*\*, \*\*\* – Statistically significant differences of the variants and control at P< 0.05; 0.01 and 0.001, respectively

Annually as well as average for the studied period, the lowest yield was obtained in the untreated control. The highest, statistically well-proven green mass yield (P<0.01) average for the period of the study was obtained in the

variant treated with dose 2,500 ml.ha<sup>-1</sup>. In the different years of the study, the green mass yield from this variant surpassed the control with 30.1% (4,162.5 kg.ha<sup>-1</sup>) to 33.5% (3,416.6 kg.ha<sup>-1</sup>).

Treatment with the dose 1,500 ml.ha<sup>-1</sup> also had a positive influence: the obtained green mass yield was on average with 15.7% (1,879.2 kg.ha<sup>-1</sup>) higher than in the untreated control. However, this was statistically proven (P<0.05) only in 2019.

In treatment of the grassland with leaf fertilizer Naturamin Plus in dose 3,500 ml.ha<sup>-1</sup> both by years, as well as average for the studied period were obtained positive differences in the green mass yield, compared to the basic variant, but they were not proven statistically.

Dry matter yield (Table 3) from natural grassland *Agropyron repens* (L.) P. Beauv. type based on leaf fertilization with the liquid organic fertilizer Naturamin Plus varied from 2,491.1 to 3,149.7 kg.ha<sup>-1</sup> in the different years of the study, and it was higher in 2019 when the combination of the climate factors - rainfall and temperatures – was more favorable for the development of the grassland. The lack of rainfall in April 2018, combined with the air temperatures, which were 3.7°C above usual

for the month, had a negative impact on the biomass productivity of first undergrowth and consequently on the annual green mass and dry matter yield for 2018.

Highest dry matter yield was obtained from fertilization with Naturamin Plus in dose 2,500 ml.ha<sup>-1</sup>. The yield was on average with 30.7% (760 kg.ha<sup>-1</sup>) higher than the untreated control. The obtained differences are well proven statistically (P<0.001) for the years of the study.

Fertilization of the natural grassland with Naturamin Plus in dose 1,500 ml.ha<sup>-1</sup> contributed to obtain higher dry matter yield but the statistical significance of the difference compared to the untreated variant was low (P<0.05).

Similarly to the results obtained for green mass, in treatment with the highest dose (3,500 ml.ha<sup>-1</sup>) of the organic fertilizer Naturamin Plus, the effect on the dry matter yield was not statistically proven.

Table 3. Dry matter yield by the years and average for the period 2018-2019 (kg.ha<sup>-1</sup>)

Variant	Dose ml.ha <sup>-1</sup>	2018		2019		Average	
		kg.ha <sup>-1</sup>	%	kg.ha <sup>-1</sup>	%	kg.ha <sup>-1</sup>	%
Control (untreated)		2,257.8	100.0	2,685.2	100.0	2,471.5	100.0
Naturamin Plus	1,500	2,520.0*a	111.6	3,245.5*ab	120.9	2,882.7*a	116.6
Naturamin Plus	2,500	2,840.8***b	125.8	3,622.2***b	134.9	3,231.5***b	130.7
Naturamin Plus	3,500	2,345.8a	103.9	3,046.1a	113.4	2,695.9a	109.1
Average		2,491.1	110.3	3,149.7	117.3	2,820.4	114.1
LSD.P< 0.05		252.2	11.2	419.4	15.6	335.8	13.6
LSD.P< 0.01		362.2	16.0	602.6	22.4	482.4	19.5
LSD.P< 0.001		532.8	23.6	886.5	33.0	709.7	28.7
SD						486.14	
CV						17.24	
SE						89.94	
Min						2,207.42	
Max						4,075.6	

\*Different letters indicate statistically significant differences among variants at P < 0.05

\*, \*\*, \*\*\* – Statistically significant differences of the variants and control at P< 0.05; 0.01 and 0.001, respectively

Leaf fertilization with Naturamin Plus has a significant influence on the botanical composition of grassland.

Perennial cereal grasses participate on average with 49.54% in the first year (Table 4), while their participation in grassland in the second year of the study increased with 15.86% and reached up to 65.44% from the grassland.

Average for the period of the study the participation of perennial cereal grasses in the grassland was 57.49%.

In second place in the composition of the grassland was the participation of weeds, where was observed the opposite tendency - decrease of their participation from the first year to the second year of the study.

Table 4. Botanical composition of the natural grassland *Agropyron repens* type, treated with leaf fertilizer Naturamin Plus, by the years and average for the period 2018-2019 (%)

Variant	Dose ml.ha <sup>-1</sup>	Groups	2018	2019	Average
Control (untreated)		Perennial cereal grasses	38.06	58.58	48.32
		Legumes	4.75	0.28	2.51
		Weeds	57.19	41.14	49.17
Naturamin Plus	1,500	Perennial cereal grasses	58.49	72.69	65.59
		Legumes	3.88	1.18	2.53
		Weeds	37.63	26.13	31.88
Naturamin Plus	2,500	Perennial cereal grasses	53.76	69.04	61.40
		Legumes	9.44	3.59	6.52
		Weeds	36.79	27.37	32.08
Naturamin Plus	3,500	Perennial cereal grasses	47.87	61.46	54.66
		Legumes	9.93	3.53	6.73
		Weeds	42.20	35.02	38.61
Average		Perennial cereal grasses	49.54	65.44	57.49
		Legumes	7.00	2.14	4.57
		Weeds	43.45	32.42	34.99

They occupied on average 43.45% in the first year and decreased to 32.42% in the second year. Perennial cereal grasses had the highest participation share after treatment with Naturamin Plus in doses 1,500 and 2,500 ml.ha<sup>-1</sup>. While weeds decreased the share of their participation after treatment with dose 1,500 ml.ha<sup>-1</sup> Naturamin Plus. These results demonstrate the positive influence of Naturamin Plus for leaf fertilization. Legumes had the smallest share in the studied grassland - average for the period of the study 4.57%, while their participation varied from 2.14% to 7.00%. The share of legumes in the obtained forage mass was bigger in the first year of the study. Each year of the study, and as average for the period, was observed that with the increase of the share of perennial cereal grasses, was increased the participation of beneficial legume plants in the grassland.

According to the obtained results, legume grasses had the smallest share in the biomass received from the untreated control and in treatment with the low dose of the biofertilizer Naturamin Plus (1,500 ml.ha<sup>-1</sup>).

Their share increased with the increase of the applied dose of the product Naturamin Plus. Leaf application of the preparation Naturamin Plus had the strongest positive influence on the share of legumes in the first year of the study.

Agronomic efficiency of fertilization characterizes the abilities of plants to increase their yield as a result of the applied fertilization.

Highest values for AE were received after application of 2,500 ml.ha<sup>-1</sup> dose fertilizer - with 21% more for green mass and 11% more for dry matter - compared to the lower fertilization dose (Table 5).

Table 5. Agronomic efficiency and partial fertilization productivity of fertilization with Naturamin plus of the natural grassland *Agropyron repens* type (kg.ml<sup>-1</sup>)

Variant	Dose ml.ha <sup>-1</sup>	Agronomic efficiency			Partial fertilization productivity		
		2018	2019	Average	2018	2019	Average
Green mass							
Naturamin plus	1,500	722.22	1,783.33	1,252.78	7,516.67	10,991.67	9,254.17
Naturamin plus	2,500	1,366.67	1,665.00	1,515.83	5,443.33	7,190.00	6,316.67
Naturamin plus	3,500	373.81	210.71	292.26	3,285.71	4,157.14	3,721.43
Average		820.90	1,219.68	1,020.29	5,415.24	7,446.27	6,430.75
Dry matter							
Naturamin plus	1,500	174.78	373.52	274.15	1,680.0	2,163.7	1,921.8
Naturamin plus	2,500	233.21	374.78	303.99	1,136.3	1,448.9	1,292.6
Naturamin plus	3,500	25.13	103.11	64.12	670.2	870.3	770.3
Average		144.37	283.80	214.09	1,162.2	1,494.3	1,328.2

Agronomic efficiency frequently lowers with the increase of the applied fertilization rate (Li et al., 2020). The results obtained in this study correspond with this and show that a high rate of fertilization (3,500 ml.ha<sup>-1</sup>) decreases AE with 77% compared to the lowest one (1,500 ml.ha<sup>-1</sup>). Agronomic efficiency of fertilization showed the same tendency for the green mass yield and for the dry matter yield.

Partial fertilization productivity is the ratio of the yield to the applied dose fertilizer. In specific conditions of growing, the efficiency of the used fertilizer in most cases decreases with the increase of the applied dose (Dibb, 2000), which was also confirmed in the present study. The highest value of partial fertilization productivity average for the period of the study was established for the lowest applied fertilization rate (1,500 ml.ha<sup>-1</sup>), surpassing with 32% the values of partial fertilization productivity for fertilization rate 2,500 ml.ha<sup>-1</sup>, and with 60% for fertilization rate 3,500 ml.ha<sup>-1</sup>.

## CONCLUSIONS

Application of liquid organic fertilizer Naturamin Plus to natural grassland of *Agropyron repens* (L.) P. Beauv. type has a positive influence on the productivity, regardless of the climate conditions during the study period. The strongest effect on the productive characteristics resulted from the treatment with dose 2,500 ml.ha<sup>-1</sup> - green mass yield increased with 31.6%, and dry matter increased with 30.7%.

Treatment with Naturamin Plus in dose 2,500 ml.ha<sup>-1</sup> leads to increase in the agronomic efficiency of fertilization. Using higher doses of Naturamin Plus, the effect on the productiveness of grassland and of agronomic efficiency of fertilization decreases.

In natural grasslands *Agropyron repens* (L.) P. Beauv. type, the largest share is occupied by perennial cereal grasses. Fertilization with the liquid organic fertilizer Naturamin Plus increases the participation of cereal and legume grasses and reduces that of weeds.

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MISCELLANEOUS



## THE INFLUENCE OF CHITOSAN TREATMENTS IN ORGANIC FETEASCA NEAGRA VINEYARD ON THE AROMATIC PROFILE OF WINES EVALUATED BY ELECTRONIC NOSE

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### Abstract

*The present study evaluates the differences in the aroma profile of wines, induced by the chitosan treatments of Feteasca neagra grapes organically cultivated. The treatments of the grapes included a variant only with Bordeaux mixture (5 kg/ha), as a control, a variant only with chitosan (5 kg/ha), and another variant treated with both chitosan and Bordeaux mixture (5+5 kg/ha). The aroma of the resulted wines produced by the classical maceration-fermentation technology was analysed by an electronic nose working on the principle of fast GC. Several organic volatile compounds with impact on aroma were identified and their relative quantities compared for each type of treatment. Esters (fruity scents) are the main aroma compounds found, but some other compounds more related to vegetal aroma were also present. The electronic nose clearly identified each type of wine in accordance to the treatment in the vineyard. The principal component analysis separated the wines based on their floral-fruity-vegetal notes (PC1) versus grassy notes (PC2). SIMCA analysis showed that, compared to the control samples with Bordeaux mixture, the samples from grapes treated with chitosan or chitosan and Bordeaux mixture placed closer in the space of odour and outside of the space of control wines. Thus, this preliminary study showed that chitosan treatment in vineyard induces measurable olfactory differences in the wines.*

**Key words:** chitosan vine treatment, electronic nose, e-nose, Feteasca neagra, wine aroma profile.

### INTRODUCTION

Chitosan is a natural compound extracted from insects, which is used for plant protection (Hadrami et al., 2010) and is also allowed in organic agriculture. Chitosan hydrochloride was found to enhance plant protection against pathogenic bacterial or fungi infections, such as *Plasmopara viticola* (Dagostin, 2006.)

It can be an alternative to the treatments based on copper that are used with high frequency in organic viticulture, which are very effective but, in the long run, lead to copper accumulation in the soil and harm the vine itself due to its phytotoxicity (Toselli et al., 2009).

Chitosan treatments can replace or, at least can contribute to the reduction of copper usage.

In the vineyard chitosan may be used for treatments based on its several documented insecticidal (Badawy and El-Aswad (2012) and fungicidal effect (Garde-Cerdán et al., 2017; Matei et al., 2019), but so far consistent

treatment plans have not been established. The effect grape treatment with chitosan has on the wine produced afterwards from these grapes was not sufficiently investigated, therefore, this type of research should be conducted to determine several aspects related to wine quality parameters. In order to achieve the stated objective, in the present study, the samples obtained with various interventions in the technology of ecological culture were evaluated in order to determine the profile of volatile substances and to identify the differences induced by the treatments. The samples were tested using an electronic nose analyzer, based on flash chromatography, by taking volatile substances from the sample by the "headspace" method (i.e. from the gaseous part above the sample in a chromatography vial). The "headspace" method allows the analysis of the compounds present in the gas phase, supposedly those compounds that the human nose would perceive when a wine sample is smelled.

## MATERIALS AND METHODS

Grapes of Feteasca neagra were cultivated at the Research Station for Viticulture and Oenology Murfatlar under 3 various types of treatments in the vineyard. More data about the vine plantation can be found in previous publications (Artem et al., 2021a; Artem et al., 2020).

The experimental variants, with the vine treatments, product quantities and number of treatments are described in Table 1. Chitosan was procured from Kitozyme SA, Belgia and the Bordeaux Mixture from SC Verdon Solution SRL, Romania.

Table 1. Treatments used for Feteasca neagra grapes used for the experimental wine variants

Variant code of wine	Type of vine treatment		Number of treatments
	Bordeaux mixture	Chitosan	
FN20-Bord (control)	5 kg/ha	0 kg/ha	12
FN20-Chit	0 kg/ha	5 kg/ha	12
FN20-ChiBo	5 kg/ha	5 kg/ha	6

The wines were produced in the autumn of 2020 from grapes of Feteasca neagra organically grown. The volatile substance profiles of the experimental wines were determined using a Heracles electronic nose (Alpha MOS, France), which works on the principle of flash gas chromatography, using two short columns with different polarities (one non-polar DB5 - 5% diphenyl, 95% dimethylpolysiloxan and one medium-low polar DB1701 - 14% cyano-propylphenyl, 86% dimethylpolysiloxan). The two columns have flame ionization detectors (FID) located at the end, providing two

chromatograms that are recorded simultaneously. More details regarding the apparatus and the method used for recording and analysing the volatile profile can be found in previously published papers (Antoce and Cojocaru, 2021; Cojocaru and Antoce, 2019; Antoce and Namolosanu, 2011).

The software AlphaSoft v12.42 is used to control the chromatograph and to record and process data. Various statistical methods are available, the ones selected for this study being principal component analysis (PCA), discriminant function analysis (DFA) and SIMCA.

Some of the volatile substances which are recorded as chromatographic peaks can be identified using an integrated database of chemical compounds, AroChemBase 2010.

## RESULTS AND DISCUSSIONS

### 1. Main volatile substances identified by the e-nose in Feteasca neagra wines

The main volatile substances were identified by the electronic nose as part of the aromatic profile of Fetească neagră wines obtained with various interventions in organic farming technology by using the AroChemBase database that the device is equipped with. Relevant volatile compounds of experimental wines are identified and presented in detail in Table 2, along with their main sensory attributes. The sensory descriptors associated with a particular volatile organic compound are based on information provided by AroChemBase and other databases, such as ChemSpider.

Table 2. Relevant volatile organic compounds identified in wine samples from Feteasca neagră obtained from ecologically treated plots with different treatments

Column DB5				Column DB1701			
Average retention time (RT)*	Kovats Indices/Sensors	Identified compound	Sensory descriptors	Average retention time (RT)*	Kovats Indices/Sensors	Identified compound	Sensory descriptors
10.43	810.80-1	ethyl lactate	fruits	10.04	820.36-2	3-mercapto-2-butanone	sulfurous, onion
10.76	819.10-1	3-hydroxy-2-pentanone	grass, truffle	14.83	941.16-2	3-methylbutyl acetate	banana, pear
12.93	873.53-1	3-methylbutyl acetate	banana, pear	16.52	981.96-2	1-hexane-ol	sweet, woody, green, herbaceous
17.77	992.23-1	ethyl hexanoate	apples, bananas, wine, pineapples	19.60	1,058.08-2	ethyl hexanoate	apples, bananas, wine, pineapples
22.40	1,108.53-1	2-phenylethanol	floral, honey, rose	28.06	1,278.58-2	2-phenylethanol	floral, honey, rose
32.82	1,387.40-1	ethyl decanoate	grapes, pears, oily, sweet, waxy, fruity, apple, brandy, soapy	34.57	1,456.03-2	ethyl decanoate	grapes, pears, oily, sweet, waxy, fruity, apple, brandy, soapy

\*average values of 3 recorded chromatograms (repetitions of the same sample); \*\*the sample code consists of the Kovats index and the column on which the chromatogram was recorded (1 = DB5; 2 = DB1701); \*\*\*Sensory descriptions for identified compounds are retrieved from AroChemBase and other public databases.

The volatile compounds identified in the aromatic profile of these red wines showed different concentrations in samples from grapes from ecologically treated plots with different treatments, which leads to a different overall flavour for each group of wines. In order to be able to highlight the differences in concentration, the peak areas corresponding to each compound identified in the samples with different treatments were compared (Table 3).

By applying the analysis of variance (ANOVA) and the Tukey test to compare the means in pairs, it was possible to determine the main significant differences that appear between the experimental variants. Thus, in the sample treated with Bordeaux mixture, FN20\_Bord, the presence of significantly increased concentrations of esters such as ethyl lactate, ethyl hexanoate and ethyl decanoate is noted, which each of them bringing, and especially in combination, a fruity note (apples, bananas, pineapple, grapes). The FN20\_ChiBo sample specifically shows more vegetal notes, due to a higher content of 1-hexanol (raw, green note) and 3-mercapto-2-butanone (onion note). These special aromatic notes are appreciated in some wines, especially in red ones, which makes this sample stand out more easily than other experimental wines.

Table 3. Chromatographic peak surface area of relevant volatile organic compounds identified in Feteasca neagră wine samples obtained from ecologically treated plots with different treatments

	FN20 Bord	FN20 ChiBo	FN20 Chit
<b>Column DB5</b>			
ethyl lactate	<b>1238±102<sup>a</sup></b>	810±155 <sup>b</sup>	1123±228 <sup>a</sup>
3-hydroxy-2-pentanone	1982±172 <sup>a</sup>	2134±292 <sup>a</sup>	1723±253 <sup>a</sup>
3-methylbutyl acetate	38059±1540 <sup>a</sup>	37308±2057 <sup>a</sup>	40835±3363 <sup>a</sup>
ethyl hexanoate	<b>13574±406<sup>a</sup></b>	11487±688 <sup>b</sup>	11071±1341 <sup>b</sup>
2-phenylethanol	3754±530 <sup>a</sup>	3048±591 <sup>b</sup>	4315±676 <sup>a</sup>
ethyl decanoate	10973±631 <sup>a</sup>	9919±655 <sup>a</sup>	10658±759 <sup>a</sup>
<b>Column DB1701</b>			
3-mercapto-2-butanone	2078±66 <sup>b</sup>	<b>2312±140<sup>a</sup></b>	2036±145 <sup>b</sup>
3-methylbutyl acetate	27262±893 <sup>b</sup>	27385±1123 <sup>ab</sup>	29786±2135 <sup>a</sup>
1-hexane-ol	636±29 <sup>b</sup>	<b>727±42<sup>a</sup></b>	618±16 <sup>b</sup>
ethyl hexanoate	<b>9791±221<sup>a</sup></b>	8241±426 <sup>b</sup>	7984±887 <sup>b</sup>
2-phenylethanol	2487±341 <sup>ab</sup>	2118±145 <sup>b</sup>	2900±336 <sup>a</sup>
ethyl decanoate	<b>8117±78<sup>a</sup></b>	7135±379 <sup>b</sup>	7862±448 <sup>a</sup>

\*The different letters show that there is a significant difference between those samples at a probability level of 95% ( $\alpha = 0.05$ ). The statistical analyses applied were the ANOVA and Tukey test. The averages with the highest value, if significantly different from those in other samples, are marked bold.

## 2. Electronic nose discrimination of Fetească neagră wine groups obtained with various interventions in organic farming technology

Another attempt to analyse the data provided by the electronic nose focused on the possibility of discriminating wine samples produced from plots treated ecologically with different substances.

The substances included in Tables 2 and 3 are all that can be identified using the electronic nose database for GC and may be present in different amounts and combinations in the experimental wine samples analysed. However, the Heracles analyser does not quantify these substances, the discrimination of the samples being made only on the basis of differences in their specific combinations of volatile organic compounds (fingerprints of each wine). This is one of the advantages of using this e-nose technology, namely that conclusions can be drawn in a faster and cheaper way, without having to invest in GC with mass spectroscopy to quantify the identified compounds. Thus, these chromatographic substances / peaks were identified only to determine if they are more correlated than others with a certain type of treatment performed in the vineyard.

However, the correlation of a certain treatment with several volatile substances in wine is easier to observe in the PCA and DFA graphs, especially in the bi-plot type, which separates the sample groups, but also presents the compounds (vectors) with the highest probability of inducing separation.

The PCA analysis shows that the experimental ecological samples of Fetească neagră obtained with various interventions in culture technology can be differentiated with the help of the electronic nose based on the profile of volatile substances with discrimination power over 0.5, the discrimination index being positive (Figure 1). Although the value of the discrimination index is not very high, there is a clear differentiation of the groups of samples, especially the samples resulting from plots treated with Bordeaux mixture (FN20\_Bord), compared to the other two groups, which had treatments that included chitosan (FN20\_ChiBo and FN20\_Chit), which are closer to each other in the two-dimensional PCA space.

The two main components comprise 97.41% of the total variance, with PC1 including 67.08% of the variance and PC2 30.33%.

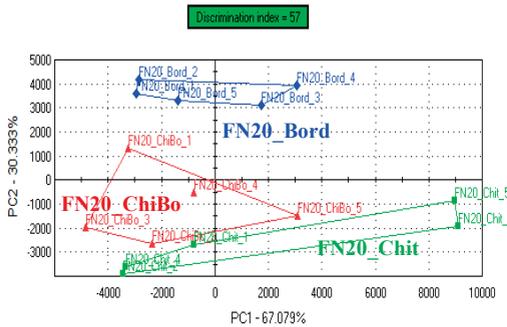


Figure 1. Analysis of the Principal Components (PC1 and PC2) for the experimental ecological wines of Fetească neagră obtained with various interventions in the culture technology (discriminative sensors with power greater than 55%)

In general, it is sufficient to include variations induced by the presence of various volatile substances in wine samples in only two main components, but sometimes, when the separation is not clear enough, a third component can be used, resulting in a three-dimensional space (Figure 2).

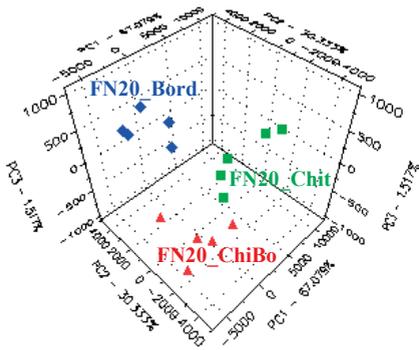


Figure 2. Separation by PCA-3D analysis of the experimental wine samples of Fetească neagră according to 3 main components (PC1, PC2 and PC3)

In the three-dimensional PCA space, the separation is clearer, noting that each wine group has an independent olfactory/aromatic identity. The 3 main components explain 98.92% of the variation of aromatic profiles (PC1 67.08%, PC2 30.33% and PC3 1.51%). However, even this separation shows that

chitosan treatments lead to sensory profiles of wines closer to each other compared to the sensory profile of control wine (FN20\_Bord). PCA analysis performed with a selection of sensors represented by chromatographic peaks whose substances have been clearly identified, compared to those based on the selection of all sensors with over 55% discrimination power, lead to a better discrimination of wines produced with technologies different vineyards. Thus, in Figure 3 it is observed that the volatile substances identified by the electronic nose and reported in Tables 2 and 3 lead to the explanation of the total variability in proportion of 95.25% (PC1 = 75.29%, PC2 = 19.96%), having a higher discrimination index (81 when taking into account the identified substances, compared to 57 when taking into account all electronic nose sensors with more than 55% discriminating power).

The principal component PC1 mainly includes substances that induce pleasant aromas in wines: 3-methylbutyl acetate (banana, pear), 2-phenylethanol (floral, honey, rose), 3-mercapto-2-butanone (vegetable), 1-hexane-ol (sweet, woody, green), while the PC2 component predominantly includes shades of raw and green such as ethyl hexanoate (green, herbaceous) and 3-hydroxy-2-pentanone (grass). Ethyl decanoate (grapes, fruity) and ethyl lactate (fruity) are equally present in both principal components. We can thus consider the PC1 axis as the floral-fruity-vegetable axis, and the PC2 axis as the one with a grassy aroma. The PC1 axis identifies two different trends: a floral-fruity area for the left quadrants of the graph and a vegetable-woody area for the right quadrants of the graph. The sample group that includes the Bordeaux mixture treatment (control, FN20\_Bord) is mainly influenced by the herbaceous component (PC2). Samples from the chitosan treatment group (FN20\_Chit) are majorly influenced by the floral-fruity component of the PC1 axis. The samples in the group treatment of Bordeaux mixture of chitosan juice and chitosan (FN20\_ChiBo) are rather correlated with the compounds on the PC1 axis that create vegetable flavours.

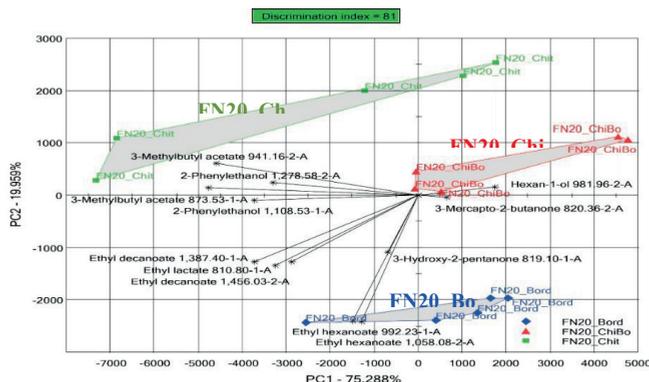


Figure 3. Bi-plot PCA for the experimental ecological wines of Fetească neagră obtained with various interventions in the culture technology

DFA analyses were also performed for the 3 groups of samples, and the results are included in Figures 4 and 5. Thus, the analysis of the discriminant factors (RA) of certain chromatographic peaks (e-nose sensors), selected by the procedure described above.

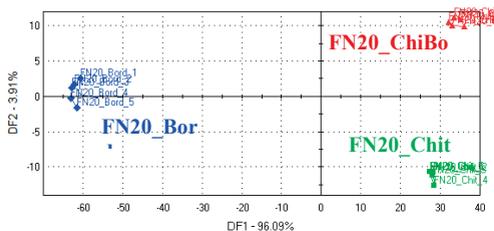


Figure 4. Analysis of Discriminatory Factors (DF1 and DF2) for discriminating groups of experimental organic wines of Fetească neagră obtained with various interventions in culture technology (discriminatory sensors with power greater than 55%)

As we can see in Figures 4 and 5, the groups of samples analyzed (FN20\_Bor, FN20\_Chi, FN20\_ChiBo) are well discriminated and separated on the DFA graph, based on the differences in concentration of the compounds that define all discriminant sensors with power greater than 55%. (Figure 4), as well as based only on the identified volatile compounds included in Tables 2 and 3 (Figure 5).

The general DFA analysis (Figure 4) shows that the first two dimensions explained in full (100%) the total variance observed for our samples, with 96.09% and 3.91% of the variance of the data explained by DF1 and DF2, respectively.

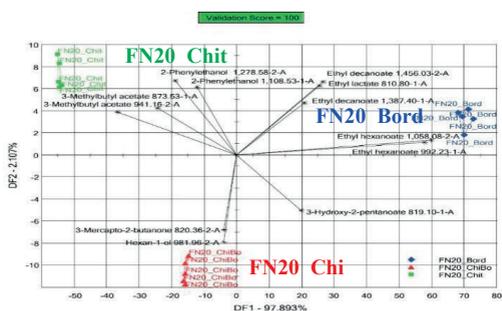


Figure 5. DFA bi-plot for the experimental ecological wines of Fetească neagră obtained with various interventions in the culture technology

The DFA bi-plot, which places the sample groups in the field containing the volatile substances identified in their sensory profile, also shows that the first two dimensions explained in full (100%) the observed variance for our samples, with DF1 = 97.89% and DF2 = 2.11%. This DFA chart also shows that wine groups tend to have different dominant flavours. Thus, it is confirmed that the control FN20\_Bor is defined by the ester aromas of ethyl hexanoate (green, herbaceous) combined with those of ethyl decanoate (grapes, fruit), FN20\_Chi samples made from chitosan-treated plots are more floral-fruity with flavours of 3-methylbutyl acetate (banana, pear) and 2-phenylethanol (floral, honey, rose), and those of FN20\_ChiBo treated with both substances have a predominant vegetal scent due to 3-mercapto-2-butanone (vegetable), 1-hexane-ol (sweet, woody, green).

In Figure 6, the SIMCA diagram also shows that compared to the control with Bordeaux mixture, the samples with treatments containing chitosan

are placed outside the olfactory space of the control, and are very close to each other.

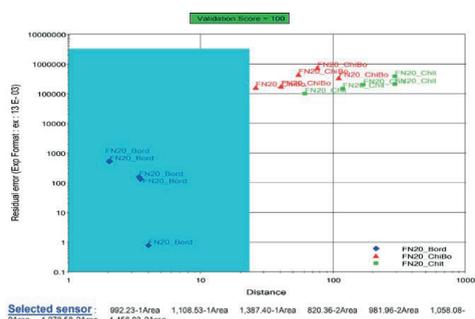


Figure 6. SIMCA diagram for determining the olfactory differences between the groups of organic Fetească neagră wines obtained with various interventions in the culture technology

## CONCLUSIONS

Electronic nose determinations indicate that samples of chitosan-treated grapes showed volatile profiles of wines closer to each other compared to the volatile profile of control wine (FN20\_Bord).

Control FN20\_Bord is defined by the ester aromas of ethyl hexanoate (green, herbaceous) combined with those of ethyl decanoate (grapes, fruit); FN20\_Chit samples, made from chitosan-treated plots, are more floral-fruity with aromas of 3-methylbutyl acetate (banana, pear) and 2-phenylethanol (floral, honey, rose); and those of FN20\_ChiBo treated with both substances have a dominant vegetable due to 3-mercaptopropanone (vegetable) and 1-hexanol (sweet, woody, green).

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## CHANGES IN THE QUALITY OF FOOD DURING STORAGE AND THE MAIN DETERMINING FACTORS

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### **Abstract**

*Food products have a vital importance for consumers because providing the energy requirements and nutrients. Through their properties, these products can improve, maintain or affect human health. Storage is the most important aspect of the food supply chain that ensures food security and round-the-year quality food supply of a country. During storage, the food quality can undergo significant changes, as most food products are characterized by a relative stability over time, influenced by both internal and external structural factors, which can modify their fundamental properties, through some degradation, alteration, chemical or microbiological processes, impurification with foreign substances, etc. Storage conditions, the microclimate and hygiene of the warehouses, environmental factors, gas composition, the nature of the packaging, the nature of other products present in the warehouse, management practices etc. affect the shelf life and quality of food products to great extent. Storage in a controlled atmosphere can slow down the loss of food quality and is an important alternative to chemical preservatives and pesticides. For example, reducing the oxygen content in a warehouse slows down the degradation of stored food. Controlled atmosphere systems maintain the organoleptic characteristics of the food and reduce losses due to pathogens.*

**Key words:** food quality, storage, chain; biochemical, microbiological.

### **INTRODUCTION**

The planet's population will reach 9.3 billion in 2050, according to a study conducted by the French Institute of Demographic Studies (INED, 2020). Facing a demographic explosion, it is essential that agriculture and the food industry find a balance between food production, energy production, resource protection and food waste reduction, while satisfying consumer demand.

The food industry is a priority area of the economy, because foods are of strategic importance (Fen, 2018; Sadiku et al., 2019). Sustainable intensification of agricultural crop production is one of the links in ensuring food security through modern genetic and biotechnological measures. The result is the sustainable production of a larger amount of food using the same area of land and at the same time reducing the negative effects on the environment, in favourable social and economic conditions (Roșculete et al., 2018; Bonciu, 2020; Bonciu et al., 2020).

Climate change is affecting food security by reducing the production of agricultural raw materials, but also by degrading agricultural

land. It is very likely that the stability of the food supply will continue to decline as extreme weather events will intensify.

Food needs to be stored in special conditions, as it is prone to rapid spoilage. The alteration consists in the change, in a negative sense, of the initial properties of the food product, so that the respective product degrades, registers losses of the nutritional value and accumulates some toxic components.

Improper food storage is one of the causes that contribute massively to the phenomenon of food waste. Proper food storage does not mean simply storing products in the refrigerator or storage space; how food is arranged in these spaces and storage conditions are important to keep food in optimal condition for consumption.

Food systems have a critical impact on people's lives, health and well-being. They also have a fundamental influence on the planet's natural resources and ecosystems. Biodiversity and food systems are highly interdependent. Biodiversity decline and mismanagement of chemicals and waste poses a serious threat to long-term food production capacity and the resilience of food systems. Urgent action is

needed to combat the direct and indirect factors of biodiversity decline in the context of food production and consumption. In this regard, it is essential to reduce pesticide dependence and nutrient overuse (Roşculete et al., 2019; Olaru et al., 2020; Partal and Paraschivu, 2020).

A systemic understanding of how agriculture, economy, consumer's health and also environmental health are interconnected is essential for food security and food safety ensuring (Paraschivu et al., 2020; Durău et al., 2021; Paraschivu et al., 2021). The Covid-19 pandemic continues to affect food systems and to endanger the global population's access to balanced nutrition. The global food crisis has caused not only major supply chain disruptions and a significant downturn in the global economy, but also the emergence of uneven steps towards recovery, which has led to an imbalance in the supply of food to the population (Paraschivu and Cotuna, 2021).

Chemical Changes During Processing and Storage of Foods provides researchers in the fields of food science, nutrition, public health, medical sciences, food security, biochemistry, pharmacy, chemistry, chemical engineering, and agronomy with a strong knowledge to support their endeavours to improve the food we consume (Rodriguez-Amaya and Amaya-Farfan, 2021).

Storage is the most important aspect of the food supply chain that ensures food security and round-the-year quality food supply of a country. During storage, the food quality can undergo significant changes, as most food products are characterized by a relative stability over time, influenced by internal and external structural factors, which can modify their fundamental properties, through some degradation, alteration, chemical or microbiological processes, impurification with foreign substances, etc.

## **MATERIALS AND METHODS**

The purpose of this paper was to point out some of the changes in the quality of food during storage and the main determining factors of these changes.

The topics followed in this research were: an overview of the changes in the quality of some main food products during storage; the main

factors that influence the safety of stored food and some modern and sustainable ways to extend the shelf life of food.

The used methods included the searching of various databases and hand searching of specialized literature with the latest publications in the field and identification of some relevant results. The main databases were Web of Science and Google Scholar as well as FAO (The Food and Agriculture Organization) and EFSA (European Food Safety Authority). Some relevant information was transposed in the form of adapted figures and tables.

## **RESULTS AND DISCUSSIONS**

The production of raw material for food and food storage at the national or global level is important due to its multifunctional roles of enhancing food access, nutrition, and income security at the national, community and household levels (Paraschivu et al., 2015; Owach et al., 2017; Paraschivu et al., 2019).

Food spoilage is caused by different types of microorganism such as: bacteria, fungus, yeasts etc. Spoiled foods usually have an unpleasant appearance, aroma, and taste. Sometimes, however, spoilage can be difficult to detect, such as when staphylococci deposit exotoxins in food or when few bacteria are present to cause a perceptible change.

Contaminating microorganisms can be transmitted to foods in several ways. Airborne pathogens can fall onto fruits and vegetables and then penetrate the product through an abrasion of the skin or rind, whereas crops carry soil borne bacterial pathogens to the processing plant. Shellfish concentrate pathogens by straining contaminated water and catching the microbes in their filtering apparatus, and rodents and arthropods transport pathogens on their feet and body parts as they move about among foods.

Human handling of foods also provides a source for transmission. For example, bacterial pathogens from an animal's intestines can be transmitted to and contaminate meat handled carelessly by a butcher (Pommerville, 2018).

Because food is basically a culture medium for microorganisms, the chemical and physical properties in and surrounding the food have a significant impact on the type of

microorganisms growing on or in the food. Several intrinsic and extrinsic factors (Figure 1) determine whether foods are likely to spoil quickly or resist spoilage (<https://microbiologynotes.org/food-spoilage-intrinsic-and-extrinsic-factors>).

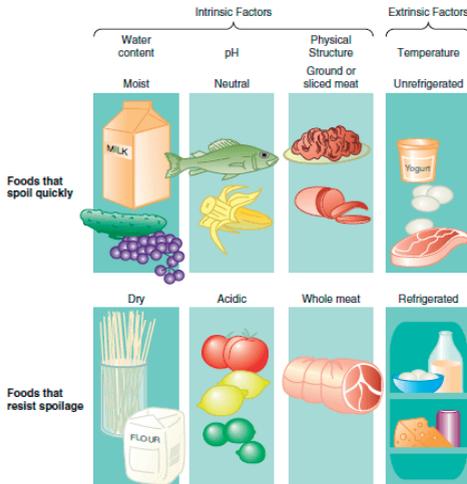


Figure 1. The intrinsic and extrinsic factors which determines foods spoilage (Pommerville, 2018)

Conditions naturally present in foods that influence microbial growth are called intrinsic factors (water, pH, physical structure, nutrients content, etc.). Thus, one of the prerequisites for all life is water. Therefore, food must be moist, with a minimum water content of 18% to 20% before contamination by microorganisms and spoilage can occur (Pommerville, 2018).

Most foods fall into the slightly acidic range on the pH scale, and numerous bacterial species multiply under these conditions. In foods with a pH of 5.0 or below, acid-loving molds often are the spoilage organisms. Extreme pH affects the structure of all macromolecules. The hydrogen bonds holding together strands of DNA break up at high pH. Lipids are hydrolyzed by an extremely basic pH. Citrus fruits, for example, generally escape bacterial spoilage but are susceptible to mold contamination (Pommerville, 2018).

The optimum growth pH is the most favourable pH for the growth of an organism. The lowest pH value that an organism can tolerate is called the minimum growth pH and the highest pH is the maximum growth pH. These values can cover a wide range, which is important for the

preservation of food and to microorganisms' survival in the stomach. For example, the optimum growth pH of *Salmonella* spp. is 7.0-7.5 but the minimum growth pH is closer to 4.2 (Jin and Kirk, 2018).

The food's nutrients can contribute to microbial growth. Fruits support organisms metabolizing sugars and carbohydrates, whereas meats support protein decomposers. Starch-hydrolyzing bacterial cells and molds often are found on potatoes, corn, and rice products. Environmental conditions surrounding the food (food storage and packaging) are extrinsic factors influencing microbial growth and food spoilage (Pommerville, 2018).

Microbial spoilage causes the loss of more than 25% of food products before consumption and wastes a considerable amount of food each year (Faour-Klingbeil and Todd, 2020). Using active packaging and antimicrobial additives for food preservation are two important target areas to protect and extend the shelf-life of perishable foods by preserving them from external environmental impacts and contamination (Faour-Klingbeil and Todd, 2020).

In the bakery and flour products units, the flour is stored in specially arranged spaces, with appropriate conditions of temperature, relative humidity and light. The storage aims at: improving the quality of the flour (as a result of the maturation process), the formation of mixtures from batches with different qualities (so as to introduce into the manufacture flour of the most homogeneous qualities, over a longer period of time), as well as ensuring the quantity necessary for the continuity of the production. The storage of the flour in improper conditions leads to the deterioration of the quality or even to its alteration, causing significant losses (Feng et al., 2020).

In normal quality flours, the microbiota is predominantly made up of bacteria and in smaller quantities yeasts and molds are found. The amount of microorganisms in the flour also depends on the conditions of transport and storage, the quality of the packaging and other factors. *Salmonella*, coagulase-positive staphylococci and other human pathogenic bacteria must be absent from the normal flour micro biota. The presence of such bacteria proves an inadequate degree of hygiene and contamination of these products by rodents.

The most common types of microbial spoilage of flour are molding, heating and the flour souring. Most common species of molds are *Aspergillus*, *Rhizopus*, *Mucor* and *Fusarium*. A significant aspect of spoilage of molds is production of mycotoxins, which may pose danger to health (Kabak et al., 2006; Duarte et al., 2010; Omotayo et al., 2019).

Compared to cereals, flour is a less microbiologically stable product. Because the flour lacks the existing defence systems in the whole grain, the representatives of the grain micro biota that reach the flour come in direct contact with the nutrients it contains and for this reason, with the appearance of favourable conditions of humidity and temperature they proliferate and this is how the flour deterioration phenomenon occurs.

Some volatile compounds formed during food photo-oxidation are toxic to humans, such as 1,4-dioxane, benzene, toluene, and lipid peroxides. Some researchers suggest that the toxic action of oxidized lipids is due to the accumulation of peroxides, aldehydes, ketones, polymers, oxypolymers, aromatic compounds and other substances that form at high temperatures (Sies, 2015; Chircă et al., 2020).

Temperature can cause many physical and chemical changes in all food constituents. Sometimes these changes can be beneficial, other times they can cause health problems. Oxidation reactions are often the cause of unwanted food changes. One such reaction is oxidative rancidity due to the peroxidation of fats and oils from various foods. In addition, many vitamins, pigments, and some amino acids and proteins are sensitive to oxygen (Chircă et al., 2020).

The packaging can control two variables in terms of oxygen and these have different effects on the rates of oxidation reactions in food. Therefore, the selection of smart packaging is necessary to ensure adequate food protection.

Meat is one of the most commonly eaten foods in a diet. Given the diversity of origin (of cattle, pigs, sheep, poultry, etc.) special attention must be paid to the receipt, storage and marketing of this product.

During storage, the dominant microbiota can cause the deterioration and release of volatile compounds or slime formation; as a

consequence, the product becomes unacceptable for human consumption (Iulietto et al., 2015). Thus, according to the cited authors, the main factors which affect the shelf-life of meat products and favour some bacterial strains rather than others, are: packaging (aerobically, vacuum or modified atmosphere), storage temperature, the composition of the products (Table 1)

Table 1. The main factors which affecting the shelf-life of meat (Iulietto et al. 2015)

Intrinsic factors	Extrinsic factors
Species, breed, age and feeding of the animal of origin	Quality management system
Initial microbiota	Packaging system
Chemical properties (pH, a <sub>w</sub> , redox potential, peroxide value)	Temperature control
Product composition	Processing conditions and hygiene
Antimicrobial components	Storage types
Biopreservation systems (bacteriocinogenic LAB cultures and/or their bacteriocin)	Relative humidity, atmospheric gas composition and ratio

Regarding the fruits and vegetables, the factors that cause alteration are: temperature, non-ionizing radiation, water, oxidation process, microorganisms and enzymes.

After harvest, fruits and vegetables continue to show vital attributes through the processes of respiration and perspiration, degradation of substances with more molecular weight as well as the synthesis of flavours. On the other hand, the lifespan of these products is limited by their composition and the interaction between its components and environmental factors (Silva, 2008).

Because of this, especially under the influence of temperature and humidity, the activity of enzyme systems leads to profound changes in the nature of chemical components, a change that results in the loss of the state of fragility and freshness, by the loss of its colour, the weakening and even disappearance of the aroma, structure and textural firmness and natural resistance to various microorganisms.

Maintaining crop quality after harvest is an important consideration for any fresh market produce grower or handler. Because of the significant effect of temperature on respiration, the amount of time a harvested product is exposed to heat should be minimized; the fruit or vegetable should be quickly brought to its optimal storage temperature (Silva, 2008).

Harvested fruits and vegetables of different plants have different rates of respiration (Table 2).

Table 2. The different rates of respiration to fruits and vegetables (Silva, 2008, after Wilson, 1999)

Respiration Rates	Types of Fruits and Vegetables
Very low	Dried fruit and nuts
Low	Apples, garlic, grapes, onions, potatoes (mature), sweet potatoes
Moderate	Apricots, cabbages, carrots, figs (fresh), lettuce, nectarines, peaches, pears, peppers, plums, potatoes (immature), tomatoes
High	Artichokes, Brussels sprouts, cut flowers, green onions, snap beans
Extremely high	Asparagus, broccoli, mushrooms, peas, sweet corn

Molds such as *Penicillium* sp., *Aspergillus* sp. and *Sclerotinia* sp., causes spoilage of fruits and vegetables, both before and after harvest. Alteration becomes faster as the microorganisms come in contact with the cell juices and as the temperature, relative humidity of the air and its oxygen content are more favourable to the living conditions of the microorganisms.

Increasing the shelf life for perishable fruit can be achieved by applying some sensor-equipped monitoring devices. In this way, concrete and ultra-fast information can be obtained about the actual conditions in which the respective foods were stored and transported. The unique identification of each food product is possible by combining sensor-based technologies with radio frequency identification (RFID) markings, which allow the interpretation of environmental data on a chip. These modern technologies allow the use of guaranteed quality declarations, the supply of ready-to-eat products to supermarkets, as well as the application of logistical concepts such as FEFO (the first to expire, the first to leave).

In the supply chains for processed and fresh food products, the partners have a shared responsibility of minimizing quality losses to deliver high-quality products to end users. In spite of their efforts, a large portion of what is produced is never consumed (Hertog et al., 2014).

Food storage should be done under conditions that prevent changes in nutritional, organoleptic and physico-chemical properties, as well as microbial contamination. For this purpose, the food will be stored in rooms or in specially arranged spaces, protected from insects and rodents, equipped with the necessary installations and equipment to ensure the control of

temperature conditions, humidity, ventilation, etc., established by normative acts in force.

In order to prevent food spoilage, an important factor is the duration of the transport, which must be as short as possible. During transport, the food must not change its qualities. Maximum efficiency is an essential condition in preventing food contamination or spoilage.

## CONCLUSIONS

In the process of cultivation, collection, transportation, storage, slaughter, processing, production of raw materials and food, their contamination can take place. In addition, improper food storage is one of the leading causes of food waste.

Foods need to be stored in special conditions, as they are prone to rapid spoilage. The alteration consists in the modification, in a negative sense, of the initial properties of the food product, so that the respective product registers losses of the nutritional value and accumulates some toxic components. Depending on the nature of the contaminants, they are classified into: physical, chemical and microbiological contaminants.

During storage, the food quality can undergo significant changes, as most food products are characterized by a relative stability over time. There are several parameters which are optimal considered to control the food quality: water activity, pH, temperature, light, and the partial pressures of oxygen and carbon dioxide.

A modern concept for preventing food spoilage is the design of smart food packaging based on these parameters. Such packaging has the advantage of helping to maintain the safety and quality of food in conditions of durable environmental protection.

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## ESTIMATION OF MEASUREMENT UNCERTAINTY FOR POTASSIUM IN ORGANO-MINERAL FERTILIZERS

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### Abstract

*Along with nitrogen and phosphorus, potassium is one of the primary nutrients that provides significant yield increases when applied in amounts necessary for crop development. In order to avoid environmental pollution and to ensure the necessary content of this nutrient, it is important to know the amount of soluble potassium in the applied fertilizers. This article presents the evaluation of some parameters for estimation of measurement uncertainty for water-soluble potassium in organo-mineral fertilizers. Potassium was determined by flame-photometric method and the expanded uncertainty (U) of the method, obtained by multiplying the combined uncertainty by the coverage factor  $k = 2$  (confidence level 95%) was 10.18%. The determinations were performed on a sample of organo-mineral fertilizer with a complex matrix and known composition in which the average of the determinations was 12.69%  $K_2O$ .*

**Key words:** measurement uncertainty, potassium, organo-mineral fertilizers, flame photometric method.

### INTRODUCTION

Knowing the composition of fertilizers is a priority for all users. Manufacturers are obliged to declare the composition and to comply with the tolerances imposed by the legislation in force. Currently, in Romania, both national (Order no. 6/2004) and European legislation (Regulation (EC) no. 2003/2003) on fertilizers provide for tolerances on nutrient composition. Currently, in Regulation (EC) no. 2003/2003 for the analysis of the fertilizers provided in Annex I List of types of EC fertilisers, for determination of potassium is provided in the standard EN 15477: Fertilisers - Determination of the water-soluble potassium content. According to this Annex, given the sources of raw materials and the types of fertilizers provided, producers need only declare the water-soluble potassium content.

Agriculture is one of the sectors that contributes greatly to the world's greenhouse gas emissions. The use of raw materials obtained from the recycling of by-products from agricultural and industrial activities allows the return to the production chain, which can be beneficial both economically and ecologically and it is part of the concept of circular economy (Crusciol et al., 2020; Liu et

al., 2020). Currently, on the market are sold fertilizers from the category "organic", "organo-mineral" with variable content of organic substances from various sources. In this context, the variety of raw materials makes it difficult to find a method for determining the composition of fertilizers. As the water-soluble potassium content is an indication of the immediate availability of this nutrient for plant nutrition, its determination is of great importance to farmers. In addition to this, we must keep in mind that potassium applied in improper amounts can lead to environmental pollution or poor nutrition.

All measurements are affected by errors and the measurement uncertainty tells us the size of the measurement error (Magnusson et al., 2017).

As defined in Regulation (EC) no. 2003/2003, a solid organo-mineral fertilizer shall contain at least one of the following declared primary nutrients: nitrogen (N), phosphorus pentoxide ( $P_2O_5$ ) or potassium oxide ( $K_2O$ ) and the content of total potassium ( $K_2O$ ) should be at least 2%. The determinations presented in this paper were made on a matrix of organo-mineral fertilizer with a content of 12%  $K_2O$ .

Potassium plays an important role in plant development (Çalışkan & Çalışkan, 2019). Potassium is an essential nutrient for plant

growth (Marschner, 1995; Nieves-Cordones et al., 2019). Potassium helps in alleviating detrimental effects of salt stress (Kumari et al., 2021) influencing the chlorophyll content (Kaya et al., 2001).

A useful measure used to demonstrate the quality of results is measurement uncertainty (Ellison & Williams, 2012), and laboratories need this parameter to verify their own measurement quality, given that estimating measurement uncertainty is a requirement in SR EN ISO 17025: 2018. All measurements are affected by errors and the measurement uncertainty provides information about the size of the measurement error. Therefore, measurement uncertainty is an important part of the reported result (Magnusson et al., 2017). Ignoring the value of the uncertainty of the composition of a fertilizer can lead to an overestimation of its efficiency (Khor & Zeller, 2014).

The use of validated methods is important for an analytical laboratory to show its qualification and competency. Method validation is done by evaluating a series of method-performance characteristics, such as precision, trueness, selectivity/specificity, linearity, operating range, recovery, limit of detection (LOD), limit of quantification (LOQ), sensitivity, robustness, measurement uncertainty (Magnusson & Örnemark, 2014).

This paper focuses on estimating the measurement uncertainty related to water-soluble potassium determination by the flame photometric method in organo-mineral fertilizers.

## MATERIALS AND METHODS

### *Reagents and solutions*

An organo-mineral fertilizer reference material with the following characteristics was used:

- assigned value:  $m = 12.69\%$   $K_2O$  and uncertainty,  $u = \pm 0.19\%$ ;
- standard deviation of the mean:  $s = 0.88\%$ ;
- tolerance range: 11.89-13.49%.

The sample was finely ground in order to obtain a representative sample for analysis.

For the preparation of the working standard solutions of organo-mineral fertilizer samples, a standard potassium solution of 1000 mg/L traceable to the NIST was used. Potassium

standard solution and  $H_2SO_4$  95-97% (used to prepare 1N solution) of analytical grade were obtained from Merck (Germany). All solutions were prepared using distilled water (purity class 2), according to the standard SR EN ISO 3696.

### *Equipments*

All weighing operations were carried out using METTLER TOLEDO AG204 analytical balance. Potassium content was determined with the Flame Photometer model Sherwood 410 with a preloaded filter stick for K.

### *Preparation of Working Standard Solutions*

Working standard solutions containing 0.00, 2.00, 4.00, 6.00, 8.00, 10.00 K  $\mu\text{g/mL}$  were prepared by proper dilutions of the 1000 mg/L potassium standard. For every 100 mL of prepared standards, 10 mL of 1N sulfuric acid was added. The volumetric flask was filled to the mark with distilled water.

### *Method description*

Flame photometry is part of atomic emission spectrometry and aims to determine the qualitative and quantitative elements based on the interpretation of emission spectra. The potassium in the sample is dissolved in boiling water. After elimination or fixation of substances that may interfere with the quantitative determination, potassium is determined based on the interpretation of the emission spectra using a flame photometer. The temperature of the flame ensures the melting and evaporation of the salt, the dissociation of the molecules and the excitation of the atoms formed.

During the determinations, the temperature was monitored using a thermo-hygrometer ( $t = 20 \pm 4^\circ\text{C}$ ).

Spray each standard solution successively into the flame of the flame photometer and note the intensity of the radiation emitted. After each standard solution, water is sprayed into the flame. For each standard solution 3 sprays are made, and as a result the average of the three indications of the emitted radiation intensity is considered.

It is known that the number of replicates of the calibration standards is an excellent way to minimize the random calibration error and then

to increase the accuracy of the values in the measurements of the samples (Raposo, 2016). In order to evaluate the parameters of the calibration line, the calibration curve is drawn, considering on the ordinate the intensity of the emitted radiation, and on the abscissa the corresponding potassium content ( $\mu\text{g/mL}$ ).

### Calibration curve

From the practical experience of the laboratory and the evaluations performed (ISO 8466-1: 1990), the linearity range in the determination of potassium is  $0.5 \div 10 \mu\text{g/mL}$  which is in accordance with the equipment manufacturer's instructions ( $0.5 \div 20 \mu\text{g/mL}$ ) (Sherwood-model-410).

## RESULTS AND DISCUSSIONS

The measurement uncertainty was calculated by following an internal procedure and international guidelines (Ellison & Williams, 2012). The evaluation of measurement uncertainty of water-soluble potassium determination consisted in: specification of the measurand, identification of uncertainty sources, quantification of uncertainty components and calculation of combined and expanded uncertainty.

### Specification of the measurand

The potassium content of the organo-mineral fertilizer, expressed as a percentage of potassium oxide ( $\text{K}_2\text{O}$ ), shall be calculated according to the following formula:

$$\% \text{ Potassium } (\text{K}_2\text{O}) = \frac{c \cdot v \cdot d_1 \cdot d_2 \cdot 100}{m \cdot 10^6} \times 1.2 \quad (1)$$

where:

v - the volume of extract from the sample, (mL);

m - mass of the sample, (g).

c - the concentration of potassium corresponding to the intensity of the emitted light read on the calibration curve ( $\mu\text{g/mL}$ );

1.2 - conversion factor for potassium (K) in potassium oxide ( $\text{K}_2\text{O}$ );

$d_1$  - coefficient of the first dilution (ratio between the volume of the intermediary dilution and aliquot 1);

$d_2$  - coefficient of the second dilution (ratio between the volume of the final dilution and aliquot 2).

### Identification of uncertainty sources

Figure 1 illustrates the cause-and-effect diagram for the standard uncertainty of the method.

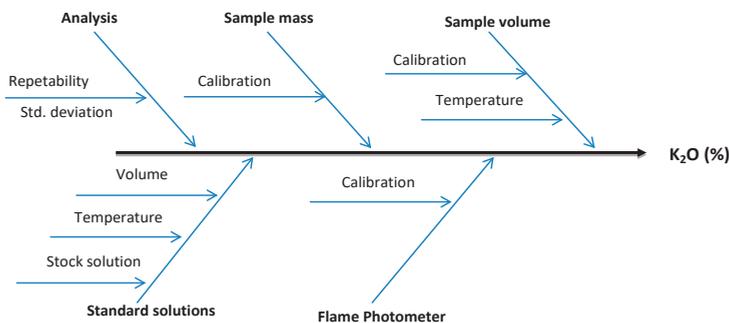


Figure 1. Cause and effect diagram for water-soluble potassium determination by flamephotometric method

As schematized in Figure 1, the following sources of were identified: (a) uncertainty from the standard and standard dilution to obtain the working calibration solutions; (b) uncertainty from the flame-photometer; (c) the dilution factor includes the contributions of uncertainties from the volumetric flasks and pipettes and the volume expansion at the

laboratory temperature; (d) uncertainty related to the sample mass/analytical balance (calibration certificate); (e) repeatability, evaluated by measuring 10 sample replicates. The measurements were conducted by the same analyst using the same flame-photometer instrument under the same working conditions.

## Quantification of individual components

### Repeatability

All steps of the measurement procedure (Figure 1) were included in the uncertainty associated with the repeatability of the overall experiment. Repeatability data is given in Table 1.

Table 1. Repeatability of the flame-photometric method for determination of potassium in organo-mineral fertilizer (OMF) (secondary reference material)

Sample	% water-soluble potassium (% K <sub>2</sub> O)
Replicate 1	12.686
Replicate 2	12.687
Replicate 3	12.920
Replicate 4	12.687
Replicate 5	12.450
Replicate 6	12.215
Replicate 7	12.686
Replicate 8	13.156
Replicate 9	13.390
Replicate 10	11.981
Statistical parameters	% K <sub>2</sub> O
Average value	12.686
Standard deviation, n = 10	0.414

### Sample mass, dilution factor and flame-photometer contribution

The uncertainty related to the calibration of all equipment was evaluated using the data from the calibration certificates. The standard uncertainty associated with the mass of the sample was calculated (Eq. 2), using the data from the calibration certificate (De Oliveira, 2016). This contribution was counted twice, once for the tare and once for the sample weight, because each is an independent observation and the linearity effects are not correlated (Ellison & Williams, 2012). Standard uncertainties  $u(x)$  were calculated according to the following equations:

$$u(m) = \sqrt{\left(\frac{U_{\text{eccentricity}}}{2}\right)^2 + \left(\frac{U_{\text{linearity}}}{2}\right)^2} \quad (2)$$

$$u(v) = \sqrt{\left(\frac{U_{\text{calibration}}}{2}\right)^2 + \left(\frac{V_{\text{temp}}}{\sqrt{3}}\right)^2} \quad (3)$$

$$u(x) = \frac{U_{\text{calibration}}}{2} \quad (4)$$

For all volumetric equipment the uncertainty consists of 2 components: calibration uncertainty and uncertainty due to the volume expansion at laboratory temperature (20±4°C), calculated by means of Eq. 5 (Ellison & Williams, 2012):

$$V_{\text{temp}} = V_{\text{calibration}} \times Dt \times 2.1 \times 10^{-4} \quad (5)$$

where:

$V_{\text{temp}}$  - uncertainty due to the volume expansion at laboratory temperature;

$V_{\text{calibration}}$  - volumetric glassware uncertainty according to calibration certificate;

$2.1 \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$  - coefficient of volume expansion for water;

$Dt$  - temperature variation in the laboratory (20±4°C).

Standard uncertainty due to sample dilution was estimated by summing the uncertainties of the volumetric glassware to the volume expansion (Table 2). The relative uncertainties due the dilution factor of the sample (volumetric flasks and pipettes) were combined into one contribution. Standard uncertainty related to the flame-photometer equipment was calculated by means of Eq. (4).

### Working standard solutions and stock solution contribution

An example for the preparation of one working standard solution is summarized in Table 3. The same procedure was repeated for all standards. Standard uncertainty associated to the stock solution was estimated by means of Eq. 4 (Table 3). The relative uncertainty related to the dilution of the standards was obtained by the sum of the relative uncertainties due to the dilution factor and stock solution.

The relative contribution of each component, as well as the combined and combined standard uncertainties are summarized in Table 4. The combined standard uncertainty of the method was estimated according to Eq. (6). The expanded uncertainty of the method (U) was obtained by multiplying the combined uncertainty with a coverage factor of 2.

$$u(K_2O) = \bar{x} \sqrt{u(r)^2 + u(m)^2 + u(l)^2 + u(c)^2 + u(v)^2 + u(e)^2} \quad (6)$$

To calculate combined uncertainty for potassium determination in organo-mineral fertilizer by flame-photometer method, Eq. (7) was used, and the value 5.09% was obtained.

$$U_c = (u_c \times 100) / \bar{x} \quad (7)$$

The extended uncertainty (U) of the method is calculated by multiplying the standard uncertainty combined with a coverage factor  $k = 2$ , which gives a level of confidence of approximately 95% (Eq. 8) and a value of 10.18% was obtained.

$$U = U_c \times 2 \quad (8)$$

The result of water-soluble potassium in organo-mineral fertilizers of measurement and the expanded uncertainty estimated by using Eurachem guidelines (Ellison & Williams, 2012) was  $12.686 \pm 1.29\%$   $K_2O$ .

Bias of the method is expressed as analytical recovery (value observed divided by value expected) and the value of 0.3% was obtained.

Table 2. Standard uncertainties related to sample mass, dilution factor and flame-photometer

Source/ Quantity	Uncertainty components		Distribution		Eq. (no)
Analytical balance					
Mass of the sample 2.5 g	Eccentricity 0.0005 g/	Linearity 0.0002 g	normal 2	normal 2	Eq. (2)
Dilution factor (volume)					
Volumetric flask (v1) 500 mL	Calibration 0.05 mL	Volume expansion 0.2425 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3)
Volumetric flask (v2) 250 mL	Calibration 0.03 mL	Volume expansion 0.121 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3)
Volumetric flask (v2) 100 mL	Calibration 0.03 mL	Volume expansion 0.0485 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3)
Aliquot 1 25 mL	Calibration 0.02 mL	Volume expansion 0.0121 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3)
Aliquot 2 10 mL	Calibration 0.023 mL	Volume expansion 0.0048 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3)
Flame photometer	Calibration 0.570 $\mu\text{g} / \text{mL}$		normal 2		Eq. (4)

Table 3. Standard uncertainties related to the preparation of working standard solutions and to the stock standard solution

Source/ Quantity	Uncertainty components		Distribution		Eq. (no)
Dilution factor (volume) <sup>i</sup>					
Burette 2 mL	Calibration 0.015 mL	Volume expansion 0.00097 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3)
Volumetric flask 100 mL	Calibration 0.03 mL	Volume expansion 0.0485 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3)
Stock solution <sup>ii</sup>					
Concentration 1000 mg/kg	Calibration 5 mg/kg		normal 2		Eq. (4)

<sup>i</sup>Standard uncertainty related to the preparation of one working standard solution

<sup>ii</sup>Standard uncertainty related to the stock standard solution

Table 4. The relative contribution of the uncertainty components and combined standard uncertainties for potassium determination in organo-mineral fertilizer by flame-photometer method

Uncertainty component	Value	Type
Potassium content mean, %	12.686	A
Relative uncertainties of the repeatability, u(r)	0.0327	A
Relative uncertainties of the calibration solutions, u(c)	0.0052	A & B
Relative uncertainties of the linearity range, u(l)	0.0151	A
Relative uncertainties of the balance, u(m)	0.00008	B
Relative uncertainties of the flask / pipette, u(v)	0.0014	B
Relative uncertainties of the flam photometer, u(e)	0.0356	B
Combined standard uncertainty ( $u_c$ )	0.6458	A+B
Combined uncertainty ( $U_c = (u_c \times 100) / \bar{x}$ ) of the method	5.09	%
Expanded uncertainty, $k=2$ ( $U = U_c \times 2$ ) of the method	10.18	%

### Uncertainty budget

The uncertainty budget as relative contributions of the different components are shown in Figure 2.

As shown in Figure 2, the main sources of uncertainty of the result of measurement were

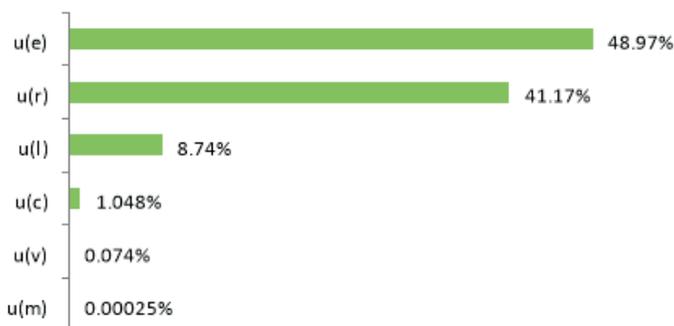


Figure 2. The uncertainty contributions as relative uncertainties (as coded in Table 4)

### CONCLUSIONS

This study evaluated the measurement uncertainty of the result of water-soluble potassium determination by flame-photometer instrument in organo-mineral fertilizers.

The identified uncertainty components were the repeatability, standard solution dilutions, flame-photometer, linearity range, sample mass (analytical balance) and sample dilution factor. It was observed that the largest contribution comes from the flame-photometer (type B) followed closely by repeatability (type A).

For the extended uncertainty (U) of the method with a coverage factor  $k = 2$ , which gives a level of confidence of approximately 95% a value of  $\pm 10.18\%$  was obtained.

### ACKNOWLEDGEMENTS

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contributions from repeatability, standard solutions preparation and flame-photometer instrument, whereas the contribution of the balance (sample mass) and volumetric glassware had no influence on the overall uncertainty budget.

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## ENTOMOFAUNISTIC STUDY ON THE SPECIES *Silphium perfoliatum* L. IN THE REPUBLIC OF MOLDOVA

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### Abstract

This article presents the results of entomofaunistic investigations on the species *Silphium perfoliatum*, known as a fodder, honey, energy, medicinal and ornamental crop. Under the climatic conditions of the Republic of Moldova, *S. perfoliatum*, starting with the 3rd year of vegetation, enters the generative phase, blooms, bears fruit and produces viable seeds. It is characterized by a staggered and long flowering period (52-63 days) that occurs in July-October. The monitoring of the entomofauna has allowed the determination of the spectrum of insects attracted by the *S. perfoliatum* plants in the flowering stage. Ten species of insects included in 6 families and 4 orders were determined. The species of the Apidae family (*Apis mellifera*, *Bombus terrestris*, *B. lapidarius*), recognized as the main species of pollinating and honey insects, had the highest frequency on flowers.

**Key words:** *Silphium perfoliatum*, development, pollinating insects.

### INTRODUCTION

The role of insects is essential in plant life and also in nature in general. Insects are among the most diverse organisms on earth and comprise more than 60% of all known species (Bidau, 2018). They are responsible for pollination, as an essential process for plant reproduction. Approximately 90% of angiosperms rely on animal pollinators (Friedman & Barrett, 2009). In recent years, the area cultivated with entomophilous plants has increased, respectively, the need for pollination has also increased. However, there is a decrease in the number of pollinating insects worldwide. In Europe, over the last 20 years, domestic honey bee populations alone have declined by 25%. The wild pollinating insects have experienced a similar situation (Kluser & Peduzzi, 2007). There are a large number of insects that participate in pollination. In addition to the familiar ones, flies (representatives of the Diptera family), bumblebees, solitary bees, butterflies, moths, beetles, stingless bees also make a special contribution. The European honey bee (*Apis mellifera* L.), described as the most common pollinator and honey producer, enjoys special attention as compared with the other almost 20,000 known bee species (Kunast et al., 2014). The bee products obtained as a

result of the activity of honey insects (honey, pollen, propolis, wax, royal jelly) are used as food, dietary, cosmetic and pharmaceutical resources, due to the rich content of biologically active substances (Eremia et al., 2002).

*Silphium perfoliatum* L. is a plant species of the family ASTERACEAE Bercht. & J. Presl (= COMPOSITAE Giseke.). It is a perennial herbaceous, polycarpic plant, native to North America, with erect stem, branched in the upper part. The leaves are light green, heart-shaped, opposite, the lower ones are petiolate, and the upper ones are fused around the stem, forming a cup that allows the efficient use of humidity and sunlight. The plant produces yellow flowers, 3-5 cm in diameter. The fruit of cup plant is a brown achene with a marginal wing used for wind dispersal. About 20-30 achenes with a length of 8-14 mm were produced in each flower head (Țiței & Cîrlig, 2020). Seed productivity reaches 290-450 kg/ha. The plant develops a tap root growing down to 3.5 m deep (Țiței & Roșca, 2021).

Multiple scientific studies have demonstrated that cup plant can be used in various fields. Knowledge about the entomofauna of cup plant is of great practical interest. To date, there are some data on harmful insects that could attack

the plants. Cup plant is described as a species less vulnerable to pests and diseases, and even if it is attacked, the production of seeds and biomass is not affected (Reinert et al., 2020). Murrell and Turner, in 2020, described the pests that can attack cup plant. The list includes harmful insects, fungi and viruses (*Silphium* virus caused by *Dahlia* endogenous plant pararetrovirus sequence (Dv EPRS) (Murrell & Turner, 2020). The useful entomofauna of cup plant is less studied worldwide, but, in the Republic of Moldova, this research is of great importance.

In recent years, cup plant has been listed among the promising honey plants, plants with high nectar productivity (58 kg/ha) (Muller et al., 2020; Muller & Bergher, 2020; Savin & Gudimova, 2019; Pastukhova, 2019), which allows collecting large quantities of honey, 150-450-560 kg/ha honey (Koltowski, 2005). Other sources mention that nectar productivity can reach up to 205.2-611.6 kg/ha, depending on the amount of mineral fertilizers used (Savin & Gudimova, 2019).

Cup plant blooms from late summer to late autumn and the flowers produce nectar and pollen, that is why plants are an important source of food for insects before the cold season, especially for bumblebees, which prepare the queen for hibernation (Amiet & Krebs, 2012; Mueller et al., 2020), being also a source of food for bees in the agricultural landscape (Decourtye, 2010). *Silphium perfoliatum* can support certain hoverfly groups when it is harvested late to ensure a flower supply through to September and when semi-natural habitats are maintained in agricultural landscapes (Muller & Dauber, 2016).

The goal of this study has been to carry out the inventory of the entomofauna detected on *S. perfoliatum*, grown under the climatic conditions of the Republic of Moldova, as a high-potential honey plant, attractive for pollinating and honey insects.

## MATERIALS AND METHODS

The research subjects were the plants of the cultivar 'Vital' of cup plant, *Silphium perfoliatum* L., which was created, registered in the Catalogue of Plant Varieties in 2012 and patented at the State Agency for Intellectual

Property (Teleuță & Țiței, 2016), grown in the experimental sector of the "Alexandru Ciubotaru" National Botanical Garden (Institute), the collection of fodder and honey plants.

The experimental sector is composed of plants belonging to different botanical families, species with different, staggered flowering periods, which can serve as a source of food for insects for a long time. This contributes to the diversification of invertebrate fauna.

The growth and development rate of the studied species, in the years 2020-2021, was analyzed according to the methodologies: *Методика изучения фенологии растений и растительных сообществ*, (Beideman, 1974). *Методические указания по семеноведению интродуцентов* 1980.

In order to determine the insect species on the *S. perfoliatum* plants, biological surveys were carried out during the generative phase of the plants, especially in the flowering stage. The entomological monitoring was performed starting with the middle of June (when the flowering stage began), with an interval of 5-8 days. In total, more than 12 records and assessments were made in the flowering stage. Emphasis was placed on the morning (9: 00-12: 00) and afternoon hours (14: 00-16: 00). The observations were made in sunny weather, with no strong wind, sometimes in light rain. The determination of the taxonomic affiliation and the morphological spectrum of the insects was based on a series of direct observations in the field, on the diagonal of the experimental sector, taken photos and sample collections. Subsequently, in the laboratory, using manuals and entomological determination guides (Talmaciu & Talmaciu, 2014; Cozari, 2010, Perju, 1995; Plavilsciov, 1994; Mamaev, 1985), the list of pollinating insects was made, the trophic specialization was determined.

## RESULTS AND DISCUSSIONS

*Silphium perfoliatum* L., under the climatic conditions of the Republic of Moldova, grows as an herbaceous perennial plant, with a growing season of about 205 days. The plants come out of dormancy in early spring when temperatures reach 3-5 C. Cup plant is propagated by transplanting the seedlings

grown indoors into the open field. *S. perfoliatum* passes into the generative phase starting with the 3rd year of development. Mature plants develop stems that can reach 250-370 cm in height. The leaves are 25-35 cm long and 16-22 cm wide (Țiței & Cîrlig, 2020). The research on the development rates made it possible to describe the phenological phases

characteristic of the plants that had acclimatized under the conditions of the Republic of Moldova. In this article, more emphasis was laid on the generative stages (Table 1), to highlight the honey potential of cup plant and to determine the spectrum of insects that visit the plants.

Table 1. The initiation of generative stages depending on climatic conditions

Stages	Budding (beginning)	Budding (full)	Flowering (beginning)	Flowering (full)	Flowering (end)
Year					
2020	16.06	23.06	22.06	04.07	25.08
2021	23.06	30.06	28.06	19.07	20.10

Based on the criteria used in beekeeping, honey plants are classified according to the time of flowering: early spring, spring, summer and autumn (Pîrvu, 2000). *S. perfoliatum* is attributed to the group with summer-autumn flowering. The budding stage begins in June. The staggered flowering extends over a period of 52-63 days, which positively influences the food supply for bees. The inflorescence (dichasium) is composed of 20-30 yellow flower heads with a diameter of 3-5 cm, each flower produces 20-30 seeds. The climatic conditions recorded in the growing seasons of 2020 and 2021 (Table 2) were favorable for plant growth and development, and any significant differences haven't been noticed.

Some changes were noticed at the onset of the generative phenological stages (budding, full flowering and end of flowering). As a result of the flowering, which occurs later in the season, the insects that are preparing for winter only benefit. The spring of 2021 was inhomogeneous in terms of temperature, but with favorable humidity levels for plant development. The autumn of 2021, as compared with that of 2020, was characterized by meteorologists as a season with an average air temperature by 2-3°C below the normal average (meteo.md). In September - November (2021), there was a deficit of rainfall, but the development of *S. perfoliatum* plants during this period was not affected.

Table 2. Meteorological indices in the studied period

Year	Indices	Months												
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	M
2020	t°C	1.5	4.4	8.4	11.8	14.4	21.8	23.7	24.0	20.8	14.6	4.8	1.8	12.7
	P. mm	9	23	19	4	69	86	85	5	75	81	32	74	562
2021	t°C	0.1	-0.3	3.8	8.5	15.3	20.2	24.0	21.7	15.7	10.2	6.8	0.8	10.6
	P. mm	39	43	36	39	101	86	114	118	6	1	12	71	666

Note: M - annual average; P - amount of precipitation

According to the production potential (kg/ha), honey plants are classified into 6 groups. Depending on the climatic conditions and the geographical area in which they grow, *S. perfoliatum* plants fall into the group 4 (101-200 kg/ha), 5 (201-500 kg/ha) and group 6 (over 500 kg/ha).

Cup plant, being known as a multi-purpose crop, is also a high potential honey plant, therefore, it is necessary to study the

pollinating honey-producing entomofauna interested in this species, especially in the flowering stage.

The research on pollinating and honey insects in the growing season of 2020, 2021, with the weather conditions corresponding to the area and the year, according to the study of the generative organs (flower buds, flowers), detected the presence of 10 species of insects on *S. perfoliatum* plants. Systematically, they are

classified into 6 families (Apidae, Halictidae, Syrphidae, Sarcophagidae, Cerambycidae, Satyridae) and 4 orders (Hymenoptera, Diptera, Coleoptera, Lepidoptera) (Table 3).

Table 3. The diversity of entomofauna on *S. perfoliatum* plants

No	Order	Family	Species	Trophic spectrum
1	Hymenoptera	Apidae	<i>Apis mellifera</i> (Linnaeus, 1758)	Pollen and nectar
2			<i>Bombus terrestris</i> (Linnaeus, 1758)	Pollen and nectar
3			<i>Bombus lapidarius</i> (Linnaeus, 1758)	Pollen and nectar
4			<i>Bombus hortorum</i> (Linnaeus, 1761)	Pollen and nectar
5		Halictidae	<i>Lasioglossum malachurum</i> (Kirby, 1802)	Pollen and nectar
6	Diptera	Syrphidae	<i>Eristalis tenax</i> (Linnaeus, 1758)	Phytophagous
7			<i>Sphaerophoria scripta</i> (Linnaeus, 1875)	Phytophagous
8		Sarcophagidae	<i>Sarcophaga carnaria</i> (Linnaeus, 1758)	Plant liquids
9	Coleoptera	Cerambycidae	<i>Agapanthia villosoviridescens</i> De Geer.	Phytophagous
10	Lepidoptera	Satyridae	<i>Maniola jurtina</i> (Linnaeus, 1758)	Pollen and nectar

They are species with phytophagous trophic specialization, which feed on nectar, pollen and other plant liquids. The entomofauna was more abundant from the beginning of July to the middle of October, period which coincides with the flowering stage of the plants, which provides favorable conditions for the nutrition and reproduction of insects.

The taxonomic classification of the entomofauna allows identifying the maximum share, which belongs to the species of the order Hymenoptera (50% of the total number of determined insects), Diptera with 30%, Coleoptera - 10% and the order Lepidoptera with 10%. The species from the Apidae family (*Apis mellifera*, *Bombus terrestris*, *B. lapidarius*) (Figure 1, 2), had the maximum frequency on flowers, being present throughout the day on the cup plant flowers. The honey bee collects food from a cup plant flower for about 20 sec. On days with high temperatures, without wind, 2-3 bees and 1-2 bumblebees can simultaneously be on a shoot.

The representative of the family Syrphidae, *Eristalis tenax* (Figure 3) is a species quite common on cup plant flowers, especially in September and October. It feeds on flower nectar and plays an important role in pollinating entomophilous plant species.



Figure 1. The species *Bombus terrestris* on a cup plant flower



Figure 2. The species *Apis mellifera* collecting pollen



Figure 3. The species *Eristalis tenax*



Figure 4. *S. perfoliatum* flower with the pollinating insect *Lasioglossum malachurum*

The species *Lasioglossum malachurum* (Figure 4) was observed less frequently on flowers, as solitary individuals, but it has an important function in the pollination process.

Several researchers from South Dakota described 3 species of insects found on *S. perfoliatum* plants that have not yet been found on other plant species (*Eucozoma giganteana*, *Acanthocaudus* n. sp., and the aphid *Uroleucon* cf. *ambrosiae*). These species, at different stages of life, can be harmful to the growth and development of cup plant, reducing the level of reproduction (because of the damage to the apical meristematic tissues, including flower buds) and productivity (Johnson, 2011, Johnson et al., 2019).

Honey bee participates in the cross-pollination of agricultural crops, and as a result, increases the quality and quantity of seeds and fruits. The value of the production obtained from agricultural crops as a result of pollination by honey bees is tens of times higher than the cost

of bee products in general (Eremia, 2002). As a result of the activity of the insects, the seed productivity of the plants increases considerably. About 20-30 seeds can develop in a cup plant flower. The weight of 1000 seeds is  $20.26 \pm 0.32$  g, and one gram contains  $53 \pm 3.61$  seeds (seeds from the 2020 harvest).

## CONCLUSIONS

Under the climatic conditions of the Republic of Moldova, *S. perfoliatum* demonstrates a high adaptive potential, with a staggered flowering period that lasts from July to October. The biological surveys have determined the spectrum of pollinating and honey insects on *S. perfoliatum* plants. The entofaunal list has been made, which includes 10 species of insects, of 4 orders (Hymenoptera, Diptera, Coleoptera, Lepidoptera) and 6 families (Apidae, Halictidae, Syrphidae, Sarcophagidae, Cerambycidae, Satyridae). These insect species have phytophagous trophic specialization. The species of the order Hymenoptera are the most numerous - 50% of the total determined insects. The research and introduction of new plant species will help increase the range of plant resources, honey plants in particular, forming a sequence of food sources for pollinating and honey-producing insects.

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## EXPLOTATION OPPORTUNITY FOR WOODEN MASS BY MULTIFUNCTIONAL MACHINERY

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### *Abstract*

*The aim of this paper was to highlight the economic and productive efficiency of the use of modern woodworking machine systems, as well as the motivation for the extension of these systems, compared to classical systems. In order to achieve these desideratums, an object of study was chosen, an exploitation parquet within the activity was made the design of the works of exploitation of the wood mass, using the proposed system. The use of Harvester and Forwarder multifunctional machines, in addition to the opportunity to make definitive assortments in the exploitation floor and a clear record of the resulting quantity, considerably reduces the damages caused to the existing natural regenerations and avoids the destruction of the exploitation roads and the damage exploitation.*

**Key words:** economic efficiency, modern machines, harvester, forwarder, operating technology.

### INTRODUCTION

The exploitation of forests is defined as the set of activities through which the timber is extracted and valued, in order to ensure optimal conditions for development and regeneration of trees and to meet the requirements of society (Ciubotaru, 2007).

The notion of forest exploitation is defined, in fact, as the two sides of the activities specific to the enhancement of wood masses: the transformation of trees marked with parts with certain characteristics and their transfer to places accessible to users. This activity can be defined in two ways, namely: the productive side - by the structure and characteristics of the production process; the scientific side - the theoretical knowledge that regulates the development of this process.

As a production process, the exploitation of wood is an integral part of the forest production process and involves the transformation of the woody part of the trees into assortments of raw or semi-finished wood and their transport for delivery or further processing (Covrig, 2013).

When choosing the means of collecting wood, it must be taken into account that the forests in our country are located largely in the mountainous area with rugged, fragile and steep slopes, where environmental factors must

be maintained within the normal limits. (Ionascu, 2012)

It is well known that cable installations, efficient and non-polluting, are the most suitable for wood collection, and yet it seems that the most appropriate operating solution is related to the use of multifunctional machines and equipment or processors. By using them, very good technical-economic results are obtained regarding: the capitalization and superior sorting of wood, labor productivity and production costs (Covrig et al., 2018).

The multifunctional wood harvesting machines involve a complex system consisting of two components: multifunctional vehicles for felling / cleaning and sectioning (harvesters), which have all the necessary functions for the application of the mechanized system of harvesting the wood mass, respectively the final assortments to "Cut - To - Length" (CTL) and specialized tractors for the approach of wood by carrying (forwarder), with which they work in tandem within the same method of exploitation. (Borz et al., 2012)

### MATERIALS AND METHODS

This research was carried out within the exploitation prosecutor's office no. 958, Valea Cutii located within the basic unit I Ulmoasa,

administered by the Municipal Forest District Baia Mare, Maramureş County.

The basic unit I Ulmoasa, with an area of 1815 ha, is located in the northern part of the country, in the land of the Eastern Carpathians, in the foothills of the Northern Volcanic Mountains, Igriş Mountains district, within the Oaş-Guţii-Ţibleş mountain range, near the municipality Baia Mare and includes the hydrographic basin of the Băiţa valley.

The forest fund that makes up the basic unit consists of two large forest bodies, namely the Hotaru-Ulmoasa body, with an area of 1574.5 ha representing 86.7% and the Căpitanu Mare body, with an area of 240.5 ha representing 13.3%.

The area under study (Figure 1) is located within the Hotaru-Ulmoasa forest body, Hotaru basin, and from an administrative point of view within the I Ulmoasa district, Bagnău canton.



Figure 1. Sketch of the Valea Cutii parcel, item 958

### Establishing the operating method

Following the analysis of the physical-geographical factors, the characteristics of the forestry technique applied in the studied prosecutor's office, respectively of the technical-economic factors that characterize the 958 Valea Cutii parcel, was chosen as the optimal exploitation method, the treatment of progressive cuttings is applied, and the volume of the average shaft is large, 1.64 m<sup>3</sup>.

The system of cars, classics that were adopted for the operation of the 958 Valea Cutii parcel, consists of:

- for downing: Stihl type chainsaw, model MS 660;
- collected by: T.A.F. model 690 PE, produced by IRUM Reghin.

The system of modern machines used in the exploitation of the wood from the 958 Valea Cutii parcel were the John Deere harvester model 1270-D, with which the harvesting operation was performed and the John Deere forwarder model 1410D, with which the whole collection process is executed.

Harvester machines are from the latest generation. They perform without intermediate handling, the other operation, respectively the felling of the tree, the cleaning of the bar and the sectioning. Another major advantage of using this machine is the simpler organization of the work, the investment is less than the purchase of machines to carry out these operations separately, reducing the number of workers required to serve these machines.

Forward-type machines are used in the process of collecting wood, usually with harvester-type machines. These tractors are equipped with a semi-trailer and a hydraulic arm with a gripper, this feature allows the movement of the fully suspended wooden masses.

For the choice of the technological variant of exploitation, the most important is the volume of the average tree mentioned above and the volume per hectare which is 30.92 m<sup>3</sup>. In addition, the direct slope of the land, which in the studied situation is 33G and the collection distance, has a direct influence.

## RESULTS AND DISCUSSIONS

### Economic analysis of operating efficiency

As the design of the operating works was carried out in two variants, it is possible to evaluate the productivity, respectively the economic profitability for the classic machine systems and for the modern machine systems. The following tables (Tables 1-4) show in antithesis the total costs and fuel consumption that were calculated for the two variants.

The use of the classic car system results in a total cost of 18.38% higher than in the case of modern car systems. This difference is mainly due to the cost of fuel and lubricants, the cost of human resources and the cost of materials needed to carry out the work of operating the parcel. The analysis of the cost structure showed that in the case of operation using modern systems and machines, the value of fuel and lubricants is 22.34% lower than in the

case of the technological process using classic systems and machines, representing 24.16% of the total.

The value of labor in both classic and modern systems has the highest share of total costs, being 36.4% for classic car systems and 32.79% for modern car systems, mainly due to the reduction in the number of required working hours. Within the modern operating system, the total time required is 42.86% lower than the classic operating system. The time difference can be explained by the automation of operations related to the technological process of collection and the technological process of the primary platform. Expenditure on materials required for the operation of the flooring operations represents 2.21% of the total expenses and 2.67% of the total I for the

modern car system, while the same category of expenses represents 2.44% of the total expenses and 2.93% of the total I for the classic car system. As it was found, the largest share in total expenditures I is represented by human resources expenditures, followed by those with fuels and lubricants. These types of expenses are higher in the case of the technological process that uses classic car systems, due to the higher fuel consumption, performance and lower automation.

The value of the categories of expenses mentioned above directly and totally influences II, these representing the share of total I, and implicitly also the sales expenses which are 21.42% lower in the case of the modern operating system compared to the classic operating system.

Table 1. Analysis of fuel requirements in the two operating variants

The need for fuels and lubricants in the technological process using modern machine systems			The need for fuels and lubricants in the technological process using traditional machine systems		
Combustible	Required quantity (l)	Value (ron)	Combustible	Required quantity (l)	Value (ron)
Gasoline	4.18	24.42	Gasoline	112.23	655.43
Diesel	1715	9689.54	Diesel	1888	10667.2
Lubricants		613.28	Lubricants		1976.50
Total fuel and lubricants		10327.24	10327.24		13299.13

Table 2. Expenditure analysis in the two operating variants

No	Modern machine systems	Value (ron)	No	Traditional machine systems	Value (ron)
1	The value of labor in the technological process	14016.90	1	The value of labor in the technological process	19060.19
2	Required fuels and lubricants	10327.24	2	Required fuels and lubricants	13299.13
3	Expenditure required for other activities required for the preparation and conduct of operations	9745.7	3	Expenditure required for other activities required for the preparation and conduct of operations	9745.7
4	Expenses for arranging the primary platform	244.77	4	Expenses for arranging the primary platform	244.77
5	Expenditure on materials required for the operation of the parquet operation	945.12	5	Expenditure on materials required for the operation of the parquet operation	1279.32
TOTAL I		35279.73	TOTAL I		43629.11
6	Own equipment repair costs (7% of Total I)	2053.29	6	Own equipment repair costs (7% of Total I)	2613.5
7	Other expenditure (6% of Total I)	1759.96	7	Other expenditure (6% of Total I)	2240.13
TOTAL II		3813.25	TOTAL II		4853.63
8	Sales and sales expenses (3% of Total II)	114.4	8	Sales and sales expenses (3% of Total II)	145.60
9	Profit (10% of Totalul I)	2933.28	9	Profit (10% of Totalul I)	3733.56
OVERALL TOTAL (RON)		42735.28	OVERALL TOTAL (RON)		52361.90
Total (ron/m <sup>3</sup> )		55.29	Total (ron/m <sup>3</sup> )		67.74

Table 3. The structure of the technological process of exploitation of the wood mass from the Valea Cutii parquet using modern machine systems

The trial Technological	Operations - phases	Symbol of the norm	Group of species	U. M.	Volume mc	Average distance (m)	N.T. ore/mc	Time required
A. Processes technological harvesting	1. Felling, sectioning, shaping, sorting with harvester John Deere 1270 D	-	Hardwood	mc	773	-	0.17	129.37
	2. STIHL MS 660 chainsaw cleaner	D64B11	Hardwood	mc	41	-	0.27	11.07
B. The technological process of collection	1. Assembled, removed, approached with forwarder John Deere 1410 D	-	Hardwood	mc	757.4	1150	0.24	178.98
C. The primary platform technological process	1. Manually stacked timber	J.20	Hardwood	mst	96	10	0.40	38.4
	2. Expedition of wood ster	-	Hardwood	mst	96	-	0.020	1.92
D. The technological process of transport	1. Ready for loaded rot wood	J.22.b.I.	Hardwood	mc	727.5	-	0.21	150.52
	2. Loaded round wood with winches mounted on vehicles	-	Hardwood	mc	727.5	-	0.13	93.18
	3. Manually loaded wood of steri	-	Hardwood	mst	96	10	0.21	20.16
Cleaning the floor of non-recoverable waste		J.6.II.B.	Hardwood	ha	25	-	21.05	526.25
Total time background								1.149.85

Table 4. Structure of the technological process of exploitation of the wood mass from the Valea Cutii parquet using classic machine systems

The trial Technological	Phase operations	The symbol of the norm	Group of species	U. M.	Volume mc	Average distance (m)	N.T. hours/mc	Time required
A. Processes technological harvesting	1. Down with STIHL MS 660 chainsaw	D65A11	Hardwood	mc	773	-	0.24	185.52
	2. Cleaning the legs with the STIHL MS 660 chainsaw	D64B11	Hardwood	mc	41	-	0.27	11.07
	3. Sectioned with STIHL MS 660 chainsaw	D64C11	Hardwood	mc	732	-	0.15	109.8
B. The technological process of collection	Gathered wood with winch mounted on TAF 690 PE	-	Hardwood	mc	757.4	50	0.20	151.48
	1. Forming and tying the load for the loved one with TAF 690 PE	J.7	Hardwood	mc	757.4	-	0.06	45.44
	2. Unloading the load from TAF in the primary platform	J.7	Hardwood	mc	757.4	-	0.024	18.17
	3. Close by semi-grinding wood material with TAF 690 PE	J.8.XIV	Hardwood	mc	757.4	2050	0.31	234.79
C. The primary platform technological process	4. Hand-picked small pieces of wood	-	Hardwood	mst	96	100	1.77	169.92
	1. Receiving, sorting, shipping roundwood	J.11	Hardwood	mc	727.5	-	0.08	58.2
	2. Handled round wood with tractor	J.21.III.b	Hardwood	mc	727.5	20	0.21	152.77
	3. Mechanically shaped sterry wood	-	Hardwood	mst	96	-	0.43	41.28
	4. Manually stacked timber	J.20	Hardwood	mst	96	10	0.40	38.4
D. The technological process of transport	5. Expedition timber shipment	-	Hardwood	mst	96	-	0.020	1.92
	1. Ready for Loaded rot wood	J.22.b.I.	Hardwood	mc	727.5	-	0.21	152.77
	2. Loaded round wood with winches mounted on Vehicles	-	Hardwood	mc	727.5	-	0.13	94.57
	3. Manually loaded wood of steri	-	Hardwood	mst	96	10	0.21	20.16
Cleaning the floor of unusable waste		J.6.II.B.	Hardwood	ha	25	-	21.05	526.25
Total time background								2.012.51

## CONCLUSIONS

After a comparative analysis of the efficiency and productivity of the timber exploitation from item 958 Valea Cutii, UBI Ulmoasa, using classic and modern machine systems, the following can be concluded: small in the case

of using multifunctional machines, the difference between the two technologies adopted being 44%.

From the point of view of labor, the use of harvester and forwarder requires lower costs, the difference being 37% smaller than in the case of operation with classic car systems.

There is an appreciably higher efficiency of fuel consumption by modern cars, the difference between the two variants being approx. 18%.

The labor force is also lower in the case of using modern machine systems by 34%, modern technology requiring the operation of equipment only by a single operator.

By using harvester and forwarder machines, some machines are removed from the structure of the technological process, because modern machines are designed to perform a multitude of operations, being able to take the place of other machines.

The reduction in the number of machines implicitly leads to a reduction in the amount of fuel needed to carry out the operation process, as well as to a reduction in the need for labor.

The arrangement of the tractor roads and the primary platform does not imply a difference in cost for the two studied variants, because the collection routes are unchanged and the dimensioning of the primary platform is done according to the volume of wood to be exploited.

The value of the materials necessary for the good development of the exploitation works is higher in the case of the solution that proposes the use of the classic machines, by approximately 30%.

The difference in costs per m<sup>3</sup> between the two variants is 18% higher in the case of adopting the classic car system.

The total cost of the operating works, which includes the achievable profit, presents a difference between the two variants for which the design of the works was made, the use of classic machine systems being more expensive than the use of modern machine systems;

## RECOMMENDATION

It is recommended to extend the use of multifunctional machines wherever possible, for the exploitation of wood, due to lower

costs, increased productivity, the time required to operate a parquet being considerably reduced, so the effect on soil and stress caused to wildlife is limited to a short time.

By using the harvester and the forwarder, most operations are performed in the parquet, their quality being higher as in the case of classic car systems. Because forwarder collection involves transporting the roundwood on trailers and not semi-crawling as in the case of TAF collection, the pieces of wood remain clean without any traces of mud or even pebbles on the ridge, which can later influence the process. log processing.

For the expansion of modern machinery in the exploitation sector, it is necessary to make the forest fund as accessible as possible by building forest roads and permanent collection routes that can be used later for cultural or protection purposes.

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## THE PROSPECTS OF CULTIVATION AND USE OF THE SPECIES PEARL MILLET, *Pennisetum glaucum*, IN MOLDOVA

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### Abstract

The goal of this research was to evaluate the quality of phytomass of the non-native species pearl millet, *Pennisetum glaucum*, cultivated in the experimental plot of the National Botanical Garden (Institute), Chișinău, Republic of Moldova. It has been found that the green mass contained 116 g/kg CP, 361 g/kg CF, 370 g/kg ADF, 606 g/kg NDF, 33 g/kg ADL, 166 g/kg TSS, 236 g/kg HC, 337 g/kg Cel, with 9.75 MJ/kg ME and 5.58 MJ/kg NEL, the prepared silage was characterized by pleasant smell, pH = 3.78, 18.0 g/kg DM lactic acid and 1.8 g/kg DM acetic acid. The pearl millet grain contained 132.8 g/kg CP, 58.5 g/kg EE, 21 g/kg CF, 764.1 g/kg NFE, 304.6 g/kg starch, 10.9 g/kg ash, 0.6 g/kg Ca, 0.7 g/kg P, 1.09 nutritive units and 11.78 MJ/kg ME, but pearl millet straw 57 g/kg CP, 487 g/kg CF, 530 g/kg ADF, 823 g/kg NDF, 74 g/kg ADL, 293 g/kg HC, 456 g/kg Cel, with 7.92 MJ/kg ME and 3.95 MJ/kg NEL. The pearl millet substrates for anaerobic digestion have C/N = 26-54 with biochemical methane potential 282-375 l/kg. The theoretical ethanol potential from structural carbohydrates of the pearl millet straw averaged 544.4l/t, Pearl millet, *Pennisetum glaucum*, can be used in many ways: as multi-purpose feed for livestock and as feedstock in the production of renewable energy.

**Key words:** biochemical composition, energy biomass, forage value, pearl millet *Pennisetum glaucum*, phytomass.

### INTRODUCTION

The most important task for the sustainable development of modern agriculture is to increase the biological diversity of cultivated crops. In recent years, due to global climate change, the possibilities of using more thermophilic crops in new agro-ecological zones have expanded. In this regard, *Poaceae* species with C<sub>4</sub> photosynthesis type are of great interest among non-traditional plant species, due to their high resilience to climate change effects: high temperature, water insufficiency and salinity stress. The cultivation of this plant species will provide an opportunity to expand the range of non-traditional crops and will be a promising renewable source of valuable plant raw materials, which will find application in various fields of circular economy: in agriculture, in the food industries and renewable energy production, as well as in

ornamental gardening. The possibility of increasing the biological diversity of *Poaceae* crops largely depends on the introduction of non-traditional plant species, simultaneously assessing the initial material for its potential implementation in economically useful purposes and the creation of varieties adapted to local conditions.

Milletts are gaining popularity especially due to their high resilience to climate change effects and acceptable productivity and nutritional value (Jukanti et al., 2016). Pearl millet, *Pennisetum glaucum* [L.] R.Br. (syn. *Pennisetum americanum*, *Pennisetum typhoides*, *Pennisetum typhoideum*, *Pennisetum spicatum*, *Setaria glauca*) is one such multi-purpose C<sub>4</sub> photosynthesis type crop, cultivated in the Indian subcontinent and African semiarid regions since prehistoric times. Currently, pearl millet is the sixth most important cereal crop after rice, wheat, maize, barley and sorghum in

the world, being grown on over 33 million hectares, accounting for approximately 50% of the total world production of millets, besides, it is a crop of major importance in arid and semi-arid regions. *Pennisetum glaucum* is a robust, strongly tillering, annual, herbaceous, grass plant, usually 1-4 m tall, with basal and nodal tillering, producing an extensive and dense root system, which may reach a depth of 1.2-1.6 m, sometimes even of 3.5 m; sometimes the nodes near ground level produce thick, strong prop roots. The stem is slender, 1-3 cm in diameter, solid, often densely villous below the panicle, with prominent nodes. The leaf sheath is open and often hairy; the ligule is short, membranous, with a fringe of hairs; the leaf blade is linear to linear-lanceolate, up to 1.5 m × 5-8 cm, and has margins with small teeth, scaberulous and often pubescent. The inflorescence is cylindrical or ellipsoidal, contracted, with a stiff and compact panicle, similar to a spike, 15-200 cm long. The spikelet is 3-7 mm long, consisting of 2 glumes and usually 2 florets. The caryopsis is globose, subcylindrical or conical, 2.5-6.5 mm long, the colour varies from white, pearl, or yellow to grey-blueish and brown, occasionally purple; the weight of 1000 seeds ranges from 2.5 to 14 g, with a mean of 8 g. The size of the pearl millet kernel is about one-third that of sorghum. The relative proportion of germ to endosperm is higher than in sorghum (Oyen & Andrews, 1996; Marsalis et al., 2012).

Pearl millet is drought- and heat-tolerant and has a considerable ability to grow and yield in poor, sandy and saline soils under arid, hot and dry climates; this is an advantage over other popular forage grasses in the region, such as fodder maize. It is also a hydrocyanic and prussic acid-free crop, which gives it nutritional superiority over sorghum and Sudan grass (Jukanti et al., 2016; Hassan et al., 2014; Toderich et al., 2016).

Pearl millet is also nutritionally superior and rich in micronutrients such as iron and zinc and can mitigate malnutrition and hidden hunger. Inclusion of minimum standards for micronutrients-grain iron and zinc content in the cultivar release policy-is the first of its kind step taken in pearl millet anywhere in the world, which can lead toward enhanced food and nutritional security. Nutrients of pearl

millet play a role in the prevention of diabetes, cancer, cardiovascular and neurodegenerative diseases. It has been reported that Pearl millet has many nutritional and medical functions, such as hypoglycaemic, cardio protective, colon cancer anticipatory and prebiotic actions. Pearl millet grain is used in different forms at a global level, for example, to make unleavened bread (roti or chapatti), porridge, gruel, desserts etc. Its flour can substitute (10-20%) wheat flour in “whole-grain” breads, pretzels, crackers, tortillas and dry and creamed cereals (Satyavathi et al., 2021)

The goal of this research was to evaluate the quality of phytomass of the non-native species pearl millet, *Pennisetum glaucum* and the prospects of its use as fodder for farm animals or as substrates for renewable energy production.

## MATERIALS AND METHODS

The non-native species pearl millet, *Pennisetum glaucum*, which was cultivated in the experimental plot of the National Botanical Garden (Institute) Chişinău, N 46°58'25.7" latitude and E 28°52'57.8" longitude, served as subject of the research, and the common oat *Avena sativa* and tall fescue *Festuca arundinacea* were used as control variants. *Pennisetum glaucum* was sown at a rate of 130 seeds per 1 m<sup>2</sup>. The green mass was harvested manually in the flowering period: *Festuca arundinacea* - on May 20, *Avena sativa* - on June 15 and *Pennisetum glaucum* - on July 24.

The leaf/stem ratio was determined by separating leaves and flowers from the stem, weighing them separately and establishing the ratios for these quantities. For this purpose, samples of 1.0 kg harvested plants were taken. The harvested green mass was chopped with a stationary forage chopping unit. The fractional composition of the chopped green mass was determined using a vibrating screen device. We used 200 mm diameter sieves, where sieves with round pores were placed one on another (in the order from the top sieve): with the diameter of 31.5 mm, 16 mm, 8 mm, 3.15 mm and 1 mm.

The dry matter content was detected by drying samples up to constant weight at 105°C. For chemical analyses, the samples were dried at 65°C.

For ensiling, the green mass (pearl millet, tall fescue) and wilted mass (oat) was chopped into 1.5-2.0 cm pieces by using a forage chopping unit, and then it was shredded and compressed in well-sealed glass containers. After 45 days, the containers were opened, and the sensorial and fermentation indices of the conserved forage were determined in accordance with standard laboratory procedures - the Moldavian standard SM 108 for forage quality analysis. Some assessments of the main biochemical parameters: crude protein (CP), crude fibre (CF), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS) have been evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research-Development Institute for Grassland Brasov, Romania. The concentration of hemicelluloses (HC) and celluloses (Cel), digestible dry matter (DDM), relative feed value (RFV), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEL) were calculated according to standard procedures. The content of crude protein (CP), crude fats (EE), crude cellulose (CF), nitrogen free extract (NFE), soluble sugars (TSS), starch, ash, calcium (Ca), phosphorus (P) in seeds - in accordance with standard laboratory procedures at the Institute of Biotechnology in Animal Husbandry and Veterinary Medicine, Maximovca. The carbon content of the substrates was obtained from data on volatile solids, using an empirical equation reported by 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 cellulose and hemicellulose into hexose (H) and pentose (P) sugars.

## RESULTS AND DISCUSSIONS

Under the weather conditions of the spring of 2020 in the Republic of Moldova, which was characterized by amounts of precipitation below the average and very high temperatures, *Pennisetum glaucum* seeds were sown on May 5, when the soil temperature in the germinative layer was over 16°C. The seedlings emerged in

the period 11-14 of May, and by the end of that month the plants developed by 3-5 leaves. In June, the growth and development rates were moderate. In the first days of July, the formation of the inflorescences was observed, and after 20 days - the full flowering of *Pennisetum glaucum* plants. At the harvest time, the plants were 132-135 cm tall and the weight of a shoot was on average 162 g, consisting of 74.6% leaves and panicle. The productivity of *Pennisetum glaucum* plants achieved 5.49 kg/m<sup>2</sup> green mass or 1.03 kg/m<sup>2</sup> dry matter, but the forage yield of *Festuca arundinacea* (first cut) was 3.78 kg/m<sup>2</sup> green mass or 0.89 kg/m<sup>2</sup> dry matter and *Avena sativa* - 2.99 kg/m<sup>2</sup> green mass or 0.94 kg/m<sup>2</sup> dry matter.

Several literature sources have described the productivity of *Pennisetum glaucum* plants. According to Medvedev & Smetannikova (1981), in the Kuban region of Russia, the green mass productivity of *Pennisetum glaucum* var. *aristatum* was 40.5-51.0 t/ha, but *Pennisetum glaucum* var. *inermis* yielded 34.0-43.0 t/ha. Shashikala et al. (2013) found that pearl millet yield recorded was 81.1 t/ha green fodder, 27.7 t/ha dry matter and 30.4 t/ha protein. Toderich et al. (2016) reported that, in some marginal lands of Central Asia, the productivity of pearl millet ranged from 42.23 to 45.12 t/ha green mass at the first cut and 27.18-31.23 t/ha green mass at the second cut, respectively, the total annual aboveground dry matter varied from 27.18 to 31.23 t/ha. As a result of a research conducted by Gurinovich et al. (2020) in the Oryol region of Russia, it has been revealed that the three years' period average yield of pearl millet Gurso variety was 65.4 t/ha green mass and Sogur variety - 62.4 t/ha green mass.

Particle size distribution of harvested chopped whole plants influences the cost of transport and particle size reduction has been an important goal in preparing livestock feed, in the process of ensiling and storage, also of different substrate pretreatments of lignocellulosic energy biomass. The results, Table 1, show that a higher amount of particles (60.2%) with a size < 8 mm was found in *Pennisetum glaucum* chopped mass, but in *Zea mays* green mass forage, on average 66.8% of the particles were larger than 8 mm.

Table 1. Particle size distribution of chopped green mass from the *Poaceae* species, %

Particle size	<i>Pennisetum glaucum</i>	<i>Zea mays</i>
3.15 mm	13.4	5.5
3.15-8.00 mm	46.8	27.2
8.00-16.00 mm	28.5	48.4
16.00-31.50 mm	11.3	18.4

The comparative analysis whole plant nutrient composition of studied *Poaceae* species, Table 2, showed that pearl millet fodder was characterized by a significantly higher content of proteins (11.6%), as compared with common oat (9.5%), but reduced as compared with tall fescue forage (12.4%). The concentration of minerals in the pearl millet is at the same level as in tall fescue forage, but higher than in oat green mass. The pearl millet green mass is richer in soluble sugar. The level of cell wall fractions were low as compared with the control, which had a positive effect on the digestibility, nutritional value and energy supply of the feed.

Different results regarding the biochemical composition and the nutritive value of the green mass from pearl millet, *Pennisetum glaucum*, whole plants are given in the specialized literature. According to Sheta et al. (2010), the forage of pearl millet contained 8.08-11.95% CP, 71.38-77.49% NDF, 40.07-45.45% ADF. Heuze et al. (2015; 2016) mentioned that the average feed value of fresh pearl millet was: 194 g/kg DM, 12.4% CP, 2.0% EE, 29.2% CF, 64.8% NDF, 34.5% ADF, 4.2% lignin, 2.7% WSC, 12.3% ash, 63.8% DOM, 17.6 MJ/kg GE, 10.8 MJ/kg DE and 8.7 MJ/kg ME, but oat fresh forage - 263 g/kg DM, 10.5% CP, 3.4% EE, 30.2% CF, 54.2% NDF, 31.0% ADF, 4.5% lignin, 7.1% WSC, 10.1% ash, 67.0% DOM, 18.0 MJ/kg GE, 11.5 MJ/kg DE and 9.3 MJ/kg ME, respectively. Anjum & Cheema (2016) remarked that the harvested fresh millet forage

contained 32.15% DM, 7.12% CP, 21.82% CF, 69.81% NDF, 42.93% ADF and 52.55% TDN. Grger et al. (2016) revealed that under the irrigation conditions during dry season in Brasil, the pearl millet fodder harvested on the 47<sup>th</sup>-67<sup>th</sup> days after seedling emergence was characterized by 12.7-17.2% DM, 20.2-24.2% CP, 10.6-12.1% ash, 52.1-55.1% NDF and 25.1-27.5% ADF. Jahansouz, et al. (2016) found that the concentrations of nutrients and the nutritive value of pearl millet dry matter were 11.85% CP, 60.47% NDF, 39.77% ADF, 9.78% WSC, 50.0% TDN, 57.91% DDM, RFV=89.1 and 1.25 Mcal/kg NEI. Freitas et al. (2017) revealed that *Pennisetum glaucum* green mass contained 280 g/kg dry matter with 10.86% CP, 2.1 % EE, 63.62% NDF, 34.47% ADF, 9.04% ash, 4.16% lignin, 30.61% Cel, 32.29% HC, 14.38% NFC, 69.9% IVDMD. Animasaun et al. (2018) evaluating of the forage quality of *Pennisetum* species collected at 10 weeks after planting, reported that *Pennisetum glaucum* dry matter yield was 4.18-6.28 t/ha with 7.51-10.35% CP, 27.30-30.12% ADF, 40.25-43.70% NDF, 8.86-9.60% ash; *Pennisetum purpureum* contained 8.67-11.67t/ha, 9.01-9.31% CP, 32.15-35.42% ADF, 51.65-53.60% NDF, 8.93-9.90% ash. Costa et al. (2018) reported that the nutritive value of pearl millet was 314 g/kg DM, 149 g/kg CP, 545 g/kg NDF, 308 g/kg ADF, 48 g/kg EE, 20 g/kg ash with 695 g/kg TDN and 692 g/kg IVDMD. Machicek et al. (2019) found that "pearl millet produced 6.29- 9.87 t/ha DM with 4.3-5.1% CP, 58.9-64.5% NDF, 38.0-39.3% ADF, 58.6-59.9% TDN, RFV 85.5-90.8. Salama et al. (2020) mentioned that pearl millet fodder contained 6.50-11.33% CP, 30.52-35.89% ADF, 61.60-68.78% NDF, 44.31-54.38% NFE, 2.94-6.74% ADL, 30.40-46.31% OMD, 32.31-42.70% TDN, 4.81-5.44 MJ/kg ME, 2.24-2.79 MJ/kg NEI.

Table 2. The biochemical composition and the economic value of the green mass from the studied *Poaceae* species

Indices	<i>Pennisetum glaucum</i>	<i>Avena sativa</i>	<i>Festuca arundinacea</i>
Crude protein, g/kg DM	116	95	124
Crude fibre, g/kg DM	361	356	368
Minerals, g/kg DM	75	65	76
Acid detergent fibre, g/kg DM	370	374	398
Neutral detergent fibre, g/kg DM	606	627	665
Acid detergent lignin, g/kg DM	33	46	35
Total soluble sugars, g/kg DM	166	167	107
Cellulose, g/kg DM	337	328	363
Hemicellulose, g/kg DM	236	258	267
Digestible dry matter, g/kg DM	600	598	570
Relative feed value	92	89	80
Digestible energy, MJ/ kg DM	11.88	11.94	11.34
Metabolizable energy, MJ/ kg DM	9.75	9.72	9.31
Net energy for lactation, MJ/ kg DM	5.58	5.53	5.46
Ratio carbon/nitrogen	28	34	26
Biomethane potential, L/kg VS	353	329	353

Table 3. The biochemical composition and the economic value of the silage from the studied *Poaceae* species

Indices	<i>Pennisetum glaucum</i>	<i>Avena sativa</i>	<i>Festuca arundinacea</i>
pH index	3.78	4.10	4.16
Content of organic acids, g/kg DM	20.1	46.7	32.5
Free acetic acid, g/kg DM	0.9	2.5	4.2
Free butyric acid, g/kg DM	0.1	0	0
Free lactic acid, g/kg DM	6.8	10.7	4.8
Fixed acetic acid, g/kg DM	0.9	3.4	5.1
Fixed butyric acid, g/kg DM	0.2	0	0
Fixed lactic acid, g/kg DM	11.2	28.1	18.4
Total acetic acid, g/kg DM	1.8	5.9	9.3
Total butyric acid, g/kg DM	0.3	0	0
Total lactic acid, g/kg DM	18.0	38.8	23.2
Acetic acid, % of organic acids	8.96	13.20	28.66
Butyric acid, % of organic acids	1.49	0	0
Lactic acid, % of organic acids	89.55	86.80	71.38
Crude protein, g/kg DM	120	102	123
Crude fibre, g/kg DM	390	393	350
Minerals, g/kg DM	93	78	99
Acid detergent fibre, g/kg DM	391	413	365
Neutral detergent fibre, g/kg DM	668	699	608
Acid detergent lignin, g/kg DM	21	40	23
Total soluble sugars, g/kg DM	99	26	81
Cellulose, g/kg DM	370	373	342
Hemicellulose, g/kg DM	277	281	243
Digestible dry matter, g/kg DM	584	567	597
Relative feed value	81	76	91
Digestible energy, MJ/ kg DM	11.58	11.28	11.81
Metabolizable energy, MJ/ kg DM	9.51	9.26	9.70
Net energy for lactation, MJ/ kg DM	5.53	5.29	5.83
Ratio carbon/nitrogen	26	31	25
Biomethane potential, L/kg VS	375	341	372

Table 4. The biochemical composition of the grains of the studied *Poaceae* species

Indices	<i>Pennisetum glaucum</i>	<i>Avena sativa</i>
Crude protein, % DM	13.28	10.30
Crude fats, % DM	5.85	4.46
Crude cellulose, % DM	2.10	13.76
Nitrogen free extract, % DM	76.41	62.69
Soluble sugars, % DM	2.61	-
Starch, % DM	30.46	-
Ash, % DM	2.36	3.74
Nutritive units/ kg DM	1.09	1.00
Metabolizable energy, MJ/kg DM	11.78	10.76
Calcium, % DM	0.06	-
Phosphorus, % DM	0.07	-

Table 5. The biochemical composition and the economic value of the straw from studied *Poaceae* species

Indices	<i>Pennisetum glaucum</i>	<i>Avena sativa</i>	<i>Festuca arundinacea</i>
Crude protein, g/kg DM	57	62	68
Crude fibre, g/kg DM	487	467	471
Minerals, g/kg DM	113	82	96
Acid detergent fibre, g/kg DM	530	499	518
Neutral detergent fibre, g/kg DM	823	800	754
Acid detergent lignin, g/kg DM	74	56	75
Cellulose, g/kg DM	456	443	443
Hemicellulose, g/kg DM	293	301	236
Digestible dry matter, g/kg DM	476	500	485
Digestible energy, MJ/ kg	9.65	10.09	9.82
Metabolizable energy, MJ/ kg	7.92	8.28	8.07
Net energy for lactation, MJ/ kg	3.95	4.30	4.08
Ratio carbon/nitrogen	54	51	46
Biomethane potential, L/kg VS	282	308	275
Hexose sugars, g/kg	82.35	80.20	79.41
Pentose sugars, g/kg	48.20	49.51	38.82
Theoretical ethanol potential, L/t	544.4	540.9	493.0

Fodder conservation is necessary in most parts of Earth to maintain feed supply during winter. Silage and haylage are the main conserved green succulent roughage fodder for domestic herbivores, their quality is key to good animal performance, reducing winter feed costs and increasing profitability during the housing period. During the sensorial assessment, it was found that, in terms of colour, the silage from pearl millet had specific dark green leaves and pink-maroon stems and panicles, with pleasant smell, specific to pickled apples, while the silage made from tall fescue- yellow stems with olive leaves, with pleasant smell, like pickled cucumbers; the oat haylage was of homogeneous olive colour with dark green hues and pleasant smell, specific to pickled vegetables. The results regarding the quality of the ensiled forage are shown in Table 3. It has been determined that the pH values and content of organic acids of the ensiled forage depended on the species, thus, *Pennisetum glaucum*

silage had lower pH value and amount of organic acids. In pearl millet silage, butyric acid was detected in very small quantities (0.3 g/kg), but the level of acetic acid was very low in comparison with tall fescue silage and oat haylage. It was found that during the process of ensiling, the concentrations of crude protein did not modify essentially, minerals increased, lignin and soluble sugars decreased in comparison with initial green mass. As compared with the initial fresh mass, the silage from pearl millet had high concentration of NDF, ADF, cellulose and hemicellulose which had a negative impact on digestibility, relative feed value and energy concentrations. In pearl millet silage, the amount of crude protein, digestible dry matter and energy concentrations was high as compared with ensiled oat, but lower nutritive value as compared with tall fescue silage.

Several studies have evaluated the potential of pearl millet as silage for ruminants. According

to Hernández et al. (2013), the chemical composition of silage was: 10.26-10.98% CP, 8.68-9.31% DP, 57.80-61.87% NDF, 35.05-37.12% ADF, 5.24-6.01% EE, 12.82-13.04% ash, 0.48% Ca, 0.17-0.18% P. Anjum & Cheema (2016) mentioned that the silage was characterized by 31.97% DM, pH 4.12, 6.18% lactic acid, 7.02% CP, 22.15% CF, 71.82% NDF, 44.15% ADF and 55.18% TDN. Costa et al. (2018) found that the pearl millet silage was characterized by pH 3.75, 47.3 g/kg lactic acid, 6.7 g/kg acetic acid, 0.1 g g/kg butyric acid, 148.1 g/kg CP, 573.2 g/kg NDF, 337.1 g/kg ADF, 47.3 g/kg EE, 16.7 g/kg ash, 689 g/kg TDN with 683.5 g/kg IVDMD. Alix et al. (2019) remarked that the pearl millet silage had pH 3.8, 55-60 g/kg lactic acid, 10-12 g/kg acetic acid, 0.33-0.46 g/kg propionic acid, 7-16 g/kg N, 100-145 g/kg WSC, 9-62 g/kg starch and 429-474 g/kg TDN.

On the basis of our observations, *Pennisetum glaucum*, under the pedoclimatic conditions of the Central region of the Republic of Moldova, achieved full seed maturity in late August - middle September. The biochemical composition of the grains of the studied *Poaceae* species has been shown in Table 4. It has been found that pearl millet grain contained higher amount of protein, fats, nitrogen free extract, starch and low concentration of crude cellulose and ash than oat grain. The esteemed nutritive value of pearl millet grains reached 1.09 nutritive units/ kg and 11.78 MJ/kg ME, while the control oat grains - 1.00 nutritive units/kg and 10.76 MJ/kg ME, respectively.

Davis et al. (2003) mentioned that the evaluated pearl millet grains had 3300-3448 kcal/kg ME and 12-14% protein, higher content than corn grains. Wu et al. (2006) stated that pearl millet grains contained 9.72-13.68% CP, 6.27-6.80% EE, 1.10-1.87% CF, 65.30-70.39% starch, 1.53-1.96% ash, but corn grains - 8.35% CP, 4.05% EE, 1.97% CF, 73.00% starch, 1.54% ash. Mustafa (2010) found that the biochemical composition of pearl millet grains used was 2.0% ash, 6.7% EE, 13.9% CP, 63.4% starch, 18.3% NDF and 4.4 % ADF, the respective values for corn grains were: 1.3% ash, 3.9%EE, 9.1% CP, 72.2% starch, 15.3% NDF and 8.2% ADF. Pearl millet grains contained 13.8% CP, 4.34% EE, 2.8% CF, 17.6% NDF, 3.42% ADF,

77.95% NFE, 64.93% starch, 1.91% ash, 4480 kcal/kg GE, 81.25% DDM, 11.77% DP, 3361 kcal/kg DE, they can completely substitute corn in diets for growing rabbits (Catelan et al., 2012).

Cereal crop residues are important feed resources for ruminants and other herbivores animals, also as feedstock for biorefineries. The biochemical composition and the economic value of the straw from the studied *Poaceae* species are presented in Table 5. The pearl millet straw as fodder is characterized by low content of crude protein and high ash and cell wall concentration which had a negative effect on feed value and energy concentration as compared with control variants. According to Bidinger & Blummel (2007) the pearl millet stover quality was 32% leaf, 0.83% nitrogen, 3.35% soluble sugars and 5.53 MJ/kg ME. Blümmel et al. (2010) found that pearl millet stover yields range from 3760 to 4930 kg/ha, stover nitrogen content from 0.62 to 1.10%, *in vitro* digestibility of dry matter from 37.6 to 46.7% and metabolizable energy from 5.26 to 6.88 MJ/kg. Hamed & Elimam (2014) reported that pearl millet straw contained 96.87% DM with 5.21% CP, 0.50 % EE, 39.99% CF, 10.0% ash, 43.50% NFE, 79.00% NDF, but sorghum stover: 97.53% DM, with 4.52% CP, 1.27% EE, 40.00% CF, 7.84% ash, 41.0.5% NFE, 67.50% NDF. Packiam et al. (2018) revealed that pearl millet biomass contained 41.60% Cel, 21.81 % HC, 21.32% lighin, 6.27% ash. Vijayanand (2016) found that pearl millet straw contained 93% DM, with 29.6% HC, 37.7% Cel, 18.8% lighin, 2.5% ash, 86.0% volatile matter, 11.5% fixed carbon, 16.4 MJ/kg caloric value, 2171.4 MJ/m<sup>3</sup> energy density. Elzaki et al (2020) reported that the chemical characteristics of *Pennisetum glaucum* whole stalks were as follows: 5.6% ash, 3.6% total silica, 47.4% Kurschner-Hoffer cellulose, 42.2% alfa-cellulose, 64.3% holocellulose, 17.0% pentosans, 21.1% lignin, 8.1% total extractives, thus representing a promising feedstock in pulp and paper manufacturing. Substituting fossil fuels with biofuels has been identified as one of the most feasible steps to reduce the agricultural greenhouse gas footprint. Conversion technologies used to produce renewable energy from biomass include combustion, anaerobic digestion (AD)

and thermochemical methods (Hall and Gifford, 2007). Anaerobic digestion of organic waste has gained improved consideration to produce methane as energy source and to address environmental challenges including effective waste disposal, besides, mineral nutrients and undigested carbon compounds can be recycled back to the land as digestate, thereby largely eliminating the need for external nutrient inputs.

The results regarding the quality of the substrates for anaerobic digestion from studied *Poaceae* species and the potential for obtaining biomethane are shown in Tables 2, 3 and 5. We would like to mention that carbon to nitrogen ratio (C/N), which constitutes a basic factor governing the correct course of methane fermentation, ranged from 25 to 54. In the tested *Pennisetum glaucum* substrates, the carbon to nitrogen ratio was C/N = 28 in green mass substrate, C/N = 26 in silage substrate and C/N = 54 in straw substrate. Essential differences were also observed between the lignin contents. The tested substrates contained acceptable amounts of hemicellulose. The biochemical methane potential of *Pennisetum glaucum* tested substrates varied from 282 l/kg VS to 375 l/kg VS, but in substrates made from the control crops - from 275 l/kg VS to 372 l/kg VS. The best methane potential was achieved in silage substrates, the lowest - in straw substrates.

Several literature sources describe the composition of cell walls in pearl millet straw and energy potential. Paritosh et al. (2019) remarked that the contents of cellulose, hemicellulose and lignin in pearl millet straw were about 36.42%, 25.31% and 15.63%, respectively. C/H = 48.87 124.1-162.4 L/kg VS methane yield.

The possibility of converting lignocellulosic biomass in bioethanol fuel is currently an area of great research interest around the world. The bioethanol yields are influenced by tissue composition, ratios of cellulose, hemicellulose and lignin. Analyzing the cell wall composition of straw substrates (Table 5), we could mention that the concentration of structural carbohydrates in *Pennisetum glaucum* straw substrate was 823 g/kg, including 456 g/kg cellulose, 293 g/kg hemicellulose and 74 g/kg lignin, but *Avena sativa* and *Festuca*

*arundinacea* substrates: 754-800 g/kg, 443 g/kg, 236-301 g/kg and 56-75 g/kg, respectively. The theoretical ethanol yield from fermentable sugars averaged 544.4 L/t in pearl millet straw substrates, compared to 493 L/t in tall fescue straw substrates and 540.9 L/t in oat straw substrates.

## CONCLUSIONS

The introduced ecotype of pearl millet, *Pennisetum glaucum*, under the climatic conditions of the Republic of Moldova, was characterized by optimal growth rates and productivity.

The green mass and silage prepared from pearl millet contain a lot of nutrients, which make them suitable to be used as a part of diverse livestock diets.

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## PRELIMINARY RESULTS REGARDING THE INFLUENCE OF SOME NUTRIENT SUBSTRATES ON THE FRUITS QUALITY IN BLACKBERRY

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### Abstract

*Blackberries are an important commercial fruit crop, widely grown in all temperate regions of the world. The blackberries contain significant amounts of polyphenol antioxidants such as anthocyanin pigments linked to potential health protection against several human diseases. In Romania, it is not cultivated on a large scale. The studied plantation was established in the spring of 2020, and the results presented refer to the fruits harvested in 2021. The crop combines different nutritive substrates, that were applied to the soil, as: manure, forest compost, semi-fermented compost and spent mushroom substrate (SMS). The experiences were set up in the field within SCDP Băneasa the experimental farm Moara Domneasca, Afumati. This paper considers a classic blackberry culture (variety: Triple Crown). The research was based on both the monitoring of the physico-chemical characteristics of the soil and the monitoring of the biometric indicators of the fruits. This paper presents preliminary results of research conducted in order to study the possible influence of nutrient substrates on the quality of blackberries fruits.*

**Key words:** blackberry cultivation, biometric indicators, preliminary results, physico-chemical characteristics.

### INTRODUCTION

Blackberry is one of the 740 species of the genus *Rubus* in the Rosaceae family and it grows spontaneously in Europe, the Middle East, North Africa and North America, being introduced into culture in America at the beginning of the XIX century and in Western Europe in the second half of the century XIX (Balan et al., 2013).

Throughout history, the blackberry has had significance in medicine and has been used in many ways. Today, the demand for blackberries is increasing, this fruit being defined as functional foods in the medical terminology and the public awareness of this issue is also growing (Eskimez et al., 2019).

Blackberries can be consumed, either fresh or processed in the form of juice, syrup, jam, sherbet, liqueur and these fruits present a rich biochemical content. Numerous studies draw attention to the properties and benefits of these biochemical constituents and provide further encouragement that selection in breeding can be utilised to increase the levels of beneficial compounds in these fruit (Cho et al., 2004; Clark and Finn, 2008; Mladin et al., 2008). Anthocyanins, ellagitannins, phenolic acids,

flavonoids, vitamins, minerals as well as other compounds contribute to blackberries high antioxidant capacity (Diaconeasa et al., 2014; Kaume et al., 2012; Vlad et al., 2019) and also have anti-carcinogens, anti-neurodegenerative and anti-inflammatory effect (Milosevic et al., 2012).

Considering the demand for these fruit is increasing and the fact that they can be processed and consumed in different ways, they can be valorised on a high price (Eskimez et al., 2019). The potential production of blackberries could be 15-30 tons/ha, depending on the variety and the duration of profitable exploitation is 12-15 years, with a rate of net annual profit of 62% (Sumedrea et al., 2014). This crop also has other advantages, among which a low ecological selectivity and regularly yields products (Clark and Finn, 2011; Eydurán et al., 2008; Eskimez et al., 2019; Milosevic et al., 2012).

The purpose of this work is to carry out studies on the potential of using the compost used by mushrooms in fruit growing, respectively in blackberry culture.

The specific objectives are:

- Comparative study of the influence of the 5 culture substrates on the soil in which the Triple Crown blackberry variety is planted;
- The study on the influence of the physico-

chemical characteristics of the soil determined by the 5 types of substrates on the quality of the fruits of the Triple Crown blackberry variety in the pedo-climatic conditions of the Experimental Base Moara Domneasca in Ilfov county.

The reason for choosing this topic was the knowledge, recovery and valorisation in Romanian agriculture of a secondary product from mushroom production, the compost used by mushrooms, allowing mushroom producers to meet environmental requirements and protect people's health.

All these organic materials from agriculture, forestry, animal husbandry are rich sources of different plant nutrients (Khan et al., 2012). Moreover, there is a continuous interest in using different organic wastes as a growth medium and nutrient source for plants due to the growing awareness of environmental issues (Grigatti et al., 2008).

Combining different practices to protect the soil and feed it with organic matter is proving to be the most effective method.

## MATERIALS AND METHODS

The study was conducted at Experimental Base Moara Domneasca, located N-E of Bucharest (in the Vlasia Plain, a subunit of the Romanian Plain), in Ilfov county, right about 17 km from Bucharest. The farm belongs to the Research and Development Station for Fruit Growing Băneasa. The experimental plot was established in the early spring of the 2020 by planting the Triple Crown blackberry variety on nutrient substrates (spent mushroom substrat, garden soil compost, semi-fermented compost, forest compost, and a mixture of the 4 substrates in equal quantities), spaced 3.0 m apart between rows and 1.0 m apart in the row.

A description of Triple Crown blackberry variety we have used in our study is:

- It is a blackberry variety approved by the Agricultural Research Service in Beltsville, Maryland - USA
- Average frost resistance
- The fruits are large (8-10 g), with a superb sweet taste; firm, with good resistance to handling and transport.
- The harvest period is from mid-July to mid-August. High fruit production is obtained (over 15 t fruit/ha).

The plantation was provided with a training system, a drip irrigation and standard cultural practices were applied.

One of the most important factors in agriculture is the environment in which the plant grows and develops. Substrates must be able to provide the necessary water, nutrients and oxygen for plants, as well as support for the whole plant (Kang and colab, 2004; Miller and Jones, 1995).

The methods used were:

- Determination of pH in aqueous suspension 1:2.5; SR 7184-13:2001; PTL 04.
- Determination of humus by wet oxidation, using the Walkley - Black - Donut method, and titrimetric dosing; STAS 7184/21-82; PTL 12.
- Determination of nitrogen (Nt) using Kjeldahl methode STAS 7184/2-85; PTL 09.
- Determination of soluble potassium was carried out in ammonium lactate acetate, according to the Egner-Riehm method, STAS 7184/18-80; PTL 22.
- Determination of soluble phosphates was done in ammonium lactate acetate extract by the Egner-Riehm method; STAS 7184/19-82 PTL 19.
- Determination of accessible Mg was done in  $\text{CaCl}_2$  0.025n (Mg).
- Determination of exchangeable calcium ( $\text{Ca}^{2+}$ ) was done in (ammonium acetate 1n, pH=7.0); STAS 7184/12-88, cap 2, PTL 26.

For the correction of the soil reaction, the degree of saturation with bases,  $V_{\text{Ab}}$ , which represents the ratio between the sum of the basic cations, SB, and the total cation exchange capacity, SB + Ah, was determined, and for estimating the amount of soil mineralized organic matter and that of N mineralized from this, the nitrogen index was determined,  $\text{IN} = \text{H} * V_{\text{Ah}}/100$ .

In order to establish the substrate in which the Thorn Free variety adapted best, for the southern part of Romania a sample of 20 harvested fruit were collected at 3 different moments from each substrate and analysed under biometric and biochemical aspects. Fruit length and width were measured with a digital calliper and the size index was calculated using the formula:  $(\text{height} + 2 \times \text{diameter})/3$  (Ancu et al., 2014). Fruits weight was measured with an electronic scale (Precisa Balance XT 620M). Soluble solid content was determined using a digital refractometer (Hanna Instruments - HI 96800) and the fruit pH with a digital pH meter.

## RESULTS AND DISCUSSIONS

The soil type at Experimental Base Moara Domneasca is a reddish preluvosoil. Several analyses were performed to the level of the soil profile, within the internal laboratory for agrochemical and biochemical analyses, to determine its physical and chemical properties.

Table 1. The granulometric composition of the soil (Experimental Base Moara Domneasca, 2019)

Horizon	Depth (cm)	Clay (%)	Coarse sand (%)	Fine sand (%)	Dust (%)	Texture
Ao	0-40	40.6	0.36	34.3	24.8	Clay loam
Ao/Bt	41-53	41.6	0.52	21.5	56.3	Clay loam
Bt	54-200	47.4	0.37	27.6	30.3	Clay loam
C	Over 200	36.2	0.42	32.0	32.0	Clay loam

The clay texture determines a low mobility of nutrients and a poor permeability of the water in the soil. The soil content in humus is good in the first 40 cm of profile, where most of the roots of young trees are located, reaching the value of 3.26%, then suddenly decreases up to 1% in the Bt horizon profile (Table 2). The pH is slightly acidic at the soil surface (6.4), reaching alkaline in the C horizon (8.3).

Table 2. Physical and chemical properties of the profile soil (Experimental Base Moara Domneasca, 2019)

Horizont Properties	Horizont			
	Ao	Ao/Bt	Bt	C
Humus (%)	3.26	1.87	1.0	1.0
Soluble Ca (mg/100 g soil)	55	32	32	30
Hydrolitic acidity (meq)	2.8	2.04	1.72	0.18
Exchangeable Bases (meq)	22.6	23.62	26.28	-
Total cation exchange capacity (meq)	28.65	28.04	30.01	-
Base saturation degree (%)	78.94	84.28	87.53	-
pH	6.4	6.6	6.8	8.3
Total N (%)	0.144	0.102	0.075	0.07
Soluble P(mg/100g soil)	50	40	40	30

Other indicators like the nitrogen index (NI), hydrolytic acidity, humus, organic carbon and so on were determined during before planting (2020) (Table 3) and after planting (2020) (Table 4).

The climate of the Moara Domneasca is temperate continental.

The following soil characteristics were determined (by granulometric analysis for determination the soil content in clay, dust and sand): a high percentage of clay ranging from 40.55% in the upper horizon 0-40 cm, to 41.63% at a depth of 41-53 cm and 47.39% at depths greater than 54 cm (Table 1).

The annual mean temperature is 12°C and the total annual amount of precipitation is ranging between 550 and 600 mm.

Table 3. Soil's characteristics determined before planting (Experimental Base Moara Domneasca, 2020)

Date	2020
Plot	Shrubs ( <i>Rubus</i> spp.) – CP1E5
Depth (cm)	0 – 20
pH	6.33
Exchangeable Bases (meq) Ca <sup>2+</sup> +Mg <sup>2+</sup> +K <sup>+</sup> +Na <sup>+</sup>	13.30
Hydrolitic acidity (meq)	5.60
Humus (%)	5.27
Organic Carbon	3.03
Total cation exchange capacity (meq)	18.9
Base saturation degree (%)	68.83
Nitrogen Index	3.63
P (ppm)	51.96

Table 4. Soil's characteristics determined after planting (Experimental Base Moara Domneasca, 2020)

Date	2020
Plot	Shrubs ( <i>Rubus</i> spp.)
pH	7.3
Exchangeable Bases (meq)	12.29
Hydrolitic acidity (meq)	2.77
Humus (%)	4.46
Organic Carbon	2.59
Total cation exchange (meq)	23.57
Base saturation degree (%)	98.35
Nitrogen Index	4.39
P (ppm)	60.1

For this study we took into account the average values of temperature, precipitation and relative humidity for the blackberry harvesting period (July - August of 2021) (Table 5, Figures 1-3).

Table 5. Average weather data of Moara Domneasca during the experimental study

Temperature (°C)			Precipitation (mm)			Humidity (%)
2021 (July - August)	2020-2021	normal	2021 (July - August)	2020-2021	normal	2021 (July - August)
25.7	14.5	12.0	6.8	623.4	600	68.3

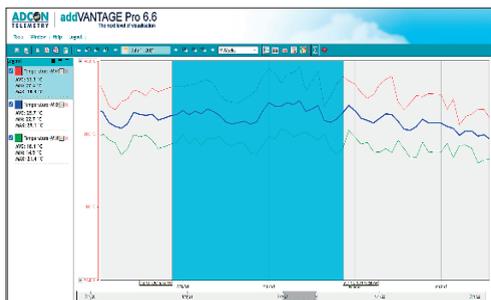


Figure 1. Average temperature dynamics during the harvest period (July - August) 2021

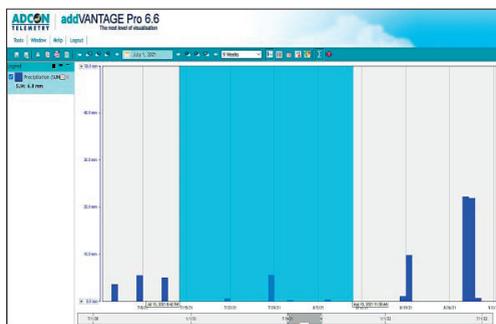


Figure 2. Average precipitation dynamics during the harvest period (July - August) 2021

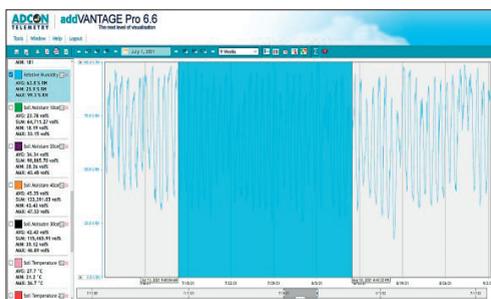


Figure 3. Average humidity dynamics during the harvest period (July - August) 2021

The highest average fruit weight was recorded for the blackberry on the substrate made of spent mushroom substrat (7.33 g), during the first harvest period (T1, 27.07.2021) (Figure 4) followed by the blackberry grown on the mixed substrate (6.55 g) and blackberry grown on semi-fermented compost; the lowest values

were registered by the fruits grown on forest compost and garden compost. At harvest 2 (T2, 03.08.2021) (Figure 5) the highest average fruit weight was recorded for the blackberry on the mixed compost substrate 6.47 g, followed by the blackberry grown on the spent mushroom substrate (6.34 g), and the lowest values were recorded for garden compost and forest compost. The same difference was seen in harvest 3 (T3, 09.08.2021) (Figure 6).

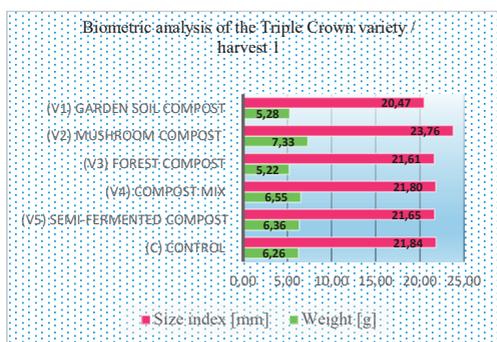


Figure 4. The biometric analysis of the Triple Crown blackberry at first harvest (T1=27.07.2021)

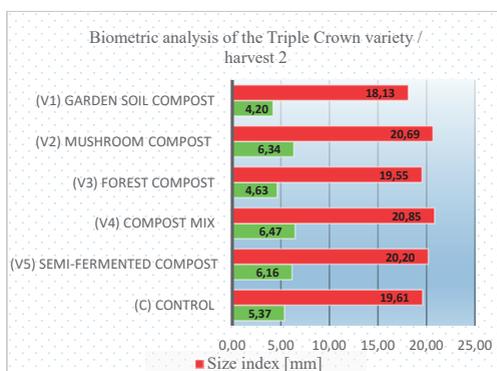


Figure 5. The biometric analysis of the Triple Crown blackberry at second harvest (T2=03.08.2021)

Regarding the size index, the highest values (23.76 mm and 20.69 mm) were recorded by the blackberry on the spent mushroom substrate at the first (T1, 27.07.2021) and the second harvest (T2, 03.08.2021), followed by blackberry grown on mixed substrate. The lowest values were

recorded for garden compost and forest compost.

The highest values of the content of soluble solids (% Brix) were recorded in the blackberry on the garden compost substrate (14.21% and 15.03%) (Figures 6, 7) at the first (T1, 27.07.2021) and the second harvest (T2, 03.08.2021) (Figures 8, 9), followed by mulberry grown on spent mushroom substrate (13.42% and 13.66%).

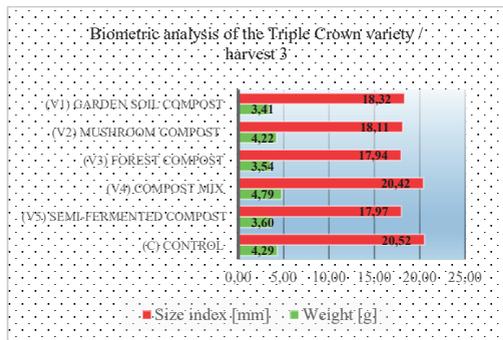


Figure 6. The biometric analysis of the Triple Crown blackberry at third harvest (T3=09.08.2021)

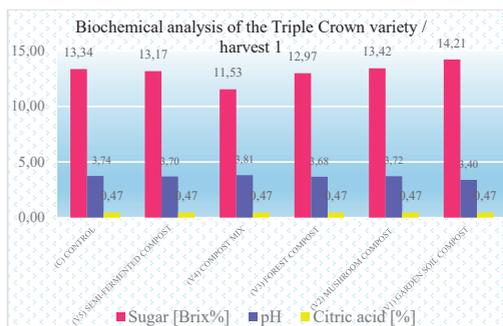


Figure 7. The biochemical analysis of the Triple Crown blackberry at second harvest (T2=03.08.2021)

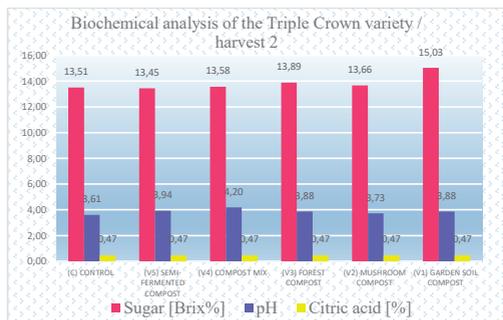


Figure 8. The biochemical analysis of the Triple Crown blackberry at second harvest (T2=03.08.2021)

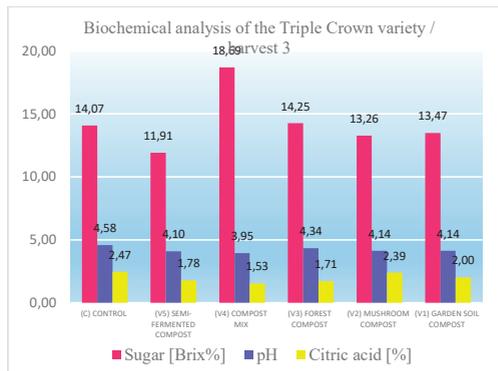


Figure 9. The biochemical analysis of the Triple Crown blackberry at third harvest (T3=09.08.2021)

## CONCLUSIONS

Preliminary results of the study showed that:

- The Thorn Free blackberry variety on the spent mushroom substrate recorded the highest values for average weight and size index, the lowest values were recorded on garden compost and forest compost;
- The highest values for the content of soluble substance were recorded for blackberry grown on garden compost 15.03%, which makes it suitable for processing.

The started study will continue with other pomological, chemical and sensory determinations, doubled by the processing and statistical analysis of the data.

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## WHICH WOODY SPECIES SHOULD BE USED FOR AFFORESTATION OF HOUSEHOLD DUMPS CONSISTING OF DEMOLITION MATERIALS MIXED WITH ORGANIC MATERIALS?

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### Abstract

Romania has a great tradition in afforestation of diverse categories of lands, including the degraded terrains. Afforestation projects are made in accordance with the technical norms approved by normative acts. In the last two decades, even if there were hundreds of afforestation projects, the forested area didn't increase significantly. This was mainly due to the fact that the targeted areas were small and due to the very bureaucratic process. In the perspective of upcoming funds from the European Union, through the dedicated component of the Romanian Recovery and Resilience Plan, it is expected that an area of 56.700 hectares of diverse lands, other than the ones already included in the national forest fund, would be forested until 2026. In this perspective, several reforms and investments are planned. Among them, a greater flexibility of the normative acts is envisaged, especially for lands situated outside the forest fund. In this context, the present paper analyzed the proposals contained in the afforestation technical norms corresponding to group no. 121, by introducing additional criteria. In AHP, three scenarios were considered. Out of the six species recommended by the technical norms, Tree of Heaven and black locust proved to be the most preferred species for afforestation of household dumps consisting of demolition materials mixed with organic materials.

**Key words:** AHP, black locust, household dump, Tree of Heaven, woody species.

### INTRODUCTION

In Romania, in the last two decades, hundreds of afforestation projects of agricultural and/or degraded lands were implemented. The vast majority of the projects were financed by the Romanian Government. For example, Ministry of Environment, Waters and Forests, through its territorial branches responsible for forestry (*i.e.* Forest Guards), and based on Law no. 100/2010 and Government Decision no. 1257/2011, is financing projects aimed at afforestation of several categories of degraded lands, unsuitable for agriculture. In the last decade, the funds were available every year, but the area afforested decreased mainly due to the very bureaucratic procedures and the lack of the attractiveness in terms of grants/financial compensations, especially for private land owners. In addition, during the last decade, hundreds of afforestation projects were financed by the Administration of the Environment Fund (Barbu, 2021) and through sub-measure 8.1 managed by the Agricultural Payments and Intervention Agency (Enescu,

2020). Thanks to the implementation of the sub-measure 8.1., 1.266 hectares were afforested (MIPE, 2021). Even so, the afforested area (*i.e.* new forests planted on agricultural/degraded lands which were included into the forest fund and managed under forest regime) in the last two decades was, on average, around 200 hectares per year (MIPE, 2021), most of the new forests being established in the sandy soils from southern-western part of Romania, mainly in Oltenia region (Enescu, 2019).

In September 2021, the European Commission adopted Romania's Recovery and Resilience Plan, based on which the country will receive 29.2 billion EUR under the European Union's Recovery and Resilience Facility. The plan has fifteen components, the second one being Forests and Biodiversity Protection (MIPE, 2021). Afforestation and reforestation represent among the main investments, with a total budget of 730 million EUR. These investments will be managed by the Ministry of Environment, Waters and Forests through the nine territorial Forest Guards and beneficiaries

could be all categories of owners, both public and private. The main targets of these investments consist in 56.700 hectares of new forests and 3.150.000 square meters of urban green spaces (MIPE, 2021).

These investments are aligned with the targets of the European Union contained in newly adopted strategic documents, such as: Biodiversity Strategy for 2030, Green Deal and Forest Strategy for 2030 (Hermoso et al., 2022).

Moreover, new forested areas are needed from the perspective of fighting against climate change or to improve the environment (Constandache et al., 2021; Korneeva, 2021; Lupănescu, 2021; Mușat et al., 2021).

Apparently, the initiative assumed by the Romanian Government (*i.e.* planting 56.700 hectares of new forests) should not be a great challenge taking into consideration the vast experience in terms of afforestation of several categories of lands in Romania (Palaghianu and Dutcă, 2017). In this context, any category of land, especially the degraded ones as a result of the human activities, may be considered.

The aim of this study was to highlight the most suitable woody species used for afforestation of the household dumps consisting of demolition materials mixed with organic materials in Romania.

## MATERIALS AND METHODS

Black locust (*Robinia pseudoacacia* L.), Tree of Heaven [*Ailanthus altissima* (Mill.) Swingle], manna ash (*Fraxinus ornus* L.), European ash (*Fraxinus excelsior* L.), Russian olive (*Elaeagnus angustifolia* L.) and sea-buckthorn (*Hippophae rhamnoides* L.) were the six woody species recommended by the Technical Norms regarding the compositions and technologies for forest regeneration and afforestation of degraded lands (MAPP, 2000), as the best option for afforestation of the household dumps consisting of demolition materials mixed with organic materials. According to the technical norms (MAPP, 2000), the afforestation composition 50 Sc 25 Cn (Mj, Fr, Sl) 25 Ct (Sc: black locust, Cn: tree of Heaven, Mj: manna ash, Fr: European ash, Sl: Russian olive, Ct: sea-buckthorn) is proposed in terrains from the steppe to the hilly regions (Group no. 121).

In order to highlight the most suitable woody species for afforestation of the household dumps consisting of demolition materials mixed with organic materials, an Analytic Hierarchy Process (AHP) was performed. Within AHP, the decision problem (*i.e.* the goal of this study) was decomposed into a hierarchy sub-problems (*i.e.* the ten criteria used), each of which can be independently analyzed (Enescu, 2018). A scale ranging from 1 to 6 was used for each criterion, namely: **criterion 1** - growth rate (from 1 - very slow growing rate to 6 - very fast growing rate); **criterion 2** - vegetative propagation (from 1 - no vegetative propagation to 6 - very intense vegetative propagation); **criterion 3** - generative regeneration (from 1 - the lowest to 6 - the highest); **criterion 4** - height (from 1 - the smallest to 6 - the highest); **criterion 5** - crown density (from 1 - rare crown to 6 - very dense crown); **criterion 6** - root system (from 1 - very less developed in depth and sidewise to 6 - very developed in depth and sidewise); **criterion 7** - demand for light (from 1 - very shade tolerant to 6 - very high demand for light); **criterion 8** - soil requirements (from 1 - very high requirements to 6 - extremely low requirements); **criterion 9** - temperature requirements (from 1 - resistant to low temperatures to 6 - resistant to high temperatures) and **criterion 10** - ornamental value (from 1 - very low value to 6 - very high value), respectively. This methodology was used in similar studies aimed at highlighting the shrub species that should be used for establishment of the field shelterbelts in Romania (Enescu, 2018) and highlighting the allochthonous tree species that should be used for afforestation of salt-affected soils across Romania (Enescu, 2020). Each criterion was analyzed by the aid of Expert Choice Desktop software (version 11.5.1683). As regards the height of the criteria within the analysis, three scenarios were considered. In scenario no. 1, all ten criteria received an equal share (*i.e.* 10%), meaning that they had an equal importance. Criteria no. 1, 6 and 8 (*growth rate, root system and soil requirements*) were considered most important in scenario no. 2 (*i.e.* with an equal share of 26.5%, while the remaining seven criteria received an equal share of 2.9%, respectively). The goal of this scenario was to

highlight the most suitable species that has simultaneous ones of the fastest growing rate and the most developed root system and they have the lowest soil requirements.

Scenario no. 3 focused on the generative and vegetative regeneration properties of the six woody species. Criteria no. 2 and 3 received an equal share of 34.6%, while the rest of the eight criteria received an equal share of 3.8%, respectively.

## RESULTS AND DISCUSSIONS

A detailed description of the six woody species was made based on the information available in the specialized manuals and studies, following the ten selected criteria.

**Black locust** has a very fast juvenile growth rate; it has a far-reaching dimorphic root system; it has a high annual production of fast-decomposing leaves which generate a high quantity of organic matter; it can be propagated both in vegetative and generative ways; its seed maturation is annual, but vegetative propagation is the most common way of reproduction (Şofletea and Curtu, 2008; Rahmonov, 2009; Muşat, 2012; Rédei et al., 2012; Enescu and Dănescu, 2013).

**Tree of Heaven** has a fast growing rate; it tolerates a wide variety of climatic and edaphic conditions; it demands a warm climate and a long growing season and it is a shade intolerant species, preferring open spaces; it produces a big amount of seeds, being reported that a mature tree can produce around 300.000 seeds in a season; it has also a vigorous re-sprouting rate, its sprouts being able to reach up to 3-4 meters in height in the first year (Hu, 1979; Pan and Bassuk, 1986; Şofletea and Curtu, 2008; Enescu, 2014a).

**Manna ash** has a well-developed root system; it is a light-demanding species; it is suitable for a broad array of degraded lands, such as those

affected by gully erosion and landslides, being able to colonize bare terrains thanks to its plasticity, fast germination and fast growth at early ages (Constandache et al., 2002; Constandache et al., 2006; Enescu, 2015; Caudullo and de Rigo, 2016).

**European ash** is a medium-size tree, growing up to 25-30 m, with a strong root system; it is a mesophile species, a strong light-demanding species and it can tolerate a broad range of nutrient and water conditions, but it prefers rich soils (Şofletea and Curtu, 2008; Beck et al., 2016).

**Russian olive** can tolerate a wide variety of environmental conditions, being resistant both to drought and frost, withstanding temperatures ranging from -45 to +46°C; it has a rapid juvenile growth rate; it can be propagated both in generative and vegetative ways, its seed dispersal being mainly done by animals and water; it can grow under a wide spectrum of soil conditions and can colonize bare lands, being suitable for several categories of degraded terrains, such as eroded soils, landslides, on tailing dumps, on mining dumps, etc. (Akbolat et al., 2008; Khamzina et al., 2009; Neţoiu, 2012; Cântar et al., 2014; Hamidpour et al., 2017).

**Sea-buckthorn** is a pioneer species, highly adapted to extreme soil and climatic conditions; it is a light demanding species; it can grow in a vast array of degraded terrains, being also adapted to the alkalinity and salinity; it can be propagated very easy both in vegetative and generative ways (Small et al., 2002; Bolibok et al., 2008; Covaci et al., 2009; Acharya et al., 2010; Vescan et al., 2010; Bolea and Chira, 2012; Enescu, 2014b).

AHP alternative ranking for the ten criteria in the case of the six woody species, based on the information available in scientific papers, specialized manuals and on the author's expertise, is given in Table 1.

Table 1. AHP alternative ranking

Criteria / Species	Black locust	Tree of Heaven	Manna ash	European ash	Russian olive	Sea-buckthorn
1. Growth rate	5	6	2	4	1	3
2. Vegetative propagation	5	6	1	2	3	4
3. Generative regeneration	1	6	4	5	2	3
4. Height	4	5	3	6	2	1
5. Crown density	4	1	3	5	2	6
6. Root system	5	6	3	4	2	1
7. Demand for light	3	6	2	1	5	4
8. Soil requirements	3	5	2	1	4	6
9. Temperature requirements	4	5	3	2	6	1
10. Ornamental value	6	1	3	4	5	2

Within scenario no. 1 (when all criteria received an equal share), the most preferred woody species were the Tree of Heaven, black locust and European ash, respectively (Figure 1). In scenario no. 2, Tree of Heaven and black locust ranked also on the first two positions, being followed by sea-buckthorn. This means that these species should be used with priority for afforestation of household dumps when in reclaiming process criteria such as growth rate, root system and soil requirements are of great interest (*i.e.* when the forest manager, land

owner or other stakeholder is interested in promoting fast growing shrub and/or tree species, with a well-developed root system and with fewer soil requirements (Figure 2). When both generative and vegetative regeneration accounted for the highest share within the decision of which species should be used (*i.e.* almost 70%; scenario no. 3), Tree of Heaven, black locust and European ash, as in the case of scenario no. 1, ranked on the first three positions. The less recommended species was manna ash.

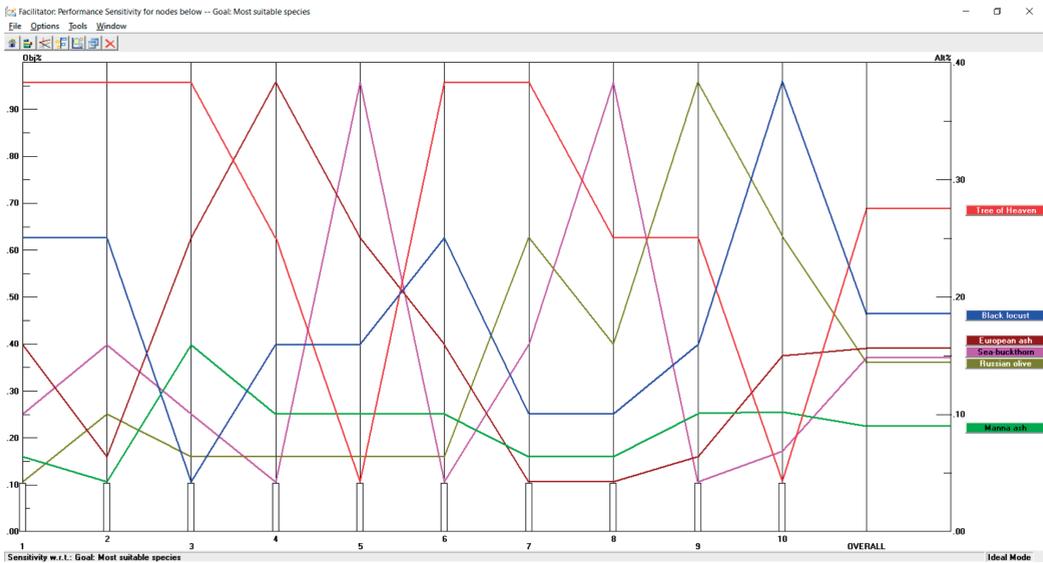


Figure 1. The ranking of the six woody species in the first scenario

In all three cases, Tree of Heaven and black locust resulted to be the best choices for afforestation of household dumps consisting of

demolition materials mixed with organic materials.

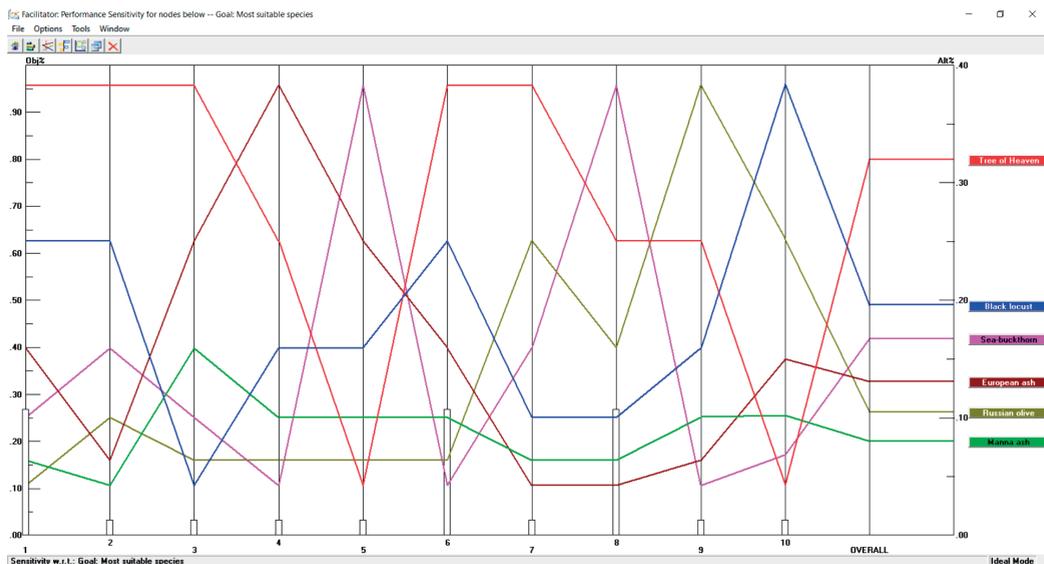


Figure 2. The ranking of the six woody species in the second scenario

## CONCLUSIONS

This study should be regarded as an attempt to provide additional criteria that could be considered when someone is planning to select the most suitable woody species out of a diverse combination.

In the perspective of afforestation of a very diverse range of degraded lands corroborated with the desire to grow the green spaces nearby localities, a flexibility in choosing the species that would be planted, but a science-based one, is more than welcome.

Future research should also include criteria that take into consideration aspects regarding functional relations of the future green spaces with the nearby ones, from integrated and sustainable development perspectives.

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## THE EVOLUTION OF THE TYPE AND MATURITY GROUP OF MAIZE HYBRIDS REGISTERED IN ROMANIA OVER TIME

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### Abstract

*Maize is the most grown crop in Romania (2.68 million ha in 2020 according to FAO database), this being used widely in human food and in animal feed. The history of hybrid maize in Romania is impressive starting in 1957 when the first foreign double cross hybrids were cultivated. The first double cross hybrids produced nationally were HD 208 and HD 405 registered in 1962 and 1963 respectively, which lead to their widespread use in only 7 years.*

*The aim of this study was to evaluate how the registration of the new maize hybrids has evolved over time in Romania in term of number and type of hybrid and to see if there is any correlation between the increase of the average annual temperature and the maturity group of these hybrids. The study led to the following main conclusions: the tendency in the last time was a reduction close to 0 of double-cross hybrids registered; there is a clear direction towards registration of single-cross and trilinear hybrids with higher productivity and heterosis effects closer to 1; there is a massive increase of newly registered hybrids following Romania's entry in E.U. in 2007; there is an increase of the number of new hybrids registered in the intermediate and late FAO groups and a fast decrease in the last 10 years of the ones in extremely early and early groups; there is an increase in the registration of late hybrids which is correlated with the increase of annual average temperature in Romania, this leading to an increase in yield without any early freeze risk.*

**Key words:** maize, hybrid type, hybrid maturity group.

### INTRODUCTION

According to FAO data, present world maize production is about 1.16 billion tonnes grain from about 202 million ha (FAOSTAT, 2020). Maize's ecological flexibility make 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. Practically, maize is grown successfully from 58° N to 40° S including tropical, subtropical, and intermediate climates (Troyer, 1996). Until our times, maize has developed into one of the most important plants for the human race, being used in a wide array of fields from animal feed, starch additives, syrups, flour and many other applications. Increase in demand of maize grain, as human population inhabiting the earth has been growing exponentially exceeding already 8 billion people, has led to a higher focus on genetics and the breeding of more and more productive cultivars of maize. The increase in world maize production in the last two decades is associated with the intensification in the use

of maize hybrid seed (Chassaigne-Ricciulli et al., 2020).

The increase in the number of newly developed maize varieties, and then hybrids, at the beginning and mid 20<sup>th</sup> century made it necessary to have an independent, state controlled, process for testing and registering these hybrids in a national register of hybrids and varieties to ensure farmers that the seed available for them is superior in terms of yield and growing characteristics and to promote a healthy competition in terms of breeding in order to invest resources only in the most productive, and thus the future easiest to sell hybrids.

In the present, maize is the most grown crop in Romania (2.68 million ha in 2020 according to FAO database), this being used widely in human food and in animal feed. In fact, Romania has a long history of maize growing, the first documented report being in the 17<sup>th</sup> century, when it became essential for the everyday food of the local population.

The history of hybrid maize in Romania is impressive starting in 1957 when the first

foreign double cross hybrids were cultivated. The first double cross hybrids produced nationally were HD 208 and HD 405 registered in 1962 and 1963 respectively, which led to their widespread use in only 7 years.

In Romania, the framework for registering new varieties and hybrids was established in 1953 by the creation of the State Committee for Testing and Homologating Varieties (HCM 3726/1953) and it has evolved nowadays into the State Institute for Testing and Registering of Varieties (ISTIS). Only varieties and hybrids registered in the Official Catalogue published annually by ISTIS can be planted in Romania (Popescu et al., 2018). Registration of new hybrids of maize needs to follow specific steps set up by ISTIS in its national network of testing centres in order to establish if they pass the D.U.S. test, which means they must be distinct from existing hybrids, they have uniform characteristics and they are stable with consistent phenotypic characteristics (ISTIS, 2020). The admission of Romania in the European Union in 2007 meant the opening of the national register to newer hybrids and varieties. European Union regulations state that a hybrid registered in the register of a member country can be automatically considered as registered in the register of any other member country i.e. Romania. This has brought the advantage of earlier access to highly developed hybrids but at the same time lost the filter of location testing in order to identify the most suitable hybrids for the growing conditions available in Romania (GEVES, 2022).

George Harrison Shull (1908) was the first maize breeder to publish a paper on the subject of crossing inbred lines of maize and the effect of heterosis in the hybrid seed exhibits. Heterosis or hybrid vigour refers to the superior performance of a hybrid relative to its parents, maize showing highly significant and positive heterosis for yield and yield components (Zaid et al., 2014).

E.M. East and D.M. Jones openly promoted the use of two-way crosses between hybrids in order to obtain four-way hybrids which meant more seed to sell by breeders, essential for survivability of early maize breeding programs, and more variability than randomly cross-pollinated varieties (Crow, 1998).

In time, disadvantages of double cross hybrids such as the need of a big number of fields in order to produce the inbred lines, each two-way cross parent and the four-way cross hybrid. This together with evolution in the field of hybrid maize breeding led to the development of more sustainable programs of single cross hybrids, as these exhibit a higher index of heterosis and thus higher yield performance together with almost perfect uniformity in the field (Beck, 2002).

Since the beginning of the 21<sup>st</sup> century the scientific world's focus has been increasingly on the potential long term negative effects of global warming. For farmers and plant breeders, the increase of the average annual temperature means the start of a race for developing and planting more late varieties of maize to take advantage of this resource. Of course, the downside of increased temperatures is the need of more and more drought tolerant hybrids in order to survive the water stress and heat and yield more than early variety maize.

The aim of this study was to evaluate how the registration of the new maize hybrids has evolved over time in Romania in terms of number and type of hybrid and to see if there is any correlation between the increase of the average annual temperature and the maturity group of these hybrids.

## MATERIALS AND METHODS

The primary data base for our research have been the Romanian Official Catalogues of the Varieties of Plant Species issued annually by ISTIS, from these being extracted the information regarding maize varieties and hybrids registered in Romania and the variation of these from year to year.

It was defined maturity groups according to FAO, respectively: 100-200, 200-300, 300-400, 400-500, 500-600, and 600>, later named extremely early, early, intermediate and late, each hybrid being assigned to its corresponding group. At the same time, the hybrids were centralized based by their type: single-cross, double-cross or trilinear.

Climate data was collected from variate sources in order to show the evolution of the annual mean temperature.

The number of hybrids registered by year was plotted into a chart in order to illustrate their evolution in time. There was also plotted the registrations of hybrids based on their type: single-cross, double-cross and trilinear in time to show the trend in evolution. The same method was used to show the evolution of hybrid registrations in time based on maturity groups. Statistical analysis was conducted through ANOVA analysis together with linear regression in order to determine the relationship between average mean temperature evolution and hybrid maturity.

## RESULTS AND DISCUSSIONS

The result of our data analysis highlighted significant interesting facts about the evolution of maize hybrid registered for cultivation in Romania. The number of hybrids registered in Romania varied a lot in the 37 years studied,

starting from 1984 with a relatively low number of 37 hybrids registered to nowadays, in 2020 with 313 hybrids registered (Table 1). This can be, of course, attributed firstly to the fact that previous to 1989, there was not a free-market economy as entry of new hybrids was strictly regulated and mostly domestically focused.

It can be observed that there are two maximum peaks regarding the number of registered hybrids: 2006 and 2016 (Figure 1). The first peak is an artificial one as it included all the hybrids including ones not used at all anymore, while after Romania's entry into the European Union in 2007, the catalogue was updated to include only active hybrids which reduced a lot the number of total registered maize hybrids. The evolution post EU membership shows us that a lot of new hybrids were registered many of them from the registers of EU member countries.

Table 1. Maize hybrids registered in Romania by maturity group

FAO Group	1984	1985	1986	1987	1989	1990	1992	1993	1995	1996	1997	1998
100-200	2	7	5	6	5	7	12	14	22	29	29	32
200-300	8	11	11	9	8	8	8	16	25	26	26	26
300-400	9	9	8	7	7	6	6	12	15	16	13	14
400-500	6	5	5	3	3	4	7	7	17	21	26	32
500-600	12	8	9	8	7	8	9	10	18	21	23	28
>600	0	4	4	5	5	4	6	8	9	12	13	17
<b>Total</b>	<b>37</b>	<b>44</b>	<b>42</b>	<b>38</b>	<b>35</b>	<b>37</b>	<b>48</b>	<b>67</b>	<b>106</b>	<b>125</b>	<b>130</b>	<b>149</b>
FAO Group	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
100-200	35	40	43	43	44	47	53	51	13	13	13	13
200-300	28	39	46	49	54	62	64	60	29	31	43	43
300-400	20	32	35	42	49	57	61	68	26	29	47	47
400-500	39	59	62	70	76	80	85	90	36	39	56	56
500-600	31	42	49	56	55	58	63	64	29	29	38	38
>600	18	24	24	27	30	35	42	42	9	9	11	11
<b>Total</b>	<b>171</b>	<b>236</b>	<b>259</b>	<b>287</b>	<b>308</b>	<b>339</b>	<b>368</b>	<b>375</b>	<b>142</b>	<b>150</b>	<b>208</b>	<b>208</b>
FAO Group	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020		
100-200	15	15	16	16	13	12	11	11	11	7		
200-300	58	58	78	82	89	85	77	84	70	68		
300-400	63	61	72	82	94	104	104	112	116	118		
400-500	75	80	95	102	116	106	86	91	76	71		
500-600	49	52	65	69	65	58	48	53	45	40		
>600	14	13	14	14	13	10	9	11	10	9		
<b>Total</b>	<b>274</b>	<b>279</b>	<b>340</b>	<b>365</b>	<b>390</b>	<b>375</b>	<b>335</b>	<b>362</b>	<b>328</b>	<b>313</b>		

From the analysis of maize hybrid registrations in Romania by their type (single-cross, double-cross or trilinear) it can be perfectly observed the evolution of breeding and the change in focus of breeding programs towards single-cross and trilinear maize hybrids in order to maximize the yield and to have the strongest heterosis effect possible (Table 2). The plot perfectly illustrates the increased focus of trilinear hybrids, in the years after joining the European Union (Figure 2).

By analysing the registration of new maize hybrids in Romania by maturity group as defined by FAO, it can be observed the tendency in registrations driven by demand of farmers, to shift from extra early and early

maize hybrids toward intermediate and late hybrids registration. If there is taken into account each maturity group as a percentage out of the total number of registered hybrids, it can be seen for example that for intermediate hybrids there is a growth from 20% in average in 1980s-early 1990s up to 37% in 2020 (Figure 3). In terms of numbers, this is also evident as in 1984 there were 9 registered 300-400 FAO maize hybrids and in 2020 there were 118, a tremendous increase which shows the shift in focus of farmers and breeders alike for this maturity group, as it seems to be the most suitable for Romania's maize growing conditions (Figure 4).

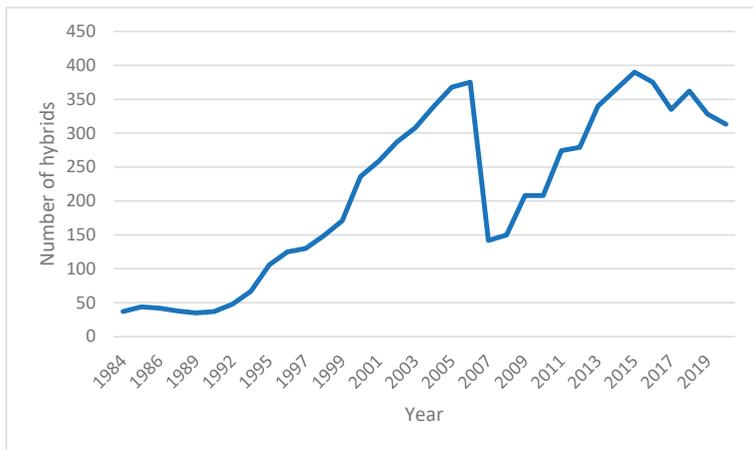


Figure 1. Evolution of the number of registered maize hybrids in Romania (1984-2020)

Table 2. Maize hybrids registered in Romania by breeding type

Hybrid Type	1984	1985	1986	1987	1989	1990	1992	1993	1995	1996	1997	1998
Single-Cross	20	20	20	17	15	17	30	45	74	88	93	109
Double-Cross	10	12	10	9	8	7	8	6	9	9	7	7
Trilinear	7	12	12	12	12	13	10	16	23	28	30	33
<b>Total</b>	<b>37</b>	<b>44</b>	<b>42</b>	<b>38</b>	<b>35</b>	<b>37</b>	<b>48</b>	<b>67</b>	<b>106</b>	<b>125</b>	<b>130</b>	<b>149</b>
Hybrid Type	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Single-Cross	126	181	195	221	238	269	295	304	119	124	173	173
Double-Cross	8	8	8	8	8	8	7	8	1	1	2	2
Trilinear	37	47	56	58	62	62	66	63	22	25	33	33
<b>Total</b>	<b>171</b>	<b>236</b>	<b>259</b>	<b>287</b>	<b>308</b>	<b>339</b>	<b>368</b>	<b>375</b>	<b>142</b>	<b>150</b>	<b>208</b>	<b>208</b>
Hybrid Type	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020		
Single-Cross	228	238	299	325	345	328	288	313	282	263		
Double-Cross	3	3	3	3	3	2	1	1	1	1		
Trilinear	43	38	38	37	42	45	46	48	45	49		
<b>Total</b>	<b>274</b>	<b>279</b>	<b>340</b>	<b>365</b>	<b>390</b>	<b>375</b>	<b>335</b>	<b>362</b>	<b>328</b>	<b>313</b>		

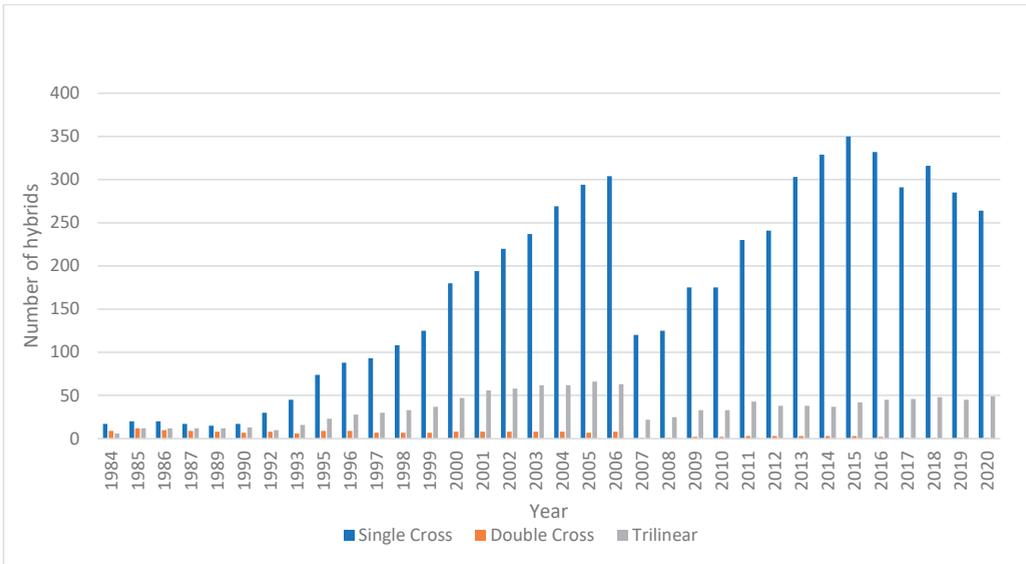


Figure 2. Evolution of registered maize hybrids in Romania by type (1984-2020)

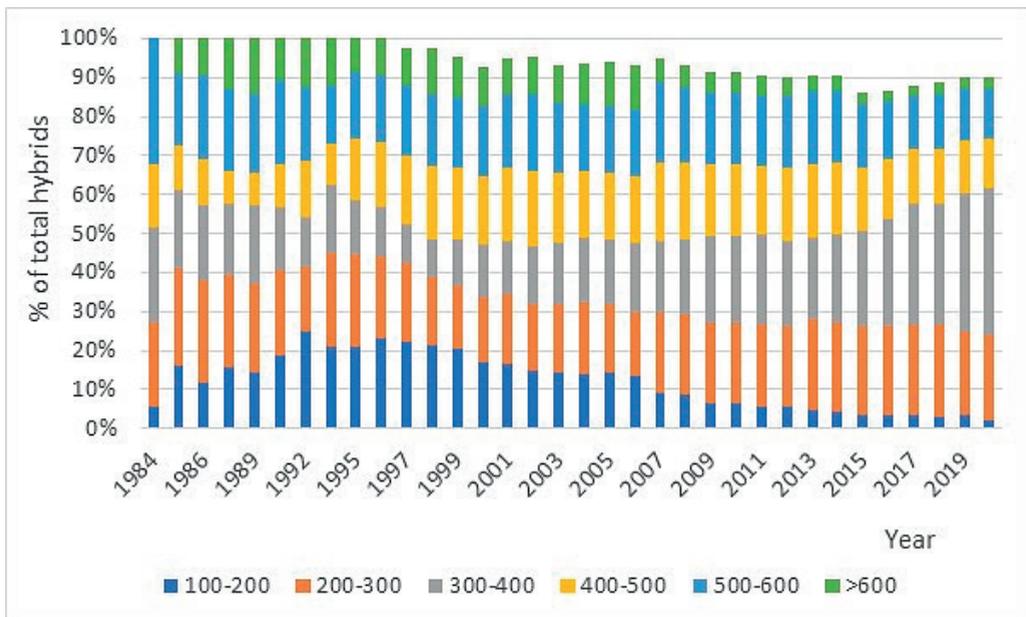


Figure 3. Maturity group evolution (%) of registered maize hybrids in Romania (1984-2020)

The average annual temperature in Romania has seen a constant growth since 1991 until nowadays, in tune with global warming as it has been observed at end of the 20<sup>th</sup> century (Figure 5). In total, there is a growth of 1.5 degrees Celsius and a constant trend of growth

in terms of heat resources to support growth of intermediate and late maturity maize hybrids in Romania. There is a growth from the 1980s when average annual temperature was 8.5 degrees on average up to over 10 degrees in the 2020 (Figure 6).

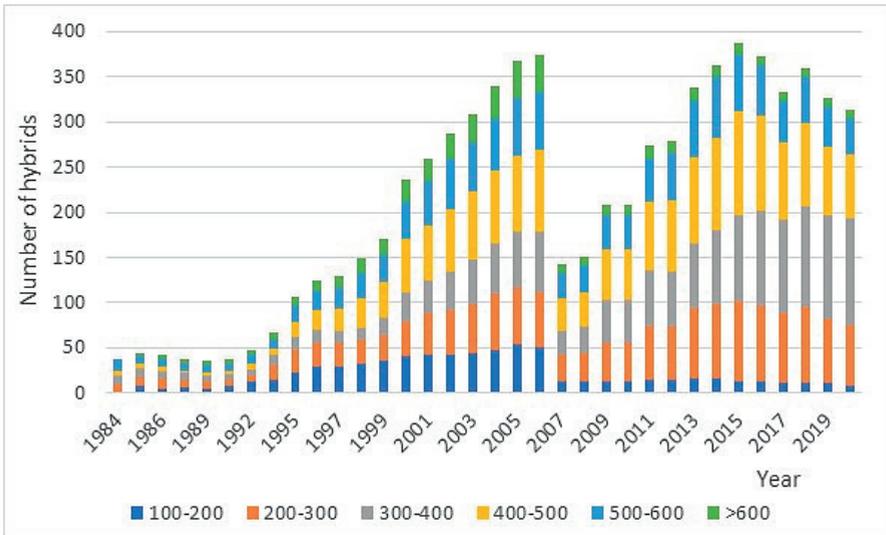


Figure 4. Maturity group evolution (number) of registered maize hybrids in Romania (1984-2020)

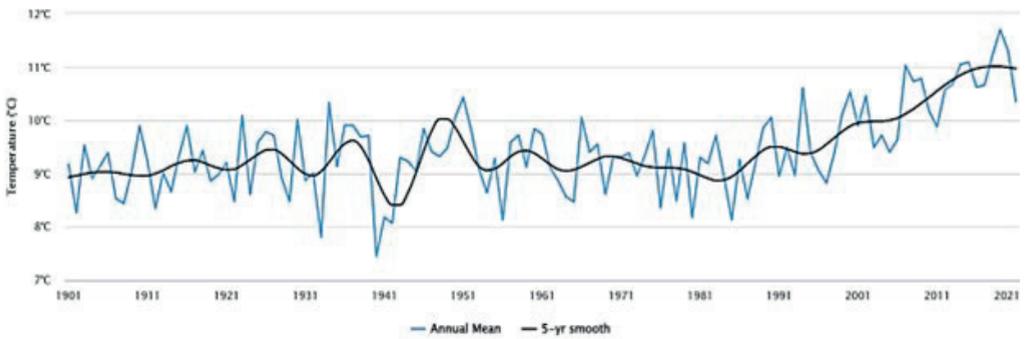


Figure 5. Historical annual mean temperature in Romania with a 5 years trailing average line (climateknowledgeportal.worldbank.org, 2021)

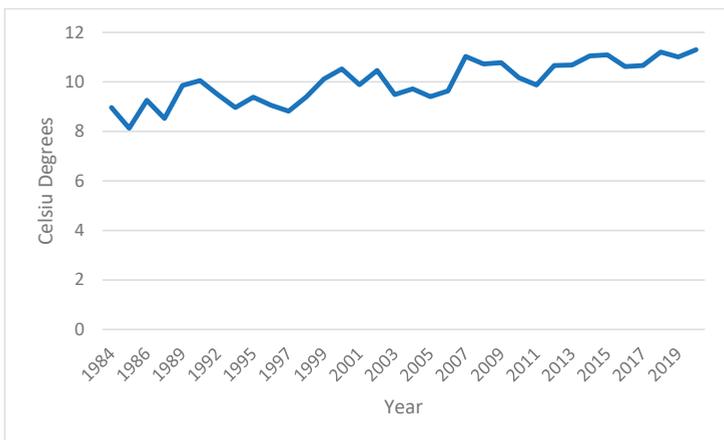


Figure 6. Average annual temperature in Romania, 1984-2020 (climateknowledgeportal.worldbank.org, 2021)

Statistical analysis performed using ANOVA and linear regression methods allowed us to research if there is any correlation and direct effect of the increase of average annual temperature, thus a higher level of the resource solar energy, and the registration of new hybrids by maturity group.

The value of R squared > 0.65 shows us that the number of hybrids registered per maturity group varies positively with the value of annual mean temperature (Table 3). The results of the statistical analysis performed confirms our supposition that there is a strong positive

correlation between the growth of annual temperatures in Romania and the maturity of registered maize hybrids. Fisher test results show us that there is random variability and our results are statistically significant. These results allow us to present the link between the growth in average annual temperature and maturity group of the hybrids, as the 1.5 degrees growth in temperature from 1984 to 2020 is correlated with the growth in intermediate 300-400 FAO maturity maize hybrids from 9 to 118, respectively from 20% of the total number of hybrids registered in 1984 to 37% in 2020.

Table 3. Regression and ANOVA statistics

<i>Regression Statistics</i>					
Multiple R	0.815974156				
R Square	0.665813823				
Adjusted R Square	0.591550228				
Standard Error	0.53860766				
Observations	34				
ANOVA					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	6	15.60533654	2.600889423	8.965547962	2.00079E-05
Residual	27	7.832651694	0.290098211		
Total	33	23.43798824			

## CONCLUSIONS

From the analysis of the data presented in this paper one can infer that the number of hybrids registered in Romania has increased tremendously since 1990 up to 2007, when Romania joined the European Union. In 2007, the Romanian Official Catalogue was updated, which reduced significantly the number of total registered maize hybrids. The evolution post EU membership showed that a lot of new hybrids were registered many of them from the registers of EU member countries.

Also one can infer that over time, the number of double-cross hybrids registered have reduced almost completely from the Romanian Official Catalogue in favour of single-cross hybrids and trilinear ones, which are more productive, having a higher heterosis index.

In terms of maturity groups, it can be observed a certain shift, especially in the last 10 years, from extremely early and early maturity maize towards more hybrids from intermediate and late maturity groups as the increase in average temperature and warmer autumns meant that

increases in yield can be achieved and early freezes have been pushed later, from late September towards early-mid October.

## ACKNOWLEDGEMENTS

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- \*\*\* <https://www.fao.org/faostat/en/#data/QCL>

## VALIDATION PROCEDURE FOR TOTAL SULPHUR DETERMINATION IN COMPLEX FERTILIZER MATRICES USED IN ORGANIC FARMING

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### Abstract

*Sulphur plays a vital role in plant nutrition. Its deficiency can have severe effects on crops, being essential in many biological processes. For an optimal supply it is necessary to know the content of elements of both the soil and the inputs used in agriculture. With the conversion from conventional to organic farming, an increasing number of analyses are needed on soil, the used inputs, and the obtained products. In general, sulphur determination methods are tedious and time consuming. This study proposes a dry combustion method for sulphur determination, which can gain ground due to the speed and ease of application. This elemental analysis method is sensitive and accurate, and can be applied for the determination of total S content in many types of sample matrices, including fertilizers. Different validation parameters were measured to ensure that the method can be used successfully applied and the procedure can be standardized, fully validated and nationally accepted.*

**Key words:** CHNS, dry combustion, method validation, organic inputs, sulphur.

### INTRODUCTION

Sulphur is one of the essential macronutrients for the good development of plants, having multiple roles in the synthesis of proteins, vitamins, enzymes or chlorophyll (Narayan et al., 2022).

The soil contains many sources of sulphur, a large part of which is present in organic matter. The decomposition of organic matter makes sulphur available for plants. Various soil minerals also contain different forms of sulphur which can be released for plant use. Sulphur deficiency can affect plant growth and development, resistance to various diseases and has a major impact on harvest quality (Kopriva et al., 2019). As a safety factor, the soil sulphur content represents an indicator of available sulphur that must be taken into account at crop establishment, in order to correct the deficiency with appropriate inputs. The growing number of analyzes required for organic crops, as well as the time required to perform them, led to the search for fast, precise and low-cost alternatives. Although it is one of the essential macronutrients, sulphur analysis is not as popular as the other macronutrients. Until two decades ago sulphur deficiency was almost non-existent due to

fertilizers and atmospheric sulphur content (Ibrahim et al., 2019), but nowadays this problem has become more and more visible due to the limitation of the use in ecological agriculture of sulphur-based pesticides, conventional fertilizers with sulphur and the increasingly strict legislation regarding atmospheric emissions in order to make agriculture more sustainable and environmentally friendly. (Jamal et al., 2010; Scherer, 2009; Koufiotis et al., 2016).

Unlike other mineral elements, the determination of sulphur is done by laborious methods. Most of the time, the soluble fraction of sulphur in certain solvents is determined by various analytical methods. A colorimetric method on an acetone extract was described by Maynard and Addison in 1985, but due to high limit of detection it was not widely accepted. Later, a HPLC analysis on a chloroform extraction was tested by Watkinson (1987) with good results. In 2015, this method was optimized by Alberta Environment and Parks, performing tests with more solvents and different equipment parameters.

The development and validation of the total sulphur determination method was successful, but many problems appeared during the tests

(such as clogging of the column due to the extraction methods). Although efficient methods and equipments have recently appeared, sulphur analysis is still problematic due to the high costs of this analysis.

Thus, elemental analysis of sulphur can be performed using the ICP-MS technique, which is still problematic using conventional single quadrupole ICP-MS.

With some optimizations, such as the additional oxygen cell or using a triple quadrupole ICP-MS (ICP-MS/MS), the method can generate very good results (McCurdy et al., 2020; Sugiyama et al., 2019).

Another method often used for sulphur determination involve the full conversion of S into sulphate followed by Ion-Chromatography detection (Rahier, 2005)

Studies have shown that the method is superior to gravimetric or colorimetric methods and the results are comparable to other modern methods (Nicolescu et al., 2017; Sapcanin et al., 2013)

The dry combustion method, a much more accessible method, has been tested in recent years with promising results.

In 2014, Bernius et al. conducted a study for the validation of the sulphur determination method by dry combustion (Dumas, 1831) for inorganic fertilizers. The method proved to have a very good accuracy, managing to determine concentrations of 1% S.

The precision of the method was acceptable, and it proved to be efficient in terms of time and resources, allowing the analysis of dozens of samples per day with a single calibration curve and far fewer hazardous reagents than other procedures. Also, the device does not require an operator during the analyses.

The same method was also tested for food, with very good results as well. The only change was that the Flame Photometric Detector (FPD) was used instead of the Thermal Conductivity Detector (TCD) (Krotz & Giuzzi, 2017).

Following the results obtained in the studies mentioned above, the aim of this paper is to develop and validate a method based on automated Pregl-Dumas technique for the quantification of total sulphur in organic fertilizers.

This method can be also optimised for other types of organic fertilizers based on biomass waste material, soils, sediments and also food.

## MATERIALS AND METHODS

### *Chemicals and reagents*

For sulphur determination, the following reagents and consumables were used:

- *CHNS combustion tube* (Elemental Microanalysis Ltd), compatible with the elemental analyzer Eurovector Elemental Analyzer EA 3100;
- *Pure O<sub>2</sub> gas* (Messer 5.0, 99,999% purity) for complete sample combustion;
- *Pure He gas* (He 6.0, 99.9999% purity), used as carrier gas;
- *Tin capsules* (8 x 5 mm) for weighing and packing the samples. These capsules also enhance the combustion. Tweezers were used to seal them in order to prevent sample loss and also to exclude atmospheric air.
- *Cystine OAS (Organic Analytical Standard)* (Certif. no. 347115, Elemental Microanalysis Ltd), used as calibration standard (S content % w/w = 26.67).
- *Ethanol 99%* was used for tweezers and spatulas washing after each sample.
- *Chromosorb W 30-60 mesh* (Elemental Microanalysis Ltd), used for liquid samples to prevent sample loss during packing.

### *Samples*

Two types of fertilizers were used as test matrices:

- CF - (Commercial liquid water-soluble fertilizer which contains N (nitrogen), P (phosphorous), K (potassium) and S;
- BIPEA Nitrogen fertilizer (Sample 04-4024)

The samples were stored according to the manufacturer's instructions and were used as such, without any preparation.

### *Elemental analysis instrument and parameters*

Elemental Analyzer CHNS Eurovector EA 3100 equipment was used for the development of the method and its validation. The following components were used:

- 80 positions autosampler;
- Mettler Toledo microbalance (precision 0.000001 g);
- H<sub>2</sub>O trap for water retention;
- PTFE GC Column, outer diameter 8 mm, length 2 m;
- Eurovector Weaver.NET 1.8.0.0 software.

The parameters used for sulphur determination method are detailed in the Table 1.

Table 1. Method parameters for S determination using elemental analyzer Eurovector EA3100

Parameter	Unit	Value
Carrier Pressure	kPa	90
Reference Pressure	kPa	20
Furnace #1	°C	950
GC Oven	°C	110
Transfer Line	°C	110
Run Time	s	600
Sample Delay	s	6
O <sub>2</sub> Volume	mL	15
O <sub>2</sub> Injection Rate		slow

The analysis lasts 600 seconds for each sample and is fully automated (sample handling, equipment control, data processing, equipment and gases shutdown).

### Method validation

The verification steps for method validations included the checking of some performance indicators:

- linear response domain (drawing calibration lines);
- accuracy (fairness) of the measurement;
- intra-day and inter-day precision ;
- standard and extended uncertainty.

## RESULTS AND DISCUSSIONS

### Method principles

The well-homogenized sample is subjected to combustion in a furnace heated to a high temperature where combustion occurs rapidly at over 900°C in the presence of pure oxygen. The combustion produces a number of gases, mainly water, carbon dioxide, sulphur oxides and nitrogen as several oxides (NyOx).

If other elements, such as chloride, also appear in the sample, they will also be transformed into the corresponding compounds, such as hydrogen chloride (HCl). In order not to interfere with the gases to be determined, these secondary combustion compounds are eliminated using special traps that retain these substances. A certain type of traps can also be used to remove some of the main elements, such as water or carbon dioxide, if their determination is not necessary. This gas mixture passes through a reduction chamber consisting of heated copper. This stage transforms nitrogen oxides into

elemental nitrogen and collects the excess of oxygen left after the combustion of the sample. The gases are carried by a carrier gas (usually pure helium) then passed through the absorbent traps that allows only to pass carbon dioxide, water, nitrogen, and sulphur dioxide. The gases are passed through a GC column where they are separated, and the total content of each gas arrives after a certain time at a thermal conductivity detector (TCD), where each amount of gas is quantified.

The interface of Weaver.NET 1.8.0.0 software allows the control of the equipment, the creation of analysis methods by establishing the running parameters, the storage of data and their subsequent processing. Also, it enables a real-time view of the analytical process during the analysis.

Once that a peak has been detected and its area has been calculated using the integration parameters, it labels the element in order to identify the detected peak as N, C, H, S or O, based on time around which the peak is expected to appear at the detector (Retention Time). For the integration parameters used in the development of the method, a chromatogram shows as in Figure 1, where the sulphur retention time is at 288 s.

Quantification of these elements is based on calibration for each element by using high purity standards such as cystine, BBOT, sulphanilic acid, etc. (AMC technical briefs, 2008).

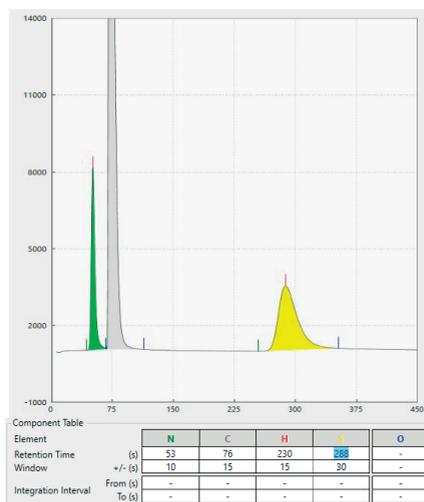


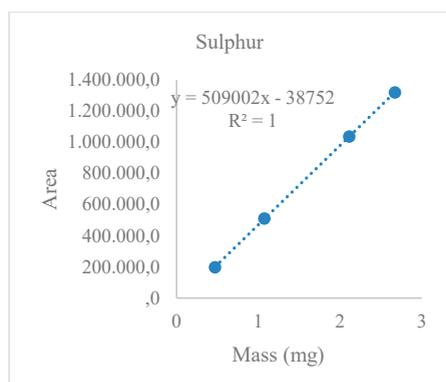
Figure 1. A typical chromatogram for CHNS analysis generated by Weaver.NET software (green peak - Nitrogen; grey peak - Carbon; yellow peak - Sulphur)

### Linearity

Linearity represents the ability of the method to provide results that are directly proportional to the amount of analyte in the sample. Linearity was determined using 4 calibration points, whose analyte concentration should range from 80% to 120% of the expected levels. Linearity report should include the slope, linear range and correlation coefficient data. Correlation coefficient must be greater or equal to 0.99 in the working range. A minimum correlation coefficient ( $R$ ) > 0.999 should be obtained in the working range.

Calibration curve was made with Cystine as a calibration standard and it was obtained from four points that covered a wide range of sulphur masses, corresponding from 0.50 to 2.50 mg of standard material.

The calibration curve was obtained from four points, using cystine (SRM) as a calibration standard. The following parameters were calculated, as shown in Figure 2: slope, linear range, correlation coefficient, residual standard deviation ( $S_y$ ) and the deviation of the analysis method ( $S_{x0}$ ), the coefficient of variation (CV). All acceptability criteria were met, according to the Eurachem Guide:  $R \geq 0.995$ ,  $CV < 5\%$ .



Equation	$ax+b$
Slope, a	509002
Corelation coef. R	1
Regression coef. $R^2$	1
y Intercept, b	-38752
$S_y=$	21553.64
$S_{x0}=$	0.04
CV %	3.35

Figure 2. Calibration curve for S determination

### Intra-day ( $RSD_r$ %) and the inter-day ( $RSD_R$ %) precision coefficients

Precision is a determination of the closeness between measurements obtained through a series of experiments under similar conditions. It must be considered that a high precision does not necessarily mean good accuracy. The precision can be considered as three indicators: repeatability, intermediate precision and reproducibility and it can be usually expressed as the variance, standard deviation or as coefficient of variation of a series of determinations. A minimum of five determinations should be carried out for accurate results.

The precision of the analytical method was evaluated based on the relative standard deviation (RSD) of the intra-day precision ( $RSD_r$  %) and the inter-day precision ( $RSD_R$  %). For determination of repeatability precision, Cystine (Microelemental Analysis), BIPEA and CF was successively analyzed 5 times as 5 different sample weights in the same day, by one analyst. Intra-laboratory reproducibility precision was determined by analyzing the same sample in 8 replicates during 4 days by two different analysts.

The intra-day (repeatability) and inter-day (reproducibility) precision was evaluated using Cystine, BIPEA and CF. The following parameters were calculated: mean ( $X_m$ ), standard deviation ( $S_r$ ), repeatability limit ( $r$ ), reproducibility limit ( $R$ ),  $RSD_r$ ,  $RSD_R$  (Tables 2 and 3).

Table 2. Intra-day precision

Analyte	Mass (mg)	$X_m$ (%)	$S_r$ (%)	$r$ (%)	$RSD_r$ (%)
Cystine	0.50-2.50	26.65	0.80	2.24	3.01
BIPEA	0.50-2.50	5.21	0.29	0.81	5.52
CF	2.50-5.00	62.60	3.13	8.77	8.77

Table 3. Inter-day precision

Analyte	Mass (mg)	$X_m$ (%)	$S_r$ (%)	$R$ (%)	$RSD_R$ (%)
Cystine	0.50-2.50	26.82	0.76	3.39	4.52
BIPEA	0.50-2.50	5.14	0.32	1.43	9.93
CF	2.50-5.00	64.84	4.57	20.46	11.27

The method has good results for the sample quantities used for tests. Smaller amounts will either suffer from sample heterogeneity or will not be detected by TCD. Larger quantities can lead to superposition of the peaks and the wrong integration of the areas. Also, large amounts can

lead to incomplete combustion or oversaturation of the TCD.

The obtained  $RSD_r$  values for standard materials are situated under 10.00%, from 3.01% for Cystine SRM to 5.52% for BIPEA, which indicated that the equipment method is repeatable (Table 2). Also, the  $RSD_r$  value for tested fertilizer was below the value indicated in the validation guide (Magnusson & Örnemark, 2014). The  $RSD_R$ % (inter-day precision) values for all three types of analytes were less than 20% which means that the method can be used in different days and by different analysts.

#### Accuracy and Bias

Accuracy is one of the most critical parameter in method validation, and it shows the degree of closeness between the 'true' value of analytes in the sample (as mentioned by the manufacturer in the certificate of conformity) and the value determined by the method to be validated.

For accuracy and bias determination, 10 samples of cystine were successively analyzed and the obtained values were compared with the values from the certificate issued by the manufacturer (Table 4). The obtained results were compared with the certificate value. Bias was expressed as the difference between the mean of the 10 samples and the real value from certificate.

$$Accuracy (\%) = \frac{\bar{x}}{\mu} \times 100 \quad (1)$$

where:

$\bar{x}$  = the mean of SRM samples;

$\mu$  = "real" value from SRM certificate.

$$Bias (\%) = \frac{\bar{x} - \mu}{\mu} \times 100 \quad (2)$$

Table 4. Accuracy and BIAS of Cystine SRM

Material	Cystine
Mass (mg)	0.50 – 2.50
Mean value (% S)	26.65
Certificate value (% S)	26.67 ± 0.12
Accuracy (%)	99.91
BIAS (%)	-0.09

The standard material obtained a very good accuracy, in the accordance with EURACHEM Guide (Magnusson & Örnemark, 2014), which recommends that the accuracy should fall within the range 70-110%. BIAS (trueness) also obtained good values, -0.09%, which is below 15% (the value mentioned in the above guide) and very close to zero, which is the optimal value.

#### Measurement Uncertainty

To perform measurement uncertainty, several sources of uncertainty were taken into account, considered to contribute the most when the extended uncertainty is calculated:

- The standard uncertainty compounded from weighing;
- Uncertainty derived from method calibration data (CV % value);
- The uncertainty derived from the internal repeatability test ( $RSD_r$  value) on the standard solution;
- The uncertainty derived from the internal reproducibility test ( $RSD_R$  value) on the matrix;
- Uncertainty derived from accuracy and bias tests.

Using the dry combustion method (Dumas) good results were obtained for the analysis of SRM and organic fertilizer (Table 5). Although the extended uncertainty had high values, the method suited below the limits accepted by the validation guide.

These values can be improved using some optimizations such as the homogeneity of the sample or using different calibration standards depending on the analyzed sample.

Table 5. Uncertainty results for reference material and CF fertilizer

Sample	Mean Conc (%)	Ustd (%)	Uext (%)
BIPEA	5.14	10.90	21.80
CF	64.84	12.14	24.28

#### CONCLUSIONS

The method development and validation revealed good values for:

- linearity,
- accuracy and bias,
- intra-day precision
- inter-day precision

The method was successfully applied to organic fertilizers, obtaining fast results, in accordance with the certificates of used materials. A variation of this method can be used also for other type of samples, as soils, to establish the level of sulphur fertilization. It must be taken into account that certain samples may require a prior preparation.

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## SUNFLOWER GRAIN YIELD AT DIFFERENT CONDITIONS OF ROW SPACING AND PLANT DENSITY

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### Abstract

*Sunflower is a major oil crop whose oil is very important in human nutrition as well as for a range of other uses. Grain yield is determined by the plant genetics and it is conditioned by a series of factors among which are counting as important ones the row spacing and plant density. Therefore, find the optimum row spacing and plant density according to cultivated sunflower hybrid and to growing conditions are of interest for sunflower growers.*

*The aim of this paper is to present the results obtained with respect to the sunflower grain yield at different row spacing and plant densities in the specific growing conditions of Romania. Research was performed under field conditions at four sunflower hybrids under different row spacing (70, 60, and 50 cm) and plant densities (50,000, 60,000, and 70,000 plants/ha). The field experiments were located in four locations in East, South-East and South of Romania in three years (2019, 2020, and 2021). The obtained data showed that under favourable growing conditions for sunflower plants, especially under good water plant supply conditions, the highest grain yields are obtained at row spacing of 70 cm, while under less favourable growing conditions for sunflower plants, the narrower rows (at 60 cm and 50 cm) seem to provide better plant growth and finally higher grain yields. Plant density has to be correlated with the growing conditions of the sunflower plants. Thus, the better growing conditions are the higher plant density should be, as to put into value the available growing factors. In the case there is no correlation between the plant density and growing conditions, the plants density too high becomes a limitative yielding factor.*

**Key words:** sunflower, grain yield, row spacing, plant density.

### INTRODUCTION

Sunflower is a major oil crop whose oil is very important in human nutrition as well as for a range of other uses. Aside from its current uses, ecosystem services (e.g. bee feeding, soil phytoremediation...) and non-food industrial uses are now expected externalities for the sunflower crop (Debaeke et al., 2021).

Sunflower crop can be characterised as being easy to manage, flexible and economic, but however demanding in terms of establishment and control to achieve a fair satisfaction of its needs (Lecomte & Nolot, 2011).

The sunflower yield is determined by the interaction of the genetics of the hybrid with the environmental factors and the crop technology the farmer can implement.

Agronomic practices in addition to high yielding varieties are important items for higher

productivity of the sunflower crop (Beg et al., 2007). In the crop technology, sowing method is one of the most important agronomic practices which affect the performance of sunflower crop by modifying it phenologically and morphologically (Bakheit et al., 2022).

Productivity per unit area of sunflower is determined by many factors including plant population (Hossam, 2012).

Plant population based on row and plant spacing is a major part of agronomic practices (Beg et al., 2007), it affecting primarily the amount of radiation intercepted per plant (Villalobos et al., 1994). One important management consideration is choosing a plant density that will maximize the yield (Holt & Campbell, 1984).

Sunflower can be manipulated over a wide range of plant density and row spacing (Vijayalakshmi et al., 1975).

Plant spacing effects are greatly prominent in various crops including sunflower because there is no option of filling gaps between plants by tillering and branching (Ali et al., 2013; Sneha et al., 2022). Sunflower crop can be grown over different row spacing conditions; for a given plant population, row spacing is determining the shape of the nutritional space (Ion et al., 2018).

Narrow rows make sunflower plants able to use in an efficient way the growing resources, respectively the solar radiation, water and nutrients, but this seems to be influenced by the specific environmental factors (Ion et al., 2015). The experimental results show that different planting patterns sometimes produced higher yield, but not always (Zarea et al., 2005). There are reports showing that reduced distance between rows could be neutral, beneficial or counterproductive (Calviño et al., 2004). Thus, there are authors who obtained higher grain yields at row spacing of 75 cm than at row spacing of 50 cm (Diepenbrock et al., 2001; Kazemeini et al., 2009) or obtained higher grain yields at row spacing of 60 cm than at row spacing of 45 or 30 cm (Nawaz et al., 2001), while other authors obtained higher grain yields at narrow rows (Zarea et al., 2005). The aim of this paper is to present the results obtained with respect to the sunflower grain yield at different row spacing and plant densities in the specific growing conditions of Romania.

## MATERIALS AND METHODS

Research was performed in field experiments under rainfed conditions in the years 2019, 2020, and 2021. The field experiments were located each year in four locations, among which one in East Romania, one in South-East Romania and two in South Romania, these locations being characterised as follows:

- Negrești, which is located in eastern part of Romania, Vaslui county, Moldavia region; the soil is a cambic chernozem, the average annual temperature is of 9.5°C, and the average annual rainfall is of 420 mm.
- Cogealac, which is located in southeast part of Romania, Constanța county, Dobruja region; the soil is a chernozem, the average

annual temperature is of 10.7°C, and the average annual rainfall is of 352 mm.

- Dâlga, which is located in southern part of Romania, Călărași county, southeast part of Romanian Plain; the soil is a chernozem, the average annual temperature is of 11.0°C, and the average annual rainfall is of 503.6 mm.
- Troian, which is located in southern part of Romania, Teleorman county, central-southern part of Romanian Plain; the soil is a chernozem, the average annual temperature is of 10.4°C, and the average annual rainfall is of 550.1 mm.

The field experiments were based on method of subdivided plots into 3 replications, with the following factors:

- Factor A - row spacing, with 3 graduations:
  - a2 = 70 cm;
  - a3 = 60 cm;
  - a4 = 50 cm.
- Factor B - plant density, with 3 graduations:
  - b1 = 50,000 plants/ha;
  - b2 = 60,000 plants/ha;
  - b3 = 70,000 plants/ha.
- Factor C - sunflower hybrid, with 4 graduations:
  - c1 = KWS Acer (early Clearfield hybrid);
  - c2 = NK Neoma (mid-early Clearfield hybrid);
  - c3 = P64LE25 (mid-early sulfonylurea resistant hybrid);
  - c4 = Subaro (mid-late hybrid).

In all locations and all experimental years, the preceding crop was winter wheat. The fertilisation was performed with 40-60 kg/ha of nitrogen and 40-60 kg/ha of phosphorus by applying 200-300 kg/ha of 20:20:0 complex fertiliser, according to soil conditions in each location. The fertiliser was applied by spreading it before seedbed preparation.

Tillage in the experimental fields consisted in ploughing in autumn, one disk harrow passage in March, and seedbed preparation before sowing made with a combinatory.

Sowing was performed in the first two decades of April except for Troian location where sowing was performed either at the end of April or at beginning of May, and Dâlga location in 2021, when sowing was performed at beginning of May (Table 1).

Table 1. Sowing data in the field experiments according to location and year

Location	Year		
	2019	2020	2021
Negrești	09 of April	06 of April	19 of April
Cogealac	11 of April	10 of April	16 of April
Dâlga	10 of April	14 of April	03 of May
Troian	03 of May	29 of April	05 of May

The weed control was made by applying the herbicide Dual Gold 960 EC (S-metolachlor 960 g/l) in a rate of 1.5 l/ha applied either before seedbed preparation or after sowing, respectively before emergence. For controlling the monocotyledonous weeds, in the vegetation period it was used the herbicide Select Super (Clethodim 120 g/l) in a rate of 0.8-1.3 l/ha, depending on the weeds (annual or perennial) identified in the experimental plot.

The sunflower heads of each experimental variant were harvested in the stage of full maturity and the grain yield was calculated in kg/ha and was expressed at 9% moisture

content. The data are presented in this paper as average values for the four studies sunflower hybrids and they were statistically processed by the analysis of variance (ANOVA).

In all locations of experimentation, the year 2020 was the warmest, while the year 2019 was the coldest except for Negrești location where the coldest year was 2021 (Table 2). Among the four locations, the highest average temperatures were registered in Cogealac and Dâlga, while the smallest average temperatures were registered in Troian location.

Also in all locations of experimentation, the year 2021 was the rainiest year, while the year 2020 which was the warmest was also the driest (Table 3). Among the four locations, the Negrești and Troian locations were the wettest and the Cogealac and Dâlga were the driest. Dâlga location was exposed to extreme rainfall, with a very dry year 2020 (340.9 mm rainfall) and a very wet year 2021 (851.7 mm rainfall).

Table 2. Average temperatures in the four locations and the three experimental years

Month	Temperatures in Negrești location (°C)			Temperatures in Cogealac location (°C)			Temperatures in Dâlga location (°C)			Temperatures in Troian location (°C)		
	2018-2019	2019-2020	2020-2021	2018-2019	2019-2020	2020-2021	2018-2019	2019-2020	2020-2021	2018-2019	2019-2020	2020-2021
Sept.	16.0	17.0	18.2	20.2	20.7	21.4	19.8	20.0	20.7	15.7	18.8	20.0
Oct.	11.5	12.7	13.2	15.7	15.2	16.6	14.8	14.6	15.3	12.5	13.2	13.4
Nov.	4.1	8.7	4.4	7.3	13.1	6.6	5.5	12.3	6.4	4.2	8.4	4.4
Dec.	-2.2	1.8	3.6	1.9	6.9	5.6	0.4	3.6	4.3	-1.6	2.1	2.0
Jan.	1.5	-0.9	-0.1	1.9	3.7	4.0	-1.8	1.3	3.9	-2.2	-0.3	-0.2
Feb.	2.8	3.7	-0.4	4.0	6.5	4.9	3.0	5.5	5.4	2.3	4.0	1.9
Mar.	10.0	6.6	3.0	8.7	8.8	5.4	9.7	8.6	6.1	8.2	6.5	3.4
Apr.	14.7	10.1	7.0	10.5	9.9	10.0	11.9	12.4	11.3	9.5	10.3	7.8
May	16.6	13.6	15.7	16.6	15.7	16.2	17.6	16.6	18.7	15.2	15.5	14.9
Jun.	21.7	22.0	20.6	24.3	20.8	19.8	25.5	22.8	21.9	21.8	19.5	19.9
Jul.	28.0	27.0	31.0	23.9	25.2	25.1	25.0	26.3	25.4	22.8	23.0	25.6
Aug.	29.8	23.5	28.6	24.4	25.0	24.6	25.8	26.4	25.7	24.2	23.7	24.3
Mean	12.9	12.2	12.1	13.3	14.3	13.4	13.1	14.2	13.8	11.1	12.1	11.5

## RESULTS AND DISCUSSIONS

In three of the four studied locations in Romania, respectively in Negrești, Cogealac and Dâlga, the highest average grain yields were registered at row spacing of 70 cm (Figure 1). Sowing at narrower rows proved to lead at a decreasing of the grain yield, the differences of the grain yield registered at

70 cm row spacing and those obtained at row spacing of 60 cm and 50 cm being the highest in the rainy year 2021. However, for Cogealac location in 2019, when in August was registered 0 mm rainfall and the plants were in the stage of grain filling, the average grain yields obtained at the three studied row spacing were quite close.

Table 3. Average rainfall in the four locations and the three experimental years

Month	Rainfall in Negrești location (mm)			Rainfall in Cogealac location (mm)			Rainfall in Dâlga location (mm)			Rainfall in Troian location (mm)		
	2018-2019	2019-2020	2020-2021	2018-2019	2019-2020	2020-2021	2018-2019	2019-2020	2020-2021	2018-2019	2019-2020	2020-2021
Sept.	37.6	41.4	46.8	3.0	24.0	13.0	15.0	31.0	70.2	25.0	2.0	27.0
Oct.	2.2	34.2	74.0	11.0	61.0	26.0	10.0	43.0	33.0	8.0	20.0	69.0
Nov.	37.7	9.7	6.7	59.0	36.0	15.0	70.0	25.5	33.5	50.0	62.0	24.0
Dec.	34.2	11.8	58.0	30.0	7.0	78.0	52.0	18.0	88.0	58.0	13.0	46.0
Jan.	37.0	5.6	26.4	45.0	26.0	83.0	30.0	2.5	94.5	31.0	7.0	113.0
Feb.	23.5	26.1	17.4	11.0	0	32.0	14.0	28.8	28.0	24.0	52.0	9.0
Mar.	0	8.4	31.2	22.0	33.0	38.0	20.5	8.0	78.5	23.0	56.0	84.0
Apr.	81.6	0	38.6	15.0	3.0	34.0	59.0	11.0	28.0	55.0	13.0	48.0
May	72.0	100.6	35.3	44.0	17.0	42.0	57.5	51.5	67.0	45.0	127.0	82.0
Jun.	121.0	112.3	118.8	48.0	64.0	141.0	73.5	53.6	250.0	137.0	107.0	48.0
Jul.	36.0	7.5	65.0	74.0	22.0	28.0	32.0	65.0	34.0	79.0	6.0	22.0
Aug.	57.0	58.5	116.0	0	47.0	50.0	43.0	3.0	47.0	0	23.0	67.0
<i>Sum</i>	<i>539.8</i>	<i>416.1</i>	<i>634.2</i>	<i>362.0</i>	<i>340.0</i>	<i>580.0</i>	<i>476.5</i>	<i>340.9</i>	<i>851.7</i>	<i>535.0</i>	<i>488.0</i>	<i>639.0</i>

At Troian location where in all studied years the sowing was delayed being performed either at the end of April or at beginning of May, the average grain yield was higher at narrow rows, respectively at 60 cm and 50 cm between rows, the smallest yields being registered at row spacing of 70 cm. The yield differences were more evident in years 2019 and 2020 characterised by drought in July and August (0 mm rainfall in August in 2019 and 6 mm rainfall in July and 23 mm rainfall in August in 2020), respectively when the plants were in the stage of grain filling. In the rainy year 2021, even the highest average grain yield was registered at row spacing of 60 cm, the yields were quite close (Figure 1).

These findings are according to those found also for Romanian growing conditions for sunflower by Ion et al. (2015), respectively that under less favourable growing conditions the narrow rows seem to make sunflower plants to use the growing factors in a more efficient way which is reflected into higher values for the yield components of the head and finely into higher grain yields.

In two of the four studied locations in Romania, respectively in Negrești and Dâlga, the average grain yields increased with increasing of plant density, the highest yields being registered at plant density of 70,000 plants/ha (Figure 2). This situation was found also at Troian location in 2019, as well as Cogealac location in 2019 but with highest yield obtained at

60,000 plants/ha. In all the other cases, the average grain yields were close at the three plant densities or even decreased with increasing of plant density. This is proving that plant density has to be correlated with the growing conditions of the sunflower plants in searching for the optimum plant density. In the case there is no correlation between the plant density and growing conditions, the plants density too high becomes a limitative yielding factor.

Generally, the increasing of plant density from 50,000 plants/ha to 60,000 plants/ha and 70,000 plants/ha determined an increased grain yield at all the three row spacing, with an evident effect in locations Negrești and Dâlga, but with a limited effect in locations Cogealac and Troian (Figure 3). There was an exception in Cogealac location for row spacing of 70 cm and 50 cm, where increasing of plant density was associated with a decreased grain yield.

Compared to the grain yield obtained at row spacing of 70 cm and plant density of 50,000 plants/ha which was taken as control variant, it has to be underline the positive differences statistically significant registered at all row spacing in Troian location in 2019 (Table 4) and in Dâlga location in 2020 (Table 5), as well as those registered in Dâlga location in 2021 for row spacing of 70 cm and for row spacing of 60 cm but only for plant density of 60,000 plants/ha (Table 6).

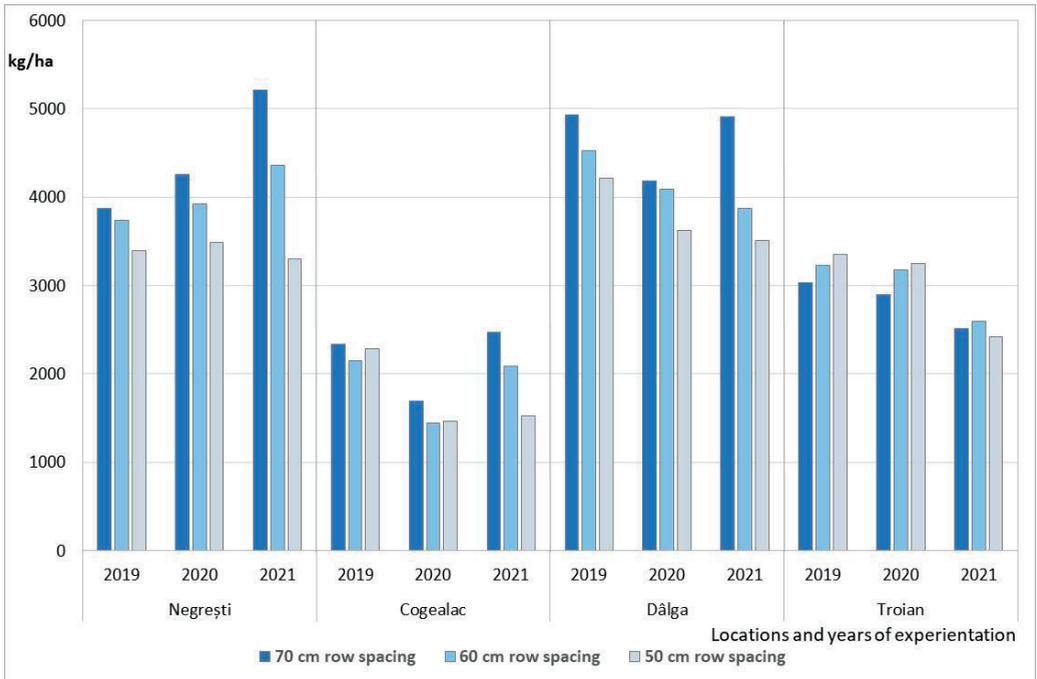


Figure 1. Sunflower grain yield at different row spacing in different years and locations in Romania

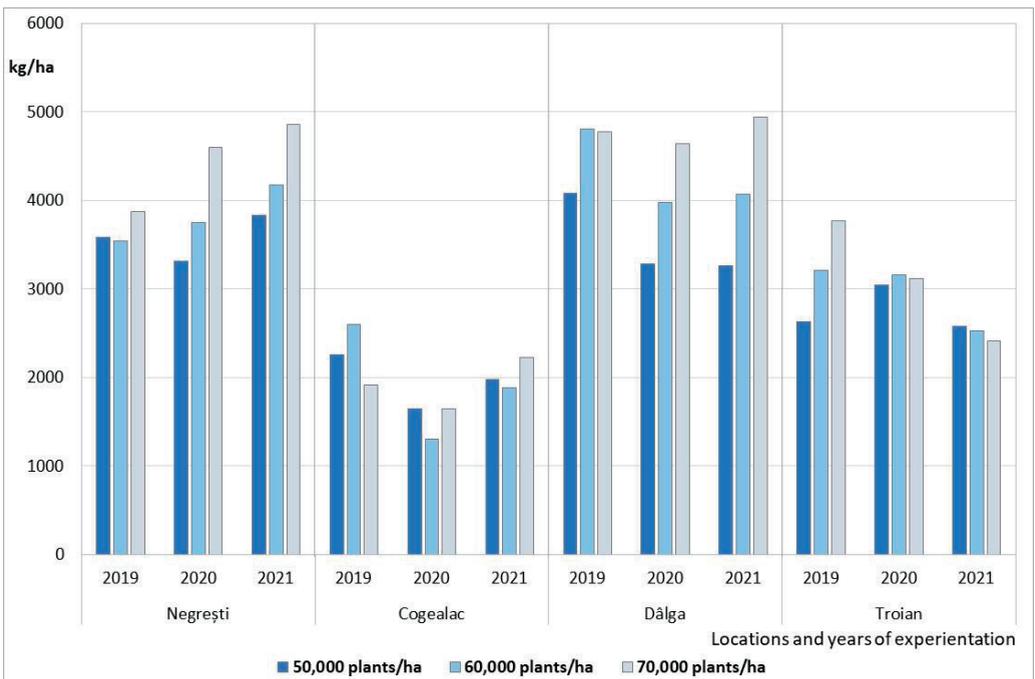


Figure 2. Sunflower grain yield at different plant densities in different years and locations in Romania

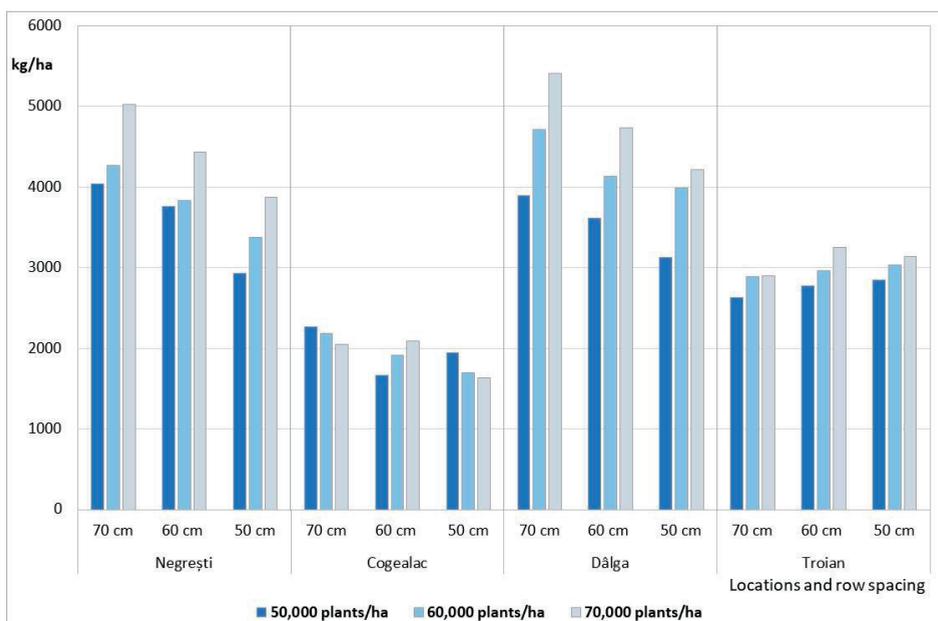


Figure 3. Sunflower grain yield at different plant densities and row spacing in different locations in Romania

Table 4. Sunflower grain yield at different row spacing and plant densities in 2019 and in different locations in Romania

Experimental factors		Negrești location		Cogealac location		Dâlga location		Troian location	
Row spacing	Plant density	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)
70 cm	50,000 plants/ha	3735	Control	2098	Control	4301	Control	2480	Control
	60,000 plants/ha	3699	-36	2726	628 *	5146	845 **	3075	595 ***
	70,000 plants/ha	4174	439	2198	100	5341	1040 ***	3523	1043 ***
60 cm	50,000 plants/ha	3544	-191	2159	61	4345	44	2593	113
	60,000 plants/ha	3721	-14	2472	374	4461	160	3167	687 ***
	70,000 plants/ha	3949	214	1818	-280	4758	457	3932	1452 ***
50 cm	50,000 plants/ha	3462	-273	2525	427	3609	-692 °°	2805	325 *
	60,000 plants/ha	3220	-515	2611	513	4800	499 *	3395	915 ***
	70,000 plants/ha	3497	-238	1713	-385	4218	-83	3860	1380 ***
DL5%		-	788.50	-	613.04	-	492.47	-	318.44
DL1%		-	1064.78	-	827.85	-	665.03	-	430.18
DL0.1%		-	1417.91	-	1102.41	-	885.58	-	572.63

Table 5. Sunflower grain yield at different row spacing and plant densities in 2020 and in different locations in Romania

Experimental factors		Negrești location		Cogealac location		Dâlga location		Troian location	
Row spacing	Plant density	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)
70 cm	50,000 plants/ha	3786	Control	1874	Control	3458	Control	2831	Control
	60,000 plants/ha	4045	259	1563	-311	4104	646 ***	3123	292
	70,000 plants/ha	4928	1142 *	1644	-230	4991	1533 ***	2727	-104
60 cm	50,000 plants/ha	3520	-266	1526	-348	3374	-84	3084	253
	60,000 plants/ha	3532	-254	1226	-648 °	4135	677 ***	3108	277
	70,000 plants/ha	4713	927 *	1564	-310	4756	1298 ***	3337	506 *
50 cm	50,000 plants/ha	2631	-1155 °	1544	-330	3015	-443 °°°	3235	404
	60,000 plants/ha	3673	-113	1133	-741 °	3678	220 *	3234	403
	70,000 plants/ha	4152	366	1726	-148	4171	713 ***	3278	447 *
DL5%		-	895.96	-	600.74	-	197.73	-	443.11
DL1%		-	1209.90	-	812.24	-	267.01	-	598.37
DL0.1%		-	1611.16	-	1080.28	-	355.57	-	796.82

Table 6. Sunflower grain yield at different row spacing and plant densities in 2021 and in different locations in Romania

Experimental factors		Negrești location		Cogealac location		Dâlga location		Troian location	
Row spacing	Plant density	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)	Yield (kg/ha)	Difference (kg)
70 cm	50,000 plants/ha	4594	Control	2833	Control	3922	Control	2591	Control
	60,000 plants/ha	5051	457	2257	-576 °	4906	984 ***	2485	-106
	70,000 plants/ha	5972	1378 **	2307	-526	5903	1981 ***	2458	-133
60 cm	50,000 plants/ha	4209	-385	1326	-1507 <sup>ooo</sup>	3114	-808 <sup>ooo</sup>	2656	65
	60,000 plants/ha	4233	-361	2040	-793 <sup>oo</sup>	3815	-107	2624	33
	70,000 plants/ha	4640	46	2880	47	4683	761 ***	2498	-93
50 cm	50,000 plants/ha	2709	-1885 <sup>ooo</sup>	1768	-1065 <sup>ooo</sup>	2759	-1163 <sup>ooo</sup>	2496	-95
	60,000 plants/ha	3238	-1356 <sup>oo</sup>	1343	-1490 <sup>ooo</sup>	3502	-420 <sup>ooo</sup>	2476	-115
	70,000 plants/ha	3968	-626	1480	-1353 <sup>ooo</sup>	4252	330 **	2292	-299
DL5%		-	951.47	-	540.40	-	215.84	-	390.03
DL1%		-	1284.86	-	729.76	-	291.47	-	526.70
DL0.1%		-	1710.98	-	971.78	-	388.14	-	701.38

## CONCLUSIONS

The obtained data showed that under favourable growing conditions for sunflower plants, especially under good water plant supply conditions, the highest grain yields are obtained at row spacing of 70 cm, which is the row spacing generalized in Romania for sunflower crop. However, under less favourable growing conditions for sunflower plants, the narrower rows (at 60 cm and 50 cm) seem to provide better plant growth and finally higher grain yields compared to the row spacing of 70 cm.

Plant density has to be correlated with the growing conditions of the sunflower plants in searching for the optimum plant density. Thus, the better growing conditions are the higher plant density should be as to put into value the available growing factors. In the case there is no correlation between the plant density and growing conditions, the plants density too high becomes a limitative yielding factor.

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## ***Datura wrightii* Regel. INVASIVE PLANT IN OLTENIA, ROMANIA**

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### **Abstract**

The heavy anthropic activity has as a result the invasion of allochtones in the natural damaged ecosystems from Romania and the whole Europe. This fact has a negative impact over the genuine evolution and development of natural biocenosis. Invasive species are the real main threats for the agro-biodiversity. *Datura* is a genus of nine species of poisonous vespertine flowering plants belonging to the family Solanaceae. All species of *Datura* are poisonous, especially their seeds and flowers. *Datura wrightii* is an invasive species whose range has expanded greatly in recent years in Romania. According to studies in our country, the species *Datura wrightii* was identified for the first time in Romania from the Galati County - Foltești, Tg. Bujor, Umbrărești. This species has an aggressive character on biodiversity and we found it in ruderal areas, garbage storage on land, vacant lots, edges of the road. The species has an important anthropogenic impact on herbaceous and woody plant communities and therefore on some types of community interest habitats. *D. wrightii* has also been used to induce hallucination for recreational purposes. Ingestion of plant material can induce auditory and visual hallucinations similar to those of *Datura stramonium*.

**Key words:** *Datura wrightii*, invasive species, ecology, corology, plant communities.

### **INTRODUCTION**

The genus *Datura* belongs to the family Solanaceae and includes nine species for Europe: *D. ceratocaula* Ortega, *D. ferox* L., *D. innoxia* Miller, *D. discolor* Bernh., *D. metel* L., *D. quercifolia* Humboldt et al., *D. leichhardtii* F. Muell. ex Benth. (syn. *D. pruinosa*), *D. wrightii* Regel and *D. stramonium* L. The species name of this plant is for Charles Wright, 1811-1885, world-wide botanical collector but mainly in Texas (1837-1852), Cuba and his native Connecticut. Extracts from this plant and its relatives are narcotic and, when improperly prepared, lethal. The narcotic properties of species have been known since before recorded history.

They once figured importantly in religious ceremonies of south-western Indians (<http://www.wildflower.org/>).

*Datura wrightii* is native species in the south-western United States and Mexico. It much resembles *Datura innoxia* in general habit and is best distinguished on the basis of stem indumentum. *Datura wrightii* is characterized by: stem indumentums dense, of very short appressed or retrorse englandular hairs (occasionally intermixed with some longer

erect glandular hairs). Stigma usually exceeding anthers. Seeds with a single marginal furrow. Corolla - 14-26 cm long (Verloove, 2008).

Following field studies conducted in Oltenia I found that *Datura wrightii* is an invasive species whose range has expanded greatly in recent years in Romania. According to studies in our country, the species *Datura wrightii* was identified for the first time in Romania from the Galați County (Foltești, Târgu Bujor, Umbrărești). Also, the species was cited by Oprea, Sirbu and Dorofteu from the Danube Delta (Crișan, Caraorman, Letea) in 2011. This species has an aggressive character on biodiversity and we found it in ruderal areas, garbage storage on land, vacant lots, edges of the road.

The species has an important anthropogenic impact on herbaceous and woody plant communities and therefore on some types of community interest habitats.

*D. wrightii* has also been used to induce hallucination for recreational purposes. Ingestion of plant material can induce auditory and visual hallucinations similar to those of *Datura stramonium*.

## MATERIALS AND METHODS

The studies on the field involved a good bibliographic documentation regarding the physical-geographical frame: the relief, the geology-lithology, the hydrographic network, the soils and the general and local climate. The plant species nomenclature follows the Flora of Romania. For the study of the vegetation, we have used the methods of phyto-sociologic research characteristic to the Central European phyto-sociologic School. For the classification of the plant communities, we have used the synthesis of Rodwell et al. (2002). Field data were collected in the period 2018-2021.

The sample areas were chosen according to the presence of the species and its abundance-dominance. Floristic and phytosociological studies have been carried out on this invasive species in several localities in Dolj, Mehedinți and Vâlcea Counties.

## RESULTS AND DISCUSSIONS

In terms of spreading, the species can be found in meadows, bushes, in weed plant communities, on the edge of meadow woodland plant communities, in agricultural crops, roadside, forest roads, trails. In urban areas this species forms population with a huge number of specimens in parks, green spaces, gardens, private courtyards, raw land (Figures 1-4). It settles very well on the roadsides, successfully managing the seeds of this species to germinate in the concrete cracks and to form well-developed bushes that produce a large number of seeds.



Figure 1. *Datura wrightii* in the ruderal plant community

Thus, in the cities of Oltenia the species is quite common. The species was identified in Râmnicu Vâlcea, Băile Govora, Bălcești, Nicolae Bălcescu, Craiova, Segarcea, Giurgiuța, Măceșu de Sus, Cârna.

In the thematic area this species grows in the floristic composition of the following ruderal plant communities: *Hordeetum murini* Libbert 1932 em. Pass. 1964; *Polygonetum avicularis* Gams 1927 (Rodwell et al., 2002; Sanda et al., 2001), *Cardarietum drabae* Timar 1950 (Rodwell et al., 2002; Sanda et al., 2001); *Digitario sanguinalis-Galinsogetum* Beck 1941, *Echinochloo - Polygonetum lapathifolii* Soó et Csürös, 1947.

The species has been identified at the border of the following woody plant communities: *Stellario nemori-Alnetum glutinosae* (Kärstner, 1938) Lohm, 1957; *Prunus spinosa-Crataegus monogyna* (Soó, 1927) Hueck, 1931.

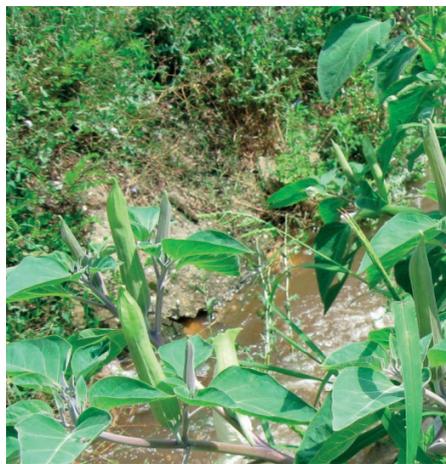


Figure 2. *Datura wrightii* in the Cotmeana Valley



Figure 3. *Datura wrightii* in the urban area

Between the localities of Segarcea and Giurgiuța, the species is frequently found in the *Polygonetum avicularis* Gams 1927 plant community, which is installed in front of the yards or on the edge of the ditches.

This plant community is very widespread in all localities in the researched territory. Knotweed phytocoenoses grow on trampled land, along roads, alleys, in yards and on roadsides. The stations where the phytocoenoses of this plant community are installed, have a pronounced mesophilic, euriterm and euriionic character.

From a phytosociological point of view, together with the edifying species, the following species also frequently participate in the composition of the vegetal carpet: *Capsella bursa-pastoris*, *Poa annua*, *Matricaria recutita*, *Hordeum murinum*, *Potentilla argentea*, *Lolium perenne* and *Datura wrightii*. The vegetation cover is between 85-95%.

In Giurgiuța locality, well-developed shrubs of *Datura wrightii* were found in the concrete ditches. It has spread rapidly from the yards or gardens where it was grown as an ornamental plant. It prefers anthropogenic, ruderal plant communities with low soils and nitrogen-rich soils.

In Măceșu de Sus, the species was also identified in the floristic composition of the *Polygonetum avicularis* Gams 1927 plant community (Table 1).

In Vâlcea County in the Cotmeana River Basin, the species was identified in the floristic composition of the *Echinochloo - Polygonetum lapathifolii* Soó et Csürös 1947 plant community.

The *Echinochloo - Polygonetum lapathifolii* Soó et Csürös 1947 (Syn.: *Echinochloo crus-galli* - *Galinsogatum parviflorae* Burduja et Florita Diaconescu, 1976, *Panico - Galinsogatum* Tx. et Becker, 1942) (Table 2) plant community vegetates on low ground or in shady places, especially on excessively moist soils.

This plant community is usually found in corn, soybean, sunflower, straw, and even vine plantations, where mechanical control could not be applied.

The floristic composition of this plant community is dominated by mesophytic species, followed by xeromesophytes, mesothermophyte and subtermophyte species, as well as euriionic and weakly acidophilic species.

From a phytosociological point of view, most of the species recorded in the surveys belong to the Chenopodietea class, the Panico - Setarion alliance and the order *Chenopodietalia albi* and *Plataginietalia majoris*.

The terophytes represent the majority of species in the floristic composition, many of which are cosmopolitan and adventitious, and almost half of all species are of Eurasian origin. In the researched territory from the Cotmeana river basin, this vegetal community also forms weeds on the bank of the Cotmeana river.

The species *Datura wrightii* was found in these weeds, in the immediate vicinity of the households in the localities, from where it probably spread after being cultivated as an ornamental plant.

Locals usually plant this species in front of households, from where it spreads very easily.



Figure 4. In the ruderal plant community

Five test areas were chosen, the species being found in only five of them. The abundance-dominance of the species within the phytocoenoses is relatively low.

Table 1. *Polygonetum avicularis* Gams, 1927

Number of relevées	1	2	3	4	5
Coverage (%)	90	95	85	95	90
Area (m <sup>2</sup> )	30	25	10	30	25
<b>Char. ass.</b>					
<i>Polygonum aviculare</i>	4-5	5	4-5	5	5
<b>Polygonion avicularis et Plantaginetaia majoris</b>					
<i>Plantago major</i>	+	-	+	-	-
<i>Poa annua</i>	+	-	+	-	-
<i>Cichorium intybus</i>	-	+	-	+	-
<i>Potentilla reptans</i>	+	-	-	+	-
<i>Rumex crispus</i>	-	+	-	+	-
<i>Matricaria recutita</i>	+	+	-	+	+
<i>Potentilla anserina</i>	-	+	-	+	+
<i>Lolium perenne</i>	1	1	+	+	-
<b>Chenopodietea</b>					
<i>Capsella bursa-pastoris</i>	+	+	+	-	-
<i>Malva pusilla</i>	+	-	+	-	-
<i>Cardaria draba</i>	-	+	+	-	+
<i>Hordeum murinum</i>	+	-	+	-	+
<i>Veronica persica</i>	-	-	+	+	+
<b>Festuco-Brometea</b>					
<i>Lotus corniculatus</i>	-	+	-	+	-
<i>Plantago lanceolata</i>	-	-	-	+	+
<i>Potentilla argentea</i>	-	+	+	+	-
<b>Molinio-Arrhenetheretea</b>					
<i>Festuca pratensis</i>	-	+	-	+	-
<i>Trifolium repens</i>	-	+	-	-	+
<i>Poa pratensis</i>	-	-	-	+	+
<i>Ranunculus repens</i>	-	-	+	-	+
<i>Leontodon autumnalis</i>	-	-	-	-	-
<i>Taraxacum officinale</i>	-	+	+	-	-
<b>Variae Syntaxa</b>					
<i>Cynodon dactylon</i>	+	-	-	+	-
<i>Arenaria serpyllifolia</i>	-	+	-	+	+
<i>Trifolium campestre</i>	-	+	-	+	+
<i>Utica urens</i>	-	+	-	-	+
<i>Potentilla reptans</i>	+	+	-	-	-
<i>Medicago lupulina</i>	-	-	-	+	+
<i>Datura wrightii</i>	+	+	-	-	+

Place and data of relevés: 1-5, Giurgița (Dolj County), 20.09.2020

## CONCLUSIONS

This species is mainly in the invasion of ruderal areas, the dense networks eliminating other herbaceous plants.

Regarding the woody habitat, the species was found only at their limit, but given the large number of seeds they produce, the vitality of the species and its ability to germinate it can spread very quickly and can conquer new territories entering the floristic composition of these plant communities.

Taking into account these considerations, it is very important that this species is constantly monitored and that the best control methods are found, without affecting the biodiversity and phytocenoses in which it develops.

Given its presence in urban areas, it is necessary that the bushes developed on the side of the streets, through parks gardens, garbage, be cleaned and burned before fruiting and seeding.

Table 2. *Echinochloa - Polygonetum lapathifolii* Soó et Csűrös, 1947

Number of relevées	1	2	3	4	5
Coverage (%)	100	90	100	90	100
Area (m <sup>2</sup> )	30	30	25	25	25
<b>Char. ass.</b>					
<i>Galinsoga parviflora</i>	2-3	2	2	2	2
<i>Echinochloa crus-galli</i>	3	1-2	1	1-2	2
<i>Polygonum lapathifolium</i>	1	2	2-3	2	1
<b>Panico - Setarion</b>					
<i>Amaranthus retroflexus</i>	+	-	+	-	+
<i>Setaria pumila</i>	-	+	-	+	+
<i>Stachys annua</i>	-	+	-	-	-
<i>Lathyrus tuberosum</i>	-	-	-	-	+
<i>Polygonum convolvulus</i>	-	-	-	+	-
<b>Polygono - Chenopodium polyspermi et Chenopodietalia albi</b>					
<i>Chenopodium album</i>	+	+	1	1	+
<i>Thlaspi arvense</i>	+	+	+	-	-
<i>Euphorbia heliosopia</i>	-	-	-	-	+
<i>Senecio vernalis</i>	-	-	+	-	-
<b>Stellarietea mediae</b>					
<i>Hibiscus trionum</i>	+	+	-	+	+
<i>Matricaria perforata</i>	+	-	-	+	+
<i>Veronica persica</i>	-	+	-	-	+
<i>Anagallis arvensis</i>	-	-	-	+	+
<i>Brassica nigra</i>	-	+	+	-	-
<i>Stellaria media</i>	+	+	-	-	-
<i>Solanum nigrum</i>	-	-	-	+	+
<i>Artemisia annua</i>	-	-	-	+	+
<i>Lamium amplexicaule</i>	-	-	+	+	+
<i>Chenopodium hybridum</i>	+	-	-	+	+
<b>Artemisietea vulgaris</b>					
<i>Equisetum arvense</i>	-	+	-	-	+
<i>Xanthium strumarium</i>	+	-	-	-	-
<b>Molinio - Arrhenatheretea</b>					
<i>Taraxacum officinale</i>	-	-	-	+	+
<i>Ranunculus sardous</i>	-	-	-	-	+
<i>Medicago lupulina</i>	-	-	-	-	+
<b>Plataginetalia majoris et Polygonion avicularis</b>					
<i>Plantago major</i>	-	-	-	+	+
<i>Malva pusilla</i>	+	-	-	-	-
<i>Matricaria recutita</i>	+	-	-	-	-
<b>Variae Syntaxa</b>					
<i>Rorippa sylvestris</i>	-	-	-	-	+
<i>Datura wrightii</i>	+	-	-	+	-
<i>Sambucus ebulus</i>	+	-	-	+	-
<i>Utica dioica</i>	+	-	-	+	-
<i>Saponaria officinalis</i>	+	-	-	+	+

Place and data of relevés: 1-5, Galicea (Vâlcea County), 14.07.2019

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## BIODYNAMIC AGRICULTURE AN ALTERNATIVE TO CONVENTIONAL AGRICULTURE: A CASE STUDY OF TRANSILVANYA AREA, ROMANIA

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### Abstract

*The delimitation of the research topic has as a starting point the problematic situation represented by the unfavorable effects on the environment and food safety caused by chemical agriculture. Biodynamic agriculture, through its peculiarities, represents an alternative for farmers concerned about environmental health and obtained products. The elaboration of a questionnaire and its application on a sample of 95 farmers from the Transylvanian Plain and Plateau, Romania has consisted an important support for revealing their perception on biodynamic agriculture. Our research results allow us to conclude, at least in this phase, that biodynamic agriculture, although little known among farmers, can help protect biodiversity and the environment, and biodynamic farms can be profitable due to the increased interest of consumers for healthy products*

**Key words:** *biodynamic agriculture, sustainability, farmers' opinion, yield, profitability.*

### INTRODUCTION

Sustainable farming methods have emerged in the 20th century throughout the entire developed world due to the imperative need to protect the environment and food safety, as a response to the negative effects of industrial agriculture (Gradi et al., 2014). According to (Crippa et al., 2021) food systems are solely responsible for a third of the global greenhouse gas emissions. Biodynamic (BA) or sustainable agriculture has become the subject of research efforts in recent decades (Turinek et al., 2009; Beluhova-Uzunova et al., 2019), which are carried out in all agricultural fields and in many places around the world, being reported worldwide as an appropriate system for the conservation or even regeneration of natural resources (Robusti et al., 2020). Biodynamic agriculture is similar to organic farming in many ways (Heimler et al., 2009) both "respect the normal functioning of ecosystems, avoiding the usage of agrochemicals and lead to food" free "of synthetic chemicals, thus healthier (Carvalho, 2006).

According to Demeter, the subject of quality has consisted an important aspect ever since its

beginning ([www.demeter.net](http://www.demeter.net)). The purpose of the two agricultural systems is to maximize the synergies between the farm and the ecosystem (Altieri, 1999); the farm resembles to nature by conserving and recycling resources, requiring minimal external inputs and thus minimizing waste and pollution (Altieri, 2012).

Organic farming is one of the most widely known sustainable models of agricultural production (Antczak, 2021). The difference between organic and biodynamic agriculture, apart from philosophical and historical aspects, is made by the use of biodynamic preparations containing specific herbs or minerals, treated or fermented with animal organs, water and/or soil (Reganold & Palmer, 1995) above these, taking care of the soil rests one of the cornerstones of bio-dynamic ideology (Kaltoft, 1999). The eight preparations prescribed by Steiner for biodynamic agriculture are numbered from 500 to 5007, the first two being used for field preparation, while the last six are used for composting (Nabi et al., 2017) all improving the harvest's yield and quality (Ram, 2019). Therefore, biodynamic farms operate in a circle that improves climate change mitigation and the future of food, plants and animals (Figure 1).

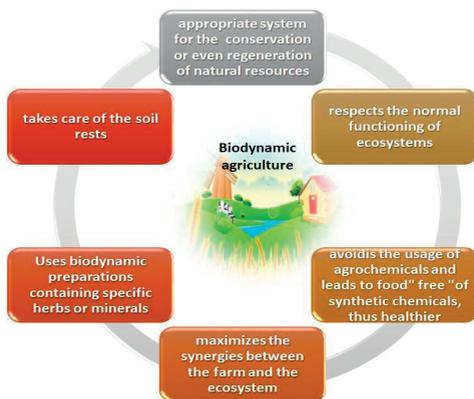


Figure 1. Synthesis of the literature review

Historically, biodynamic agriculture dates back to the early 1920's when Rudolf Steiner, the undisputed founder of the biodynamic method, became concerned with environmental pollution and the exploitation of natural resources (Diver, 1999). The editors of *Alchemy of the Everyday*, the catalog of the retrospective exhibition of Steiner's work, describe Steiner as "one of the most influential - but also controversial - reformers of the twentieth century" (Vege sack, 2010). The biodynamic approach is based on eight lectures for farmers ("Spiritual foundations for agriculture renewal: a series of lectures.") Given by Rudolf Steiner in 1924 at the Koberwitz farm near Wrocław (formerly Breslau) (Steiner, 2004). Compared to organic farming, biodynamic farming is more of a life philosophy, a healthy lifestyle that cannot exist without a direct and peaceful connection with nature) (Ponzio et al., 2013). As pointed out by (Bloksma & Struik, 2007), a healthy organism from a biodynamic perspective is not only a "healthy", resilient and sustainable agroecosystem, locally adapted, but also includes the socio-cultural and spiritual dimension, because agriculture itself represents an interaction between human and environment and is being embedded in a cultural environment (Brock et al., 2019).

In farms from Western Europe, biodynamic agriculture represents a new trend, with chances that, in the coming years, to make forgotten organic farming, especially since the new farming community is all about the integrated concept with a peaceful link between agricultural production and nature (Turinek et

al., 2009). Completely eliminating chemicals from farm life, makes possible solving environmental pollution, and the yields obtained, although smaller, are offset by the fact that they present superior biological and nutritional qualities (Papacostea, 1993; Stan, 2005) and they can be traded at higher prices compared to those obtained from conventional agriculture.

Nowadays, biodynamic agriculture (Figure 2) is practiced by approximately 6,400 farmers (www.demeter.net) in 55 countries (30.0%) of the 186 countries reporting organic farming and in a subgroup of 251,842 certified biodynamic hectares (0.35%) of the total global of 71,514,583 certified organic hectares (Paull & Hennig, 2020) and the agricultural method has a very good reputation among the consumers of organic products (Brock, 2019).



Figure 2. Map of biodynamic agriculture

(Adapter after <https://www.freeworldmaps.net/download/maps/world-outline-map.jpg>, and <https://database.demeter.net/oppub>)

Currently, in Romania there are very complex agricultural systems, their complexity being determined by natural and socio-economic conditions, the developmental level of science and technology among the evolution of human society. According to (Gradi et al., 2014) the most widely used agricultural systems are the conventional and organic one, the last one being practiced on only 3% of the total agricultural area of Romania (www.madr.ro). Research regarding the perception of Romanian farmers on organic farming are consistent (Petrescu, 2017; Ionela, 2016; Petrescu-Mag et al., 2019; Roșca et al., 2012) while those on biodynamic agriculture are non-existent, probably because biodynamic agriculture is an agricultural method challenged or even rejected, being considered unscientific.

Neither the literature regarding the practice of biodynamic agriculture and food safety is very

consistent, there are some research related to: agricultural systems practiced in Romania ([www.madr.ro](http://www.madr.ro)), the conversion from conventional to organic and biodynamic agriculture system (Buhler & Constantinescu, 2020) autumn wheat culture technology (Tomsa & Morar, 2013), and support for food safety and security provided by biodynamic farms (Negrei, 2017).

Although, biodynamic farmers are still a minority in Romanian agriculture, only 5 farmers being certified by Demeter or in the conversion period, of which two are found in the research region (Figure 3) ([www.demeter.net](http://www.demeter.net)), the fact that our country still has free/virgin lands that have not been yet cultivated or chemically treated and by founding the Association for the Development of Biodynamic Agriculture in 2021 (<https://biodinamica.ro/>) we hope to increase the interest in such agricultural practices in other farms as well.



Figure 3. The situation of biodynamic farms in Romania in 2021 (<https://database.demeter.net/oppub/all/all/all/all/all/all/ro>)

## MATERIALS AND METHODS

While the conversion from conventional to organic agriculture was targeted by many authors, researches regarding biodynamic agriculture are still in the pioneering phase in many countries, and especially in our country. Therefore, evaluating the opinion of the Romanian farmers, and especially of those from the Transylvanian Plain and Plateau (Figure 4) regarding the biodynamic agriculture represents the main contribution of our work.

The questionnaire based research method aimed to collect data from respondents. The sample consisted of farmers from the Transylvanian Plain and Plateau, Romania.

A questionnaire of 18 questions was applied in the period 2020-2021 in online mode due to the

pandemic situation given by SARS-CoV-2 virus, but also in order to save time and money.

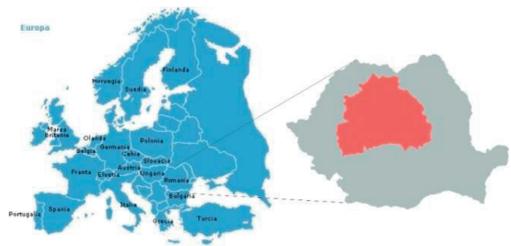


Figure 4. Transylvanian Plain and Plateau

A total of 95 questionnaires were validated, and further on, were analyzed. In order to analyze the data, we used the methods related to descriptive statistics, respectively the analysis of frequencies, in order to understand group tendencies. In order to identify the general trend and variability, the related parameters were calculated: for the general trend (average, median, sum of series values), and in order to calculate the parameters of the central trend of distribution and spreading - standard deviation. The questionnaire is divided in four main parts, involving: questions related to the agricultural situation of the respondents; questions regarding the concept, the intention to convert to biodynamic agriculture and the environmental implications deriving by practicing this type of agriculture; questions related to product quality and sales market, yield and profitability, and the fourth part was represented by questions related to socio-demographic aspects of the respondents.

## RESULTS AND DISCUSSIONS

The main aspects that were taken into account in the present research were grouped into five categories of results.

Respondents profile.

The socio-demographic characteristics of the respondents are presented in Table 1. From the total number of respondents (Table 1) 71.1% are included in the age category of 18-30 years old, have graduated from university studies (78.9%), possess as well other specialized studies (68.4%), practice farming in the conventional agricultural system (74.7%) and are mostly residents (43.3%) from Cluj County.

Table 1. Socio demographic data

Characteristic	Category	Percent %
Age	18-30 years old	71.1%
	31-40 years old	16.5
	41-50 years old	4.2
	Over 50 years old	8.2
Level education	Vocational studies	2.1
	High school	18.9
	University degree	78.9
Possess other specialized studies in the field	Yes	68.4
	No	31.6
County of origin	Cluj	68.4
	Mures	31.6
	Bistrita	
The agricultural farming system practiced	Biodynamic agricultural system	1.1
	Conventional system	74.7
	Ecological system	17.6
	Conventional + ecological	3.9

Results regarding the agricultural situation of the respondents.

In order to establish the agricultural situation presented by the respondents, some information considering the surfaces situation of the owned holdings were requested on. There have been identified five categories of holdings which are summarized in Table 2. This decision, to have a diverse sample of holdings, was made from the desire to generate as diverse responses as possible. As mentioned in the Material and Method section, respondents were residents from the Transylvanian Plain and Plateau. These regions produce a diverse range of agrifood products in Romania (Oroian, 2018) due to the geographic characteristics that they possess.

Table 2. The share of owned holdings

Category	Percent
Field crops	62%
Vegetable crops	36%
Orchards	20%
Vine	12%
Zoototechnics	55%

Respondents opinion regarding the biodynamic agricultural system.

Biodynamic agriculture is a recognition of the basic principles that exist in nature and at the same time an approach to agriculture that takes these principles into account in order to bring balance and healing, without the need for chemical interventions (Vlahova & Arabska, 2015a). Deepening the survey by analyzing the

knowledge of the biodynamic agriculture concept among the surveyed farmers we note that only 37.9% have information about this type of agriculture, while 62.1% are not familiar with the concept (Table 3). This may be due to the lack of information sources considering this type of agriculture.

Table 3. The knowledge of the biodynamic agriculture concept

Variable	Freaquency	Percent
Yes	36	37.9
No	59	62.1
Total	95	100.0

By analyzing the intention to adopt the principles of biodynamic agriculture, it was found that 18.9% of respondents want to adopt this agricultural system, while 81.1% are not interested. The explanation of this phenomenon can be given by the fact that when conventional lands are transformed into organic, respectively biodynamic, the farm becomes less efficient. The analysis of socio-demographic data at 2 groups level using chi-square test and Fisher's Exact test did not find significant differences between the two groups in terms of socio-demographic characteristics, except for the currently practiced cultivation system (Table 4). In the case of the group that wants to adopt the biodynamic agriculture system, the share of those who cultivate in a conventional system is 61.1%, compared to 77.9% in the case of the second group that does not want to adopt the principles of the biodynamic agricultural system. The share of those who cultivate in the ecological system is higher in the case of the second group (18.2%), compared to 16.7% in the case of first group. It was also found that 16.7% of those from the first group practice both conventional and ecological systems, while in the case of the second group only adopted the mixed system.

A study conducted by (Trujillo - Barrera et al., 2016) revealed that risk perception has a negative effect on the adoption of sustainable practices, especially when one's existence is at stake. Studies have found that organic and biodynamic farmers are more prone to risk than conventional farmers (Flatten et al., 2015; Gardebroek, 2006). However (Pechrová, 2014) states that if farms are subsidized, farmers are motivated to switch to biodynamic agriculture,

as they see subsidies as a significant addition to their income. Regarding other determinant factors, (Lohr & Salomonsson, 2000) highlighted that access to multiple outlets and sources of information are important for farmers and replace the level of payment according to farmer's utility. Thus, they concluded that services, rather than subsidies, can be used to encourage the transformation into organic, respectively biodynamic farming.

Further was analyzed the perception of those who want to pass to the biodynamic agricultural system regarding the principles of this crop system (Table 5). Thus, a set of 6 statements were evaluated. It was found that 72.2% of the respondents agree that the biodynamic agricultural system respects diversity and

biodiversity by stimulating the recycling of substances and chemical elements ( $4.56 \pm 0.784$ ), while 66.7% totally agree that the farm represents a unitary organism that integrates plants, animals and humans ( $4.50 \pm 0.786$ ). Respondents are less in agreement with the statements according to which cosmic rhythms influence plant growth ( $3.78 \pm 1.437$ ), respectively that biodynamic preparations emanate forces through which the elements inside plants and animals are organized ( $3.72 \pm 1.274$ ).

The research continued by identifying farmers' opinion on the implications of practicing biodynamic agriculture on the environment (Table 6).

Table 4. Intention to convert to the biodynamic farming system

Characteristic	I intend to adopt the biodynamic agriculture principles	I do not intend to adopt the biodynamic agriculture principles	Chi-square test/Fisher's Exact test	
	N=18 (18.9%)	N=77 (81.1%)		
Age	18-30 years	10 (55.6%)	58 (75.3%)	$\chi^2=3.104$ , $df=3$ , $p=0.373$
	31-40 years	5 (27.8%)	10 (13.0%)	
	41-50 years	1 (5.6%)	3 (3.9%)	
	>50 years	2 (11.1%)	6 (7.8%)	
Level of education	Vacational studies	0 (0.0%)	2 (2.6%)	Fisher's Exact test $p>0.05$
	High school	3 (16.7%)	15 (19.5%)	
	University degree	15 (83.3%)	60 (77.9%)	
Study in the field	YES	13 (72.2%)	52 (67.5%)	$\chi^2=0.149$ , $df=1$ , $p=0.700$
	NO	5 (27.8%)	25 (32.5%)	
Agricultural system	Biodynamic agriculture	1 (5.6%)	0 (0.0%)	Fisher's Exact test $p<0.05^*$
	Conventional	11 (61.1%)	60 (77.9%)	
	Ecological	3 (16.7%)	14 (18.2%)	
	Mixed	3 (16.7%)	3 (3.9%)	

Table 5. Statements regarding the principles of biodynamic agriculture

Statements	Appreciation scale (%)					Mean	St. dev.
	1-total disagreement						
	5- total agreement						
	1	2	3	4	5		
Cosmic rhythms influence plant growth	12.2	16.6	14.6	22.0	36	3.56	1.432
Quality is a priority element that influences organisms	0	4.9	14.6	24.4	56.1	4.31	0.906
The farm is considered to be a unitary organism that integrates plants, animals and people	0	0	22.0	26.8	51.2	4.29	0.813
Diversity and biodiversity are respected by stimulating the recycling of chemical substances and elements, as well as the exclusion of polluting elements.	0	7.3	22.0	19.5	51.2	4.14	1.013
Biodynamic preparations emanate a force through which the elements inside plants and animals are organized	9.8	14.6	22.0	29.3	24.0	3.43	1.285
Rational farming techniques are applied, whether they are new or old	0	4.9	31.7	24.4	39.0	3.97	0.961

Thus, over 58% of the respondents believe that biodynamic agriculture influences the environment very much, while over 23% believe that it influences much the environment. The influence of biodynamic agriculture on the environment consists in maintaining the well-being of the environment, there is an increase in biodiversity and a sustainable development. Greenhouse gas emissions are responsible for global warming and climate change (Aishwath, 2007). Compared to conventional agriculture, the practices of biodynamic farming have proven to be more resistant to environmental challenges, to encourage a diverse biosphere and to be more energy efficient. Biodynamic farming has an increasing importance in the face of climate change (Lichtfouse, 2018), energy deficit and population growth (Padmavathy & Poyyamoli, 2011). Pergola et al., 2016, when making an assessment on life cycle and energy in integrated and biodynamic apricot orchards, noted that biodynamic production had a lower impact on the environment and requires a lower energy demand. A lower impact on the environment was also noted in biodynamic viticulture compared to the conventional system, which may be related to the preference for manual work over mechanical work, reduced use of lubricants and diesel compared to the conventional system (Villanueva-Rey et al., 2014).

Table 6. To what extent does the practice of biodynamic agriculture influence the environment

	Frequency	Percent
Very much	56	58.9
Much	22	23.2
Neutral	10	10.5
Little	7	7.4
Total	95	100.0

Results regarding the quality of the products and the sales market.

Achieving high quality food is one of the main goals of biodynamic agriculture. Analyzing the opinion of the respondents regarding the quality of the products obtained in the biodynamic agriculture system we notice that more than 30% of them consider that they are of superior quality (Table 7). This topic is at high interest compared to other research topics in biodynamic agriculture. The statement regarding the quality of the products can be strengthened by the

researches made by other authors. Jarienè et al., 2017, when analyzing the concentration of total phenolic compounds and the antioxidant activity of DPPH in potato tubers (*Solanum tuberosum*) of the variety Red Emmalie and Blue Congo observed that they increased significantly after the application of the biodynamic preparation of horn silica. Also, Bavec, et al., 2012, when making a comparison between potatoes in biodynamic and conventional agriculture on by quality indices, dry matter content, taste quality, relative proportion of pure proteins and the value of biocrystallization, recorded positive values in those from biodynamic agriculture. Moreover, Vaitkevičienė et al., 2012, detected that the starch content of red-skinned potatoes (*Solanum tuberosum*) was significantly increased by the combined application of manure and horn silica. Bavec et al., 2012 respectively *Fragaria spec.*, D'Evoli et al., 2010 found more ascorbic acid in biodynamic cabbage and strawberries compared with organic and conventional ones. Heimler et al., 2011, identified a lower content of polyphenols in *Batavia lettuce* plants cultivated in the conventional agricultural system compared to the plants content in organic and biodynamic agriculture. Masi et al., 2017, have revealed that in terms of the color analysis, biodynamic apples (BIO-A) showed the brightest skin. Kusche et al., 2015, observed when comparing milk from different management systems, that the highest share of nutritionally valuable fatty acids are found in milk provided from biodynamic systems. In conclusion, as stated by Nabi et al., 2017, the consumption of biodynamic foods increases vitality, reduces allergic reactions and brings an overall improvement in health.

Table 7. Opinions regarding the quality of products obtained in the biodynamic agricultural system

Variable	Frequency	Percent
Yes	29	30.5
No	3	3.2
I don't know	63	66.3
Total	95	100.0

Sales market, yield and crop profitability from biodynamic agriculture

The sales market and the prices of biodynamic products were also investigated in the present research. Unlike conventional farms, marketing plays a major role in biodynamic farms the

biodynamic market being much smaller than the organic one in Romania, and especially in the researched area. Thus, the surveyed farmers stated that by educating the population and encouraging the consumption of quality food, demand will increase and consequently the market will develop. For example, studies conducted by Mann et al., 2012, on the consumption of biodynamic wine have shown that people with higher incomes and urban residents have a more positive attitudes towards this range of wine.

When analyzing farmers' opinions concerning the yield and profitability of biodynamic agriculture compared to organic or conventional agriculture, respondents stated that it can be just as profitable, even if production is lower, as consumers become increasingly interested in consuming healthy products even if that means a higher price for the product. They also stated that profitability can be increased by using elements within the farm in a high percentage and by adding low external inputs, which will result in lower expenses. A number of 14 articles from our database are related with issues regarding the growth and profitability of biodynamic crops. While (Garcia-Yzaguirre et al., 2011; Pechrová, 2014; Vereijken, 1990) revealed that the yields of the studied biodynamic crops were lower compared to conventional plots, (the prices of biodynamic products were up to 25% higher than the prices of conventional products), Nabi et al., 2017, and Jat et al., 2018, reported a significant increase in yields and nutritional characteristics in biodynamics compared to organically and conventionally grown vegetables, respectively cereals. Also, Jakop et al., 2017, found that the production of pumpkin seed oil (*Cucurbita pepo*) in the biodynamic system could compete with that obtained in the conventional production. Maneva et al. 2017, by comparing the yield of organically grown wheat (*Triticum turgidum* subsp. *Polonicum* L.) with biodynamically grown wheat observed significantly higher yields in biodynamic processing. In Germany a study conducted by Reinken et al., 1984, reported that all vegetable crop yields obtained for a period of six years were lower on biodynamic plots compared to conventional plots. Prices for biodynamic products were however higher, leading to higher

profits for most biodynamic vegetables. Economic studies have shown that biodynamic agricultural systems could achieve a profitability close to that of conventional or organic farms (Holmes, 1993). By examining 28 different experiments in Germany it was revealed that the use of biodynamic sprays increased crop yields in years when yields were low (Raupp & König, 1996). Schlüter, 1985 analyzed the yields and profitability of 16 biodynamic farms in seven regions of Baden Württemberg and compared the results with statistics from the German Ministry of Agriculture. He concluded that the yields of all cereal crops grown on biodynamic farms were lower. Milk productivity on biodynamic farms was almost 15% lower compared to conventional farms. However, because biodynamic farms had lower costs than conventional ones, their profit was higher (Koepf, 1986). Lampkin & Padel, 1994, 1994b have recorded similar results in terms of the economic performance of biodynamic farms.

## CONCLUSIONS

The study regarding farmer's opinion from the Transylvanian Plain and Plateau by examining the way they perceive the concept of biodynamic agriculture represents an original contribution of the present paper, which enriches the specialized literature. Although our sample is limited and the number of respondents who know or are willing to convert to this type of agriculture is low, we believe that providing more information about the potential and benefits of this type of agriculture could increase the number of biodynamic farms in the studied area. This conclusion is also reinforced by the claims related to the superior quality of these products. Also, the results obtained in this research show that respondents believe that there may be profitability and the market as a result of consumer preferences for healthy products even in conditions of a higher price of the product.

Under the present and future scenario of climate change and consumer preference for ecological food, it is imperative to explore research on biodynamic agriculture.

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## MACROMYCETES RECORDED IN THE CAMPUS OF THE UNIVERSITY OF AGRONOMIC SCIENCES AND VETERINARY MEDICINE OF BUCHAREST: PRELIMINARY DATA

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### Abstract

Basic components in the stability of natural ecosystems, with an important role in nature conservation, macromycetes are indicators of different substrates and specific habitats. In this preliminary survey, some of the macroscopic fungi (Basidiomycota: Agaricomycetes) collected from "Agronomie-Herăstrău" campus of the University of Agronomic Sciences and Veterinary Medicine of Bucharest were morphologically described, in terms of cap (pileus), gills (lamellae), stipe (stem), ring (annulus) and spores. Also, some aspects concerning edibility were discussed. The following species were subjected to the research: *Coprinellus micaceus*, *Agaricus arvensis*, *Macrolepiota excoriata*, *Hypholoma fasciculare*, *Scleroderma citrinum*, *Xerocomellus porosporus* and *Marasmius oreades*.

**Key words:** macrofungi, mushroom, morphology, spores, edible, Bucharest.

### INTRODUCTION

Mushrooms are very important both from a practical and scientific point of view. Preservation of the gene pool of fungi is necessary to deepen the evolution and diversity of species and to better understand their morphology related to ecological adaptations (Tănase et al., 2009). The knowledge regarding mycodiversity is relatively poor despite its immense significance in natural ecosystems as recyclers of organic matter (Loguercio-Leite et al., 2009).

Macrofungi species form a major component of the biodiversity of Romania (Tănase and Pop, 2005). Since the urban regions including parks are propitious habitats for wild growing mushrooms, the picking of edible species is of interest, but at the same time there is a risk that some of them will be contaminated with toxic elements (Zsigmond et al., 2018).

The aim of this study was to report and describe the features of macroscopic fungi (Basidiomycota) species as part of biodiversity encountered in the campus of the University of Agronomic Sciences and Veterinary Medicine (UASVM) in Bucharest - an area often frequented both by students and academic staff

but also by people from the surroundings, as leisure-time activity.

### MATERIALS AND METHODS

Located in sector 1 of Bucharest, with an area of 38 ha, "Agronomie-Herăstrău" campus is home to six of the seven faculties of UASVM. Gathered from several green areas of the campus in November 2019, mushroom species were photographed, identified, classified and described based on their macroscopic and microscopic features (Bielli, 1999; Locsmandi & Vasas, 2013) in the laboratory of the Faculty of Agriculture.

The main elements of the mushroom morphology were characterized herein: cap (pileus); gills (lamellae) and spores; ring (annulus); stipe (stem).

For analyzing the spores, wet mount slides and photographs were performed using a binocular microscope model Motic Panthera S (20x and 40x magnification). Also, some observations and images were obtained using a Krüss Optronic binocular microscope.

The following species were characterized: *Coprinellus micaceus*, *Agaricus arvensis*, *Macrolepiota excoriata*, *Hypholoma*

*fasciculare*, *Sclerderma citrinum*, *Xerocomellus porosporus*, *Marasmius oreades*. For each macroscopic fungi, taxonomic lineage was listed in accordance with NCBI (National Center for Biotechnology Information), EOL (Encyclopedia of Life) and First Nature - Fungi Identification online databases. When applicable, the common names were given both in English and Romanian.

## RESULTS AND DISCUSSIONS

Seven species of macromycetes were reported and described within the study period, as follows:

**1. *Coprinellus micaceus*** (Bull.) Vilgalys, Hopple & Jacq. Johnson 2001 (Figure 1a) - Glistening inkcap; Mica cap; Romanian common names: “burete de mică”; “balegă strălucitoare”; “bureți de rouă”.

Phylum Basidiomycota  
Subphylum Agaricomycotina  
Class Agaricomycetes  
Subclass Agaricomycetidae  
Order Agaricales  
Family Psathyrellaceae

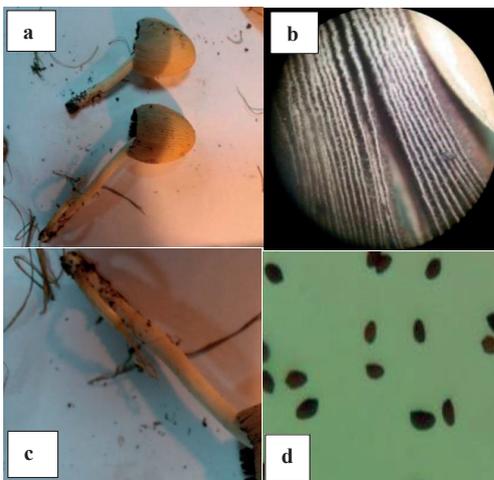


Figure 1. *Coprinellus micaceus*

The cap is thin and fragile with a diameter between 0.5-1.5 cm, color varies from white-yellow, yellowish-brown to brown-brown. At the beginning it is ovoid and at maturity it takes on a conical shape (bell), the edge growing radially and turning upwards.

The gills are thin, high, arranged very close (Figure 1b). At first they are white, then they turn brown, as the spores mature, later becoming black.

The stem (Figure 1c) has a height of 1.5 - 4 cm, hollow inside, cylindrical in shape, with no visible ring. It doesn't smell. The spores are dark brown to black (Figure 1d), fusiform, showing a sharp tip.

This mushroom is considered edible (Tersoo-Abiem et al., 2019), but potentially poisonous if collected from polluted areas (First Nature database). Mogildea (2008) has recorded a frequent occurrence of *C. micaceus* in another urban ecosystem from Bucharest - Cișmigiu Park.

**2. *Agaricus arvensis*** Schaeff., 1774 (Figure 2a) - Horse mushroom; Romanian common names: “ciupercă de câmp”; “șampionionul oilor”; “ciupercă de braniște”; “ciuperca calului”.

Phylum Basidiomycota  
Subphylum Agaricomycotina  
Class Agaricomycetes  
Subclass Agaricomycetidae  
Order Agaricales  
Family Agaricaceae

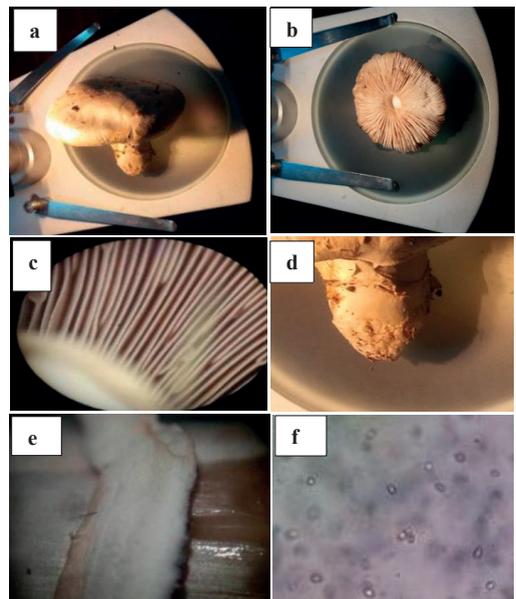


Figure 2. *Agaricus arvensis*

The cap has a diameter between 6 and 10 cm, is globular, with a soft, white cuticle that later becomes cream-whitish-yellowish (Figure 2b).

Initially, the edge of the cap is slightly curved inwards, and at maturity it flattens.

The gills (Figure 2c) are smooth, velvety, initially whitish, becoming brownish-black at maturity.

The stem (Figure 2d) is cylindrical with a fibrous tissue. It has a length of 8-12 cm and a diameter of 1-2 cm. At the base it is more swollen and has an almost glued ring reflected down on the stem (Figure 2e).

The spores are smooth, slightly elongated, dark brown (Figure 2f).

This mushroom is an edible species, however it can accumulate heavy metals such as Cd and Hg (Mogîldea, 2016).

**3. *Macrolepiota excoriata*** (Schaeff.) Wasser, 1978 (Figure 3a) - Romanian common name: “ghebă de pășune”.

Phylum Basidiomycota

Subphylum Agaricomycotina

Class Agaricomycetes

Subclass Agaricomycetidae

Order Agaricales

Family Agaricaceae

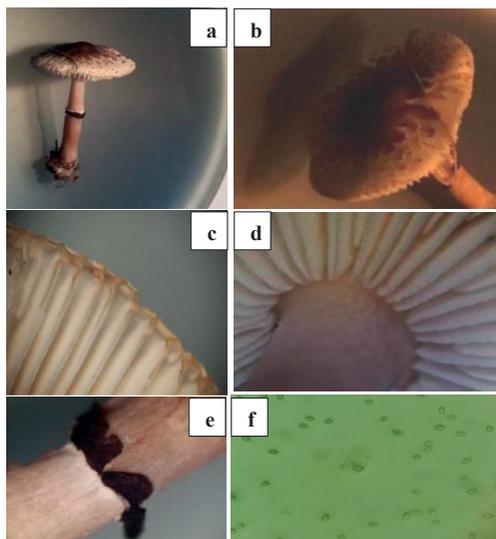


Figure 3. *Macrolepiota excoriata*

The cap is round, flattened (Figure 3b) with a diameter between 8 and 10 cm. The edges are slightly twisted inwards. On the central surface, it is smooth, with a large brown spot in the middle. The color of the cap varies from light brown to dark brown.

The cuticle is fringed at the edges; between the fringes there are white stripes (Figure 3c).

The gills (Figure 3d) are initially white, at maturity browns, crowded, fluffy and free.

The stem is cylindrical, hollow on the inside, with a slightly dilated base, has a length of 12 cm, is white with a rough surface at maturity. The annulus (Figure 3e) is narrow, ragged, dark in color. It is arranged approximately in the middle of the stem. Above the annulus, the stem is thicker.

The spores (Figure 3f) form a white powder. They are translucent, ellipsoidal in shape.

*M. excoriata* is an edible mushroom, but some studies have reported copper accumulated in this species over the maximum admitted level (Georgescu et al., 2016).

**4. *Hypholoma fasciculare*** Huds., 1778 (Figure 4a) - Sulphur tuft; Romanian common names: “gheba pucioasă”; “bureți de lemn”.

Phylum Basidiomycota

Subphylum Agaricomycotina

Class Agaricomycetes

Subclass Agaricomycetidae

Order Agaricales

Family Strophariaceae

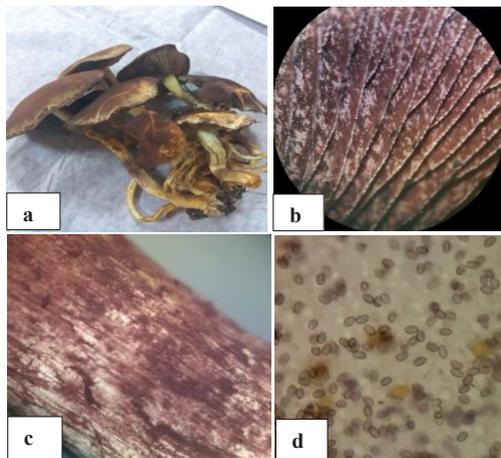


Figure 4. *Hypholoma fasciculare*

The cap with an unpleasant smell is thin, flattened at maturity, with a central tip on the surface.

The cuticle is smooth and glossy (in the darker central area) with various shades, from yellow-green to dark brown at maturity.

The gills are adherent to the stem, being at first yellowish and then, when mature, they turn dark brown (Figure 4b).

The stem (Figure 4c) has a length that varies between 3 and 10 cm. It is cylindrical curved, fragile.

The spores are very small, ellipsoidal in shape and brown to purple (Figure 4d).

*H. fasciculare* is an inedible mushroom, with a very bitter taste (First Nature database).

**5. *Scleroderma citrinum*** Pers., 1801 (Figure 5a) - Common earthball; Romanian common names: “buretele cerbilor”; “impermeabil fals”.

Phylum Basidiomycota  
Subphylum Agaricomycotina  
Class Agaricomycetes  
Subclass Agaricomycetidae  
Order Boletales  
Family Sclerodermataceae

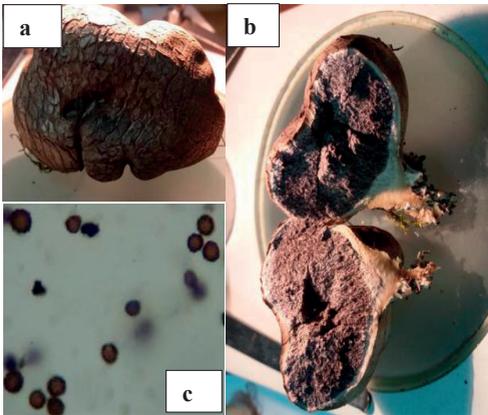


Figure 5. *Scleroderma citrinum*

The stem is a thick, oval, rigid fruiting body with rough black scales on the surface. The inside of the body is initially whitish, then becomes blackish-brown, powdery with an unpleasant odor.

At maturity, the fungus sheath breaks irregularly to allow spores to release (Figure 5b).

It has no stem or lamellae. It is fixed to the ground with the help of extensions of the thick cover layer.

The spores (Figure 5c) are rounded, brown, with a reticulated surface.

The members of Sclerodermataceae family are considered to be poisonous mushrooms (Sato et al., 2020).

**6. *Xerocomellus porosporus*** Imler ex Watling 1968 (Figure 6a) - Sepa bolete; unknown Romanian common name.

Phylum Basidiomycota  
Subphylum Agaricomycotina  
Class Agaricomycetes  
Subclass Agaricomycetidae  
Order Boletales  
Family Boletaceae  
Subfamily Xerocomoideae

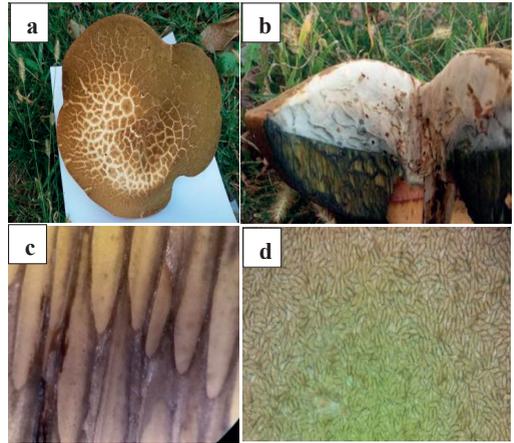


Figure 6. *Xerocomellus porosporus*

The cap of 6-9 cm in diameter, at first convex, with a turned edge, at maturity becomes flat, velvety, with a cracked cuticle and a brown color. The interior is whitish, soft and slightly elastic (Figure 6b). If it is cut, it turns blue for a short time.

The gills (Figure 6c) are fleshy, yellowish, of different sizes. At maturity it turns reddish-brown due to spores.

The stem is thick, smooth, 8-10 cm long, cylindrical and narrow at the base. It has no annulus. The spores are ellipsoidal and form a brown olive powder (Figure 6d).

Edible but not recommended, *X. porosporus* is bland and mushy (Phillips, 2013; <https://phas.ubc.ca/>).

**7. *Marasmius oreades*** (Bolton) Fr. 1836 (Figure 7a) - Fairy ring champignon; Romanian common names: “burete de rouă”; “buresciori de rouă”.

Phylum Basidiomycota  
Subphylum Agaricomycotina  
Class Agaricomycetes

Subclass Agaricomycetidae  
 Order Agaricales  
 Family Marasmiaceae



Figure 7. *Marasmius oreades*

The cap (Figure 7b) has a diameter between 1 and 4.5 cm; at first is conical and later becomes flat, notched on the edges and sticky. The cuticle is smooth, reddish-brown.

The gills (Figure 7c) are spaced apart, wide and not joined to the stem.

The stem is cylindrical, tall and thin with a slightly thickened base. It has a height between 5 and 7 cm.

The spores (Figure 7d) are ellipsoidal, transparent and form a white-brown powder.

This very common mushroom seems to thrive in public lawns and parks and is widely regarded as good edible, sweet-tasting (First Nature database).

During the study period, rainfall was abundant and favored the development of *Coprinellus micaceus*, *Agaricus arvensis*, *Macrolepiota excoriata*, *Hypholoma fasciculare*, *Xerocomellus porosporus*, in a humid and shady environment.

Future research will focus on documenting the biodiversity of these mushrooms species over several seasons, in relation with environmental conditions.

## ACKNOWLEDGEMENTS

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## CONCLUSIONS

The observations on the diversity of wild macromycetes on the campus of the University of Agronomic Sciences and Veterinary Medicine in Bucharest have highlighted so far the presence of the following species belonging to Phylum Basidiomycota, Class Agaricomycetes: *Coprinellus micaceus*, *Agaricus arvensis*, *Macrolepiota excoriata*, *Hypholoma fasciculare*, *Scleroderma citrinum*, *Xerocomellus porosporus* and *Marasmius oreades*.

Of these, only *A. arvensis*, *X. porosporus*, *M. excoriata* and *M. oreades* are unanimously considered edible mushroom species, although it worth mentioning that several studies have been reported in this species the detection of heavy metals.

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- \*\*\*<https://phas.ubc.ca/~birger/vmstable.pdf>

## QUANTITATIVE AND QUALITATIVE DIFFERENCES IN OAT PRODUCTION (*Avena sativa* L.) GENERATED BY THE TYPE OF COMPOST USED AS FERTILIZER

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### Abstract

*Interest in composting biodegradable organic waste has grown significantly in recent years in Romania. An important role in stimulating this interest was played by the integration in the European Union (EU) and the acquisition of the European legislation on waste management but also the pressure of the civil society to improve the environment quality. Thus, more and more composting centers were developed, and a composting law was adopted. Composting of the organic fraction of municipal solid waste (OFMSW) is of great interest. However, composts can present certain risks (heavy metals and even pathogens) coming from raw materials or the way the composting process is conducted. Therefore, a rigorous approach to compost quality is needed in relation to specific standards, especially when they are to be used as amendments to agricultural soils. This paper presents the results of a study in which the effects of six composts on a test plant, oat (*Avena sativa* L.), were compared in order to evaluate the impact of the waste collection method and the composting method on the composts quality and their agronomic value.*

**Key words:** *compost quality, heavy metals, composting method, source separate collection.*

### INTRODUCTION

According to Directive 2018/851/PE, CE, “municipal waste” means “mixed waste and separately collected waste from households, including paper and cardboard, glass, metals, plastics, bio-waste...”. Therefore, an important component of them is represented by „bio-waste”, which means “biodegradable garden and park waste, food and kitchen waste from households, offices, restaurants, wholesale, canteens, caterers and retail premises and comparable waste from food processing plants” (Directive 2018/851/PE, CE). Bio-waste represents the largest component of municipal waste in the European Union (EU) reaching 34%, and its recycling is essential to meet the EU objective of recycling 65% of municipal waste by 2035 (EEA, 2020). The fulfilment of this objective is intended to contribute to “protecting and improving the quality of the environment to protect human health, to ensure the prudent, efficient and rational use of natural resources, to promote the

circular economy principles, to increase the use of renewable energy, to increase energy efficiency, to reduce the Union's degree of dependence on imported resources, to create new economic opportunities and to stimulate long-term competitiveness” (Directive 2018/851/PE, CE).

In Romania, the concern of the population and the authorities for the separate waste collection and their sustainable management has grown more and more in recent years. At the same time, a growing interest is paid to the prevention of waste production, the reduction of amounts, their reuse and recycling in the energy and material resources diminishing conditions.

According to statistics, in 2020, in Romania a municipal waste production of 287 kg/capita/year was registered, i.e. a decrease of 13.34% compared to 2005 production (383 kg/capita/year), way below the European average (28 + 3) of 2020, which reached 505 kg/capita/year (EUROSTAT, 2022a). However, in the same year 2020, the municipal waste

recycling rate was only of 13.7% (EUROSTAT, 2022b).

The biodegradable organic fraction is an important component of municipal solid waste, having a complex composition, seasonally unstable, which is dependent on people's lifestyle, with differences especially between urban and rural lifestyles. Bio-waste has a high potential to be integrated into the circular economy. It can be recycled through anaerobic digestion (with biogas production) and through composting (aerobic digestion). Composting has become a key element of integrated waste management (Vaverkova et al., 2020) being one of the friendliest technologies for managing the organic fraction of municipal solid waste (Barrena et al., 2014) as well as a cost-effective (Soobhany, 2018; Vaverkova et al., 2020) and efficient method (Jara-Samaniego, 2019). It leads to obtaining a valuable material compost, which is rich in organic matter and nutrients (Duong et al., 2013; Viaene et al., 2016) and can be used for improving soil physico-chemical properties and fertility (Solaiman et al., 2019; Vaverkova et al., 2020). Moreover, the increase in the inorganic fertilizers production costs, as well as the effects of their excessive use on the environment and the climate (Solaiman et al., 2019) increase the interest in bio-waste composting and the agronomic value of compost.

Currently, in Romania, there are several integrated waste management centres that managed to implement composting projects on an industrial scale, but which face obstacles that limit the quality of the final product (Siles-Castellano et al., 2020), as well as certain barriers to the compost utilization. One of the most important obstacles, especially in large urban agglomerations, is the centralized collection, without waste separation at source. In centralized collection, OFSMW is difficult to separate, and the separation operation is very expensive. At the same time, biodegradable waste alters the state of the other types of waste that can be recycled in different sectors. Finally, in order to use the compost in agriculture, regulated quality standards are

necessary. On the other hand, in the rural environment, where people have gardens and spaces where they can organize and handle bio-waste for composting and where collection can be done with a thorough separation at the source, this method of managing biodegradable waste, efficient and sustainable (Vasquez and Soto, 2017) can be a way to reduce the administrative costs generated by centralized waste management, which would reduce the pressure on waste management centres and lead to obtaining a very good quality compost. Nowadays, few measures were taken to guide and raise the awareness of the rural population. There is, however, a growing interest in OFSMW composting.

The quality of the compost can be affected by biological factors such as the presence of human and animal pathogens and by chemical factors that can cause phytotoxic effects (Barral and Paradelo, 2011; Paradelo et al., 2020). Among the chemical factors, the presence of heavy metals (cadmium-Cd, copper-Cu, chromium-Cr, nickel-Ni, lead-Pb, zinc-Zn) is frequently mentioned (Barrena et al., 2014; Kupper et al., 2014; Cesaro et al., 2019; Siles-Castellano et al., 2020). These factors could affect the agronomic value of the compost and its agricultural utilization and would increase the risks for the environment, people, and animals. Achieving a good level of compost quality depends on several factors, and source separation is one of the most important (Rodrigues et al., 2020). That is why the process must be managed in such a way so that a high-quality compost is obtained and that its use is accompanied by benefits and not by negative effects on the environment (Barrena et al., 2014).

The purpose of this study is to evaluate the quality and test the agronomic value of some composts. The effects of composts on plants (plant growth and development, epigeic biomass production, heavy metal content) and soil (pH, organic carbon, macro- and micronutrient and heavy metal content) were monitored in the study.

## MATERIALS AND METHODS

**Compost.** In this study, 6 types of compost were used, as follows:

1. Poultry manure + wheat straw compost (3:1) - PMWSC - (12-month-old) produced in a household composter;
2. Poultry manure + vegetable food waste compost (3:1) - PMFWC - (12-month-old) produced in a household composter;
3. Industrial compost resulted from OFMSW after centralized separation within an integrated waste management centre - C1\_OFMSW (9-month-old);
4. Experimental compost resulted from OFMSW after centralized separation - C2\_OFMSW (15-month-old); small amounts were composted, in specially constructed containers (approx. 500 kg in a container);

5. Experimental compost resulted from OFMSW after centralized separation - C3\_OFMSW (18-month-old); small amounts were composted, in specially constructed containers (approx. 500 kg in a container);
6. Household compost resulted from source separated bio-waste (biodegradable waste from garden and kitchen) - HHC, produced on a rural household platform (12-month-old).

**The soil** used in these experiments is of red preluvosoil type, coming from Moara Domnească Teaching and Research Station of the University of Agronomic Sciences and Veterinary Medicine (UASMV) of Bucharest, located in a Sylvosteppe ecological area of Romanian Plain. Before making the mixtures, soil and compost samples were taken for their physico-chemical characterization (Table 1).

Table 1. The physico-chemical characteristics of soil and compost used within experiments

Soil and compost parameters	Soil	PMWSC	PMFWC	C1 OFMSW	C2 OFMSW	C3 OFMSW	HHC
pH	5.89	7.14	7.1	9.18	8.26	8.49	8.36
Moisture (%)	19.3	47.21	42.69	22.22	31.75	41.86	44.64
Dry matter (%)	80.7	52.59	56.31	77.78	68.25	58.14	55.36
C <sub>org</sub> (%)	2.02	14.92	13.62	18.19	15.46	15.8	9.71
C:N	9.35	11.39	10.72	13.38	10.24	9.88	9.23
Nt (%)	0.22	1.31	1.27	1.36	1.51	1.6	1.04
N-NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> d.m.)	28.67	229	136	19	195	197	910
N-NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> d.m.)	9.12	59.33	53.67	422	28	35	86
P (%)	0.16	1.47	1.62	0.57	0.82	0.9	0.46
K (%)	na*	1.23	1.48	3.38	2.32	2.53	1.48

\*not analyzed.

The soil had a pH (5.89) that characterizes a weak-acidic reaction (Blaga et al., 2008), and the C:N ratio was 9.35. Four of the studied composts had pH values that characterize a neutral to slightly alkaline reaction (Blaga et al., 2008), in ascending order, PMFWC<PMWSC<C2\_OFMSW<HHC which is in agreement with the values mentioned by De Bertoldi (1983), and two of them had a reaction from moderately alkaline (Blaga et al., 2008) to alkaline (Mustin, 1987), C3\_OFMSW<C1\_OFMSW. The dry matter content was over 50% in all composts, organic amendments having to respect a minimum of over 30% dry matter (NF U 44051, 2006).

After analysing the N-NH<sub>4</sub><sup>+</sup> content as an index of maturity (Brinton, 2000), it can be said that four of the composts (PMWSC, PMFWC, C2\_OFMSW and C3\_OFMSW), which had N-NH<sub>4</sub><sup>+</sup> contents between 28 and 59.33 mg kg<sup>-1</sup>d.m., can be considered very mature, and the other two, which had a N-NH<sub>4</sub><sup>+</sup> content of 86 mg kg<sup>-1</sup>d.m. (HHC) and, respectively, 422 mg kg<sup>-1</sup>d.m. (C1\_OFMSW), can be considered mature. The maturity of the composts is also revealed by the C:N ratio. The C<sub>org</sub> content was different in the 6 composts, the lowest being of 9.71% (HHC), and the highest of 18.19% (C1\_OFMSW), while the total nitrogen content (Nt) exceeded 1% of the

dry matter in all composts, which corresponds to the interval mentioned by Azim et al. (2018) but also to the French norms (NF U 44051, 2006) for organic amendments. Unlike the rest of the composts, the compost obtained under domestic conditions had a N-NO<sub>3</sub><sup>-</sup> content of 910 mg kg<sup>-1</sup>d.m.

The results of the chemical analysis of the heavy metal content (Table 2) of the 6 composts were compared with the limits imposed by the German standards for Class I and Class II (Biowaste ordinance) and with the limits imposed by Regulation 2092/91/EC. The following were revealed: i) the method used for Cd analysis did not detect Cd presence in any of the composts; ii) PMWSC and PMFWC

composts can be included into Class I of quality according to German standards, except for the Cr content that exceeds even the Cr<sub>tot</sub> content limit imposed for Class II; iii) all three composts obtained from OFMSW exceed the limits imposed by German standards for Class II for Cu, Cr<sub>tot</sub> content, and C2\_OFMSW and C3\_OFMSW also exceed the Zn content limit for the same class; iv) household compost resulted from bio-waste (biodegradable waste from garden and kitchen separately collected) – HHC belongs to Class I; v) all evaluated composts had Ni content below the limit imposed by the German standards for Class I and Pb content below the limits imposed by EC Reg. 2092/1991.

Table 2. Heavy metals content in soil and compost used within experiments

Soil and compost parameters	Soil	PMWSC	PMFWC	C1_OFMSW	C2_OFMSW	C3_OFMSW	HHC	Biowaste ordinance (Germany) ***		EC Reg. 2092/1991 (compost from source separated Biowaste) ***
								Class I	Class II	
Cd (mg kg <sup>-1</sup> d.m.)	nd**	nd	nd	nd	nd	nd	nd	1	1.5	0.7
Cu (mg kg <sup>-1</sup> d.m.)	26.8	44.1	47.9	133	119	121	34.5	70	100	70
Cr <sub>tot</sub> (mg kg <sup>-1</sup> d.m.)	32.5	183.33	126	132	311	341	34.7	70	100	70
Ni (mg kg <sup>-1</sup> d.m.)	19.6	19.5	26.83	22.1	26.7	24.9	19.9	35	50	25
Pb (mg kg <sup>-1</sup> d.m.)	12.3	9.93	19.2	20.1	12.6	19.7	10.5	100	150	45
Zn (mg kg <sup>-1</sup> d.m.)	661	285.67	257.33	400	462	413	351	300	400	200

\*\*not detected with the analysis methods used;

\*\*\*Heavy metal limits for European compost standards. Final Report. ANNEX 2; JUNE 2004. Table A2-4. Heavy metal limits for European compost standards. Compost from source separated Biowaste.

**Experiment organization.** The experiment was carried out in greenhouses belonging to UASMV of Bucharest in the spring of 2022, between March and May. All the composts, as well as the soil, were initially sieved through a sieve with 10 mm diameter holes, and a second sieving was carried out with a 6.3 mm diameter sieve. Three mixtures of compost and soil were made for each type of compost, respectively with 25%, 50% and 75% according to the scheme below, as well as a control variant only with soil. A variant with 100% compost was also made for HHC. This resulted in 20 variants organized in 2 replicates.

V1\_Soil - 100%

V2\_25% soil +75% (g/g) PMWSC

V3\_50% Soil + 50% (g/g) PMWSC

V4\_75% Soil + 25% (g/g) PMWSC

V5\_25% Soil +75% (g/g) PMFWC

V6\_50% Soil + 50% (g/g) PMFWC

V7\_75% Soil + 25% (g/g) PMFWC

V8\_25% Soil + 75% (g/g) C1\_OFMSW

V9\_50% Soil + 50% (g/g) C1\_OFMSW

V10\_75% Soil + 25% (g/g) C1\_OFMSW

V11\_25% Soil + 75% (g/g) C2\_OFMSW

V12\_50% Soil + 50% (g/g) C2\_OFMSW

V13\_75% Soil + 25% (g/g) C2\_OFMSW

V14\_25% Soil + 75% (g/g) C3\_OFMSW

V15\_50% Soil + 50% (g/g) C3\_OFMSW

V16\_75% Soil + 25% (g/g) C3\_OFMSW

V17\_25% Soil + 75% (g/g) HHC

V18\_50% Soil + 50% (g/g) HHC

V19\_75% Soil + 25% (g/g) HHC

V20\_100% HHC.

In the mixtures made, oat (*Avena sativa* L.) was sown on the same day. The seeds germinated after approx. 4 days, and the complete emergence and the beginning of the elongation of the plant stem occurred in 7 days,

approximately (Photo 1). Throughout the vegetation period, the growth and development of the plants was monitored, and a sufficient moisture was constantly ensured.



Photo 1. Greenhouse experiment with soil and different composts mixtures grown with oat

**Measurements and analysis.** The plants were harvested after grain formation and filling and the amount of epigeic biomass was evaluated on variants and repetitions. Plant samples were taken from each variant and chemically analysed for nutrients (N, P, K, Ca, Mg) and heavy metals (Cd, Cu, Cr, Ni, Pb, Zn). Soil samples were taken from the vegetation pots (soil and compost mixtures corresponding to each variant) and the physico-chemical properties were evaluated (pH, moisture, dry matter, organic carbon, N, P, K,  $\text{N-NO}_3^-$ ,  $\text{N-NH}_4^+$  and heavy metals in total forms - Cu, Cr, Ni, Pb, Zn). Since no Cd content was detected during the initial physico-chemical analysis of the composts, it was not subsequently analysed in the oat biomass or in the soil and compost mixtures. All chemical analyses, both for soil and for plants, were carried out in the Soil Pollution Control Laboratory of the Institute for Pedology and Agrochemistry in Bucharest according to its own methodology specific to each type of analysis.

**Data analysis.** All data were analysed as the average of the two replicates performed for each variant.

## RESULTS AND DISCUSSIONS

**Oat biomass production.** All the studied compost and soil mixtures, regardless of the proportion of the compost in the mixture, led to biomass productions above the level of those achieved in the control variant in which only soil was used. The biomass production registered in the variants with compost were expressed in g/pot, and differences compared to the control variant in percentages (Figure 1 a, b, c, d, e, f). Thus, a descending order ranking of the differences registered in the oat epigeic biomass production in the variants where composts were used, compared to the control (soil), looks as follows:

1. in the variants with 75% compost and 25% soil:  $\text{PMFWC} > \text{C3\_OFMSW} > \text{HHC}$   
 $\text{PMWSC} >> \text{C1\_OFMSW} > \text{C2\_OFMSW}$ ;

2. in the variants with 50% compost and 50% soil: PMFWC > PMWSC > HHC > C3\_OFMSW > C1\_OFMSW > C2\_OFMSW;

3. in the variants with 25% compost and 75% soil: PMFWC > PMWSC > HHC > C3\_OFMSW > C1\_OFMSW > C2\_OFMSW.

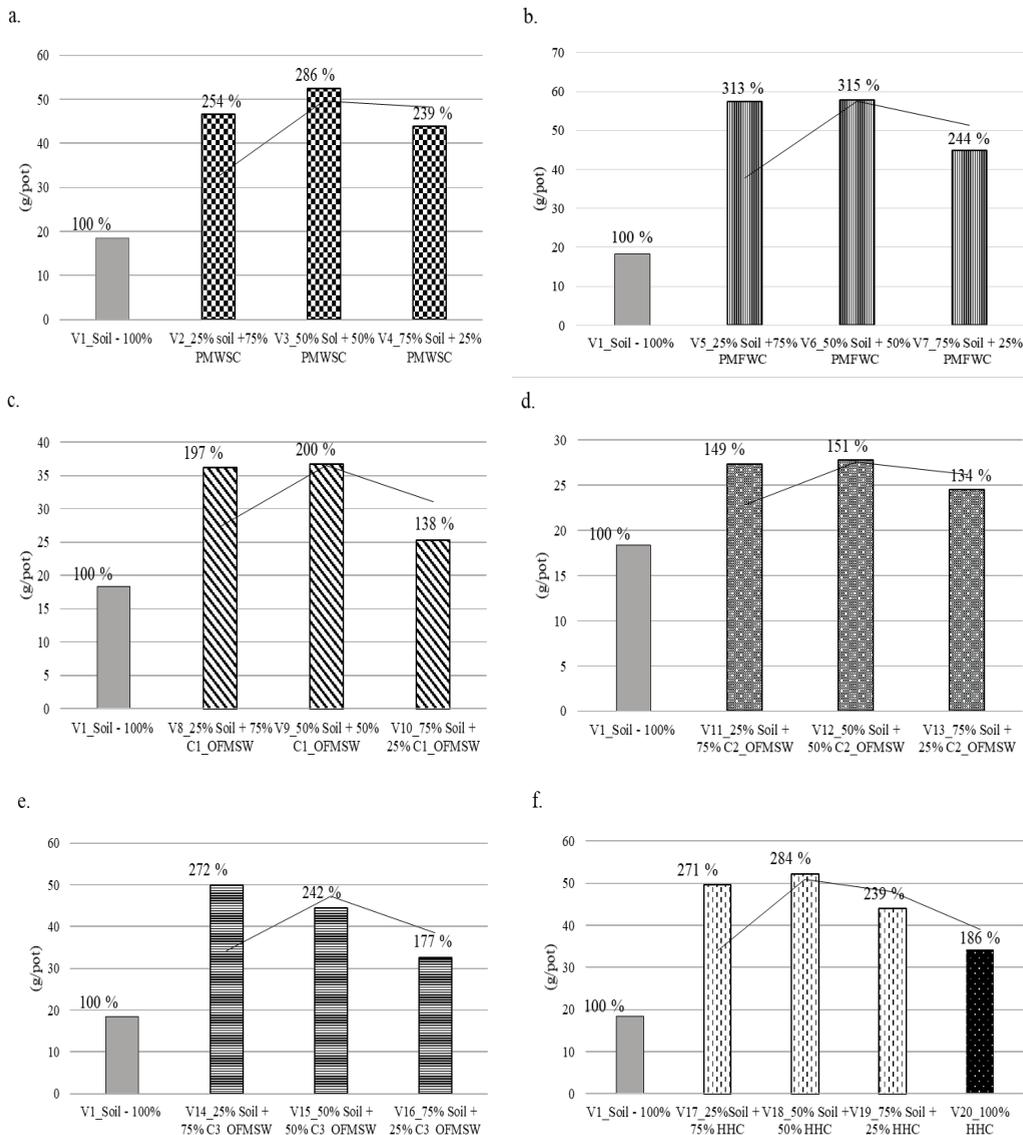


Figure 1 (a, b, c, d, e, f). Epigeic biomass production of oat grown on different soil and compost mixtures

**Heavy metal content in epigeic biomass.** The results of the chemical analysis carried out on the samples of fresh oat biomass (Figure 2 a., b., c., d.), taken immediately after harvesting (grain filling phase), are presented as the average of the variants. In general, the heavy metal content in plant biomass tended to

increase with the proportion of compost in the soil and compost mixture. Thus, the highest Cu content was registered in the two composts based on poultry manure (Figure 2 a) reaching approx. 29 mg kg<sup>-1</sup>d.m. in the V2 variant (75% PMWSC). However, in most variants, the Cu content in plant biomass was below 20 mg

kg<sup>-1</sup>d.m. and below the control level (100% soil). The lowest Cu content was registered in the variants with C3\_OFMSW and in those with HHC. The normal Cu content in plants ranges between 2 and 20 mg kg<sup>-1</sup>d.m. (Dhaese, 1979; Graham, 1981), and above 20 ppm it becomes toxic. The highest Cr content (Figure 2 b) in plant biomass was registered in the V11 variant (75% C3\_OFMSW mixed with 25% soil), respectively approx. 4 mg kg<sup>-1</sup> d.m., while in the control variant a Cr content below 1.5 mg kg<sup>-1</sup>d.m. was registered. In most variants, the Cr content was below 3.5 mg kg<sup>-1</sup>d.m. Chromium levels between 0 and 0.5 mg kg<sup>-1</sup>d.m. are considered normal, and those above 1.3 mg kg<sup>-1</sup>d.m. are considered phytotoxic (Dhaese, 1979). The plants grown in the control variant (100% soil) reached a Ni content (Figure 2 c) of 7.63 mg kg<sup>-1</sup>d.m., and contents below this level were registered in the plants grown in V2 (75% PMWSC), V3 (50% PMWSC), V5 (75% PMFWC) and V15 (50% C3\_OFMSW). The role of Ni in the plants mineral nutrition has been described quite recently (Eskew et al., 1983; 1984). However,

its normal content in plants would be between 0 and 8 mg kg<sup>-1</sup>d.m. (Dhaese, 1979), and at concentrations of 40 mg kg<sup>-1</sup> d.m., Ni is toxic (Impens, 1992). Regarding the Zn content in plants, the samples taken from plants harvested in all compost variants, regardless of its type, had contents that exceeded the control variant. At the same time, the Zn content was correlated with the percentage of compost in the nutrient substrate mixture (Figure 2 d). The highest Zn content in plants was registered in V20 (100% HHC) - 75.2 mg kg<sup>-1</sup>d.m., V14 (75% C3\_OFMSW) - 70.5 mg kg<sup>-1</sup>d.m., V5 (75% PMFWC) - 58.7 mg kg<sup>-1</sup>d.m. and V15 (50% C3\_OFMSW) - 57.0 mg kg<sup>-1</sup>d.m. In a study regarding different composts obtained from the biodegradable organic fraction of their municipal solid waste, Paradelo et al. (2020) highlight contents in ryegrass plants of 103 and 133 mg kg<sup>-1</sup>d.m., while the reference values range between 25 and 47 mg kg<sup>-1</sup>d.m. (Kabata-Pendias & Pendias, 1984). Other authors mention an average-normal Zn content in plants, ranging between 20 and 150 mg kg<sup>-1</sup>d.m. (Jones, 1972).

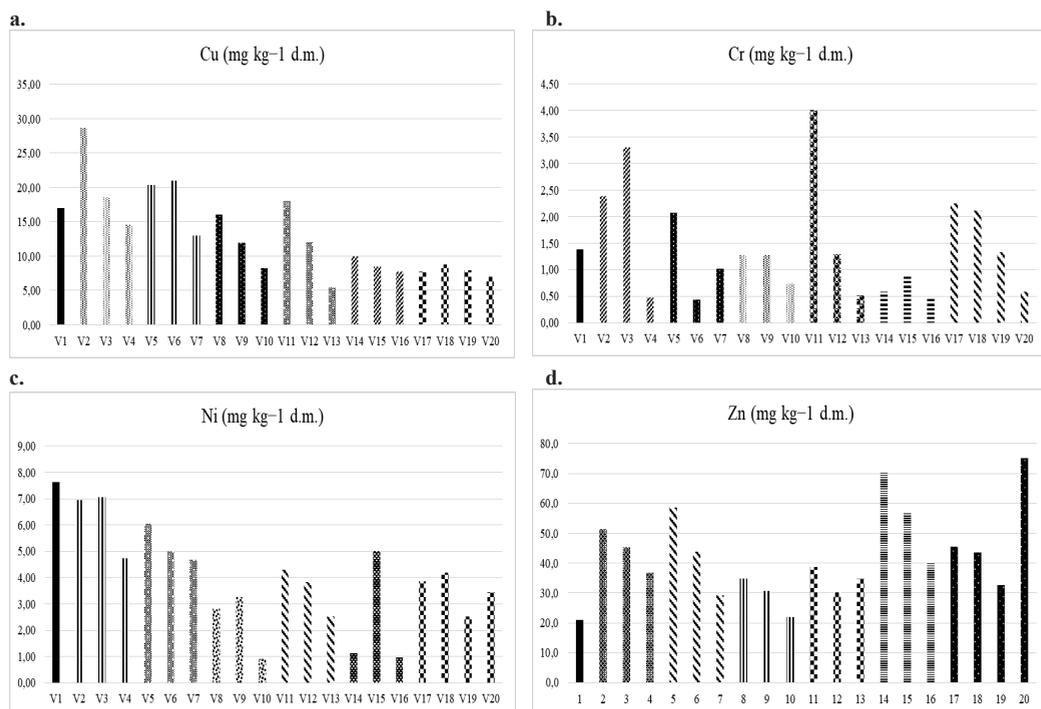


Figure 2 (a, b, c, d). Heavy metal content in the oat (*Avena sativa* L.) epigeic biomass

**Physico-chemical characteristics of soil and compost mixtures after harvesting oat plants.**

The C<sub>org</sub> content in soil and compost mixtures (Figure 3) increased in all variants compared to the control, regardless of the proportion of composts in the mixture. However, the most relevant contents were

registered, in general, in the variants in which the proportion of composts was of 75% and 50%. Also, the composts contributed to increasing the pH value (Figure 4) of soil and compost mixtures so that, in most variants, the pH had values that indicate a neutral to weak-alkaline reaction (Blaga et al., 2008).

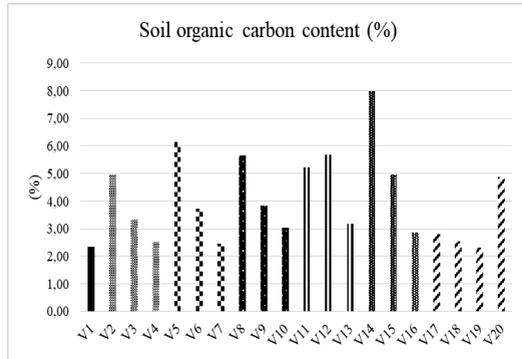


Figure 3. Organic carbon content in soil and compost mixtures after harvesting oat biomass

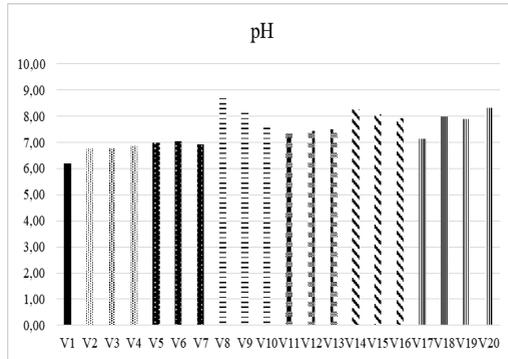


Figure 4. pH values of oil and compost mixtures after harvesting oat biomass

The macronutrients (N, P and K) content in soil and compost mixtures was, in most variants, above that of the control variant and, in general, it increased simultaneously with the proportion of composts in the soil and compost mixtures (Figure 5). However, N content was registered below the level of the control variant (V1 - 0.31% N) in almost all the variants in which the composts were in a proportion of 25% g/g in the composition of the mixtures.

The P content was above the control variant level (V1: 0.23% P) except for the V19 variant (0.19% P). The K content was also above the level of the control variant (V1: 1.30% K) in most variants except V2 (1.15% K) and variant V19 (1.25% K). The highest K content was registered in the composts obtained from OFMSW followed by HHC, PMFWC and PMWSC.

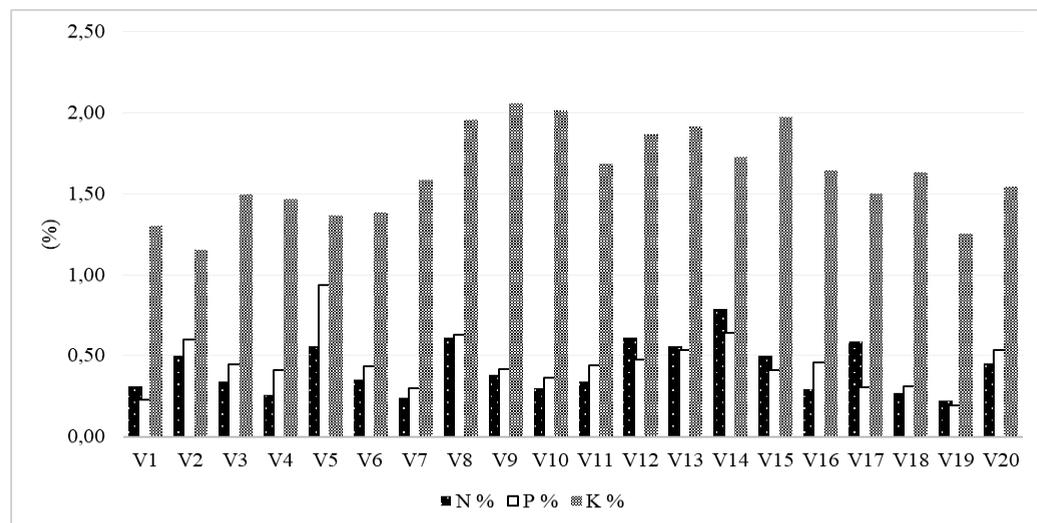


Figure 5. Macronutrients (N, P, K) in soil and compost mixtures after harvesting oat biomass

**Heavy metal content in soil and compost mixtures after harvesting oat plants.**

The Cu content (Figure 6 a) in soil and compost mixtures was influenced by the proportion of the compost in each variant and exceeded the content of the control variant, but it was below the limit of 100 mg kg<sup>-1</sup>d.m. accepted by Romanian legislation (ORDER no. 344/2004). The compost that had the highest intake was C3\_OFSMW in a proportion of 75% in the mixture.

The Cr content (Figure 6 b) was also above the level of the control variant in all variants in which composts were used in mixtures. The Cr content did not necessarily correlate with the level of compost intake. The order of magnitude of the Cr content depending on the type of compost in the mixture was as follows:

C3\_OFSMW>C2\_OFSMW>HHC>C1\_OFSMW>PMFWC>PMWSC.

The Cr content in soil and compost mixtures was below the limit imposed by the Romanian legislation (100 mg kg<sup>-1</sup> d.m.). The Ni content in soil and compost mixtures (Figure 6 c) was below the level registered in the control variant and also below the limit imposed by Romanian legislation (50 mg kg<sup>-1</sup> d.m.). The Zn content (Figure 6 d) in soil and compost mixtures after harvesting the oat plants was above the level registered in the control variant and, in most variants, below the limit imposed by the Romanian legislation (300 mg kg<sup>-1</sup> d.m.). However, in two of the variants, contents over Romanian limits were highlighted (389 mg kg<sup>-1</sup>d.m. in V20 – 100% HHC and 412 mg kg<sup>-1</sup> d.m. in V14 – 75% C3\_OFSMW).

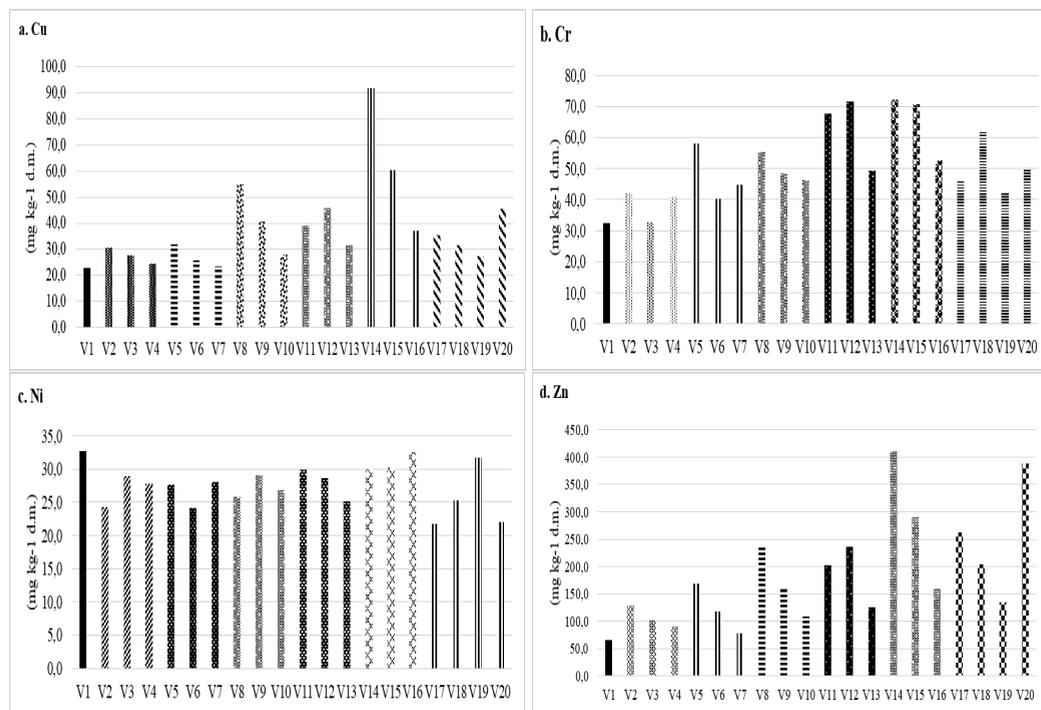


Figure 6 (a, b, c, d). Heavy metal content in soil and compost mixtures after harvesting oat plants

Table 3. The maximum admissible values for heavy metal concentrations in soils where sludge is applied (mg/kg of dry matter in a representative soil sample with pH > 6.5) according to Romanian legislation (ORDER no. 344 from August 16, 2004)

	Cd (mg kg <sup>-1</sup> d.m.)	Cu (mg kg <sup>-1</sup> d.m.)	Ni (mg kg <sup>-1</sup> d.m.)	Pb (mg kg <sup>-1</sup> d.m.)	Zn (mg kg <sup>-1</sup> d.m.)	Hg (mg kg <sup>-1</sup> d.m.)	Cr (mg kg <sup>-1</sup> d.m.)
Romanian legislation (ORDER no. 344/2004)	3	100	50	50	300	1	100

## CONCLUSIONS

The source-separated collection of bio-waste at is essential for the development of a composting chain integrated into the circular economy. This allows a good control of the raw materials for composting, a good management of the composting parameters and the composting process, as well as obtaining a good quality compost.

For the rural environment in Romania, given the conditions generated by the current energy crisis, but especially to reduce the waste management costs of rural communities, to contribute to reducing the impact of organic waste on the environment and to produce useful and good quality organic amendments to improve soil physico-chemical properties and its fertility, source-separated collection and home composting of bio-waste can be a sustainable solution.

For the agricultural use of composts resulting from bio-waste, the doses to be used must be established according to the intake of nutrients, especially N and according to their heavy metal content.

The composts tested in the study have an important agronomic value considering the biomass production achieved in all experimental variants. However, the highest productions were registered for composts obtained from bio-waste collected separately at the source, and the most relevant soil and compost mixture could be 50% soil + 50% compost for potted crops, also in correlation with the chemical composition of compost (macro- and micronutrients and heavy metals).

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## RESULTS OBTAINED BY APPLYING A BIOSTIMULANT TO TOMATO AND WHEAT CROPS

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### Abstract

*The paper presents a biostimulant (codified Bios) containing algae extract (Ascophyllum nodosum), hydrolysed proteins, as well as humic substances with effects on plant growth and mineral nutrition, thus reducing the negative effect of climate and technological stress. The application of the biostimulant indirectly stimulated the plants for the additional absorption of nutrients as well as the increase of the photosynthesis process.*

*The foliar application of the Bios product (as a 0.5% solution) led to the increase of the yield of wheat and tomato crops compared to the unfertilized control. The production increases were 36.46% for wheat and 50.12% for tomatoes. The stimulation of the photosynthesis process for both crops tested is evidenced by the increases of the concentrations of chlorophyll a and b measured in the treated leaves as compared to the control. The same trend was found for the carotene content. The nitrogen, phosphorus and potassium content of the leaves were also determined, and there were found significant increases compared to the unfertilized control.*

**Key words:** tomato, wheat, biostimulant, *Ascophyllum nodosum*, hydrolysed protein, humic substances.

### INTRODUCTION

Climate change and environmental degradation are a real threat to the world. In order to reduce the effects, the European Green Pact aims to transform the EU into a modern, competitive and resource-efficient economy, in which:

- to reach zero greenhouse gas emissions by 2050;
- dissociate economic growth from resource use;
- no person or place should be left behind (european-green-deal).

There is a current consensus that we need to make the transition from an economy based on the consumption of oil, coal and lignite to a bio-economy through the development of a sustainable circular economy (Kapoor et al., 2021). The European model of agriculture is based on the promotion of a competitive, market-oriented sector, but taking into account the needs of current society but also future generations aiming at the integration of agriculture and civil culture with the environment.

Applying the principles of the circular economy can significantly reduce the negative impact of the extraction and use of resources on the

environment and can contribute to the restoration of biodiversity and natural capital in Europe (Bruxelles, COM(2020) 98).

In this context, the identification of sustainable sources that, through minimal processing, can be sources of nutrients (especially nitrogen, phosphorus and potassium), is a global priority. The use of organic substances with crop stimulating effects in agriculture is necessary and encouraged in the context of European sustainable development policies.

The use of biostimulators can be a viable solution for sustainable agriculture which improves the condition of crops without causing adverse side effects (du Jardin, 2015). Due to the positive effects on the growth of agricultural production, the market for these products has grown in recent years, which is why the authorities felt the need to introduce this category of products in the new Regulation (EU) 2019/1009 on fertilizers. Improving the effects of abiotic stress is the most commonly mentioned benefit of using biostimulators, as 60-70% of agricultural losses are attributed to abiotic stress (Colla et al., 2017; Yakhin et al., 2017; Ertani et al., 2013).

The application of biostimulants on plants leads to the accumulation of a higher content of nutrients in their tissue and to positive metabolic changes. For these reasons, the development of new biostimulants has become a point of scientific interest (Nardi et al., 2016).

The role of biostimulators is well defined by the new European legislation, Regulation (EU) 2019/1009 being described as a product that stimulates plant nutritional processes regardless of the nutrient content of the product improving one or more of the following characteristics of the plant or the plant rhizosphere:

- (a) nutrient use efficiency;
- (b) tolerance to abiotic stress;
- (c) quality traits;
- (d) availability of confined nutrients in soil or rhizosphere.

In specialized studies, substances with biostimulator role are divided into 4 main categories: humic substances, protein hydrolysates, algae and microorganisms (Du Jardin, 2015).

Algae have been recognized for centuries for their beneficial effects on plants, but their market potential still seems to be underestimated. They are a renewable source with a complex composition and whose mechanisms of action on plants (Marti et al., 2004).

Numerous studies have shown the beneficial effects of algae on improving plant growth. Algae-based products improve seed germination, seedling development, increase plant tolerance to environmental factors and lead to increased production and plant quality (Zhang and Ervin, 2004; 2008; Zodape et al., 2008; Livingston et al., 2009).

Depending on the formulation, algae-based products could act as organic fertilizers or as a component of organo-mineral fertilizers, soil improvers, biostimulators and even pesticides. Due to the positive effects on the growth of agricultural production, the market for these products has grown in recent years, which is why the authorities felt the need to introduce this category of products in the new Regulation (EU) 2019/1009 on fertilizers.

Polysaccharides and oligosaccharides in green, brown and red seaweeds (marine macroalgae) corresponding to ulvans, alginates, fucans, laminarins and carrageenans have been found to

stimulate the defence and protection responses of plant pathogens. Oligosaccharides obtained by depolymerization of seaweed polysaccharides induce protection against viral, fungal and bacterial infections in plants (Li et al., 2021; Drobek et al., 2019; Sharma et al., 2014).

Thus, it was observed that in tobacco plants (Xhanti NN cultivar) treated with macroalgae oligosaccharides and infected with tobacco mosaic virus (TMV), the number of necrotic lesions decreased significantly. Performing 4 weekly treatments with a solution containing polyguluronic acid (Poly-Gu), sulphated galactan (Poly-Ga) or polymannuronic acid (Poly-Ma) oligosaccharides prepared at a final concentration of 500  $\mu\text{g mL}^{-1}$  of each, led to a significant decrease in the number of necrotic lesions compared to the untreated control (Laporte et al., 2007).

Alginate is the predominant polysaccharide component found in cell walls and the intercellular matrix, with a degree of polymerization of 2 to 25 and accounting for 17 to 45% of the dry weight of algae in brown macroalgae (Li et al., 2021; Vera et al., 2011).

Protein hydrolysates represent another category of organic substances with the role of biostimulation of crops. They can be obtained from plant or animal sources and are an important source of nitrogen and other active biomolecules for use in agriculture.

The average nitrogen content of protein hydrolysates is in the range of 10 and 29.9% N and differs depending on the source and production method (Corte et al., 2014).

Application of protein and amino acid products have the role of providing plants with an organic source of nitrogen, thus making it possible to reduce the amount of mineral fertilizers applied (urea, ammonium nitrate).

Peptides and amino acids form complex combinations with micronutrients (Cu, Fe, Mn and Zn), thus contributing to increasing their bioavailability for the plant (Du Jardin et al., 2015).

Currently, the bulk of the market for protein hydrolysate biostimulators is represented by products resulting from the acid hydrolysis of animal proteins, the rest coming from the enzymatic hydrolysis of plant-derived proteins. Globally, most protein hydrolysates used in agriculture are produced by companies in Italy,

Spain, the United States, China and India. Some of these companies were developed by the leather and meat processing industry as a way to capitalize on by-products resulting from the manufacture of biostimulators and fertilizers.

Several studies have shown that many commercial products derived from protein hydrolysates cause hormone-like activities (auxin and gibberellin), favoring the growth of roots and shoots (Colla et al., 2017; Ertani et al., 2013).

Foliar and root application have been shown to improve the absorption and efficiency of the use of macro and micronutrients (Colla et al., 2017). Improved nutrient uptake performance of protein hydrolyzed plants has been largely associated with changes in root architecture (density, length and number of lateral roots), as well as increased nutrient availability in the soil solution resulting from their complexation by peptides and amino acids and improving microbial activity (Colla et al., 2017; Du Jardin, 2015). Protein hydrolysates can also help plants in unfavorable nutrient conditions by increasing the efficiency of their consumption. According to literature, certain amino acids (e.g., asparagine, cysteine, and glutamine) and peptides (e.g., glutathione and phytochelatin) may play an important role in plant tolerance to some toxic metals (Cu, Zn, As, Cd and Ni) by chelating and binding them (Colla et al., 2017). Moreover, small peptides are thought to play an important role in the biological activity of protein hydrolysates. However, only a limited number of bioactive peptides have been characterized. Therefore, more studies are needed to identify the peptides responsible for the biostimulatory activity of protein hydrolysates. These findings may also help to streamline the production process of protein hydrolysates and, implicitly, bioactive peptides (Colla et al., 2017).

Another class of substances that have a role in stimulating nutrition and which are increasingly used, are humic substances.

Humic substances can be used to improve the quality and structure of the soil, having special physical and chemical properties that act effectively to combat soil erosion, improve plant development and remove soil and environmental pollutants. These characteristics indicate that humic substances play an important role in phytoremediation technologies in

degraded or polluted areas, in wastewater treatment, and use as organic fertilizers for agricultural soils (Elbehiry et al., 2020; Steverson, 1994).

Conventionally substances extracted in an alkaline solution are called humic substances (HS). In this way HS can be extracted from a wide variety of sources, including sub-bituminous coal, lignite (brown coal), peat, soil, compost and some crude organic waste (Rose et al., 2014; Steverson, 1994).

The use of HS as a biostimulant for plant growth is a beneficial approach for both farmers and the environment and is part of the circular economy concept focused on conversion to a new resource (Jindo et al., 2020).

The beneficial effects on HSs crop productivity have been elicited by the content of a variety of oxygen-containing functional groups that lead to changes in soil acidity (Nardi et al., 2002; Steverson, 1994).

There are studies that indicate that one of the major impacts of HS on plant growth is a better nutrient uptake and elongation of lateral root growth, often recognized as “auxin-like effect” (Jindo et al., 2020; Muscolo et al., 2007).

The application of HS is believed to allow plants to resist abiotic stress, such as excessive heat and salts from growing media more effectively, leading to increased yields in many crops (Suddarth et al., 2019; Abdellatif et al., 2017).

In order to evaluate the effects of plant biostimulants, it is advisable to have results obtained under controlled conditions (laboratory, greenhouse, growth chamber, phenotyping, etc.) and/or in the field (field studies) (Ricci et al., 2019).

The plant biostimulants are applied to a wide range of cultures, having no practical restrictions in this respect.

Wheat (*Triticum aestivum* L.) is the most important and cultivated crop in the world alongside maize, rice, sunflower and soybean (FAOSTAT, 2019). The EU-27's harvested production of fresh vegetables (including melons) was 60.9 million tons in 2019, about 1.1 million tons more than in 2018 and within the group of fresh vegetables, the harvested production of tomatoes was 16.5 million tons in 2019 (EUROSTAT, 2020).

The evolution of wheat areas and average production (kg/ha) in Romania (Figure 1) indicates

a steady increase in the period 2010-2019 ([www.madr.ro/culturi-de-camp/cereale/grau](http://www.madr.ro/culturi-de-camp/cereale/grau)). The evolution of average production in the period 2010-2019 shows that average of wheat production has increased through the application of efficient management, which includes the use of fertilizers and plant protection products (Figure 1).

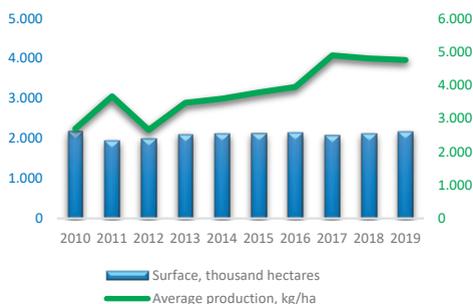


Figure 1. Trends in wheat areas and production in the period 2010-2019, in Romania

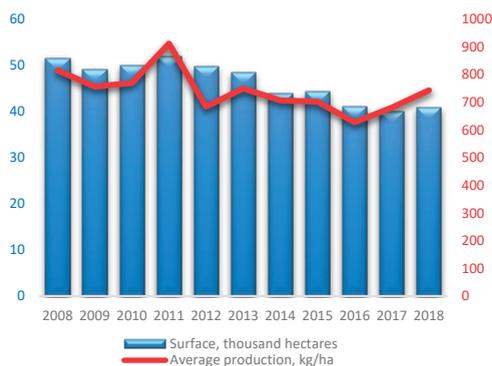


Figure 2. Trends in areas and production of tomatoes in the period 2008-2018, in Romania

Analysing the data presented in Figure 2 it can be observed a tendency of decreasing of the areas cultivated with tomatoes. In the last 3 years of the mentioned period there is a growing trend of the average production per hectare, which can be explained by the higher consumer demand for these vegetables, which has led to the use of fertilizers and thus to increased yields. ([www.madr.ro/horticultura](http://www.madr.ro/horticultura)).

The EU is largely dependent on imports for most of the mineral fertilizers (Fertilizers in the EU, 2019) and for this reason there are attempts to supplement them with products from the plant

biostimulants category. This category of products is increasingly used because they lead to an increase in the yield of agricultural crops between 5-10% according to the evaluation by The European Biostimulants Industry Council (EBIC).

## MATERIALS AND METHODS

### Plant materials and treatments

This study focuses on the effect of Bios biostimulant plant on two crops: tomatoes ("Precos" hybrid) in a protected area (solarium, drip irrigation) and wheat ("Izvor" cultivar) in non-irrigated field.

The experimental researches were carried out in the period 2018-2019 on unfertilized soil and were located as follows:

- in the field, on an unirrigated Chernozem for the autumn wheat crop;
- in the solarium, on an Anthrosol drip irrigated for tomato crop;
- during the vegetation period, the temperatures were higher than average with  $+0.6^{\circ}\text{C}$  ( $10.2-9.6^{\circ}\text{C}$ ), with a positive deviation ( $+0.5^{\circ}\text{C}$ ) compared to the average per years ( $17.9-17.4^{\circ}\text{C}$ );
- the vegetation period of 2019 year was poorer in precipitation than the average per years ( $337.5-282.1$  mm) by 55.4 mm.

In terms of the soil enzymatic activity, catalase was determined by titrimetric method, while the dehydrogenase, invertase, and urease were determined by spectro-photo-colorimetric methods after the incubation of the soil samples at  $37^{\circ}\text{C}$  during 24 hours.

The foliar fertilization with the plant biostimulant was developed as follows: application dose -  $2.5\text{ L ha}^{-1}$ ; concentration of the applied solution - 0.5%; number of treatments - 3. All three treatments were developed by the fine spraying on the whole foliage surface, during the vegetation period.

The applications with the "Bios" for both crops were chosen as follows:

- for wheat, the first treatment - in the bellows phase, the second treatment - in sprouting and the last treatment - in flowering in conditions of non-irrigation in the field;
- for tomatoes, the first treatment - 10 days after planting; the second treatment - at flowering and the last treatment - in the phase of formation and

growth of the fruit in conditions of drip irrigation in the solarium.

To perform the analysis, plant material (leaves) was harvested 7 days after the last treatment.

Agrochemical experiments for non-irrigated wheat cultivation in the field were performed on a soil with the following characteristics: total nitrogen (Nt) - 0.133%; mobile phosphorus (PAL) - 32 mg/kg; mobile potassium (KAL) - 228 mg/kg; humus (%) -2.61; pH - 6.2;

The tomato crop was placed in the protected area on a drip irrigated soil with the following characteristics: total nitrogen (Nt) - 0.221%; mobile phosphorus (PAL) - 51 mg/kg; mobile potassium (KAL) - 208 mg/kg; humus (%) - 3.0%; pH - 6.1.

The soil enzymatic activities correlated with the morphological, physical and chemical soil properties and the different management highlighted the following: high values of catalase activity in Chernozem (0.50-0.66 mL 0.02 mol/L KMnO<sub>4</sub>/g soil/h) and Anthrosol (0.85-1.04 mL 0.02 mol/L KMnO<sub>4</sub>/g soil/h); low values of dehydrogenase activity in Chernozem (0.5078-0.4116 µg TPF/g soil/h) correlated with the reduction of bioaccumulation processes and the destruction of active microbial cells under anaerobic conditions; higher values of dehydrogenase activity in Anthrosol (0.9444 µg TPF/g soil/h); high values of invertase activity in Chernozem (0.0669-0.0615 µg glucose/g soil/h) and Anthrosol (0.0618 µg glucose/g soil/h) correlated with the presence of loessoid deposits as parent material and a higher capacity of bacteria to use the glucose in soils formed on such parent materials; low values of urease activity (0.0249-0.0231 µg NH<sub>4</sub><sup>+</sup>-N/g soil/h) in Chernozem because of the deficient aerohydric regime that led to a slower mineralization of organic matter in the unirrigated chernozem from the Ezăreni farm; higher values of urease activity (0.4634 µg NH<sub>4</sub><sup>+</sup>-N/g soil/h) in Anthrosol.

### Obtaining and characterizing of the plant biostimulant “Bios”

A plant biostimulant formula has been developed for experimental testing. The fertilizer was obtained in the laboratory using raw materials that can be used in organic farming and was coded Bios for presentation in this paper. This product contains secondary nutrients

and micronutrients (B, Cu, Fe, Mg, Mn, S, Zn) in a matrix of organic matter from algae extract (*Ascophyllum nodosum*), hydrolysed protein and humic substances. The analysed parameters for the test cultures were: yield, and nitrogen, phosphorus, potassium, chlorophyll pigments and carotene contents in the leaves.

### Statistical analysis

Different lowercase letters mean significantly difference from other treatments at the level of p<0.05 according to the least significant difference (LSD) tests.

## RESULTS AND DISCUSSIONS

Tested on a chernozem in a non-irrigated field, with a weak acid reaction, low nitrogen content, medium phosphorus and high potassium contents, the “Bios” biostimulator led to an increase in wheat production of 52.12% compared to the unfertilized control (Figure 3). The foliar application of the Bios product to the wheat crop (*Triticum aestivum* L.) Romanian cultivar “Izvor” led to a significant increase in production of 36.46% compared to the production obtained by untreated control (Figure 3).



Figure 3. Wheat production (kg/ha) obtained by applying the Bios product compared to the control

The tomato crop was tested on a drip-irrigated Anthrosol which showed a weakly acidic reaction of the pH value, medium nitrogen content, low phosphorus, and medium potassium contents. Under these conditions, the application of the Bios biostimulator led to statistically positive results for tomatoes yields (Figure 4).

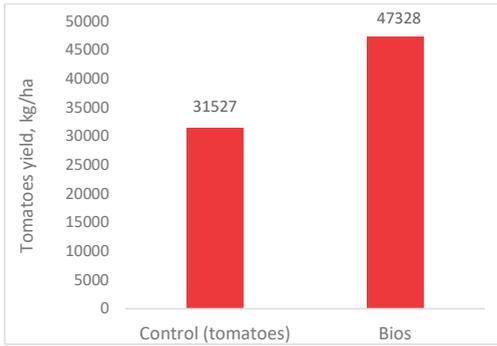


Figure 4. Tomatoes production (kg/ha) obtained by applying the Bios product compared to the control

In addition to the increase in wheat and tomato yields obtained per unit area, there was also an increase in the content of nitrogen, phosphorus and potassium, but also in the content of chlorophyll pigments in the leaves.

The accumulation of these elements in the leaves is in line with the increased production yields.

Applying plant biostimulants to different wheat cultivars can lead to increased production but also to improved crop quality (Macra and Sala, 2021).

For both cultures, the NPK content in the leaves increased significantly (Figures 5, 6), an increase that was also reflected in the chlorophyll content.

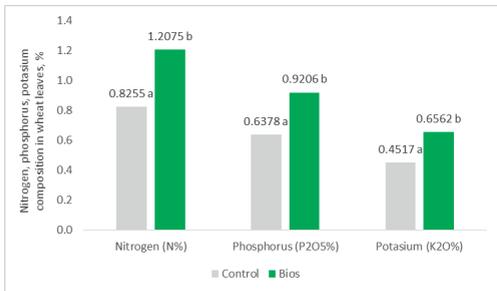


Figure 5. Nitrogen, phosphorus and potassium content (%) in wheat leaves

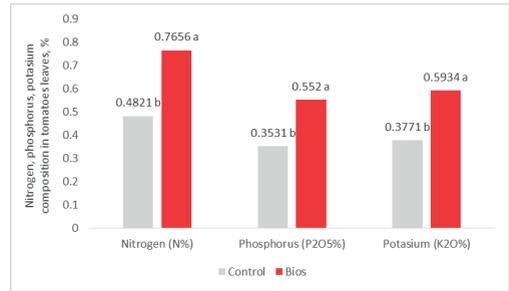


Figure 6. Nitrogen, phosphorus and potassium content (%) in tomato leaves

The foliar application of the biostimulant plant Bios, indirectly stimulated the plants for the additional supply of nutrients as well as the increase of the photosynthesis process.

Similar results were obtained by Popko et al. (2018) who tested the effect of plant growth biostimulants based on amino acids on yield and grain quality of winter wheat.

It has also been observed that the application of biostimulants to tomatoes can maintain high and economically convenient yields, even if the dose of NPK fertilization is decreased (Klokić et al., 2020; Khan et al., 2019)

The stimulation of the photosynthesis process is highlighted by the yields but also by the concentrations measured for chlorophyll a and b and carotene, both for wheat crop (Figure 7) and for tomato crop (Figure 8).

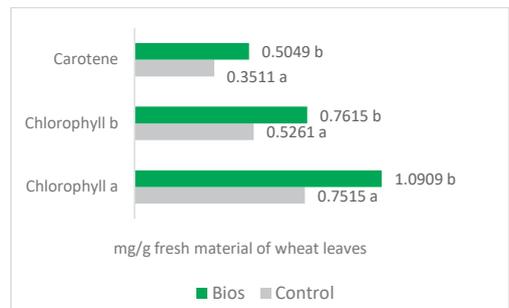


Figure 7. The content of chlorophyll pigments (mg/g fresh material) in wheat leaves

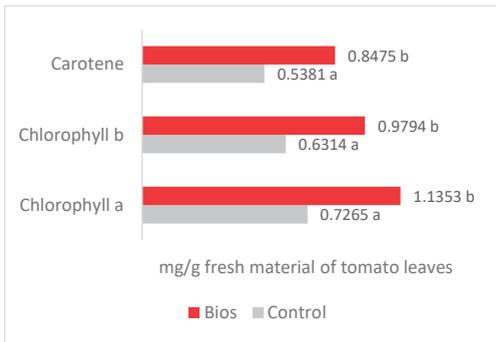


Figure 8. The content of chlorophyll pigments (mg/g fresh material) in tomato leaves

Plant biostimulants through their compounds have the ability to modify the physiological processes in plants.

There are many studies that show that the application at tomato of plant biostimulants leads to increased chlorophyll concentration and photosynthetic activity, which automatically leads to increased yield (Koleška et al., 2017), a fact also proved in this study.

Also, our results regarding the influence of the foliar fertilization on the photosynthesis showed that the percentages of increased photosynthesis are higher than the production increases because not all photosynthetic yield was directed to increase production. Part of the photosynthetic yield was maintained by the plant cell in order to accumulate reserve substances.

## CONCLUSIONS

Bios product was tested by foliar application to tomatoes and wheat, obtaining statistically significant increases in production compared to the unfertilized control.

The production increase obtained compared to the unfertilized control was 36.46% for tomato cultivation and 50.12% for wheat.

By foliar application of the Bios product, the total content of chlorophyll pigments increased significantly for the tested crops, an increase also manifested in the case of NPK content in the leaves.

The soil enzymatic activities correlated with the morphological, physical and chemical soil properties, as well as with the soil management, respond to these soils and their management in the investigated agroecosystems.

## ACKNOWLEDGEMENTS

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## EFFECTS OF COMPOST ON PLANT AND SOIL: STUDY CASE IN SUCCESSIVE CROPS

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### Abstract

*Composting is a biotechnology that can turn waste into a product (compost). It can help to improve the condition of waste materials and thus increase the sustainability of the bioeconomy. Source separation is very important for the compost quality. This paper presents the results obtained in a study that involved three composts obtained from biodegradable waste. Two of these were obtained from the organic fraction of municipal solid waste (FODMS) collected together and separated within the integrated waste management center, and the third one resulted from biodegradable household waste, separated at source. Soil and compost mixtures were made of 25%, 50% and 75% compost. A 100% soil control was used for comparison. The study was conducted in a greenhouse, in pots. Three experiments were carried out: the first one involved ryegrass (*Lolium perenne* L.), the second one, lettuce (*Lactuca sativa* L.) and the third one, after lettuce, involved radish (*Raphanus sativus* L.). The effects of compost on plants growth and development, their production and chemical composition, as well as the effects on soil were analyzed.*

**Key words:** *compost, circular economy, plant production, chemical composition, soil properties*

### INTRODUCTION

Demographic growth also leads to an increase in the agricultural and food production. At the same time, both people and various industries and services produce large amounts of biodegradable wastes or bio-waste (Directive 2018/851/PE, CE) that can substantially contribute to the environment pollution and, therefore, they must be managed in a sustainable manner. Under the conditions generated by the energy crisis, the reduction of resources, the human impact on the environment and the climate, etc. it is necessary to identify sustainable alternative solutions and develop innovative and efficient technologies and processes to cover the deficits of conventional energy resources, materials necessary for agriculture, such as fertilizers and others.

Bio-wastes can be integrated as raw materials to obtain new products, such as biogas and compost, and the circular economy can contribute to minimizing their environmental impact and to maximizing their recovery

(Velvizhi et al., 2020). In this context, the separate collection of bio-wastes is a *sine-qua-non* condition to make the processes more efficient. On the other hand, increasing the amount of bio-waste integrated into the circular economy through biogas production or composting can contribute to reaching the European Union (EU) target of recycling 65% of municipal waste by 2035 (EEA, 2020).

Composting is a bio-oxidative process of organic matter (OM) stabilization in the presence of air under the action of microorganisms (bacteria, fungi, actinomycetes). Considering the very diverse composition and characteristics of the raw materials (Siles-Castellano et al., 2020), the composting process must be conducted in such a way as to obtain a high quality product in order to eliminate the risks of phytotoxicity and to transfer some harmful compounds for plants, animals and humans in the food chain. In order to be used as a fertilizing material in agriculture, the compost must have a dry matter content (DM)  $\geq 30\%$ , a OM content  $\geq 20\%$  of the raw matter (RM), as well as a limited heavy

metal content, such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), copper (Cu), zinc (Zn), etc.(NF U44-051, 2006).

This paper presents the results of a study that integrated three experiments with three composts obtained from biodegradable wastes. It aimed at highlighting the agronomic value of the composts and their quality so that they can be used as fertilizers for agricultural soils. To this end, test plants were cultivated that were able to absorb the heavy metals present in the compost, where applicable.

## MATERIALS AND METHODS

### *Study establishment*

The study was organized in the greenhouse of the University of Agronomic Sciences and Veterinary Medicine (USAMV) in Bucharest between October 1, 2021 and March 11, 2022 and included three experiments with compost and soil mixtures. The first two experiments

(one in which ryegrass *Lolium perenne* L. was grown and another in which lettuce - *Lactuca sativa* L. was grown) were set up on October 1 (Photo 1), 2021, and the third one was set up on January 11 (Photo 2 and 3), 2022 after lettuce was harvested and radishes (*Raphanus sativus* L.) were cultivated instead.

**The composts** used for substrate mixtures were the following: 1) - *experimental compost* resulted from OFMSW after centralized separation - C1 (15-month old); small quantities were composted in specially constructed containers (almost 500 kg in a container); 2) - *experimental compost* resulted from OFMSW after centralized separation - C2 (18-month old); small quantities were composted in specially constructed containers (almost 500 kg in a container); 3) - *household compost* resulted from source separated bio-waste (biodegradable waste from garden and kitchen) - C3, produced on a household platform (12-month old).



Photo 1. Lettuce and ryegrass experiment after six weeks from planting/seeding



Photo 2. Radish at seeding

Photo 3. Radish plant one month after seeding

**The soil** used in these experiments is of red preluvosoil type coming from Moara Domnească Teaching and Research Station of the USAMV of Bucharest and located in a sylvosteppe ecological area of the Romanian Plain. Both the composts and the soil were previously analyzed from a physico-chemical point of view, and their characteristics are presented in Table 1. The soil had a slightly acidic reaction, with pH value of 5.89, and the composts had a neutral - slightly alkaline reaction, with pH values between 8.26 and 8.49 (Blaga et al., 2008). The dry matter content was well over 50% in all composts. The  $N-NH_4^+$

content of the composts ranged between 86 and 28 mg kg<sup>-1</sup> d.m. According to Brinton (2000), taking N-NH<sub>4</sub><sup>+</sup> as the maturity index, they can be considered mature and even very mature. The degree of compost maturation is also revealed by the C: N ratio.

Table 1. Soil and compost physico-chemical characteristics

Soil and compost parameters	Soil	C1	C2	C3
pH	5.89	8.26	8.49	8.36
Moisture (%)	19.3	31.75	41.86	44.64
Dry matter (%)	80.7	68.25	58.14	55.36
C_org. (%)	2.02	15.46	15.8	9.71
C:N	9.35	10.24	9.88	9.23
Nt (%)	0.22	1.51	1.6	1.04
N-NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> d.m.)	28.67	195	197	910
N-NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> d.m.)	9.12	28	35	86
P (%)	0.16	0.82	0.9	0.46
K (%)	na*	2.32	2.53	1.48

\*Not analyzed

The heavy metal content (Table 2) of the 3 composts was compared to the limits provided

Table 2. Heavy metals content of soil and compost used within experiments

Soil and compost parameters	Soil	C1	C2	C3	Biowaste ordinance (Germany)***	
					Compost class I	Compost class II
Cd (mg kg <sup>-1</sup> d.m.)	nd**	nd	nd	nd	1	1.5
Cu (mg kg <sup>-1</sup> d.m.)	26.8	119	121	34.5	70	100
Cr_tot (mg kg <sup>-1</sup> d.m.)	32.5	311	341	34.7	70	100
Ni (mg kg <sup>-1</sup> d.m.)	19.6	26.7	24.9	19.9	35	50
Pb (mg kg <sup>-1</sup> d.m.)	12.3	12.6	19.7	10.5	100	150
Zn (mg kg <sup>-1</sup> d.m.)	661	462	413	351	300	400

\*\*Not detected with the analysis methods used;

\*\*\*Heavy metal limits for European compost standards. Final Report. ANNEX 2; JUNE 2004. Table A2-4. Heavy metal limits for European compost standards. Compost from source separated Biowaste.

## RESULTS AND DISCUSSIONS

### *Lettuce and rye-grass experiments*

**Production.** The lettuce and ryegrass plants had a good evolution during the growing season, no phytotoxicity phenomena were observed and no plant losses were recorded (Photos 4 and 5). Lettuce plants were harvested 10 weeks after planting and weighed as the resulting mass of lettuce plants in each variant, in all 4 repetitions of each variant. Lettuce production was analysed as an average for each variant (Figure

by the German standards for Class I and Class II (Biowaste ordinance). The following were revealed: i) the method used for the Cd analysis didn't detect Cd presence in any of the composts; ii) C1 and C2 exceed the limits provided by the German standards for Class II for Cu, Cr\_tot and Zn content; iii) C3 falls into Class I, except for the Zn content, which recommends it for Class II; iv) all evaluated composts had Ni content below the limit imposed by the German standards for Class I.

In each of the three experiments from this study, the following substrate mixtures were used: Control - soil 100%; V1 - 75% soil + 25% C<sub>1</sub>; V2 - 50% soil + 50% C<sub>1</sub>; V3 - 25% soil + 75% C<sub>1</sub>; V4 - 75% soil + 25% C<sub>2</sub>; V5 - 50% soil + 50% C<sub>2</sub>; V6 - 25% soil + 75% C<sub>2</sub>; V7 - 75% soil + 25% C<sub>3</sub>; V8 - 50% soil + 50% C<sub>3</sub>; V9 - 25% soil + 75% C<sub>3</sub>. All variants were made in four repetitions.

During the growing season, the necessary moisture for the plants was ensured by periodic sprinkling with water. The plants benefited from light conditions specific to the greenhouse during the winter.

1). In almost all variants in which the composts were used in the mixture, the salad production was below the control variant (100% soil). Only in the variant with 25% C<sub>1</sub>, the lettuce production exceeded the control. Analysing separately the variants with compost, it can be observed that in general the highest lettuce productions were obtained in the variants with 25% and 50% compost in the substrate mixtures.



Photo 4. Lettuce - 9 weeks from planting



Photo 5. Ryegrass - 9 weeks from seeding

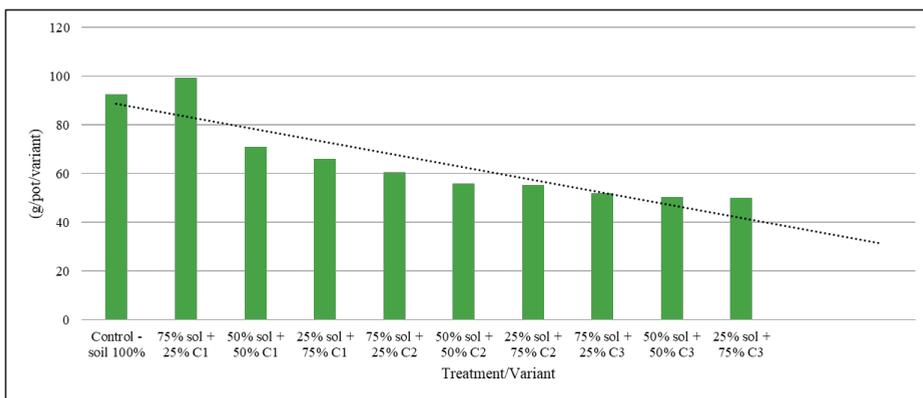


Figure 1. Average biomass of lettuce (g/pot/variant)

The production of ryegrass obtained in the variants in which the three types of compost were used in the substrate mixtures was above the one from the control variant (Figure 2). In the variants where C1 was used in the mixtures, the highest production of ryegrass was obtained at an intake of 50% compost followed by an intake of 75% compost. In the variants in which C2 was used in the mixtures, the highest production of ryegrass was obtained at an intake of 25% compost, followed by the variants with 75%, respectively with 50% compost. In the variants in which C3 was used in the substrate mixtures, the highest production of ryegrass was obtained at an intake of 25% compost followed by 50% and 75% compost respectively. Thus, as in the case of lettuce, it can be appreciated that, in general, an intake of more than 50% compost in the substrate mixture does not correlate with an increase in ryegrass biomass production.

*Macronutrients and heavy metals content of lettuce plants* (Table 3). The contents in N, P and K of lettuce plants obtained in all variants

with compost exceeded the control variant (100% soil). In variants with C1, the N content exceeded the control variant by 18.46% (V1 - 25% C1), 12.81% (V2 - 50% C1) and 9% (V3 - 75% C1). In variants with C2, the N content exceeded the control variant by 9.2% (V4 - 25% C2), 7.7% (V5 - 50% C2) and 11.2% (V6 - 75% C2). In variants with C3, the N content of lettuce plants exceeded the control variant by 13.4% (V7 - 25% C3), 8.1% (V8 - 50% C3) and 4.5% (V9 - 75% C3). The highest P contents of lettuce plants, above the content reached in the control variant (100% soil) were recorded in V2 (50% C1) > V5 (50% C2) and > V8 (50% C3). The differences compared to the control variant were over 36.2% > 31.9% and > 10.1%. Compared to the control variant, the K content of the lettuce plants was much higher in all variants in which compost was integrated, regardless of the proportion, except for V2 (50% C1) in which it was below the level of the control variant. The highest K contents in lettuce plants were recorded in the C2 and C3 variants.

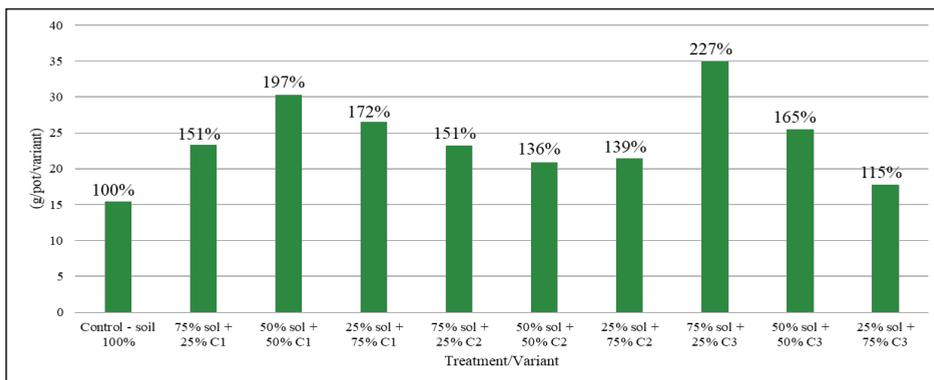


Figure 2. Average plant biomass of ryegrass at harvest

Regarding the heavy metals content, as it can be seen in Table 2, no Cd, Cr and Ni contents were determined in lettuce plants. In the variants with compost, the Cu content was between 5.3 mg/kg (V9 - 75% C3) and 9.5 mg/kg (V1 - 25% C1). The control, however, had a Cu content of 9.8 mg/kg. Normal average Cu contents in plants are between 5 and 20 mg/kg relative to dry matter (Jones, 1972). The Pb content in lettuce plants was below 5 mg/kg in all variants, and a

normal Pb content would be  $\pm 10$  mg/kg relative to dry matter (Camerlynck and Velghe, 1979). The Zn and Fe contents of the lettuce plants were within the normal limits cited by the literature (Jones, 1972). Thus, Zn recorded contents between 62 and 93 mg/kg, the normal average contents being included between 25 and 150 mg/kg relative to dry matter. Fe recorded contents between 46 and 83 mg/kg, while the normal average contents are between 50 and 250 ppm relative to dry matter.

Table 3. Macronutrients and heavy metals content of lettuce plants

Treatment	N %	P %	K %	Cd mg/kg	Cu mg/kg	Fe mg/kg	Cr mg/kg	Ni mg/kg	Pb mg/kg	Zn mg/kg
Control - soil 100%	5.31	0.69	9.8	nd	9.8	83	nd	nd	2.4	67
75% sol + 25% C <sub>1</sub>	6.29	0.82	10.0	nd	9.5	75	nd	nd	3.0	93
50% sol + 50% C <sub>1</sub>	5.99	0.94	9.5	nd	8.7	58	nd	nd	2.9	74
25% sol + 75% C <sub>1</sub>	5.79	0.80	13.5	nd	7.9	49	nd	nd	2.3	76
75% sol + 25% C <sub>2</sub>	5.80	0.80	12.6	nd	8.9	54	nd	nd	1.8	69
50% sol + 50% C <sub>2</sub>	5.72	0.91	13.6	nd	8.4	57	nd	nd	1.5	80
25% sol + 75% C <sub>2</sub>	5.92	0.78	17.0	nd	7.4	56	nd	nd	1.7	80
75% sol + 25% C <sub>3</sub>	6.02	0.75	14.3	nd	7.9	59	nd	nd	2.4	62
50% sol + 50% C <sub>3</sub>	5.74	0.76	15.0	nd	7.2	55	nd	nd	2.3	68
25% sol + 75% C <sub>3</sub>	5.55	0.72	12.5	nd	5.3	46	nd	nd	2.2	62

The results of chemical analyses regarding the content of ryegrass plants in macronutrients and heavy metals are presented in Table 4. The highest N contents (4.72%; 5.28%; 5.31%) of ryegrass plants were recorded in the variants with compost C3 where an increase somewhat correlated with the proportion of compost in the substrate mixture was also observed. In the other variants, the N contents of the plants were below 5% and not necessarily correlated with

the proportion of compost in the substrate. Unlike the lettuce plants, the P and K contents of the ryegrass plants were much lower. Regarding the Cd content, as in the case of lettuce, the presence of this element was not detected. The Cu, Ni, Pb and Zn contents of ryegrass plants were low, far below the limits proposed in the literature and mentioned above in the case of lettuce.

Table 4. Chemical composition of ryegrass plants after harvest

Treatment	N %	P %	K %	Cd mg/kg	Cu mg/kg	Ni mg/kg	Pb mg/kg	Zn mg/kg
Control - soil 100%	1.89	0.47	5.20	nd	3.29	1.55	1.28	5.04
75% soil + 25% C <sub>1</sub>	2.73	0.68	6.10	nd	1.89	0.43	0.14	3.46
50% soil + 50% C <sub>1</sub>	4.62	0.46	6.85	nd	1.25	0.39	0.31	5.22
25% soil + 75% C <sub>1</sub>	4.85	0.51	7.93	nd	5.31	0.26	0.27	6.04
75% soil + 25% C <sub>2</sub>	2.39	0.52	6.28	nd	0.90	0.26	0.15	3.82
50% soil + 50% C <sub>2</sub>	3.28	0.41	5.85	nd	0.80	0.29	0.19	3.48
25% soil + 75% C <sub>2</sub>	2.89	0.50	6.68	nd	1.53	1.38	1.26	5.36
75% soil + 25% C <sub>3</sub>	4.72	0.40	6.10	nd	1.20	0.53	0.19	3.59
50% soil + 50% C <sub>3</sub>	5.28	0.37	5.38	nd	0.80	0.42	0.17	3.63
25% soil + 75% C <sub>3</sub>	5.31	0.51	7.28	nd	0.93	0.45	0.19	4.20

As presented in the "Materials and methods" chapter, after lettuce harvesting, a radish crop was established, knowing that this species, which is part of the *Cruciferae* family, like other species from this family, is a heavy metal accumulator. Radishes were harvested two months after seeding, and chemical analyses regarding the content of heavy metals were

made on leaf and root samples (Table 5). Thus, no Cr, Fe and Pb contents were detected in the radish leaves, and the Cd, Cu, Fe and Zn contents were below 0.027 mg/kg f.m. for Cd, below 0.48 mg/kg f.m. for Cu, below 4.77 mg/kg f.m. for Zn and below 10.85 mg/kg f.m. for Fe.

Table 5. Heavy metals content of radish leaves

Treatment	Cd mg/kg f.m.*	Cu mg/kg f.m.	Cr mg/kg f.m.	Ni mg/kg f.m.	Fe mg/kg f.m.	Pb mg/kg f.m.	Zn mg/kg f.m.
Control - soil 100%	0.027	0.21	nd	nd	6.57	nd	3.18
75% soil + 25% C <sub>1</sub>	0.011	0.35	nd	nd	7.97	nd	3.50
50% soil + 50% C <sub>1</sub>	0.022	0.35	nd	nd	9.44	nd	3.85
25% soil + 75% C <sub>1</sub>	0.018	0.32	nd	nd	4.96	nd	4.05
75% soil + 25% C <sub>2</sub>	0.015	0.38	nd	nd	8.30	nd	4.02
50% soil + 50% C <sub>2</sub>	0.013	0.42	nd	nd	10.85	nd	4.00
25% soil + 75% C <sub>2</sub>	0.012	0.43	nd	nd	6.92	nd	4.77
75% soil + 25% C <sub>3</sub>	0.023	0.48	nd	nd	8.09	nd	3.17
50% soil + 50% C <sub>3</sub>	0.014	0.45	nd	nd	9.02	nd	3.92
25% soil + 75% C <sub>3</sub>	0.013	0.41	nd	nd	9.97	nd	3.49

\*fresh matter.

In radish roots, as in the case of leaves, no Cr, Ni and Pb contents were detected (Table 6). The Cd contents were much lower than those recorded in the leaves, with values between 0.019 mg/kg f.m. and 0.022 mg/kg f.m. According to Jackson and Allozay (1990), the

Dutch regulations provide a maximum permissible concentration of Cd in fresh vegetable fruits (i.e. tomatoes) of 0.10 ppm. Also, the contents of radish roots in Cu, Fe and Zn were much lower than those recorded in their leaves.

Table 6. Heavy metals content of radish roots

Treatment	Cd	Cu	Cr	Ni	Fe	Pb	Zn
	mg/kg f.m.	mg/kg f.m.	mg/kg f.m.	mg/kg f.m.	mg/kg f.m.	mg/kg f.m.	mg/kg f.m.
Control - soil 100%	0.022	0.17	nd	nd	4.49	nd	2.44
75% soil + 25% C <sub>1</sub>	0.019	0.11	nd	nd	4.41	nd	1.88
50% soil + 50% C <sub>1</sub>	0.016	0.12	nd	nd	2.82	nd	1.75
25% soil + 75% C <sub>1</sub>	0.004	0.11	nd	nd	2.10	nd	1.41
75% soil + 25% C <sub>2</sub>	0.003	0.06	nd	nd	6.95	nd	2.06
50% soil + 50% C <sub>2</sub>	0.006	0.08	nd	nd	3.23	nd	1.39
25% soil + 75% C <sub>2</sub>	0.003	0.07	nd	nd	1.44	nd	1.85
75% soil + 25% C <sub>3</sub>	0.026	0.12	nd	nd	6.81	nd	1.39
50% soil + 50% C <sub>3</sub>	0.003	0.12	nd	nd	3.15	nd	1.47
25% soil + 75% C <sub>3</sub>	0.004	0.11	nd	nd	2.75	nd	1.59

## CONCLUSIONS

The study allowed the highlighting of important aspects for the recycling of bio-waste through composting and their use as fertilizers for agricultural soils. Thus, it is essential to collect bio-waste separately and avoid the risk of mixing it with materials that could affect the quality of the compost.

At the same time, composting must be conducted in such a way that the compost reaches a good state of maturity in order to be included in quality classes that allow agricultural use.

Establishing the doses of compost or the proportions that will be combined with the soil or with other materials to create mixtures for culture substrates must be done depending on the chemical composition of the compost, especially depending on the presence of some heavy metals (Cd, Cu, Ni, Cr, etc.).

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## OBTAINING POTATO MICROTUBERS UNDER THE INFLUENCE OF OSMOTIC AGENTS IN DIFFERENT CONCENTRATIONS

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### Abstract

*In order to investigate the effect of osmotic agents on the induction of microtubers, a study was carried out in the Laboratory of Vegetable Tissue Cultures of NIRDPSB Braşov. Bifactorial experience included the following factors: experimental factor A, variety, with three graduations: a1 - Marvis; a2 - Castrum; a3 - Ervant (as a control, the average of the values obtained for the three varieties was taken into account) and experimental factor B, microtuberization medium (with 4 graduations): b1 - classical microtuberization medium without osmotic agent (control medium); b2 - microtuberization medium with mannitol; b3 - microtuberization medium with sorbitol; b4 - microtuberization medium with PEG. The determinations were made for number of microtuber/plantlets and weight of microtubers/plantlets, in function of experimental factors. Compared to the control medium, osmotic agents added to nutritive medium had a positive effect on studied parameters. Therefore, osmotic agents in low concentrations can be introduced as a stimulator of microtuberization.*

**Key words:** potato, genotype, osmotic agents, microtubers.

### INTRODUCTION

Microtubers have become an important mode of rapid multiplication for pre-basic stock in seed tuber multiplication as well as germplasm exchange (Chandra et al., 1992).

Microtubers are particularly convenient for handling, storage and transport of germplasm. Also, unlike *in vitro* propagated plants, they do not need a hardening period in a greenhouse and may be adapted to some form of large-scale mechanized planting (Ranalli et al., 1994).

According to the study by Naik and Karihaloo (2007), microtubers are small tubers (average weight being 100-150 mg) formed *in vitro* respecting the conditions of microtuberization.

In addition to growth hormones, type of media is also important for microtuberization efficiency (Kumlay, 2014; Yagiz et al., 2020, cited by Astarini, 2021).

Sucrose is the decisive factor in the formation of tubers *in vitro* (Wang and Hu, 1982; Abbot and Belcher, 1986, cited by Donnelly et al., 2003). Sucrose is a source of energy, at higher

concentrations, favouring the formation of microtubers (Perl et al., 1991; Simko, 1994; Struik and Wiersema, 1999, cited by Donnelly et al., 2003).

Dodd's et al. (1992) showed that for the microtubers formation the optimal concentration of sucrose was between 60-80 g/l.

Sucrose has a dual role in microtubers development. First, it could be a suitable carbon source, easily assimilated by microplants and transformed into starch and developing microtubers. Also, sucrose, with a concentration of 80 g/l, offers a favorable osmolarity for microtubers formation (Khuri & Moorby, 1995; Yu et al., 2000).

Sugars such as mannitol and sorbitol have been shown to induce *in vitro* regeneration, further studies revealed that mannitol mainly acts as an osmoticum rather than be uptaked or metabolized as an energy or carbon source (Altindal and Karado, 2010, cited by Motallebi-Azar, 2012).

Inorganic salt and sugar solutions, components of the culture environment besides the fact that they having a purely nutritious effect, they also

influence the growth of plant cells through their osmotic properties (George, 1993).

At slight stress conditions, potato plants utilize the strategy to generate more tuber. Therefore, the lower concentrations of PEG can be introduced as a stimulator of microtubers (Jamshid et al., 2020).

## MATERIALS AND METHODS

In order to investigate the effect of osmotic agents on microtubers induction, a study was carried out within the Laboratory of Vegetable Tissue Cultures of NIRDPSB Brasov. The technique of obtaining microtubers consisted in applying the liquid microtuberization medium in special recipients, which contain developed potato plantlets (Figure 1). The mini-cuttings from the uninodal segmentation of plantlets were inoculated on the propagation medium, 15 segments with a single node and a single leaf / culture vessel. The recipients were transferred to the growth chamber, where they had a growth and development regime of 22-25°C, with a photoperiod of 16 h, and in the culture vessels with these plantlets the microtuberization medium was applied.

The recipients were incubated in dark conditions, at a temperature of 18°C for 90 days.

The harvested microtubers (Figure 2 are sterilized) to prevent possible infections, exposed to drying, after which they are kept in cold conditions (4-5°C) for a good preservation.



Figure 1. Applying liquid medium in recipients with developed potato plantlets

In this study, two factors were analyzed: the variety and the microtuberization medium (the classic microtuberization medium, without osmotic agents considered as the control medium) and in addition to this, three osmotic agents considered as the control medium) and

in addition to this, three osmotic agents were added: mannitol, sorbitol, PEG (in two concentrations 1 and 1.5%, for each one).

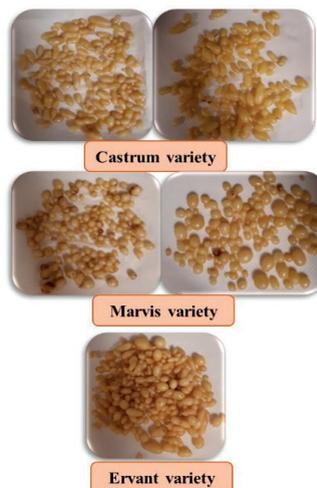


Figure 2. Microtubers harvested

To identify the osmotic agent with a higher efficiency in microtuberization, the values obtained for the two concentrations were averaged for each element studied. Thus, the bifactorial experience (3 x 4), on 3 repetitions had the following factors: experimental factor A - variety, with three graduations: a1 - Marvis; a2 - Castrum; a3 - Ervant (for this factor, as control we reported to mean values obtained for the studied parameters) and experimental factor B - microtuberization medium (with 4 graduations): b1 - classical microtuberization medium (without osmotic agent); b2 - medium with mannitol added; b3 - medium with sorbitol added; b4 - medium with PEG. The number of microtubers/plantlet and microtubers weight of/plantlets were determined at harvest. In this study, results were processed by analysis of variance, used to establish the significance of the differences between the variants.

## RESULTS AND DISCUSSIONS

From the analysis of variety influence on microtubers production, Ervant variety (Table 1) is distinguished, with a high microtuberization capacity, determining obtaining of a distinctly significant positive difference (0.221) and a value of 1.308 microtubers/pl. At the opposite pole is Marvis variety, with a low rate

of microtuberization (0.95 microtubers) and a distinctly significant negative difference (-0.137 microtubers), followed by the Castrum variety which recorded a significant negative difference (-0.084). For the second parameter taken into analysis, microtubers weight/plantlets, the values obtained for the three varieties were very close, without any significant differences, compared to all values mean (considered control), the highest value being obtained for the variety Ervant (0.33 g).

By comparing the experimental differences with the calculated limit differences over influence of the microtuberization medium on the number of microtubers obtained/plantlet (Table 2), the beneficial effect of PEG and mannitol is noted, which led to statistically assured results with very significant positive differences (0.281 and 0.215). Also, sorbitol addition to microtuberization medium had a positive influence, leading to a distinctly significant positive difference (0.207).

Examination results on microtubers weight/plantlet shows the positive influence of sorbitol and PEG, expressed by very significant

positive differences (0.162 and 0.127 g). Also, for microtubers weight, the sugar alcohol, mannitol, is notable, which led to a distinctly significant positive difference (0.091 g) compared to the control medium.

Using these osmotic agents strongly influences the microtubers formation for Castrum variety, whose very significant positive differences stand out from the control medium (0.62, 0.59 and 0.49 for mannitol, PEG and sorbitol). Applying PEG in the microtuberization medium positively influenced the formation of microtubers for the Ervant variety, causing a significant positive difference (0.26 g) and a value of 1.43 microtubers/pl. (Table 3).

The combined influence of variety and microtuberization media on microtubers weight/plantlets (Table 4) highlights Castrum variety for all osmotic agents, with very significant positive differences (0.42; 0.36; 0.36 microtubers for sorbitol, mannitol and PEG). The Ervant variety is distinguished with a high value of microtubers weight (0.402 g) by using sorbitol, leading a significant positive difference (0.133 g).

Table 1. Variety influence on microtubers obtained/plantlet and on their weight (g)/plantlet

Variety (a)	Microtubers number/pl.	Diff./Sign.	Microtubers weight/pl.(g)	Diff. (g)/Sign.
Castrum	1.003	-0.084 o	0.31	0.00 ns
Marvis	0.950	-0.137 oo	0.30	-0.01 ns
Ervant	1.308	0.221 **	0.33	0.02 ns
Mean (Ct)	1.087		0.31	

LSD 5% = 0.08; 1% = 0.133; 0.1% = 0.248. LSD 5% = 0.05 g; 1% = 0.08 g; 0.1% = 0.15 g.

Table 2. Influence of the microtuberization medium on microtubers number and on their weight/plantlet (g)

Microtuberization medium (b)	Microtubers number /pl.	Diff./Sign.	Microtubers weight/pl.(g)	Diff. (g)/Sign.
Classical microtuberization medium (Ct)	0.911	-	0.216	-
Medium with mannitol	1.126	0.215 ***	0.307	0.091 **
Medium with sorbitol	1.119	0.207 **	0.377	0.162 ***
Medium with PEG	1.193	0.281 ***	0.342	0.127 ***

LSD 5% = 0.112; 1% = 0.153; 0.1% = 0.209. LSD 5% = 0.057 g; 1% = 0.078 g; 0.1% = 0.106 g.

Table 3. Combined influence of the variety and microtuberization medium on microtubers number/plantlet

Variety (a)/ Microtuberization medium (b)	Castrum (a <sub>1</sub> )		Marvis (a <sub>2</sub> )		Ervant (a <sub>3</sub> )		a <sub>1</sub> -a <sub>3</sub> / Sign.	a <sub>2</sub> -a <sub>3</sub> / Sign.
	Microtubers number/pl.	Diff./Sign.	Microtubers number/pl.	Diff./Sign.	Microtubers number/pl.	Diff./Sign.		
Classical microtuberization medium (Ct)	0.58	-	0.98	-	1.18	-	-0.60 ooo	-0.20 ns
Medium with mannitol	1.20	0.62 ***	0.87	-0.11 ns	1.31	0.13 ns	-0.11 ns	-0.44 ooo
Medium with sorbitol	1.07	0.49 ***	0.98	0.00 ns	1.31	0.13 ns	-0.24 o	-0.33 oo
Medium with PEG	1.17	0.59 ***	0.98	0.00 ns	1.43	0.26 *	-0.27 o	-0.46 ooo

By studying the influence of the microtuberization medium on the number of microtubers/plantlet, the beneficial effect of mannitol in low concentration (1%) is noticed, followed by sorbitol (1.5%) and PEG (in both concentrations) obtaining higher values of the number of microtubers (1.25, 1.24, 1.23 and 1.16) compared to the control medium (0.91). When using as osmotic agents 1.5% mannitol and 1% sorbitol were equal values (1.00), lower than those mentioned above, but higher than the control medium (Figure 3).

Microtubers weight mean values/plantlet examination showed a positive influence of

sorbitol at both concentrations, with the highest microtubers weight (0.38 g). It is also noted that 1% mannitol in the microtuberization medium determining a high value obtaining (compared to the control medium) of microtubers weight (0.36 g). This value is followed by the results obtained when using PEG (0.35 g and 0.34 g both concentrations). The lowest value of the microtubers weight for all osmotic agents was when applying 1.5% mannitol, but this value is higher than the value of the microtubers weight obtained for the control medium (0.22 g) (Figure 3).

Table 4. Combined influence of the variety microtuberization medium microtubers weight / plantlet (g)

Variety (a <sub>1</sub> )/ Microtuberization medium (b)	Castrum (a <sub>1</sub> )		Marvis (a <sub>2</sub> )		Ervant (a <sub>3</sub> )		a <sub>1</sub> -a <sub>3</sub> / Sign.	a <sub>2</sub> -a <sub>3</sub> / Sign.
	Microtubers weight/pl. (g)	Diff./Sign.	Microtubers weight/pl. (g)	Diff./Sign.	Microtubers weight/pl. (g)	Diff./Sign.		
Classical microtuberization medium (Ct)	0.10	-	0.276	-	0.270	-	-0.17 o	0.01 ns
Medium with mannitol	0.36	0.26 ***	0.243	-0.034 ns	0.320	0.051 ns	0.04 ns	-0.08 ns
Medium with sorbitol	0.42	0.32 ***	0.308	0.032 ns	0.402	0.133 *	0.02 ns	-0.09 ns
Medium with PEG	0.36	0.26 ***	0.355	0.079 ns	0.312	0.043 ns	0.05 ns	0.04 ns

LSD 5% = 0.10; 1% = 0.14; 0.1% = 0.18.

LSD 5% = 0.11; 1% = 0.16; 0.1% = 0.23.

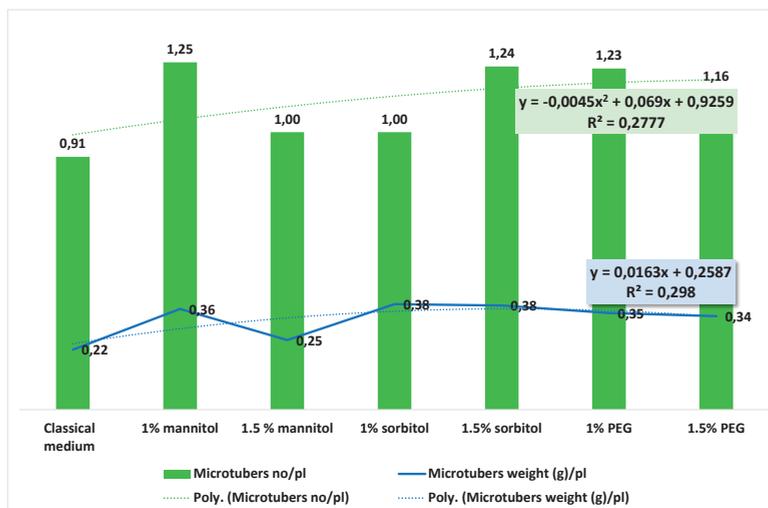


Figure 3. Microtubers number and weight in function of osmotic agents and their concentration

Examination of results regarding the number of microtubers / plant suggests the high capacity of Ervant variety for microtubers production, the highest value being recorded when 1.5% sorbitol is used in the microtuber medium (1.53

microtubers). This is followed by the Castrum variety, by using 1% mannitol (1.44), a value equal to that obtained by the Ervant variety, when 1% PEG was used (Figure 4).

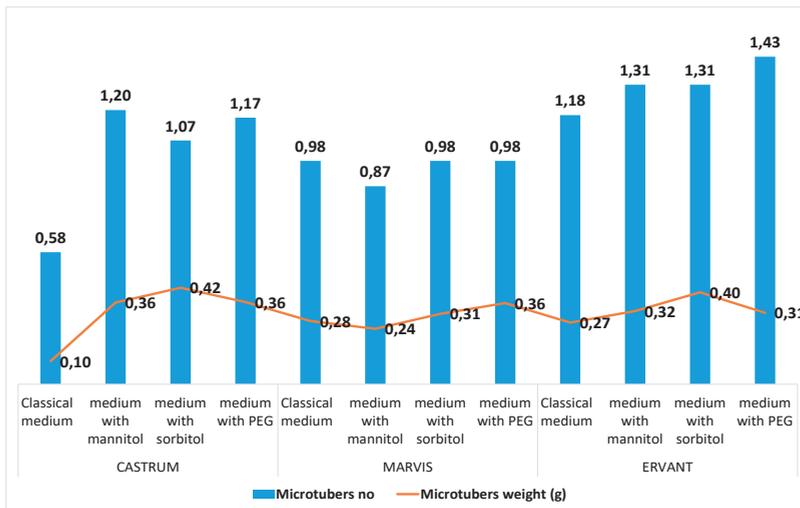


Figure 4. Microtubers number and weight in function of variety and osmotic agents

Regarding the weight of the microtubercles, the highest values are obtained for the Castrum variety, by using 1% mannitol (0.44 g) and 1.5% sorbitol (0.43 g).

## CONCLUSIONS

From this study it can be concluded that by using low concentration of sugar alcohols and PEG can be an effective method of producing microtubers. PEG and mannitol introduction into medium strongly influenced the microtuberization process for numbers (1.193 and 1.126), leading to very significant positive differences. Sorbitol also showed a beneficial effect in microtubers production (1.119), causing a distinctly significant positive difference. The beneficial effect of mannitol in low concentration (1%) was noticed, followed by sorbitol (1.5%) and PEG (in both concentrations) obtaining higher values of microtubers number of microtubers (1.25, 1.24, 1.23 and 1.16) compared to the control medium (0.91). The Ervant variety showed a superior behaviour to the other varieties in microtubers production, the highest value being registered when the sorbitol 1.5% (1.53 microtubercles) was used in the microtuberization medium. This is followed by the Castrum variety, by using 1% mannitol (1.44), a value equal to that obtained by the Ervant variety, by applying 1% PEG.

In microtubers formation as a number, PEG introduction in medium had a pronounced positive influence, obtaining the highest value.

For the second parameter studied, the highest values were obtained by applying sorbitol and PEG in the medium (0.377 g and 0.332 g) with very significant positive differences.

Mannitol utilisation, also improved the microtubers weight/plant with a high value (0.307 g) compared to the control medium (0.216 g) and resulted in a distinctly significant positive difference.

Regarding the microtubers weight, the positive influence of sorbitol was observed for both concentrations, obtaining the highest value of microtubers weight (0.38 g).

For microtubers weight, the highest values were recorded for the Castrum variety, using 1% mannitol (0.44 g) and 1.5% sorbitol (0.43 g). Supplementation with sugar alcohols and PEG has enhanced the microtuberization process. Under conditions of slight osmotic stress, the potato plantlets produced a higher number of microtubers, compared to the classical environment.

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project Research on the production of minitubers under specific isolation conditions.

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## RESEARCH ON THE BEHAVIOR OF CORN CULTURE ON THE ACTION OF DIFFERENT TYPES OF FERTILIZERS (CHEMICAL VS. BIOLOGICAL)

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### Abstract

*The processes of interaction between the plant and the active soil microbiome are the determining factors of plant welfare and health, crop productivity and soil fertility. The new technologies applied in the agricultural field have as role the promotion of the growth and development of the plants, the sustainability of the agricultural crops, the maximization and the yield of the agricultural crops. The bacteria used in the composition of fertilization or plant protection products are selected bacteria, bacteria accredited by international gene banks, these bacteria being determinants and plant health. The associativity of bacterial cultures has the role of developing and supporting the growth of plants and their protection from certain diseases or pests, through various mechanisms.*

*The use of live bacterial cultures in agriculture has different characteristics. The most important characteristics that these biopreparations have are represented by: the biological fixation of atmospheric nitrogen in the soil, the solubilization of phosphates, the acceleration of the ACC deaminase process, the production of siderophores and phytohormones, the growth and development of plants. The present paper aims to present the role on which the combinations of bacterial cultures that are used in agricultural ecosystems, bacterial cultures that can replace chemical fertilizers as well as some plant protection products. This article presents, in addition to the biology of each bacterium - the role, action and benefit that these bacterial cultures have in the activity of the soil microecosystem. The positive impact of biofertilizer "BioWais" on plant growth and development, enhancement of resistance of bacterized plants to hypothermia and pathogenic infection, increase of carotid content were demonstrated at the stations where we done the experiments.*

**Key words:** biofertilizers, live bacterial cultures, nitrogen fixation.

### INTRODUCTION

The use of fertilizer products in agricultural crops is a beneficial source of supplementing the nutrients needed for the growth and development of both plants and an increase in agricultural production. However, often the fertilizer doses applied per hectare to agricultural crops are not respected. Failure to comply with the applied fertilizer doses will lead to the occurrence of negative phenomena for soil, environment and agricultural crops, implicitly for human and animal health. Increasing the fertilizer doses per hectare and not respecting them will lead to the occurrence of soil acidification (Jugenheimer, 1976). The decrease of the bacterial colonies in the soil will bring with it a decrease of the humification

processes, of the decomposition and solubilization processes of the complex compounds in the soil as well as favoring the leaching and appearance of the complex compounds in the soil (in large quantities). The increase of complex compounds in the soil will lead to a decrease in pH (below pH 7), which will lead to an increase in soil acidity. On acidic soil, crops will not reach their maximum potential in productivity. The use of bacterial biopreparation technologies in agricultural crops plays an important role in plant protection. Some bacterial cultures give plants a protection against pedo-climatic stress, a resistance to the attack of diseases and pests as well as conferring a protection on environmental factors (drought, heavy rainfall, cold, etc.). The use of these bacterial products as fertilizers as well as plant

protection products has been shown to have great potential in growing, developing, maximizing agricultural production, in restoring and greening the soil and its beneficial flora, the role of these biological fertilizers being to address a sustainable agriculture and achieving high, healthy, nutrient-rich productivity, beneficial to human and animal health (Hallauer et al., 1988). Soil is the basis of agriculture. The soil is a mediator of processes mediated by microorganisms, both at the surface of the soil and at the root system of plants (Mureşan, 1973). The rhizosphere is the area of maximum activity of microorganisms. At this level, the activity of bacterial cultures is carried out in optimal parameters, the rhizosphere being the part of the soil that includes the surface of the root to the top of the absorbent hairs. The rhizosphere is an appropriate environment for the growth, development and interactions of microorganisms with soil, plants and the environment (Figure 1A) (Stammen, 2005). At the rhizosphere, plants through the roots, more precisely at the pylorus (calytra) release a range of phytohormones that are involved in the process of fusion with soil microorganisms (beneficial soil microorganisms communicate with plants based on phytohormone emission, transmission, recovery, decoding and response from both parties involved - plants and microorganisms) (Vary et al., 2007). The role of soil bacteria is to recolonize the bacterial fauna of the soil, to increase plant growth, increase agricultural production and ensure protection against diseases, pests, abiotic stress and fluctuations in soil and climate conditions. The mechanisms of action of bacterial cultures involve processes of availability of nutrients derived from genetic processes in plants (photosynthesis and chemosynthesis) and other related processes such as biological nitrogen fixation, solubilization of insoluble compounds in soluble compounds, production of siderophores (Ardelean, et al., 2007). Root processes are symbiotic, endophytic and associative processes (Figure 1B) that have the role of colonizing the roots (by bacteria), which creates a favorable environment for development, growth, agricultural productivity and soil greening (Alves et al., 2004; Adesemoye et al., 2009; Hungary et al., 2010).

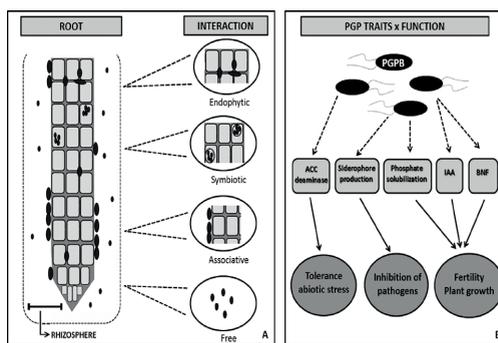


Figure 1A. The interaction between the rhizosphere and soil bacteria

Figure 1B. Colonization and association of bacteria with plant roots

The use of new fertilization technologies in Corn crops as well as an appropriate treatment of Corn seed will bring with it a number of benefits both for the soil and, implicitly, for the plants. The use of innovative fertilizers for Romanian agriculture (fertilization products based on live bacterial cultures) as well as combinations of bacterial cultures have the role of growing and developing the plant mass of the plants and, implicitly, supporting the harmonious growth and development of cobs so as to a maximized yield of agricultural Corn production on a given area is obtained. At Suceava Research and Development Station for Agriculture, a series of experiments were carried out on the cultivation of corn with different types of fertilizers in order to prove the effectiveness of each of them.

## MATERIALS AND METHODS

At SCDA Suceava, a series of experiments on plant fertilization were carried out on the *Millenium* corn crop, as follows (Table 1):

V1 - unfertilized control lot;

V2 - chemically fertilized lot (Complex Azomures NPK 20-20-0, 300 kg/ha - in autumn) + 100 kg nitrocalcar in spring;

V3 - biological fertilized lot produced Rom-Agrobiofertil NP (30 liters/ha).

In each lot, a series of biometric determinations were made to present the differences between the types of fertilizers used in the tests (colour, number of leaves, number of absorbent hairs, plant height, productivity per hectare, etc.).

Table 1. Variants of the experiment

Fertilization type	Experimental variants		
	V1	V2	V3
The unfertilized lot (control lot)		Chemically fertilized lot (Complex Azomures NPK 20-20-0, 330 kg/ha-in autumn) + 100 kg nitrocalcar in spring	Biological fertilized lot produced Rom-Agrobiofertil NP (30 liters/ha) 01.05.2020 - first treatment, 20.05.2020 - second treatment

As materials used in the research carried out, we can mention the following: Complex Azomures NPK 20-20-0 300 kg, Nitrocalcar and the biological fertilizer product, based on live bacterial cultures Rom-Agrobiofertil NP.

Methods of crop observation, determinations of established parameters, statistical methods and methods of quantitative and qualitative analysis were used as methods.

The technology of cultivation and tillage of the corn crop (of the experimental lots) within SCDA Suceava was carried out with the help of mechanized equipment (tractor, seed drill, disc, MET).

## RESULTS AND DISCUSSIONS

Following the application of the previously mentioned technologies, the following results were obtained for the corn crop at SCDA Suceava (Table 2).

Table 2. Production sheet

Parameters	Experimental variants			Differences			
	V1 - The unfertilized lot (control lot)	V2 - Chemically fertilized lot (Complex Azomures NPK 20-20-0, 330 kg/ha - in autumn) + 100 kg nitrocalcar in spring	V3 - biological fertilized lot produced Rom-Agrobiofertil NP (30 liters/ha)	V2 vs V1	V3 vs V1	V3 vs V2	V2 vs V3
No. grains/row	31	36	43	16.129	38.710	19.444	-16.279
TGW, g	251	274	303	9.163	20.717	10.584	-9.571
Grain yield %	81.7	89	98.9	8.935	21.053	11.124	-10.010
HM, kg	72.7	78.7	80.4	8.253	10.591	2.160	-2.114
Production, kg/ha	4587	6478	8753	41.225	90.822	35.119	-25.991
Humidity, %	16.6	16.4	16.6	-1.205	0.000	1.220	-1.205

It can be seen that there are a number of major differences between each even batch. In particular, we can observe that the batch fertilized with the biological product recorded the highest production. Although a good part of the corn crop was affected by hail, the action of the bacteria in the fertilization product led to the support of agricultural productivity and the restoration of both the root system of the plants and their leaf surface.

Due to the bacterial activity in the biological fertilizer, a good part of the complex compounds in the soil structure were decomposed, which led to an increase in the macroelements necessary for the growth and development of plants and agricultural productivity: nitrogen, phosphorus and potassium. At the same time, a significant amount of microelements also resulted from the decomposition.

Increasing the amount of micro and macro elements in the soil and stimulating the nutrition of corn plants with the help of bacteria led to their growth and development.

A significant increase and a much better development of the plants brought with it a high productivity per plant and, implicitly, per hectare.

The development of plants brought with them growth and stimulation of fruiting. As such, the yield (number of grains per cob) of the plants was much higher in the biologically fertilized lot compared to the chemically fertilized lot or the unfertilized control.

Also, at harvest, compared to the chemically fertilized batch and the non-fertilized control, the grain moisture was much better in the biologically fertilized batch.

From the culture sheet we can see that the biologically fertilized batch led to the acceleration of seed germination, better rooting of the plants. Also, a reduction in plant fall before vegetation can be observed, but the most important aspect is the number of plants fallen during harvesting: 0 (Table 3).

Table 3. Observation sheet

Experimental variants	Corn crop sowing date	Corn crop emergence date	The date of the appearance of the third leaf	No. days between sowing and sunrise	Flowering date	Date of appearance of silk	Physiological maturity date	Plant height (cm)	Fallen plants during the vegetation period (%)	Fallen plants at harvest (%)
V1	31.04	07.05	12.05	8	09.07	11.07	10.08	175	7	4
V2	31.04	07.05	10.05	8	06.07	08.07	15.08	220	3	1
V3	31.04	07.05	08.05	6	02.07	04.07	10.08	237	1	-

Compared to the non-fertilized and chemically fertilized control, it is observed that the plant size increased significantly in the biologically fertilized lot, followed by the chemically fertilized lot, the treatment with the organic fertilizer Rom-Agrobiofertil NP contributing much more to the thickening of the package and the increase of leaf mass (Table 4).

Also, the action of the phytohormones produced by the bacteria in the biological fertilizer had a positive effect on plant growth, seed germination, flowering date, silk emergence date, plant maturity date, their height, as well as on the fall of plants in vegetation and harvesting. These aspects were identified in the batch fertilized with the product based on microorganisms, in parallel with the two batches.

Thus, we can affirm the fact that the microorganisms contained in the biological fertilizer acted as a support for the corn culture, and through the biological processes, along with

the stimulation of the physico-chemical processes of the plants and the soil, a production surplus was created as seen in Table 3.

From the Anova analysis (Table 4) we can see that the biological fertilizer stimulated the physico-chemical and biological processes and allowed a link between soil, environment and plants. Through this "mediation", the increase in production in the biologically fertilized batch was much higher than the other two batches (chemically fertilized and unfertilized control). We can affirm the fact that the bacteria contained in organic fertilizers have the role of stimulating the growth and development of plants.

This growth is beneficial for the soil and plants because, based on it, an increase in production can be obtained with a minimal investment, an increase in production which, for the farmer, represents a profit.

Figures 2-7 present different aspects from the experimental variants.

Table 4. Production sheet - Anova: Two-Factor without replication

Anova: Two-Factor Without Replication						
<i>SUMMARY</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
No. grains/row	3	110	36.67	36.33		
TGW, g	3	828	276.00	679.00		
Grain yield, %	3	269.6	89.87	74.52		
HM, kg	3	231.8	77.27	16.36		
Production, kg/ha	3	19818	6606.00	4351177.00		
Humidity, %	3	49.6	16.53	0.01		
V1 - The unfertilized lot (control lot)	6	5040	840.00	3376633.35		
V2 - Chemically fertilized lot (Complex Azomures NPK 20-20-0, 330 kg/ha - in autumn) + 100 kg nitrocalcar in spring	6	6972.1	1162.02	6790708.65		
V3 - biological fertilized lot produced Rom-Agrobiofertil NP (30 liters/ha)	6	9294.9	1549.15	12465198.72		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Rows	105971658.5	5	21194331.69	29.47	0.000011	3.326
Columns	1512921.348	2	756460.67	1.05	0.384923	4.103
Error	7191045.119	10	719104.51			
Total	114675624.9	17				



Figure 2. Corn crop - V3 organic fertilizer lot Rom-Agrobiofertil NP (15 liters/ha x 2 applications)



Figure 5. Corn crop - fertilizer complex fertilizer (control lot)



Figure 3. Corn crop - V3 biologically fertilized lot produced Rom-Agrobiofertil NP



Figure 6. Corn crop - V3 biologically fertilized lot produced Rom-Agrobiofertil NP - after hail



Figure 4. Corn crop - fertilizer complex fertilizer (control lot) NPK 20-20-0 (300 kg/ha autumn) + 100 kg nitrocalcar in spring



Figure 7. Corn crop - fertilizer complex fertilizer (control lot) NPK 20-20-0 (300 kg/ha autumn) + 100 kg nitrocalcar in spring - after hail

## CONCLUSIONS

The agricultural year 2018-2019 was a very difficult one from a climatic point of view, with large rainfall deficits for spring crops and pedological drought recorded in the periods April-May and July-August, which caused delays in plant growth and development.

There were also two hail showers, which affected plant development in June 2019, and weeding was also very high, affecting crop plants in the second half of the growing season.

The experimental results showed that both the treatment with the organic fertilizer Rom-Agrobiofertil NP, in Corn cultivation positively influenced the growth and development of plants, both in Corn with very significant positive results in terms of quantity and quality of agricultural products, despite the fact that the crop was affected by hail.

Treatment with organic fertilizer Rom-Agrobiofertil NP has a positive influence on the growth of leaf area and maintaining the turgidity of plant tissues, prolonging the vegetation period, which contributes to the accumulation of reserve substances in large quantities, compared to untreated foliar control.

Production increases were on average 2.275 tons compared to the chemically treated Corn variant.

Complex beneficial association with soil microbiomes is necessary for plant growth, nutrition, stress tolerance and antagonizes their pathogens.

The ability of plants to synthesize substances with bactericidal and fungicidal effects in order to control diseases and pests in agricultural crops is very important for farmers.

Bacteria give plants a tolerance and a resistance of plants to unfavorable environmental factors (heavy metal pollution of the soil, soil salinization and drought).

Also, bacteria give a biocontrol and remediation system for the main functions of the plant, it present a intensification of the process of photosynthesis and chemosynthesis of plants and -increasing agricultural production.

However, the beneficial effects of the bacteria mentioned above lead to growth of the plant mass, height, number of fruits per plant, growing shoots on the plant, increasing the number of flowers per plant, increasing the number of fruits

and also increasing agricultural productivity per hectare (for biologically fertilized lots).

Decomposition of complex compounds in the soil and release of mineral elements necessary for plant growth and development is a significant increase in agricultural productivity which will also lead to a large increase in seed material and increasing the root system of the plant will lead to a better absorption of mineral elements from the soil.

The activity of bacteria on the soil has a positive effect on the decomposition of complex compounds in the soil as well as on better elasticity of the plant (wind resistance, better grip of the plant in the soil (increase of its root system).

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## THE MAIN FUNGAL DISEASES IN STRAWBERRIES CROP - REVIEW

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### Abstract

Strawberry cultivation is one of the most important fruit crops in the world from a commercial point of view, but it is constantly threatened by fungal pathogens, which cause significant economic losses. The decline of strawberry plants is also seen as a severe threat to strawberry production in Romania. Due to recent restrictions on pesticide use and the lack of effective and efficient alternatives, achieving consistently high yields has become difficult. The most important diseases that cause significant economic losses are: *Botrytis cinerea*, *Phytophthora cactorum*, *Colletotrichum fragariae*. They affect all parts of the plant: flowers, fruits, leaves and roots causing significant annual losses: 40% for *Phytophthora cactorum* attack, 50% for *Colletotrichum fragariae* and up to 80% for *Botrytis cinerea* attack. This review provides an overview of the latest studies on the main fungal pathogens, the measures and control strategies currently available, the risk factors influencing the development of pathogens and what strategies we can address to limit the economic losses of the strawberry crop.

**Key words:** strawberries, pathogens, control, strategies.

### INTRODUCTION

The strawberry cultivated with large fruits (*Fragaria* × *ananassa* Duchesne) has its origins in Europe in the 18th century. Cultivated strawberry (*Fragaria* × *ananassa* Duchesne), is a hybrid of two mostly dioecious octoploid species, *Fragaria chelonensis* Duchesne and *Fragaria virginiana* Duchesne. It is a short-lived perennial herb that grows predominantly in temperate climates. Its fruits are a rich source of vitamins and minerals (Sharma et al., 2009).

Most strawberries (*Fragaria* × *ananassa* Duchesne) in Romania are produced either in the south of the country (the area with the highest strawberry production being Hotarele in Giurgiu County) or in the North-West (Satu-Mare). Fresh fruit is usually shipped to cities and areas where soil or temperatures do not answer to crop requirements. Therefore, Romanian growers need varieties that produce attractive and aromatic fruits and in order to reach these qualities, the appearance of pathogens in the culture must be avoided. Crops and fruit quality are usually reduced when strawberry plants are infected with fungi. In the last ten years, the pathogens that cause significant losses in culture are: Gray mould (*Botrytis cinerea*), Collar rot of apple

(*Phytophthora cactorum*) and Anthracnose (*Colletotrichum fragariae*).

### STRAWBERRIES PATHOGENS

#### 1. Gray mould (*Botrytis cinerea*)

Gray mould is a necrotrophic pathogen that causes significant damage to strawberry crops. *Botrytis* fruit rot, commonly known as gray mould, is caused by the fungus *Botrytis cinerea* and is one of the most serious strawberry pathogens in Romania and across the world. The fungus affects all aerial sections of the plant. The majority of economic damage occurs when flowers and fruit are damaged, resulting in yield losses of up to 80% in some cases. *Botrytis cinerea* is also a post-harvest pathogen because infections that start in the field continue to grow during storage and transportation at refrigeration temperatures (Mertely et al., 2018).

**Symptoms.** The manner and timing of infection differ based on the plant type and plant parts infected. *Botrytis* disease symptoms vary widely depending on the host and plant section affected. A grey to brown discolouration, water soaking, and fuzzy white grey to brown mould (mycelium and conidia) forming on the surface of damaged tissue and

isolated lesions are symptoms. The pathogen can infect fruits through the bloom persisting in the very early stages of growth and developing just after harvest when the fruit is fully ripe. This method of infection is significant in strawberries and is thought to be the primary cause of deterioration in ripe fruit after harvest. Blossom blights frequently precede and contribute to fruit and stem end rots. The fungus frequently spreads from the fading petals across the inflorescence and develops on the fruit, causing blossom-end rot. Conidia can infect the fruit directly through growth cracks, cut stem scars, insect wounds, or lesions caused by other pathogens. Infected fruits develop water-soaked, yellowish green or greyish brown irregular lesions that might be mushy and spongy in texture (Elad et al., 2007).

**The host.** In Romania, the majority of strawberries are May-bearing varieties cultivated as perennials in matted rows for 2-5 years. Strawberries are typically mulched with wheat straw in the fall to give winter protection, and the straw is removed or placed between the rows in the spring. Plant regrowth occurs in April, followed by overlapping blooming and fruit development periods in May and June. The crop is harvested between mid-May and mid-June and then restored. Renovation may include leaf mowing, tilling between rows, row narrowing, plant thinning and fertilizer and pesticide application.

Gray mould also attacks other crop plants, being identified by the anamorphic form of *Botrytis cinerea* (Cristea, 2005; Docea et al., 2012; Gheorghies & Cristea, 2001; Docea E. & Cristea Stelica, 2003).

**Infection and colonization.** *Botrytis cinerea* can infect strawberry leaves, flowers, fruits, and crowns. Infected tissues are usually asymptomatic, and colonization does not progress until the tissues senesce, ripen, or die, but lesions can form on the calyces, petals, stamens, or green receptacles.

The interactions between *Botrytis cinerea* occurrences are crucially dependent on strawberry leaves. Although the pathogen does not cause symptoms on the foliage, it can be recovered from surface sterilized infected green leaves and it frequently sporulates on the leaves immediately after death. Recent discoveries

have put a spotlight on some of the previously enigmatic relationships between leaf growth and infection and colonization. The receptivity of field grown plant leaves to *Botrytis cinerea* it was found to be high for developing leaves, zero for mature leaves and low for senescent leaves.

Infection of the flowers by *Botrytis cinerea* conidia is a crucial precursor to the attack on the fruit. Senescent sepals, petals, and stamens are key sources of mycelium capable of penetrating strawberry fruit, debunking the commonly held belief that the fruit is generally infected directly by conidia. Just 1% of fruit infections to conidia, with the vast majority attributed to mycelium in detached petals, decomposing berries, and receptacles sticking to the fruit, or in diseased floral portions that remained connected to the receptacles. Conidia germinated easily in stigmatic fluid and infected stigmas in the absence of dew or other extra water, but it took 4-6 weeks to develop along the styles, making fruit invasion an implausible method. On the other hand, the infection frequently develops along the filaments of infected stamens and into the receptacle. Mycelium growth into green receptacles from diverse floral parts is fast until after the fruit ripens, giving rise to the fruit's distinctive stem-end rot (Sutton & John, 1990). Grey mould in strawberries can be caused by *B. cinerea* infections of open flowers (primary infections) or by penetration of fruit receptacle tissues (secondary infections). *B. cinerea* infects flower organs during or just after flowering, causing hyphae to develop into the receptacle. Primary inoculum sources include overwintering sclerotia as well as conidia or mycelium from infected neighboring plants. Primary infections in fruit can be facilitated by infected senescent petals, stamens, and calyces. Despite the fact that styles are commonly infected, histological investigations demonstrate that fungal development appears to be highly prevented and never reaches the receptacle. Fungal development in colonized stamens, on the other hand, can reach the receptacle in some cultivars (Bristow et al., 1986).

Following *B. cinerea* infection of the unripe receptacle, fungal growth normally stops and a symptomless quiescent phase ensues. The processes that because dormant infections are

yet unknown. Proanthocyanins (PAs) appear to promote *B. cinerea* quiescence in unripe fruit by inhibiting the activity of fungal enzymes such as polygalacturonases (PGs), which are required for aggressive host infection (50% inhibition in unripe fruit compared to 8% inhibition in ripe fruit). Despite the fact that the PA concentration of the fruit stays constant during ripening, increased polymerization of PAs results in reduced inhibitory action in mature fruit. Similarly, anthocyanins may postpone or promote *B. cinerea* infections. Strawberries exposed to white fluorescent light, had higher anthocyanin levels and delayed the growth of grey mold (van Baarlen et al. 2007). The fungus begins the necrotrophic phase without quiescence during secondary infections. Conidia for secondary infections can be found in a variety of places, including senescent leaves and infected fruit. Secondary inoculum is mostly obtained from conidia found in *B. cinerea*-infected floral sections. It is believed that organic pieces that come into touch with the fruit, such as petals and stamens, cause more than 64% of strawberry infections. Secondary infections can also occur as a result of nesting, which is characterized by direct penetration of mycelia growing on neighboring plant parts such as diseased leaves and fruit. Secondary infections, in general, progress quickly, and *B. cinerea* can complete germination and infection as quickly as 16 hours after inoculation (Jarvis, 1962). Because of the availability of high-quality reference genome sequences, the infection mechanisms of *B. cinerea* have been examined in model organisms and further defined. By using a range of virulence mechanisms, the fungus is known to actively enhance plant sensitivity. *B. cinerea* uses sRNAs and effector proteins to prevent premature host cell death and immunological responses in the early stages, allowing the fungus to establish itself inside the host and amass biomass prior to the necrotrophic phase (Veloso & Van Kan, 2018). *B. cinerea* Dicer-like proteins DCL1 and DCL2 have been shown to create sRNAs that are released from fungal hyphae and translocated to the plant cell, where they interact with host RNAi processes to silence host immune response genes in arabidopsis and tomato leaves (Weiberg et al., 2013).

**Detection and Quantification.** Late stage infections are easily identified in symptomatic material by the emergence of grey/brown conidial clusters on infected material's surfaces. It is difficult to detect infections in non-symptomatic material, such as in the early stages of infection or latent and endophytic infections. Surface sterilization, followed by freezing of tissues or berries for 2 hours at 12°C or dipping in 300 g/ml paraquat, followed by air drying and incubation in a humid atmosphere on wet filter paper or selective media for 7-10 days, has been widely used (Fillinger & Elad, 2016).

Using GC-MS, Van den Driessche et al. (2012) identified a number of volatile biomarkers in strawberries infected with *B. cinerea*. They demonstrated that while these markers may be used to track infections, they cannot detect pre-symptomatic infections. If the sensitivity of such non-destructive procedures could be enhanced, they may be extremely valuable in the food sector. Semi-selective and differential media are based on the selective suppression of competing bacteria, the promotion of target organism development, and/or the expression of a *Botrytis*-specific trait. Viable conidia germinate and form colonies on a variety of media in the presence of free water, nitrogen, and phosphate, although they do not always sporulate. A microscopic investigation is required to confirm the development of any *Botrytis* species (Dewey, 2000). A variety of immunoassays have been applied to detect and to a lesser degree quantify, *Botrytis* infections in plants. Enzyme-linked immunosorbent tests, particularly plate trapped antigen-immunosorbent assays, are by far the most prevalent (PTA-ELISAs). Ricker et al. (1991) showed that PTA-ELISAs may be used to assess levels of *Botrytis* antigens in juice from diseased grapes using rabbit antisera. Recently, the same approach was used to identify and quantify *B. cinerea* in grape juice, wines, pear stems, and strawberries using the genus-specific monoclonal antibody BC-12.CA4 (Dewey, 2000).

**Life cycle.** Gray mold typically begins in contaminated plant debris from previous crops that has been left in the field. When temperatures rise, such as in early spring, the

mycelium in the debris begins to grow. Mycelium begins to form structures known as conidiophores when exposed to bright light. Conidiophores produce spores named conidia, which are subsequently dispersed via the air and can come into touch with crop leaves or stems. They germinate and begin to attack there. For the spores to be released from the conidiophores, there must be a quick drop in humidity and an increase in temperature. This is frequently the case in the early hours of the morning. Raindrops splashing on an infected plant can also aid in the spread of the spores. Insects are another mode of transmission for conidia from one infected plant to another, and they are a major source of infection. Infected crops and gardens in the area might also be a source of infection.

Moisture and nutrients must be present on or near the plant for the spore to germinate. Moisture can occur through condensation on the plants, which is generated by air humidity levels of more than 95%, or from any plant sap that escapes as a consequence of damage to the plant's exterior surface. Germ tubes emerge from the spore when it germinates. An appressoria at the end of these tubes generates an infection peg that penetrates plant tissue. The peg does not penetrate the plant tissue immediately. It must initially produce enzymes that contribute in the elimination of the plant's first cellular barrier (Williamson et al., 2007).

Because the cuticle of healthy tissue is frequently quite strong, the fungus has a better chance of penetrating damaged, weak, or senescent tissue. Stomata and wounds can potentially be entry points for infection. That is why *Botrytis* commonly appears after a caterpillar attack, because the fungus will use the damage created by the insect bites to penetrate the plant. *Botrytis* secretes proteins and phytotoxic chemicals that cause the collapse and death of cells close to the host. The plants immune system also influences the rate at which infection occurs. The plants defenses are occasionally weaker in the autumn, allowing the half-dormant *Botrytis* to do serious damage (AbuQamar et al., 2016). Fungal spores germinate and spread by wind and/or water all across the spring when the weather is cold and rainy. Heavy rain or overhead irrigation, along with cold

temperatures, promotes *B. cinerea* development. When strawberries or flowers stay wet for 24 hours or more, the infection risk reaches 90%. The pathogen that causes fruit infection is mostly induced by blossom infection, however the disease can remain dormant until the fruit ripens (Mattson & Daughtrey, 2019).

**Ecology.** *Botrytis cinerea*, like many fungus, is only harmful to a host when particular environmental conditions are satisfied. If these conditions are not satisfied, the fungus will generally remain a saprophyte inside the crop, feeding on senescing or dead tissue. *Botrytis cinerea* may be present on all continents, but because to the 9 distribution of economic hosts, the disease it causes are mostly linked with cool temperate and warm-temperate zones (Mehli et al., 2005).

**Management.** Despite significant efforts, strawberry resistance to gray rot has yet to be improved, and the mechanisms of tolerance to the pathogen *Botrytis cinerea* attack are still poorly known and investigated.

Currently, strawberry gray rot control is focused on cultural, chemical and biological approaches. As a result, the yellowed, discolored, and dried leaves will be removed in the spring. Mulching is done with clean straw to minimize fruit contact with the soil and soiling during the wet season. To avoid crushing the seedlings or the leaves, the transit through the culture should be done with extreme caution. Plants that are susceptible to botrytis (lettuce, tomatoes) should be avoided in close proximity to strawberries. Chemical protection begins when the inflorescences are spread. *B. cinerea* infections that cause postharvest decay typically emerge before harvest, in the field, and can stay latent until fruit storage. Because there are no infection symptoms and no reliable ways for predicting the risk of this disease, preventative measures should be taken before symptoms appear. Field applications of synthetic fungicides during the crop growth cycle have traditionally been used to reduce gray mold infestation. The fungicides are administered around strawberry plant flowering and are repeated till harvest, depending on the weather and the preharvest

period for the different formulations (Feliziani & Romanazzi, 2013).

*Botrytis cinerea* is a classic "high-risk" pathogen in terms of fungicide resistance, therefore chemical treatment of gray rot is a constant issue, with new fungicides prone to resistance.

With the development of new fungicides, *Botrytis cinerea* control has become increasingly successful. Over the last four decades, a standard practice has emerged in which frequent chemical management has become a must. Fungicide programs remain the foundation of *Botrytis cinerea* control, although they have lately become less successful and increasingly unpopular with the public. Environmental authorities and consumers in many nations are worried about pesticide usage in agriculture, and as a result, there is a growing demand for organically farmed foods. This environmental issue has sparked much study and the development of alternate approaches or systems for controlling *Botrytis cinerea* in strawberries.

In the struggle against gray rot, biological control is an excellent option. Biological control agents are known to be mostly bacteria and yeast that are "antagonistic" to the pathogens that cause postharvest strawberry fruit deterioration. They compete with the pathogens for nutrients and space, or they parasitize the pathogens by creating antibiosis pressures, such as the production of volatile compounds that are noxious or unpleasant to the pathogens, or they can induce resistance in the host tissue to strengthen the plant's defenses against the pathogens. Numerous experiments have been conducted in recent years to isolate and test microorganisms that can fight strawberry infections (Jamalizadeh et al., 2011).

Several microorganism-based products are already registered against *B. cinerea*, and not just for strawberry. *Pseudomonas syringae* (BioSave; JET Harvest Solutions, Longwood, FL, USA), *Bacillus subtilis* (Serenade; Bayer, Leverkusen, Germany), *Candida sake* (Candifruit; IRTA, Lleida, Spain) and *Metschnikowia fructicola* (Shemer; Bayer, Leverkusen, Germany) are some of the most well-known commercially available biofungicides. Inorganic salts have been proven

to be active antimicrobial agents against a variety of phytopathogenic fungi, with bicarbonates being offered as a safe and effective alternative way of controlling postharvest rot of fruits and vegetables, including strawberry fruit. In addition to being non-toxic and having a low environmental impact at their effective quantities, these salts are affordable. Calcium-rich formulations can be used to fortify the central lamellae of strawberry fruit cells, increasing their resilience to mechanical and biological harm (Karabulut et al., 2004). A modified storage atmosphere with carbon dioxide enrichment, to reduce the incidence and severity of decay and thus to extend the postharvest life of strawberry fruit, is one of the most commonly used postharvest treatments to control fungal growth and reduce the respiration rate of strawberry fruit. However, negative impacts on color and flavor have been recorded in certain cases following exposure to extremely low oxygen and extremely high carbon dioxide levels. Very high oxygen conditions have been studied as an alternative to standard modified environment packing of fruits and vegetables, including strawberry fruit, since very high oxygen inhibits grey mould growth and can avoid unwanted anoxic fermentation (Allende et al., 2007). Among the different techniques of reducing plant gray rot, the use of plant extracts is an option, characterized by: lack of toxicity to humans and the environment, selectivity, biodegradability, and a high chemical diversity, having a wide range of metabolites. Secondary, many of them have not yet been explored in connection to pesticidal efficacy. *Capsicum* plant extracts such as *Capsicum annuum* (pepper), *Capsicum chinense* (habanero pepper), and *Capsicum frutescens* (hot pepper) have demonstrated in vitro, antifungal efficacy against the pathogen *B. cinerea*, eventually limiting spore germination (Wilson et al., 1997).

Cloves (*Syzygium aromaticum*) include natural chemicals that have antifungal activity against gray rot. In addition, the water and ethanol garlic extract (in vitro) acts as an antifungal by totally suppressing mycelial development and spore germination of *Botrytis cinerea* (Šernaitė et al., 2020). Although the volume of experimental research on the antibiotoxic action

of various plant extracts is remarkable, quite a few conditioned products based on such extracts have been delivered for agricultural practice.

These include BM-608 products, based on essential oils from *Melaleuca alternifolia* (Adebayo et al., 2013) or *Gloves Off*® (commercial disinfectant produced by Planet People and Laboratoire M2, INC, Sherbrooke, QC, Canada) from oils from *Thymus* (Gebel & Magurno, 2014). This direction of investigation and development being open to future research.

**Biocontrol Tests.** To be effective, microorganisms introduced into a crop to manage a disease must be able to interact effectively with the pathogen, the host, and other organisms within the prevailing microclimatic circumstances. The biocontrol system is very dynamic, including host growth and development, pathogen infection cycles and serial dispersals, quantitative alterations in biocontrol agent and indigenous organism populations, and microclimatic oscillations. In general, epidemics cannot be well reproduced in the laboratory, growth room, or greenhouse, and biocontrol experiments performed in these settings should be interpreted accordingly. Under regulated settings, biocontrol testing can be used efficiently for preliminary screening of organisms and for supplementing agricultural operations.

*Gliocladium roseum* provides a number of benefits for effective biocontrol of *Botrytis cinerea* in strawberries. Because it is effective in leaves, flowers, and fruits, it can successfully be targeted against *Botrytis cinerea* at the inoculum source in the leaves or to directly protect the flowers and fruits. The ability of *G. roseum* to penetrate and survive in the leaves results in extraordinary antagonist persistence in the foliage for weeks or months after application, in contrast to many biocontrol agents that are active against pathogens mainly on the phylloplane and rapidly decline in number and activity after application.

*G. roseum*, when administered once when the leaves are green, has the ability to suppress *Botrytis cinerea* in each leaf flush due to its persistence in green leaves and biocontrol action after the leaves senesce and die. In contrast to chlorothalonil, *G. roseum* is less

effective or ineffective when applied to senescing or dead leaves, according to field studies. *G. roseum* inoculum generation can easily scaled up on low-cost substrates like as wheat grains, which may improve the cost-effectiveness of biocontrol (Sutton, 1995).

## 2. Collar rot of apple (*Phytophthora cactorum*)

In strawberry crop, *Phytophthora cactorum* is one of the most damaging pathogens. It is a disease that has spread widely in Western Europe and the United States, destroying, in some years, large areas cultivated with strawberries or reducing production in infected plots. It is a specific root disease, but it attacks all the organs of the plant resulting in yield losses of up to 40% in some cases.

*Phytophthora cactorum* is a polyphagous organism found in temperate region soils. It causes disease in 200 crop species from 60 families. This fungus primarily affects ornamental plants (such as rhododendron, pansy, pelargonium, and begonia), fruit shrubs and trees (such as gooseberry, currant, strawberries, peach, raspberry, apple tree, and cherry) and forest trees (such as birch, black alder, common beech, common ash, and spruce) causing phytophthorosis (Hantula et al., 2000).

The remains of infected plants left over from previous plantings, water used for irrigation from neighboring ponds or rivers, irrigation systems, contaminated soil, or infected seedlings may be the source of *P. cactorum* on plantations. Oospores of *P. cactorum* are thick-walled spores and they may survive in soil for up to 6 years (Orlikowski et al., 2017).

Strawberry is resistant to the majority of *P. cactorum* strains, with just a few specialist *P. cactorum* isolates capable of causing crown rot. On the other hand, many popular strawberry cultivars are vulnerable to specialist *P. cactorum* crown rot isolates, and just a few cultivars regularly show resistance. According to Shaw et al., (2008) resistance to the crown rot pathotype is a polygenic characteristic. Aside from the genetic background, the physiological condition of the plant is important: cold-stored and/or damaged plants are especially sensitive to the disease, and

young plants are more susceptible than older ones.

**Symptoms.** Disease outbreaks frequently begin with isolated areas of diseased strawberry plants. They grow in size, especially down slopes where water dispersion may fast cause extensive regions to be impacted. Symptoms can appear on the roots as early as late autumn, but they usually do not appear on the above-ground sections of the plants until late spring or early summer, when it can be difficult to uncover confirming evidence of the pathogen in the roots. Symptoms usually appear on the upper parts of plants that come under stress in late spring or early summer, especially in low-lying, wet areas. Plants frequently do not develop or grow in a stunted manner. They may collapse shortly before ripening or yield only a few small fruits. Younger leaves might be blue-green, whereas elder leaves can be yellow or red. Digging up the plants shows a rotting and underdeveloped root system.

Lateral feeder roots are typically rotten and destroyed by the time plants are dug. The adventitious roots decay from the tips upwards and frequently have a grey to brown coloration at their distal ends. Cutting apart the top, white, unrotted sections of such roots reveals steles that range in color from wine-red to brick-red, thus the term red core. In extremely sensitive varieties, the color can spread fairly far beyond the rotten regions of the roots, all the way into the crown.

*Phytophthora cactorum* may survive in soil as sexual oospores that germinate under moist circumstances to develop mycelium and sporangia, which release motile asexual zoospores that infect the plant collar and fruits. The infection appears as dark staining inside the collar, and subsequent wilting leads to the plant's death (Poimala et al., 2021).

**Infection.** Several *Phytophthora* species are classified as hemibiotrophs because they retain a biotrophic interaction with their host for a period of their life cycle. Prior to penetration of a host cell, sporangia, zoospores, cysts, and germinating cysts with germ tubes are generated, all of which are necessary for plant infection and disease development. Infection by root pathogenic oomycetes like as *P. cactorum* begins with the production of motile, wallless

zoospores, which encyst on host surfaces after a chemotactic stage before host penetration occurs. These pre-infection structures are expected to be rich in chemicals important in infection establishment and elicitation of plant defenses (Xiaoren et al., 2011).

Wet conditions promote infection. Oospores in the soil and infected transplants are the principal sources of inoculum. Once introduced, the disease can live for many years on certain farms, whilst other farms do not have recurring issues year after year. Oospores generate zoospores, which typically infect stolon stumps, rhizomes, or freshly cut runners through wounds. Frigo plants, or plants that have been stored in the cold, are highly sensitive to crown rot. The manifestation of disease is regulated by the time of planting as well as environmental circumstances. Because the fungus demands warm temperatures and sustained wetness, early plant collapse can begin within one month of sowing in the fall, with subsequent plant collapse occurring the following spring when regrowth starts and especially as fruit develops. Water allows the virus to travel (Pettitt & Pegg, 1994).

*P. cactorum* may persist in soil for extended periods of time at the water content and temperatures that are typical throughout the growth season. They sustain viability even when soil is held at - 10°C for several hours, indicating that they are evolved for long-term survival in the soil environment. While oospores may certainly live in soil for longer periods of time than sporangia due to their form and nature, sporangia must be considered as a more essential short-term inoculum unit. Sporangia are formed when oospores germinate. Sporangia can germinate directly and possibly infect a host, or they can create new sporangia at the ends of the germ tubes, or they can discharge zoospores under conditions favorable for indirect germination (Erwin & McCormick, 1971). Zoospores are the most likely to cause infections, and a recent study shown that they may survive in soil for several weeks. Sporangia and oospores both germinate in soil with a water content less than its field capacity (McIntosh, 1972).

Because of their fast lysis, mycelium and hyphal fragments appear negligible as inoculum or in pathogen persistence in soil.

Except in wet soil as sporangiophores sprouting from the tissue, it is unusual that hyphae grow to any degree on the surface of infected root tissue (Sneh & McIntosh, 1974).

The ideal temperature for germination is 10-15°C, however it can happen at 20°C as well as very slowly at 5°C. Temperatures between 10 and 17°C are ideal for infection. Infection can develop at temperatures as low as 2°C but not at temperatures as high as 25°C. It moves more slowly below 10°C, but more secondary inoculum is produced over longer periods of time, explaining why the disease is more severe following a rainy winter (Liu et al., 2018).

### **Methods used to determine levels of infection**

Strawberry crown rot induced by *P. cactorum* is a difficult subject to quantify. Previous studies measured disease, pathogen virulence, and host resistance in terms of percentage mortality or the period of manifestation of the first symptoms.

Duncan (1985) isolated *P. fragariae* oospores from infected strawberry root tissues by comminution, followed by filtration and thorough washing, primarily for germination experiments. Harris (1985) retrieved *P. syringa* oospores in fallen apple leaves by comminution and selective sifting, followed by haemocytometer counts. Although these approaches are useful for estimating the inoculum potential in affected tissues, they do not provide a direct estimate of the amount of invading mycelium. The chemical measurement of chitosan is another method for determining the mycelial concentration of diseased plant tissues. However, due to the lack of chitin in the hyphal walls of this species, such chemical techniques cannot differentiate between living and non-viable mycelium and would be unsuitable for *Phytophthora* spp. (Bartniki-Garcia, 1966). Another method for measuring pathogen quantity is to apply an enzyme-linked immunosorbent test, which has been effectively used to identify *P. infestans* mycelium in potato leaf tissue (Harrison et al., 1990).

**Detection of *P. cactorum*.** Culture-based isolation methods are still widely used for detecting *P. cactorum* in strawberries, but with

proper optimization and validation, PCR may offer significant benefits over culturing and other methods for monitoring this pathogen. Culture-based detection of *Phytophthora* species takes about a week and specific mycological expertise, whereas PCR-based detection, including DNA extraction, takes around 2-3 days and general molecular biology knowledge. There are enzyme-linked immunological assays available for detecting *Phytophthora* spp., however they are not species-specific (Olsson, 1995).

Diagnostic PCR techniques and primers for *Phytophthora* species that infect strawberries, such as *P. cactorum* and *P. fragariae* var. *fragariae*, have been developed. The nested PCR primers ADF1 and ADR1 were found to amplify a 520-bp DNA fragment from the ITS sections of the *P. cactorum* rRNA gene in strawberry root samples. PC1/PC2, developed as SCAR markers from a particular random amplified polymorphic DNA fragment to produce a PCR product of roughly 450 bp, were shown to be specific, sensitive, and robust for identifying *P. cactorum* in different host plants, including strawberry. Primers Ycac1F and Ycac2R, which amplify a 192-bp segment of the ras-related protein gene Ypt1, were recently shown to be specific for *P. cactorum* (Bhat & Browne, 2010).

**Management.** In agricultural fields, soil fumigation and good cultural methods offer effective *Phytophthora* management. Using certified seedlings, avoiding poorly drained soils, and preparing fields to allow sufficient soil drainage during wet weather are all examples of appropriate cultural practices. Because *Phytophthora* can be spread by water that has been drained from contaminated fields, avoid utilizing runoff water for irrigation or watering down field roadways for dust management. Even with tolerant cultivars, it is critical to adhere to excellent cultural practices (Koike & Gordon, 2018).

Mefenoxam (Ridomil Gold) and metalaxyl (different formulations) have been shown to be beneficial when applied by drip irrigation. These fungicides are most effective when sprayed in the fall, shortly after planting and after all overhead watering for plant establishment has been completed. In most

circumstances, an early spring spray is also beneficial as new plant development begins to accelerate. The time of the fall and spring seasons coincides with strong root development. Additional treatments can be done in areas with significant disease pressure in the spring (Frank et al., 2019).

Chemical compounds are commonly utilized to minimize *P. cactorum* infection, despite the fact that this species and other *Phytophthora* spp. have showed the potential to develop resistance to such chemicals. *P. cactorum* has been shown to be resistant to dimethomorph, metalaxyl (mefenoxam), cymoxanil, and mancozeb. Resistance can develop quickly in certain populations, the frequency of *P. cactorum* strains resistant to metalaxyl reached up to 80% as early as four years after metalaxyl was first used in strawberry plant protection (Utkhede & Gupta, 1998).

Copper-based chemicals (fungicide group M1), such as the Bordeaux combination, have been used for a long time and are still effective. Copper hydroxide, copper oxide, basic copper sulfate, copper oxychloride, and copper ammonium carbonate are some other copper-based protectant fungicides. The Cu<sup>++</sup> ion is the active agent against *Phytophthora* in each case. Some of these may leave residues on the leaf of plants. They are most commonly used during the dormant season. Acidic situations, such as tank mixing with phosphorous acids, will release an excessive amount of copper ions, causing plant damage (Hoitink & Powell, 1990).

Although the use of fungicidal chemicals remains an efficient technique of protecting plants in both systemic and eradicant applications, the possibility of resistance to such compounds has prompted research to identify alternate methods of plant protection that do not offer such a risk. Other ways of plant protection are being studied, frequently based on live biological control agents (BCAs) or their metabolites, in addition to the use of proven antiresistant approaches, which reduce the rate of resistance formation in the practical use of fungicides.

Fungi of the genus *Trichoderma*, *Aureobasidium pullulans* (yeast fungus), *Bacillus*, namely *B. amyloxylophilus*, *B. megaterium*, *B. subtilis*, *B. velezensis*,

*B. licheniformis*, and *B. cereus*, *Pseudomonas*, specifically *P. fluorescens* and *P. syringa* and the use of arbuscular mycorrhizal fungi from the genera *Glomus* and *Claroideoglomus* proved effective against *P. cactorum* (Pánek et al., 2021).

The very promising effect of *Trichoderma* spp. and solarization against *P. cactorum*, as well as cultural practices to reduce or eliminate standing water, the ability of the biocontrol agent to proliferate in field soil, and the increase in marketable yield, suggest that there may be future alternatives to traditional chemicals for strawberry disease control (Porras et al., 2007).

Some fertilization programs have been applied to control *Phytophthora* spp. They include using organic materials that release ammonia and nitrous acid, using sulfur-based fertilizers and amendments that reduce pH to less than 4 for acid-tolerant plants, reducing pH to less than 5 in high-aluminum soils (for plants with a tolerance for aluminum), applying foliar nutrients to compensate for rotting fibrous roots' loss of uptake, and avoiding excessive nitrogen fertilization, which makes the resulting succulent foliage more susceptible (Pscheidt & Ocamb, 2015).

Nursery transplants subjected to aerated steam in a closed chamber at 37°C for 1 hour followed by 44°C for 4 hours were substantially less likely to die from *Phytophthora crown rot*, according to studies conducted at the UF/IFAS GCREC. As a result, thermotherapy of transplants may be a viable option for nurseries in managing *Phytophthora* populations in plant stock (Natalia & Juliana, 2019).

### 3. Anthracnose (*Colletotrichum fragariae*)

Strawberry anthracnose is a serious disease that affects all parts of the plant, including the fruit, crowns, leaves, petioles, and runners. *Colletotrichum acutatum*, *C. gloeosporioides*, and *C. fragariae*, three related species of the fungus *Colletotrichum*, have been correlated to anthracnose. However, *C. acutatum* is the primary pathogen involved with the anthracnose fruit rot phase resulting in yield losses of up to 50% in some cases (Gunnell & Gubler, 1992).

**Symptoms.** Lesions of anthracnose fruit rot develop as dark, sunken lesions on contaminated fruit. Anthracnose lesions on green fruit are small, firm, deep, and dark brown or black. Ripening fruit lesions are bigger, harder, deep, and brown to dark brown. When it rains, the lesions get covered with a sticky, light orange liquid consisting of millions of spores (conidia) in a viscous liquid structure. Numerous lesions almost cover the fruit when conditions are favourable for infection, and lesions may form on petioles. When strawberry flowers become infected, they turn brown and remain attached to the plant. Flowers infected with the gray mold fungus *Botrytis cinerea* may present symptoms similar to those described above. Flower infections can also cause small dark spots on green button-sized fruit ( Mertely et al., 2012).

**Disease development.** Despite various species of fungus in the genus *Colletotrichum* can produce anthracnose, *Colletotrichum acutatum* is the most prevalent species causing fruit rot in Romania. According to recent study, the fungus may grow and develop spores on the surface of seemingly healthy leaves.

Once the infection has established in the field, the fungus may survive in winter on affected plants and plant debris, such as dead leaves and mummified fruit. Warm, humid temperatures and rain promote spore formation and germination, and strawberry fruit infection. Spores are abundant on previously infected plant debris in the spring and early summer. Splashing rain, wind-driven rain, and people or equipment moving through the field all spread the spores. Because they are not airborne, they do not go a long distance in the wind. Spores need free water on the plant's surface to germinate and infect.

Temperatures between 25 and 30°C are ideal for infection on both immature and mature fruit. On infected fruit, the fungus generates secondary spores under favorable conditions. Rain spreads these spores, resulting in secondary infections throughout the growing season. Disease development can proceed at a rapid speed. Within a week or less, up to 90% of the fruit can become infected. Infection can occur in both immature and mature fruit;

however, the disease is most prevalent in ripening fruit (Michael et al., 2016).

**The host.** The fruit rot produced by *C. acutatum*, which also infects many other fruit and vegetable crops such as apples, tomatoes, peppers, peaches, blueberries, blackberries, and grapes, causes the most economic losses on strawberries crops. The pathogen has been found on strawberries in practically every region of the world where they are farmed. Infected plants often do not flourish following transplantation and provide few berries at harvest (Smith, 2008).

Conidia germinate by creating sessile appressoria or germ tubes and terminal appressoria. Appressoria melanize and create pores through which the host can enter. There are two types of infections that can occur: intracellular hemibiotrophic infections, in which infection vesicles or swollen hyphae form within epidermal cells, and subcuticular, intramural necrotrophic infections, in which cuticular penetration is followed by hyphal proliferation within the cuticle and epidermal cell walls, but without penetration of the cell lumen. Both types of infections may lie dormant for a long time before substantial cell loss, colonization, and symptom development occur (Leandro et al., 2001). Without host plants, *Colletotrichum acutatum* may persist in soil for at least 9 months (Boldá et al., 2018).

Curry et al. (2002) used light and electron microscopy to investigate the infection of strawberry petioles and stolons by *C. acutatum* and *C. fragariae*. Both fungal species entered the host tissue similarly; however, *C. fragariae* infiltrated the plants faster than *C. acutatum*. Both species used an appressorium to enter the cuticle, and their hyphae developed within the cuticle and cell walls of epidermal, subepidermal, and subtending cells. They began their invasion with a brief biotrophic phase in which they attacked live cells before transitioning into a longer necrotrophic phase in which they multiplied amid dead cells. Acervuli emerged as a stroma immediately under the outer periclinal epidermal walls after the cortical tissue was substantially disturbed.

**Detection and identification.** *Colletotrichum* species have traditionally been classified based on morphological characteristics such as conidial and appressorium morphology,

pathogenicity tests, and physiological and biochemical techniques (Munoz et al., 2000). Molecular genetic research is helping to understand the systematics of the genus *Colletotrichum*. Isozyme comparison, mitochondrial DNA restriction fragment length polymorphisms (mtDNA RFLPs), arbitrarily primed polymerase chain reaction (PCR), and ribosomal DNA (rDNA) restriction analyses have managed to make the most significant contributions to the molecular characterization of *Colletotrichum* species causing strawberry anthracnose (Martinez-Culebras et al., 2003).

**Management.** Any cultural practice that reduces rain splash, such as spreading straw mulch around the margins of and between rows of plants, would assist. Only a few cultivars have substantial anthracnose tolerance. Day-neutral types are more vulnerable. Because inoculum may be transported about on harvesting equipment and harvesters' hands, it makes sense to begin operations in less severely afflicted regions before moving on to locations where the disease is more severe. Low tunnels significantly reduce anthracnose occurrence, whereas high tunnels almost eradicate fruit symptoms even without the application of fungicides, making them very valuable for organic producers (Kathy & Timothy, 2019).

Anthracnose in strawberries has been controlled substantially with germicide operations. Since the 1990s, the most generally used pesticides have been benzimidazole pesticides similar as carbendazim and thiophanate methyl (Zhang et al., 2020).

Control of *C. acutatum* is heavily reliant on regular and many treatments of multi- and single-site fungicides. Multisite fungicides, such as captan, provide adequate control of *C. acutatum* but have no curative effect, necessitating weekly treatments throughout the season (Forcelini & Peres, 2018).

Early in the season, azoxystrobin (Quardris<sup>®</sup>), boscalid + pyraclostrobin (Pristine<sup>®</sup>), and cyprodinil + fludioxonil (Switch<sup>®</sup>) consistently offered efficient anthracnose control. Before transplants are planted in producing fields, they should be dipped in fungicides or rinsed with clean water to remove spores or other

propagules from the planting stocks, allowing disease management (Daugovish et al., 2009). Biocontrol using beneficial microorganisms like *Streptomyces* has been used successfully to control fruit postharvest diseases. In a research by Li et al. (2021), strain H4 shown strong antifungal activity against *C. fragariae*, which was isolated from *Dichotella gemmacea* in the South China Sea's Xisha islands. A preventative therapy based on strain H4 extracts greatly decreased the severity and incidence of anthracnose disease while also preserving the hardness and color of harvested strawberry fruits. In vitro, extracts efficiently inhibited *C. fragariae* mycelial growth and spore germination. Pathogenic fungi's mycelial structure showed deformation, shrinkage, collapse, and tortuosity.

Trichoderma is also known to be effective in controlling certain plant pathogens. For instance, in experiments conducted with TRICHODEX, various isolates of the fungus were able to control anthracnose and grey mould in strawberry (Freeman et al., 2004).

Li et al. (2021) discovered that strain QN1NO-4 obtained from noni (*Morinda citrifolia* L.) fruit has excellent antifungal activity against *C. fragariae*. Strain QN1NO-4 belongs to the genus *Bacillus* based on its physicochemical characteristics and phylogenetic tree of the 16S rRNA genome. The average nucleotide identity (ANI) computed by comparing two standard strain genomes was less than 95-96 percent, indicating that the strain is a unique *Bacillus* species designated *Bacillus safensis* sp. QN1NO-4. Strawberry anthracnose of harvested fruit was substantially decreased by its extract.

Based on research findings, it can be inferred that thyme, cinnamon bark, and clove bud essential oils exhibit antifungal properties against *C. acutatum*. In disease-friendly settings, thyme EO volatiles fully prevented pathogen penetration and growth on strawberry fruit, whereas cinnamon bark EO volatiles decreased pathogen penetration and development. As a result, thyme and cinnamon bark may be evaluated as promising fumigant options in the management of strawberry anthracnose (Duduk et al., 2015).

To reduce anthracnose in strawberries, a minimum of nitrogen and potassium should be

included in any fertilizer used, with no more than 15 and 10 units of nitrogen and potassium, respectively. On average, strawberry plants treated with ammonium nitrogen had more disease than those treated with nitrates, but the differences were not statistically significant (Nam et al., 2006).

## CONCLUSIONS

The qualities of strawberries: special taste and aroma, their high content of vitamin C, suitability for home processing, as well as impressive ecological adaptability, given the high potential for genetic variability, have led to a large expansion of cultivated areas with strawberries worldwide. The expansion of cultivated areas and the widespread use of chemicals have created problems in the context of the management of the main diseases of the strawberry crop. Therefore, the solution to these problems is integrated control, which aims to reduce the number of chemical treatments, the use of low-toxic and selective pesticides, and the use of biological methods.

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## DETERMINING OF THE PARAMETERS FOR MOVEMENT ON A VACUUM SOWING APPARATUS OF SEEDER SECTION FOR PNEUMATIC PRECISION SEED DRILL

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### *Abstract*

*The parameters necessary for driving the working bodies from the sowing section of the precision seed drill are substantiated. With these machines, the seeder disc is mechanically driven. The seeds are retained in its holes on seed disk by the force created by the vacuum. The stepless mechanical drive allows for the realization of different sowing rates (pieces of seeds per decare). The force required to hold the seed to the sowing disc varies to some extent due to differences in weight. In the present work are researched and determined the power for driving the sowing disc and the pneumatic units for creating a vacuum. The obtained results were used to determine the elements of a device for mechatronic control of the operation on a seed section from a precision seed drill.*

**Key words:** *pneumatic seed drill, power, vacuum, corn.*

### INTRODUCTION

In the cultivation of agricultural crops sowing is one of the most responsible operations, as correct the chosen technology, the exact adjustment of the sowing rate depending on the specific soil and climatic conditions, determine future yields (Kuvaytsev et al., 2014; Kuvaytsev et al., 2014).

The growing trend of increasing power worldwide and as a result and the energy saturation of tractors has a detrimental effect on environmental situation (Lovkis et al., 2016). The repeated passing of the equipment in the field during the cultivation of plant production leads to intensive compaction of the arable and subsoil layers of the soil, as a result of which the yields are reduced and the energy consumption during tillage is increased. At the same time, in the case of powerful tractors with an internal combustion engine of 300-350 hp, there is insufficient efficiency in their use due to the impossibility of realizing all their power in the the existing ones drives of the working bodies (PTO, towing from the support wheels, etc.).

The solution to these problems is the development and use of combined tillage and sowing units, combining operations and using

machines with active working bodies. One of the ways to increase the efficiency when using the tractor is to apply different types of drives to the working bodies of agricultural machinery - pneumatic, hydraulic, electric or combined. In the case of seed drills, one of the most promising and reliable, with good quality indicators and precise adjustment of the sowing rate, is the mechanical drive of the seed drills by means of reducers or variators.

Currently, one of the most common ways to change the speed of rotation of seed drills is the use of replaceable gears and reducers (Antonov and Laryushin, 2011). Unfortunately, this method has a number of disadvantages that affect sowing and ultimately the harvest. Thus, one of the main disadvantages is the difficulty in regulating the sowing rate due to the abrupt change in gear ratio of the gearbox.

The change of the sowing rate with the help of a reducer has some significant shortcomings, which affect the deterioration of the accuracy of its adjustment, reduce the uniformity of seed distribution, increase the traumatized seeds, which leads to reduced yields and increased seed costs (Kuvaytsev et al., 2014; Kuvaytsev et al., 2014). The operation of seed drills with a variator is the most promising, but is currently poorly studied.

The variator (from Latin *variātor*) is a device that transmits torque and is able to smoothly change the gear ratio in a certain range of regulation (Nezhizhimov, Kushnarev and Savostina, 2018). The gear ratio is changed automatically, according to a set program or manually. The adjustment range (ratio of the largest to the smallest gear ratio) is usually 3-6 and less often 10-12. The variator is characterized by high reliability and simplicity in its manufacture (Klenin and Sakun, 1994).

At the present time (Kuvaytsev, Laryushin and Mamonov, 2015) in the Penza Agricultural Academy was designed, manufactured and tested hump-lever variator for driving the seed drills. When using it, the following result is obtained - when it is positioned as a driving element of machines and mechanisms, it is possible to set the output shaft virtually any law of motion, as there is a possibility to smoothly adjust the speed of rotation of the output shaft of the variator. its uniformity of rotation, the vibrations in the variator are reduced, the kinematics of the drive is simplified due to the small number of details.

To adjust the sowing rate, the gear ratio in the seed drill system must be precisely determined. An interesting methodology for this offers (Lysy, 2015).

Increasingly, work is being done to replace the mechanical gearbox for driving the shaft of seed drills with electric drives (Gorobey and Tarimov, 2009). In this case (developed stand) the shaft is driven by a stepper motor and a driver for the motor (with modulations up to 200 step pulses in s). It also contains a simulator on the seed drill wheel, powered by an electric motor and a microprocessor control panel. On the model, with the help of specially developed tachometers, the speed of rotation of the seed drill wheel and the speed of rotation of the shaft of the seed drills are measured. The forward speed (respectively the speed of rotation of the wheel) varies from 2.5 to 12 km/h (11.3 to 54 min<sup>-1</sup>). The stepper motor sets a different speed of rotation of the seed drill shaft and realizes a different gear ratio (from 0 to 70). Studies have shown that with a gear ratio of up to 30, the relationship between the speed of rotation of the wheel and the shaft of the seeders is linear, which is desirable when adjusting the sowing rate.

A frequency converter can be used to drive and adjust electric motors. At low engine speeds, cooling problems occur. At high speeds this problem is not observed, but there is a risk of rapid damage to the bearings. By using special motors designed for operation at different speeds, the above problems are avoided, but the cost of the drive rises. The variator allows asynchronous motors to operate in rated mode. Its disadvantage is its smaller control range (e.g. 6 vs. 10 for a frequency converter).

The introduction of continuously variable transmission at the expense of frequency converters and special motors in the drive of conveyors and other equipment provides the following advantages: the main part of the drive remains and consists of standard mechanisms; the use of a variator allows the application of standard asynchronous motors that will operate in rated mode. It is economically advantageous to use such a drive because the motor life remains the same and the value of the variator is lower than that of the frequency converter and other devices.

When sowing oilseeds, the use is promising (Brichagina, Palvinsky and Orlov, 2017) of sowing apparatus controlled by an electronic system. It was found that for sowing rapeseed and other small-seeded crops, a seeder equipped with an electronic seed metering device meets all agronomic requirements and works much better than those equipped with a mechanical transmission system. Practically does not damage the seeds, allows very precise adjustment of the required sowing rate, ensures sustainable sowing and even distribution of seeds in rows and between them.

## MATERIALS AND METHODS

The corn seeds used are of the KC 4568 variety with an average weight per 1000 grains (MKV), 316.1 g (Petrovska and Dimova, 2012).

The sowing disc is green, 30/5 (30 holes with a hole diameter of  $d = 5$  mm) for corn seeds weighing 1000 g and up to 320 g.

The study is in two stages.

The first stage is to determine the required torque to drive the drill seed drill according to the following diagram shown in Figure 1.

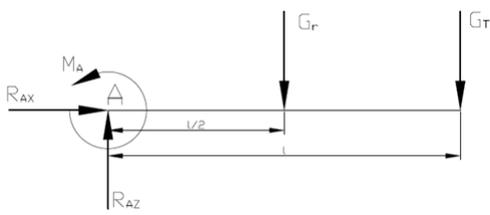


Figure 1. Scheme for determining the torque for driving the seed drill

After weighing the weights  $G_r$  and  $G_T$ , the moment is calculated by the formula:

$$M = g \cdot \left( G_r \cdot \frac{l}{2} + G_T \cdot l \right) \quad (1)$$

To meet different sowing rates (number of seeds per decare) and sowing step (distance between the seeds in a row) is determined the required range of speed change ( $n_{SD}$ ) of the seed disc relative to the speed of the seed drill. The gear ratio of the gear ( $i_{red}$ ) between the seed disc and the drive shaft of the drill section is determined. The values obtained are used to calculate the range of variation of the speed of the electric drive motor ( $n_{el.m}$ ).

For the calculations of the power ( $P_1$ ) required to drive the section, the largest (highest) revolutions are taken from the drill ( $n_{el.m MAX}$ ).

$$P_1 = M \cdot \omega, [W], \quad (2)$$

where:  $M$  [Nm] - torque for driving the sowing disc;  $\omega$  [rad / s] - angular velocity of the drive shaft of the drill section.

Превръщането от обороти  $n_{el.m MAX}$  [ $\text{min}^{-1}$ ] в ъглова скорост  $\omega$  [rad/s] е по следната формула:

The conversion from speed  $n_{el.m MAX}$  [ $\text{min}^{-1}$ ] to angular velocity  $\omega$ , [rad/s] is by the following formula:

$$\omega = \frac{2 \cdot \pi \cdot n}{60} \quad (3)$$

To determine the power of the electric drive motor, the power reserve factor ( $K_3$ ) is selected according to tabular data (Trifonov, 2015) and is calculated by the formula:

$$P_{el.m} = P_1 \cdot K_3, W \quad (4)$$

With the obtained results a standard gear motor with electronic speed control (the nearest higher standard value) is selected.

The second stage is to determine the parameters of the vacuum fan. The largest values of the

weight of the maize seed with which the sowing disc is intended to work are taken, namely 320 g per 1000 corn seeds. It follows that the mass of 1 seed will be  $M_{\text{corn grain}} = 320/1000 = 0.32$  g.

The disk has 30 holes, 26 of which are under the influence of vacuum.

The mass of the sucked grains is determined:

$$M_a = (M_{\text{corn grain}} \cdot 26)/1000, \text{ kg} \quad (5)$$

For subsequent calculations, the kilograms are converted into newtons ( $1 \text{ kg} \approx 10 \text{ N}$ ):

$$G_a = M_a \cdot 10, \text{ N} \quad (6)$$

Determination of air consumption  $Q_B$  by the formula:

$$Q_B = \frac{G_a}{3600 \cdot \mu \cdot \gamma_B}, \text{ m}^3/\text{s} \quad (7)$$

where  $G_a$  [N] is the weight of the seeds sucked on the sowing disc;  $\mu$  - weight concentration of the mixture;  $\gamma_B = 12.4$  [ $\text{N}/\text{m}^3$ ] - bulk density of air.

They are selected from the reference literature (Krasnikov, 1981).

According to calculation data, a standard fan with electronic speed control (the nearest higher standard value) is selected.

## RESULTS AND DISCUSSIONS

On an experienced path were established values of  $G_r = 122$  g and  $G_T = 197$  g. According to formula 1, calculate the required torque to drive the seed disc ( $M$ ).

$$M = 9.81 \cdot \left( 0.122 \cdot \frac{0.615}{2} + 0.197 \cdot 0.615 \right)$$

$$M = 1.56 \text{ Nm}$$

The speed of the input shaft of the sowing section was measured -  $n_{\text{input shaft}} = 3.347$  (the angle of rotation  $\alpha_{\text{input shaft}} = 1205^\circ$ ) with one full rotation of the sowing disc -  $n_{SD} = 1$  ( $\alpha_{SD} = 360^\circ$ ). The gear ratio between the input shaft and the seeding disc has been determined -  $i_{red}$ .

$$i_{red} = \alpha_{\text{input shaft}} / \alpha_{SD} = 1205/360 = 3.347$$

$$i_{red} = n_{\text{input shaft}} / n_{SD}$$

$$n_{\text{input shaft}} = i_{red} \cdot n_{SD} = 3.347 \cdot 1 = 3.347$$

The required sowing step  $s$  varies from 12 to 26 cm.

Given that the sowing disc has 30 holes, the distance traveled by seed drill S for 1 revolution ( $n_{SD} = 1$ ) will depend on the sowing step s.

The linear speed of the seed drill V varies from 4 to 16 (18) km/h. Due to the peculiarities of the field it is limited in the range from 6 to 12 km/h.

For the calculations it is necessary to convert the speed of the seed drill V from km/h to m/min.

After comparing the data for the sowing step s, the seed drill speed V, the gear ratio  $i_{red}$  and the number of holes in the seed disc (30 pieces), the range of variation of the input shaft speed is determined. The results are shown in Table 1.

Table 1. Input shaft speed  $n_{input\ shaft}$  [ $min^{-1}$ ] depending on travel speed V and step s

Sowing step s		Traveled way from the seed drill S for 1 revolution of the seeding disc	Seed drill speed V									
			km/h	5	6	7	8	9	10	11	12	
			m/min	83.33	100.00	116.67	133.33	150.00	166.67	183.33	200.00	
cm	m	m										
12	0.12	3.6		77.48	92.97	108.47	123.96	139.46	154.95	170.45	185.94	
13	0.13	3.9		71.52	85.82	100.12	114.43	128.73	143.03	157.34	171.64	
14	0.14	4.20		66.41	79.69	92.97	106.25	119.54	132.82	146.10	159.38	
15	0.15	4.50		61.98	74.38	86.77	99.17	111.57	123.96	136.36	148.76	
16	0.16	4.80		58.11	69.73	81.35	92.97	104.59	116.22	127.84	139.46	
17	0.17	5.10		54.69	65.63	76.57	87.50	98.44	109.38	120.32	131.25	
18	0.18	5.40		51.65	61.98	72.31	82.64	92.97	103.30	113.63	123.96	
19	0.19	5.70		48.93	58.72	68.51	78.29	88.08	97.87	107.65	117.44	
20	0.20	6.00		46.49	55.78	65.08	74.38	83.68	92.97	102.27	111.57	
21	0.21	6.30		44.27	53.13	61.98	70.84	79.69	88.54	97.40	106.25	
22	0.22	6.60		42.26	50.71	59.16	67.62	76.07	84.52	92.97	101.42	
23	0.23	6.90		40.42	48.51	56.59	64.68	72.76	80.85	88.93	97.01	
24	0.24	7.20		38.74	46.49	54.23	61.98	69.73	77.48	85.22	92.97	
25	0.25	7.50		37.19	44.63	52.06	59.50	66.94	74.38	81.82	89.25	
26	0.26	7.80		35.76	42.91	50.06	57.21	64.37	71.52	78.67	85.82	

From the data given in Table 1 it can be seen that the minimum speed should be 42 ( $n_{input\ shaft\ (min)} = 42\ min^{-1}$ ) and the maximum 186 ( $n_{input\ shaft\ (max)} = 186\ min^{-1}$ ).

The power for driving the sowing section  $P_1$  according to formulas 2 and 3 is determined and the maximum revolutions ( $n_{input\ shaft\ (max)} = 186\ min^{-1}$ ) are taken.

$$\omega = \frac{2 \cdot 3.14 \cdot 186}{60} = 19.48\ rad/s$$

$$P_1 = 1.56 \cdot 19.48 = 30.39\ W$$

$$(P = 0.03\ kW)$$

According to tabular data the power reserve coefficient  $K_3 = 2.0$  is determined. The required

power of the electric motor is calculated by formula 4:

$$P_{el,m} = 30.39 \cdot 2.0 = 60.78\ W$$

$$(P_{el,m} = 0.061\ kW).$$

To determine the parameters of the vacuum fan, the weight of the suction grains according to formulas 5 and 6 is calculated:

$$M_{corn\ grain} = 0.32\ g$$

$$M_a = (0.32 \cdot 26)/1000 = 8.32 \cdot 10^{-3}\ kg$$

$$G_a = 8.32 \cdot 10^{-3} \cdot 10 = 83.2 \cdot 10^{-3}\ N$$

Necessary flow rate on air  $Q_B$  [ $m^3/s$ ] is calculated by formula 7.

According to tabular data:

$$\gamma_B = 12.4 \text{ [N/m}^3\text{]}; \mu = 20$$

$$Q_B = \frac{83.2 \cdot 10^{-3}}{3600 \cdot 20 \cdot 12.4} = 0.0932 \cdot 10^{-6} \frac{\text{m}^3}{\text{s}}$$

## CONCLUSIONS

On the basis of the above, can be formulating the following conclusions:

The methodology for converting the drive of a drill section for precise sowing from mechanical (group) to electrical (individual) has been developed. The parameters of the driving electric motor are determined - power 0.061 kW and revolutions - from 30 to 190.

The required air flow rate  $Q_B$  for individual supply of the seed drill with vacuum has been calculated.

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