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# NO-TILLAGE IMPACT ON HYDRO-PHYSICAL PROPERTIES OF THE SOIL ON CORN CROP

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**Abstract**

Water content is essential for all soil processes. It functions as a transfer medium for nutrients in the soil solution, as well as a source of water needed for plant growth and development. The practice of no-tillage, compared to conventional tillage, has a positive influence on the continuity of the soil pore system, with direct effects on water and air circulation. The present scientific research investigates the dynamics of hydro-physical properties in tillage systems practiced in the experimental field of the Soil Management Department in Ezăreni Farm, Iasi University for Life Sciences, Romania. It is geomorphologically located on the transition Coast of Iasi, in a plateau area, with a cambic chernozem soil, with clay-loam texture. Soil samples were collected from the reference plots in undisturbed natural settlement, using 100 $cm^3$ metal cylinders to determine the hydraulic conductivity. Soil samples were also taken at a depth of 0-90 cm for gravimetric moisture determination and WatchDog sensors (SM100) were placed at a depth of 10-30 cm at the beginning of the growing season for volumetric moisture measurement. In the field, the infiltration rate of water into the soil was determined using the double ring infiltrometer. The results confirm that the no-tillage system conserves more water in the soil, has higher saturated hydraulic conductivity values, from 0.196 at 30-40 cm depth to 1.209 (cm/s x $10^{-2}$) at 20-30 cm depth, and the infiltration rate determined in the field is 4% higher compared to the conventional system.

**Key words**: soil moisture, infiltration rate, saturated hydraulic conductivity

Conservation tillage is associated with noninversion of soil, resulting in the maintenance of at least 30% of the soil surface coverage with crop residues after seeding. In particular, conservation tillage measures vary with depth and width of cultivation, and are classified as no-till, ridge-till, strip-till, mulch-till, and reduced-till techniques. In Europe, conservation tillage is practiced on 26% of the total arable land (Madarasz B. *et al*, 2021), in Germany, 34% of all farms are managed under reduced tillage, and only 1% of the arable land is cultivated in no-tillage systems (Zikeli S. *et al*, 2017).

Conservation tillage is well-recognized as a potential strategy to increase the agricultural sustainability by preserving soil and water resources and improving the production economy, among other agroecosystem services (Van den Putte A. *et al*, 2010).

Due to accumulating plant residues on soil surfaces, no-tillage generally has a more intense and immediate effect on topsoil than on subsoil properties. For instance, no-tillage has generally resulted in a greater wet aggregate stability (Bottinelli N. *et al*, 2017), macro-aggregation (Kibet L.C. *et al*, 2016), and water content (Parkin G. *et al*, 2013) at surface soil layer compared with conventional tillage.

Some studies have reported inconsistent results when soil hydro-physical properties were compared between no-tillage and reduced tillage systems. For example, compared with reduced tillage, no-tillage resulted in higher (Acar M. *et al*, 2018; Al-Kaisi M. *et al*, 2014), lower (Alam M. *et al*, 2017; Villamil M.B. *et al*, 2015), or similar (Bottinelli N. *et al*, 2017) aggregate stability and size distribution. Contradictory results can also be found in soil hydraulic properties and crop yield when comparing between reduced and no-tillage systems (Alam M. K. *et al*, 2017).

Conservation tillage, which includes a variety of reduced and zero tillage techniques that leave at least 30% crop residue on the soil surface, has increasingly been adopted as the agricultural best management practice to reduce soil erosion. These tillage practices significantly affect surface hydrologic properties, leading to increased infiltration and reduced runoff (Singh A. *et al*, 2009; Van Wie J.B. *et al*, 2013).

Variable soil bulk density due to human disturbances and environmental effects is an important factor causing temporal and spatial

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variations in soil hydraulic properties (Zhang M. *et al*, 2017; Tian Z. *et al*, 2018a). After tillage, soil bulk density tends to increase with time under the influences of gravity, rainfall and water flow into the soil (Tian Z. *et al*, 2018b), which results in a substantial decrease in the saturated soil hydraulic conductivity. The unsaturated soil hydraulic conductivity response to bulk density variation induced by traffic compaction or tillage was shown to exhibit complex behaviors in space and time (Strudley M.W. *et al*, 2008).

Several studies have evaluated the effects of conservation agriculture on the physical parameters of soil (Bhattacharya P. *et al*, 2020; Ajayi A.E. *et al*, 2021). The main improvements reported are in terms of aggregate stability, infiltration rate (Blanco-Canqui H. *et al*, 2018), and air permeability (Vogeler I. *et al*, 2009).

Saturated hydraulic conductivity (Ks) is an important soil property that determines the ability of soil to transmit water under saturated conditions, is potentially affected by management practices such as tillage that impact physical properties, and plays an integral role in many soil, environmental, and hydrological processes. Previous studies showed that Ks can vary considerably over space and time within agricultural fields, indicating spatial and temporal variability (Kargas G. *et al*, 2021).

Blanco-Canqui H. (2018) evaluated the effect of no-tillage, chisel plow, disk and moldboard plow tillage practices on total porosity, water infiltration, saturated hydraulic conductivity, and water retention characteristics under continuous corn after 35 years in silty clay loam soils in eastern Nebraska. They reported no significant differences in any of these soil hydraulic properties among the four tillage systems. Meanwhile, numerous studies suggested that tillage increases saturated hydraulic conductivity as a result of increasing soil macroporosity and disturbance induced by mechanical effects of tillage processes (Schluter S. *et al*, 2020; Haruna S.I. *et al*, 2018).

Haruna (2018) studied the influence of tillage and cover crops on soil hydraulic properties including saturated hydraulic conductivity. They concluded that tillage increased the proportion of coarse soil mesoporosity by 32%, resulting in 87% greater saturated hydraulic conductivity compared to under no-tillage practices.

There is not a singular conservation tillage system that can satisfy all agroecosystem services for soil quality, environmental sustainability, and economic outcome across soils, climates, and crops. Hence, there is a need for region-specific designs of tillage and cropping systems to obtain the maximum outcome within certain climatic and terrestrial boundaries. Our research is based on a field experiment established in 2014 on a cambic chernozemic soil with a rotation of winter wheat, maize, sunflower and pea, where maize crop was studied. Our research objective was to determine some hydrophysical parameters such as bulk density, total porosity, hydraulic conductivity, infiltration rate, gravimetric moisture, volumetric moisture and electrical conductivity.

## MATERIAL AND METHOD

In this study, the dynamics of hydro-physical properties during the agricultural year 2022-2023 in corn crop are investigated. The experimental field (*figure 1*) where the study was conducted belongs to the Soil Management Department, being located in Ezăreni farm, Iasi University for Life Sciences, Romania. From a geo-morphological point of view, the farm is located in the transition coast of Iasi, in a plateau area, with cambic chernozem soil (SRTS 2012, a clay-loam texture and 6.8 pH units.

The total area is 16 ha, of which 8 ha are cultivated in conventional system (CT) and 8 ha in no-tillage (NT), using the Fabimag FG – 01 seed drill (*figure 2*). The maize crop area is 4 ha, divided into two plots, one for each tillage system.


Figure 1 **The experimental field**

Figure 2 **Seed drill Fabimag FG-01**

The experimental field has been cultivated in the no-tillage system since 2014, with a 4-year rotation of winter wheat, maize, sunflower and peas.

The experimental site has a annual average temperature 10.8˚C and precipitation of 691 mm and 432.8 mm in 2021 and 2022, respectively. In 2023, until the second decade of September, 336.4 mm of precipitation was recorded, with a maximum of 136 mm in July and a minimum of 5.6 mm in March. In terms of temperature variation in 2023, they ranged from -16.4 ºC (9 February) to 38.8 ºC (28 August), data recorded by Ezăreni weather station (*figure 3*).

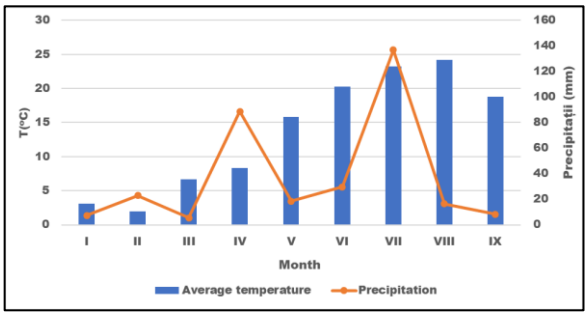



Figure 3 **Precipitation amounts and average monthly temperatures for January-September 2023**

For the measurements of bulk density (BD) and saturated hydraulic conductivity (Ks), undisturbed soil samples were taken, after sowing and during the growing season using stainless-steel cylinders with a height of 5.1 cm and diameter of 5.0 cm (volume: approximately 100 $cm^3$) on 4 depths between 0 and 40 cm.

For BD determination soil samples were transferred to the laboratory, placed in an oven at 105 °C and dried to a constant weight. The weight of the soil was recorded and the BD was calculated using Eq. 1(Canarache A., 1990):

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{weight of oven dried soil}}{\text{volume of the soil}} \quad \text{(Eq. 1)}$$

Ks was measured using a falling-head permeameter (Hauben water permeameter, Eijkelkamp Agrisearch Equipment) under conditions of instationary flow. For the Ks measurements, the samples were pre-saturated for two days in a container and then flooded for the measurement. The Ks was calculated by Equation (2) (Lal R. *et al*, 2004, Eijkelcamp, 2017).

$$k = \frac{a * l}{A * t} * \ln \frac{h1}{h2} \quad \text{(Eq. 2)}$$

where a is the burette area ($cm^2$), l is the height of the stainless-steel cylinders (cm), A is the area of the stainless-steel cylinders ($cm^2$), t is the time interval (s), and h1 and h2 are the water level at the start and end points of the measurements (cm).

The double ring infiltrometer (DRI) method was used to measure the infiltration rate of water in soil. The DRI consists of two open stainless-steel rings, of which one is placed inside the other. The inner and outer rings are 300 mm and 550 mm in diameter, respectively. By partially driving these into the soil and filling the rings with water, an above-ground reservoir is thus formed that is directly located above the set of soil that is being tested. The outer ring limits the lateral spread of water after infiltration so that one-dimensional, vertical flow is promoted beneath the inner ring (ASTM, 2009).

Infiltration rate measurements were carried out in July, in the second decade, on two consecutive days, in order to have similar soil moisture conditions in both systems. The infiltration rate was calculated by Equation (3) and (4):

$$v(t) = \frac{1}{2} * S * t^{-\frac{1}{2}} + A \quad \text{(Eq. 3)}$$

$$K = \frac{A}{m} \quad \text{(Eq. 4)}$$

where v(t) is infiltration rate, s is sorptivity, t is time, K is field saturated hydraulic conductivity, A is a parameter and m is a constant equal to 0,66667.

To determine the gravimetric soil moisture content in the experimental field, soil samples were taken in six intervals up to 90 cm depths (0–10, 10–20, 20–30, 30–50, 50–70, and 70–90 cm) with three replicates at each interval. Soil (20–25 g) from five points were collected individually from each plot in aluminium vials. Moisture content was determined in the laboratory by the gravimetric method, which is considered the standard method for calibrating moisture metres due to its high accuracy.

Spectrum Technologies Water Scout SM100 soil moisture sensors (Spectrum Technologies, Aurora, IL) were installed on 31 May 2023 at 10, 20 and 30 cm soil depths to monitor soil moisture. At a depth of 30 cm, SM300 sensors were installed to monitor soil moisture, temperature and electrical conductivity. Soil moisture content was recorded as a percent volumetric water content at 60 min intervals continuously throughout the study period by Watch Dog 1000 micro weather field station (*figure 4*). Data were downloaded monthly to a laptop computer using Spectrum technologies SpecWare 9 pro software. The mean for every day was calculated from 60 min interval data and graphed for the whole study period. The manufacturer specifications provided by Spectrum technologies for Waterscout SM100 sensor indicated that the sensor resolution for volumetric moisture measurement is 0.1% with an accuracy of ±3% at EC <8 mS/cm and 0.5 - 80 ˚C temperature range (Spectrum Technologies, 2019).


Figure 4 **Watch Dog 1000 Micro Weather Field Station with moisture senzor**

The data results are expressed as the means ± standard error (SE). One-way analysis of variance (ANOVA) was used to see the influence of tillage systems on soil compaction. The significant differences between treatments were established using Tukey's post-hoc test with a degree of confidence of 95% using SPSS ver. 26.

## RESULTS AND DISCUSSIONS

### Bulk density (BD) and total porosity (TP)

The influence of soil tillage system on BD is observed by a lower variation over time due to the non-working and less passes with agricultural machinery that cause soil compaction.

The BD has significantly different values between 1.21 and 1.42 g/cm$^3$ in the conventional system, with a maximum at a depth of 30-40 cm, which remains high during the growing season (*table 1*). In the NT system, the lowest values are recorded at the soil surface, in the 0-10 cm layer, of 1.24 at sowing and 1.26 g/cm$^3$ in vegetation, with a maximum registered at sowing of 1.45 g/cm$^3$ in the 10-20 cm soil layer, a density that decreases slightly during the vegetation period. With the exception of samples taken after sowing, the NT has a lower BD value in the topsoil layer. These results are in accordance with results of Gao *et al*, (2019) that reported significant higher BD value, in the 0–10 cm layer in CT than that of NT. A recent review paper synthesizing results from 62 studies indicated that NT can either increase or decrease soil BD, depending on the longevity of tillage management (Blanco-Canqui H. *et al*, 2018). In both tillage systems the BD is low (1.19-1.31 g/cm$^3$) to medium (1.32-1.45 g/cm$^3$) (I.C.P.A., 1987).

The magnitude of differences in BD among tillage systems can also be affected by the time of sampling. BD is generally the lowest immediately after tillage operation and gradually increases during the growing season, due to climatic factors and the mechanical load exerted on the soil surface (Leik F. J. *et al*, 2002).

TP has significantly different values by depth at the beginning of the growing season under both systems, with a maximum value in the 0-10 cm soil layer of 54.68 % v/v in CT and 53.41 % v/v in NT and a minimum value of 47.39 % v/v in the 30-40 cm soil layer in CT and 45.73 % v/v in the 10-20 cm soil layer in NT.

During the growing season, TP values decrease in all depths studied, with the exception of the 10-20 cm soil layer, where porosity is improving in NT, and on the 20-30 cm layer in CT. A study conducted by Shittu K. A. (2017) reported that soil porosity is not influenced by tillage practices significantly. This result disagreed with the study of Amin M (2014), reported that NT system reduced of TP.

Tabel 1

**Bulk density (BD) and total porosity (TP) as affected by tillage systems at depths between 0 and 40 cm**

| | BD (g/cm$^3$) | | TP (% v/v) | |
|---|---|---|---|---|
| | Sowing | Vegetation | Sowing | Vegetation |
| **Tillage treatments** | | 0 – 10 cm | | |
| **CT** | 1.21 ± 0.04 a | 1.33 ± 0.15 ns | 54.68 ± 1.39 b | 50.26 ± 5.42 ns |
| **NT** | 1.24 ± 0.03 a | 1.26 ± 0.02 a | 53.41 ± 1.20 b | 53.07 ± 0.67 b |
| | | 10 – 20 cm | | |
| **CT** | 1.29 ± 0.03 ab | 1.38 ± 0.05 ns | 51.51 ± 0.95 ab | 48.51 ± 1.82 ns |
| **NT** | 1.45 ± 0.02 b | 1.41 ± 0.03 b | 45.73 ± 0.85 a | 47.29 ± 1.12 a |
| | | 20 – 30 cm | | |
| **CT** | 1.36 ± 0.03 b | 1.27 ± 0.03 ns | 49.02 ± 1.21 a | 52.70 ± 1.15 ns |
| **NT** | 1.37 ± 0.02 b | 1.38 ± 0.02 b | 48.63 ± 0.90 a | 48.22 ± 0.81 a |
| | | 20 – 30 cm | | |
| **CT** | 1.40 ± 0.03 b | 1.42 ± 0.03 ns | 47.39 ± 1.01 a | 47.12 ± 1.04 ns |
| **NT** | 1.38 ± 0.03 b | 1.42 ± 0.02 b | 48.24 ± 1.02 a | 46.98 ± 0.65 a |

Note: CT – conventional system; NT – No-tillage system. Mean ± standard error of each column is reported in correspondence with each experimental treatment. Within each column: values associated with the same lower-case letters are not statistically different at $p \leq 0.05$ according to Tukey's test.

### Saturated hydraulic conductivity (Ks)

The Ks values after sowing are higher in the 0-10 and 10-20 cm depths due to seedbed preparation. Later, during the growing season, the Ks decreases in the CT system at depths 0-10 and 10-20 cm, but increases at depths 20-30 and 30-40 cm (*table 2*). In the NT the Ks improved during the growing season, with higher values compared to the CT. Similar results were obtained by Lavinia Burtan (2020), who performed a determination in September obtaining a higher conductivity in the NT (2.4 x $10^{-2}$ cm/s) compared to the CT system (1.9 x $10^{-2}$ cm/s). Contradictory results have been found regarding the soil tillage effects on Ks. In some situations, higher conductivity values have been observed in NT than CT (Alvarez R. *et al*, 2009; Singh P. *et al*, 2014), whereas the opposite have been reported in others (Soracco C.G. *et al*, 2012). De Moraes (2016) showed greater Ks for NT than CT at 10 cm depth.

Table 2

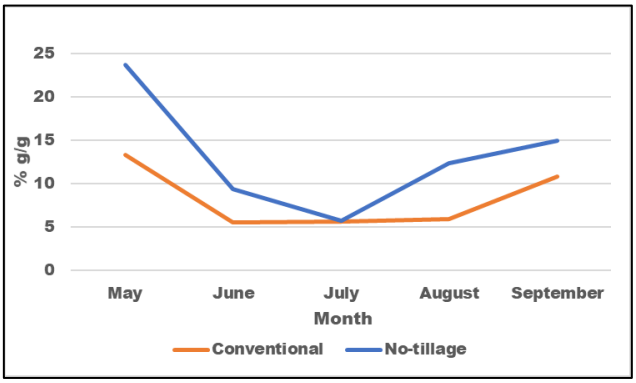
**Effects of soil tillage on Saturated hydraulic conductivity (Ks)**

| ***Specification*** | **Sowing** | | **Vegetation** | |
|---|---|---|---|---|
| | (x $10^{-2}$ cm/s) | | | |
| *Tillage system* | CT | NT | CT | NT |
| *0-10 cm* | 2,386 ± 0.09 c | 1,992 ± 0.06 b | 2,081 ± 0.05 b | 2,524 ± 0.04 a |
| *10-20 cm* | 2,079 ± 0.05 b | 1,653 ± 0.01 a | 1,906 ± 0.03 a | 2,569 ± 0.03 a |
| *20-30 cm* | 1,807 ± 0.08 a | 2,510 ± 0.12 c | 2,022 ± 0.03 b | 3,244 ± 0.06 b |
| *30-40 cm* | 1,914 ± 0.05 ab | 2.055 ± 0.04 b | 2,357 ± 0.07 c | 2,629 ± 0.05 a |

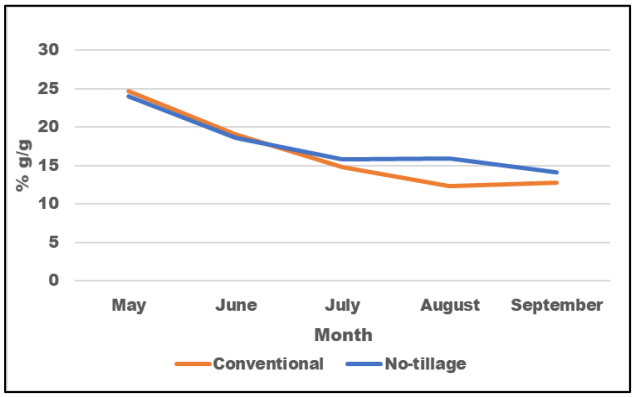
Note: CT – conventional system; NT – No-tillage system. Mean ± standard error of each column is reported in correspondence with each experimental treatment. Within each column: values associated with the same lower-case letters are not statistically different at $p \leq 0.05$ according to Tukey's test.

**Gravimetric soil water content**

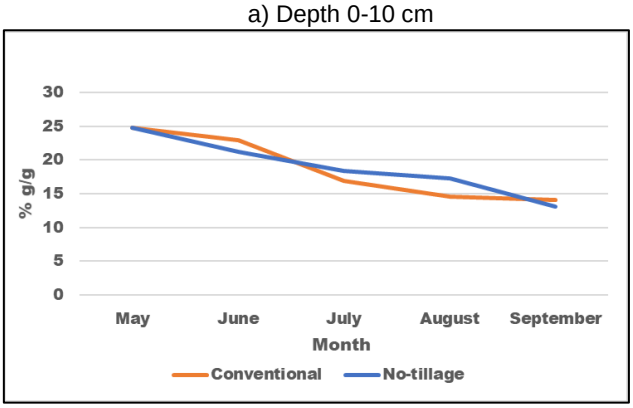
Moisture content was affected by tillage systems, especially in the upper soil layer (*figure 5a*). At 0-10 cm depth (*figure 5b*), the NT system has a higher water content throughout the growing season with differences ranging from 2% in July to 78% in May. NT reduces water evaporation and increases water availability for plant growth (Wazzan F. *et al*, 2022).



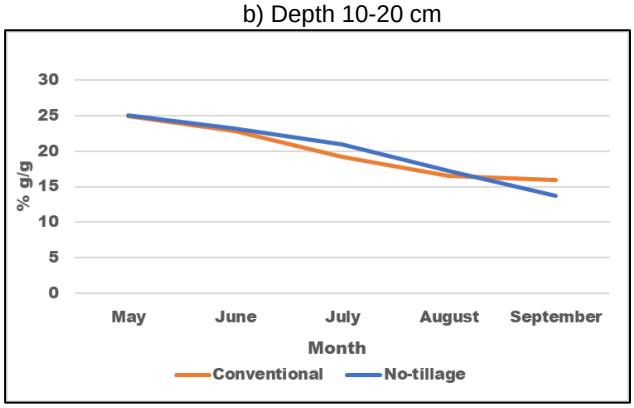

a) Depth 0-10 cm



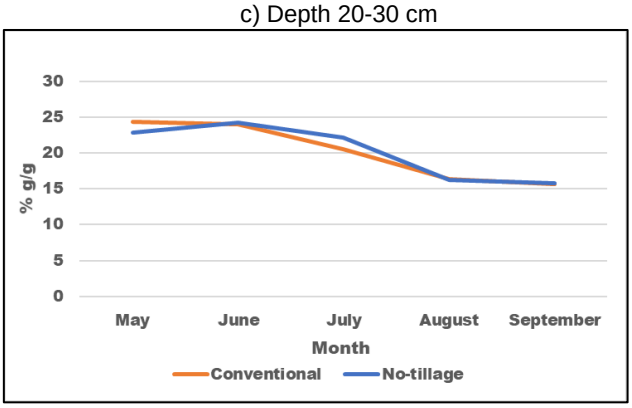

b) Depth 10-20 cm



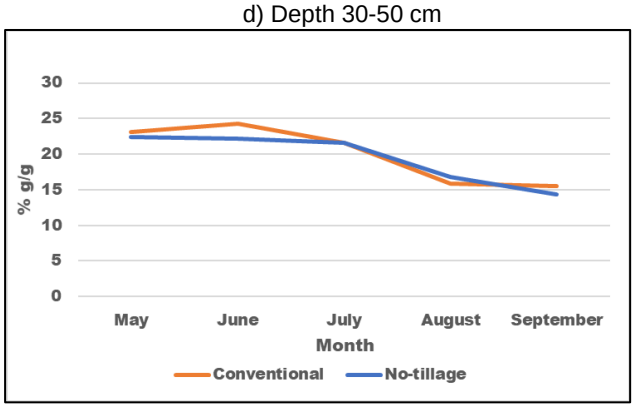

c) Depth 20-30 cm




d) Depth 30-50 cm




e) Depth 50-70 cm




f) Depth 70-90 cm

Figure 5 **Influence of tillage systems on gravimetric soil moisture**

**Infiltration rate**

The soil infiltration rates of different treatments decreased with increased infiltration time, and the changes of infiltration rates with time for all treatments were best displayed as a graphic. The average soil permeability values were 5.35 in NT and 5.14 cm/hour in CT, which shows that soil permeability was faster in the conservative system (*figure 6)*. Permeability value shows the ability of soil to pass water either laterally or horizontally. Permeability is influenced by the porosity and bulk density of the soil and closely related to soil texture (Arora V.K. *et al*, 2011).

Blanco-Canqui, H. (2018) compared 24 studies on water infiltration, and in only 15 studies the NT had a higher water infiltration compared to the CT. However, a few studies have indicated that NT may decrease or have no effect on water infiltration, suggesting that NT does not always increase water infiltration. Reduced till often has intermediate values of water infiltration between NT and CT.

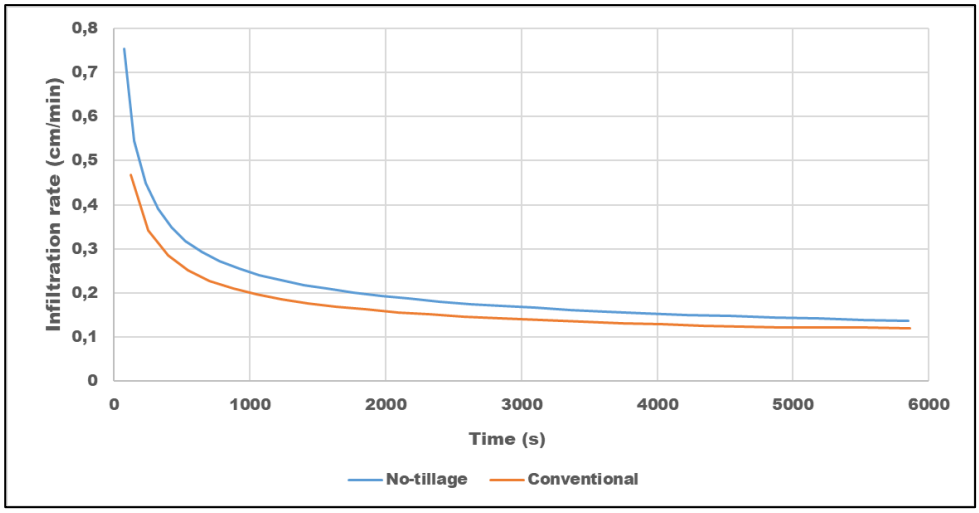



Figure 6 **Infiltration rate**

For the depth of 10-20 cm in May and June the soil moisture is slightly higher in the CT, and for the rest of the growing season the NT has a significant higher moisture content compared to the CT. The NT has a higher moisture value in July and August for the soil layer between 20 and 90 cm, and for the rest of the season the moisture is basically the same or slightly lower.

It has reported by Romaneckas K. (2009) and Slawinski C. (2012) that NT is important for saving the moisture content of the soil. The reason is that in the NT the crop residue is maintained on the ground surface that reduces water evaporation and more infiltration rate (Shittu K.A. *et al*, 2017). In the case of CT, the soil moisture content will be fluctuated after the periods of rainfall.

**Volumetric soil water content**

The soil moisture dynamics of the study field were determined at four soil depths from 31 May to 28 September 2023 (*figure 7)*.

According to the results obtained, the NT had a higher moisture content throughout the growing season for every depth studied. The exception was the 20 cm soil layer where the CT had a slightly higher moisture content in June.

Volumetric moisture values varied at each depth as follows, at 10 cm depth the CT system recorded a minimum of 15.54 % v/v on 14 June and a maximum of 40.36 % v/v on 8 July, and the NT system had a minimum moisture on 28 September of 24.7 % v/v, while the maximum on 8 July was 49.18 % v/v. At a depth of 20 cm the CT system recorded values between 28.9 (28 August) and 46.3 % v/v (8 July), compared to 36.2 (4 July) and a maximum of 49.5 % v/v (8 July). Finally, on the last depth studied, moisture values ranged from 24.45 (24 September) to 39.59 % v/v (8 July) in the CT system and from 27.8 (4 July) to 51.65 % v/v (8 July) in the NT system.

The most significant increase in moisture was recorded in the first decade of July, when the weather station on the farm recorded 97.4 mm.

During the growing season, following the significant amounts of precipitation, the moisture in the soil surface in the CT exceeded for a short time the NT.

Similar results were reported by Agbede T.M. (2010), who found significantly higher SWC under NT compared to CT. The same results were obtained by Semenikhina Julia (2020) in a study performed for a period of 2 years, where a higher moisture content was obtained in the NT using SM100 sensors.

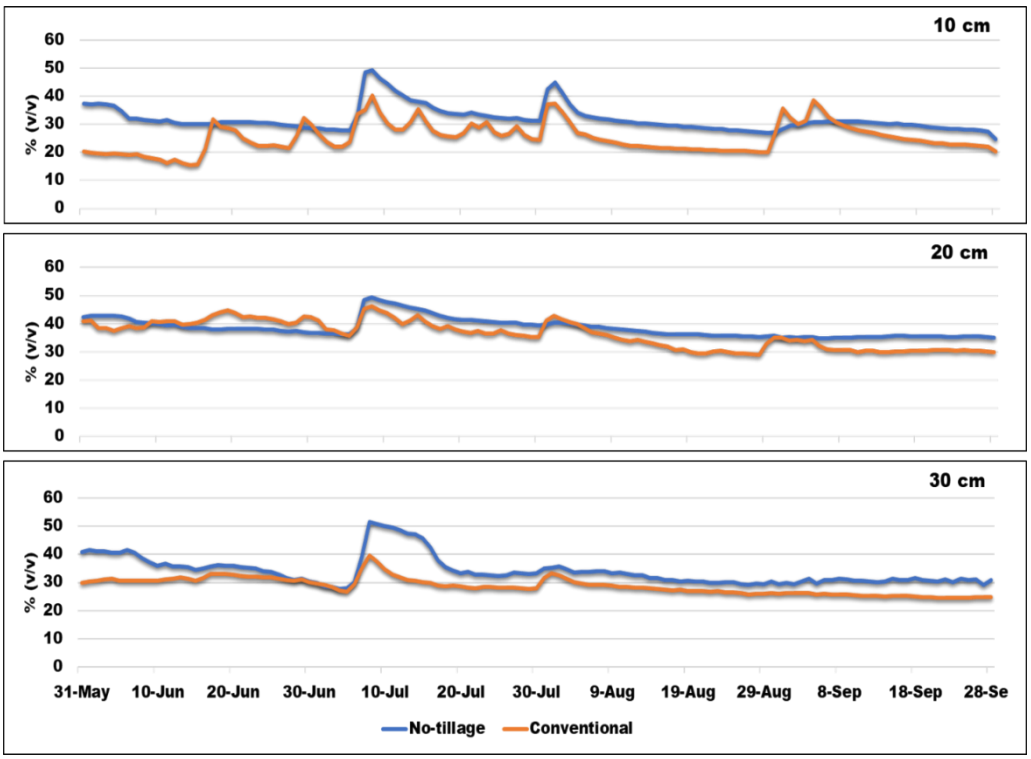



Figure 7 **Influence of tillage systems on the volumetric soil moisture**

**Soil electrical conductivity (Ec)**

Ec was measured using SM300 type sensors installed in both tillage systems at a depth of 30 cm. The results (*figure 8*) indicate a significantly better EC in the NT throughout the growing season, with a minimum of 0.38 mS/cm on 5 September and a maximum of 0.79 mS/cm on 7 July, except for the period from the beginning of the growing season between 1 and 10 June, when the CT recorded a higher Ec.

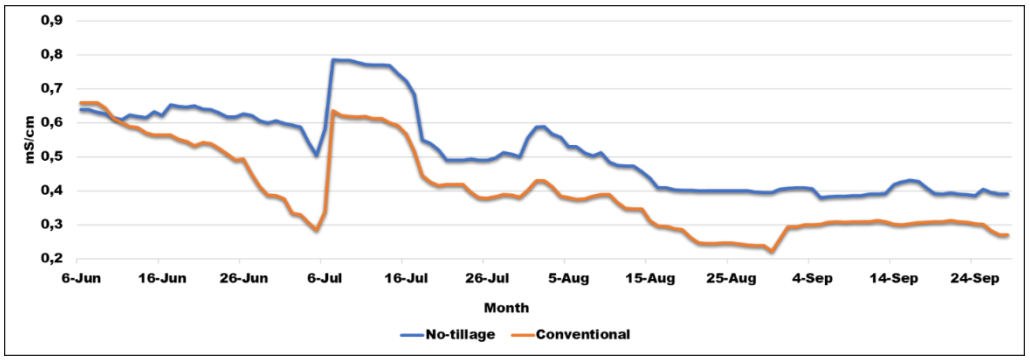



Figure 8 **Influence of tillage systems on the soil electrical conductivity**

## CONCLUSIONS

The NT has a substantially impacts on soil hydro-physical parameters. The implementation of the NT on cambic chernozem soil in the climatic conditions of the Ezăreni farm, in an agricultural year affected by the current climatic problems, the most serious being drought, has better results compared to the CT. Increased BD values, higher Ks in vegetation period by 8% in 30-40 cm to 58% in 20-30 cm soil layer, higher soil moisture content, 4% better water infiltration rate and improved Ec throughout the growing season were reported.

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# INFLUENCE OF CONSERVATION TILLAGE ON THE MAIN SOIL PHYSICAL PROPERTIES OF WINTER PEA CROP IN CONDITIONS OF EZARENI FARM, IAȘI, ROMANIA

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**Abstract**

The aim of this study is to investigate the effects of different tillage on the main soil physical properties in the conditions of Ezareni Farm, Iasi, Romania. The study was conducted at the Ezareni Farm within the Didactic Station of the University of Life Sciences ,,Ion Ionescu de la Brad" Iasi. The study was carried out on a cambic chernozem soil type. Two tillage systems, the conservative or no- tillage (NT) and the conventional one (CT), have been evaluated. The soil bulk density, the soil moisture and water stable aggregates were determined for each tillage system. The bulk density was determined measuring both the soil weight and volume at sampling. These soil samples were taken from four different layers: 0-10, 10-20, 20-30 and 30-40 cm. Regarding the water stability of soil aggregates, the soil samples were also taken at different depths and the soil moisture content was evaluated. Soil samples were taken down to a depth of 90 cm. Sampling was carried out in three replicates for each depth. The no-till system had a highly significant effect on the bulk density of the soil, particularly at a depth of 10-20 cm (NT2), with 1.50 g/cm$^3$ being the highest value obtained. The same effect was observed for soil aggregate stability under the same treatment and at the same depth, where the highest value was 90.16%. Regarding to soil moisture, it took the second year of study to obtain a significant effect.

**Key words**: No-tillage, soil physical properties, winter pea, Ezareni

Today, there is great concern about food security and environmental conservation, as it is projected that food production would need to increase by at least 70% before 2050 to support the world's population. Increased demand for food has led to the use of intensive plowing systems to achieve food security (Foley J.D *et al,* 2011; Connor D.J, Mínguez M.I, 2012; Maharjan G.R *et al,* 2018). The response to this growing demand for food production is in fact based on soil conservation systems and also on crops that provide high yields and at the same time preserve the soil, the environment and biodiversity. However, intensive agricultural use is frequently associated with environmental impacts, such as contamination of the freshwater through nitrogen leaching and increased soil erosion (Maharjan G.R *et al,* 2018; Gholizadeh A., Kopackova V, 2019). It is therefore of prime importance to use tillage systems that ensure high crop yields and at the same time conserve soil, water and biodiversity (Franchini J.C *et al,* 2012).

Among the available tillage systems, conventional tillage (CT) plays an important role in the evolution of modern agriculture, contributing to high yields, physico-chemical and biological soil improvement and weed control.

In practice, CT is characterised by the complete inversion of the soil furrow by ploughing. In contrast, conservation systems include no-till (NT). These systems consist of planting crops with minimal or no soil disturbance, leaving at least 30% mulch cover (Giller K.E *et al,* 2015). Compared to CT, NT systems have gained worldwide popularity due to reduced fossil fuel consumption and improved soil carbon content, soil structure and water infiltration, which can increase farm sustainability and optimize productivity over time (Deubel A. *et al,* 2011; Ciric V. *et al,* 2012; Villamil M.B, Nafziger E.D; 2015; Zhao X. *et al,* 2017).

In Romania and abroad, research in the field of agricultural tillage systems has been directed towards finding ways to improve soil structure, reduce soil compaction, improve water and air regime, improve organic matter content, soil quality, or air quality. In order to improve the activity of microorganisms and mesoorganisms in the soil, knowledge of the changes caused by the chemicals used, the critical limits of pollution (Sin Gh., 2013; Gus P. *et al*, 2003; Ailincăi C. *et al,*

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2004). In Romania, several studies have also been conducted on the influence of different tillage systems on the physical, chemical and biological properties of the soil under wheat (*Triticum aestivum*), maize (*Zea mays*), soybean (*Glycine max*), beans (*Phaseolus vulgaris L.*) or sunflower (*Helianthus annuus),* (Țopa D. *et al,* 2010, 2011; Răus L. *et al,* 2005, Jităreanu G. *et al,* 2006, 2008, 2018), but little or none under pea (*Pisum sativum*) cultivation. We can, however, mention the researches of Marin D.I. *et al.* (2011) on the influence of tillage system on pea, wheat and maize crops in the area of Moara Domneasca - Ilfov in Romania and Bucur G. (2018) on the effect of tillage systems on certain elements and conditions of soil fertility, crop productivity in the maize for seed, pea for seed, in "Chetrosu" area, central area of Republic of Moldova.

The need to increase agricultural production in the face of population growth requires the modernisation of agriculture. Also, the need to preserve and/or improve the good condition of the soil adopts the modernisation of agriculture, but at the same time it should face these influences which are not only positive. An equation with two emerging unknowns and the subject of the study. The physical properties of the soil are influenced from one tillage system to another. To this end, it is important to understand how tillage systems influence soil physical properties.

## MATERIAL AND METHOD

The study was conducted at the Ezăreni Farm within the Didactic Station of the University of Life Sciences ,,Ion Ionescu de la Brad" Iasi, Romania. The study was carried out on a cambic chernozem soil. This soil type is characterized by a loamy clay texture with loessososide deposits. Its humus content varies between 2.7 and 3.4% and has a slightly acidic pH, according to the Romanian Soil Taxonomy System (SRTS 2012).

This study consists of two successive phases. The first phase is the collection of soil samples from the two tillage systems studied, conservative and the conventional tillage systems. These samples were taken from four different soil layers: 0-10, 10-20, 20-30 and 30-40 cm. The samples were then transported to the laboratory for various analyses in the Soil Physics Department of the Research Institute for Agriculture and Environment - Iasi (ICAM).

Before sowing in the conventional system, an autumn ploughing was carried out at a depth of 30 cm with the Valtra T190 tractor and the Lemken Opal 140 reversible plough. In spring, the seedbed was prepared with a T190 tractor in aggregate with a Kompaktor and then sown.

In the conservative system, meaning without ploughing, there was no preliminary tillage and therefore seeding was done directly with the T190 tractor in aggregate with the FABIMAG FG-01 seed drill. The bulk density is defined as the weight per unit volume of absolutely dry soil with natural settlement. In our study we used steel cylinders with a height of 5.1 cm and a diameter of 5 cm. In addition to the cylinders, we also used the sampling knife and labels. Once this surface has been identified and prepared, soil samples can be taken either vertically or horizontally. Cylinders are then placed on this prepared surface and with the aid of a protective ring by tapping with a hammer, the cylinders are driven into the soil to the top edge. The ring was then removed and, using a knife, the edges of the soil in the cylinder were straightened. The ready cylinders were cleaned, labelled and placed in kits for transport to the laboratory. Soil moisture is related to the amount of rainfall, the evaporation rate, the water-holding capacity of the plants and, above all, the type of tillage system the soil is subjected to. To evaluate the soil moisture content, soil samples were taken from six soil layers to a depth of 90 cm.

This sampling was carried out in three replicates for each depth. Samples were taken at two intervals throughout the growing cycle, that is, from sowing to harvesting. Evaluation of the water stable aggregates of soil, is defined like the capacity of these aggregates to resist the action of water. Assessing the capacity of these aggregates involves several methods. The one used in our research laboratory is the Kemper and Rosenau.

## RESULTS AND DISCUSSION

### Bulk density

*Tables 1* and *2* show the average soil bulk density values at different levels and depths after the tillage operation for the two tillage systems considered. Soil bulk density was significantly higher in NT and at different depths, particularly 10-20 cm compared to CT. Similar results also reported by (Osunbitan J.A. *et al,* 2005; Shokoofeh S.K *et al*, 2018; Burtan L. *et al*, 2020), who respectively showed in their study that the highest bulk densities were obtained with the no-till tillage system and at depths of 10-20 cm. On one hand, this difference between tillage systems is explained by the fact that, in the conventional system, ploughing is applied as a basic tillage, reducing the apparent density of the soil due to soil disturbance, while in the conservative system there are no such changes. On the other side, this result can be explained by the presence of plant residues or residues of previous crops in the conservation system, which provide protection against the dispersive action of rainfall and therefore prevent the destruction of soil aggregates. In the conventional system, there is no plant cover, the

soil is carried away by rainwater, with a destructive effect on the aggregates.

It should be noted that the average bulk density values in the second year of the study show an increase in the conventional system to 1.52 g/cm$^3$ and are higher than in the no-till system (1.51 g/cm$^3$). This observation is in line with that of Bucur G. (2018) in his study. This phenomenon can be explained by the frequent passage of tractors and agricultural equipment, consecutive and repeated tillage at the same depths, which causes the soil to settle, contributing to the increase in the bulk density.

Table 1

**Effect of tillage operations on Bulk Density (g/cm$^3$)**

| Parameters | Bulk density at sowing (g/cm$^3$) | Bulk density at harvest (g/cm$^3$) |
|---|---|---|
| **CT1 (0- 10 cm)** | 1.13 ± 0.09 a | 1.13 ± 0.05 a |
| **CT2 (10- 20 cm)** | 1.24 ± 0.12 ab | 1.30 ± 0.12 bc |
| **CT3 (20-30 cm)** | 1.15 ± 0.09 ab | 1.32 ± 0.16 bc |
| **CT4 (30- 40 cm)** | 1.29 ± 0.15 bc | 1.52 ± 0.16 d |
| **NT1 (0- 10 cm)** | 1.25 ± 0.11 ab | 1.21 ± 0.08 ab |
| **NT2 (10- 20 cm)** | 1.49 ± 0.06 d | 1.50 ± 0.05 d |
| **NT3 (20-30 cm)** | 1.46 ± 0.07 d | 1.38 ± 0.06 cd |
| **NT4 (30- 40 cm)** | 1.42 ± 0.06 cd | 1.42 ± 0.08 cd |
| **P** | 7.91e$^{-12}$ | 5.86e$^{-11}$ |
| **P** | *** | *** |

a, b, c, d, e: Means for the same line with different letters are significantly different at the 5% threshold. * : P<0.05; ** : P<0.01; *** : P<0.001.

Table 2

**Effect of tillage operations on Bulk Density (g/cm$^3$)**

| Parameters | Bulk density at sowing (g/cm$^3$) | Bulk density at harvest (g/cm$^3$) |
|---|---|---|
| **CT1 (0- 10 cm)** | 1.29 ± 0.09 abc | 1.22 ± 0.04 ab |
| **CT2 (10- 20 cm)** | 1.34 ± 0.12 ad | 1.34 ± 0.09 cd |
| **CT3 (20-30 cm)** | 1.25 ± 0.14 ab | 1.29 ± 0.06 bc |
| **CT4 (30- 40 cm)** | 1.33 ± 0.13 ad | 1.37 ± 0.05 ce |
| **NT1 (0- 10 cm)** | 1.22 ± 0.12 a | 1.16 ± 0.07 a |
| **NT2 (10- 20 cm)** | 1.47 ± 0.09 d | 1.45 ± 0.08 e |
| **NT3 (20-30 cm)** | 1.42 ± 0.09 cd | 1.39 ± 0.08 ce |
| **NT4 (30- 40 cm)** | 1.41 ± 0.07 bd | 1.44 ± 0.03 de |
| **P** | 0.0000649 | 9.02e$^{-14}$ |
| **P** | *** | *** |

a, b, c, d, e: Means for the same line with different letters are significantly different at the 5% threshold. * : P<0.05; ** : P<0.01; *** : P<0.001.

**Soil moisture**

*Table 3* shows the results for soil moisture at seeding in the first year of the study. These results show that there was no significant difference between the treatments evaluated (NT and CT). However, a significant difference was observed between these treatments at harvesting. And the highest soil moisture values were obtained with the conventional tillage (CT) system. This result can be explained by the low level of soil compaction and the increased space between aggregates in the conventional tillage system have led to an increase in soil water content. Knowing the high organic matter content in the no-till system and the effect of the presence of crop residues and plant cover in contributing to the increase in water retention capacity and therefore moisture, one would expect the opposite effect: to obtain high moisture in this treatment. But the results showed that the effects of reducing soil compaction on soil moisture are very high within a short period of time. This result obtained in this first year of study is in line with those obtained by (Rahimzade R., Navid H., 2011; Martins R.N., 2021). As for the results obtained in the second year (*figure 1*), we note a slight contradiction. The highest soil moisture values were registreed in the no-till system (NT). These results are consistent with those obtained by (Ussiri D.A.N., Lal R., 2009) in their study This result could be explained by the fact that crop

residues were maintained on the soil surface and increased between the first and second years of our study. In fact, this condition firstly reduces evaporation and surface water runoff, then promotes soil porosity and, finally, stimulates an increase in soil water content. In addition, the regular passage of tractors and agricultural machinery on the conventional system would increase the level of soil compaction, hence the drop compared with the first year of study (*table 1*).

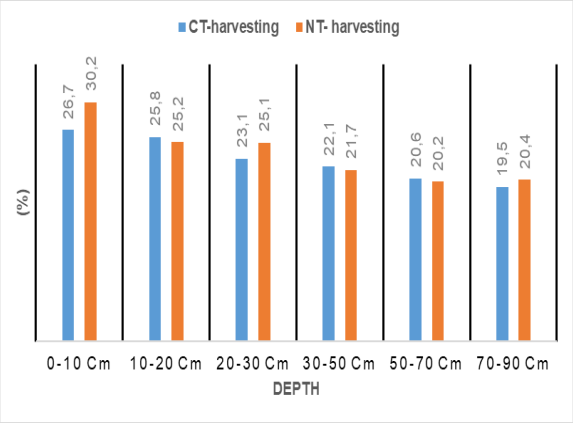


Figure 1 **Influence of conservation tillage on soil moisture**

Table 3

**Effect of tillage on soil moisture (%)**

| Parameters | *Soil moisture at sowing* (%) | *Soil moisture at harvesting* (%) |
|---|---|---|
| **CT1 (0- 10 cm)** | 16.55 ± 6.13 a | 19.32 ±1.95 b |
| **CT2 (10- 20 cm)** | 16.10 ± 3.90 a | 22.63 ± 0.89 e |
| **CT3 (20- 30 cm)** | 15.75 ± 1.85 a | 22.10 ±1.67 de |
| **CT4 (30- 50 cm)** | 16.78 ± 5.77 a | 20.29 ± 1.73 bcd |
| **CT5 (50- 70 cm)** | 15.75 ± 2.80 a | 19.47 ± 1.47 |
| **CT6 (70- 90 cm)** | 15.43 ± 1.20 a | 16.41 ± 1.27 a |
| **NT1 (0- 10 cm)** | 15.12 ± 3.98 a | 18.79 ± 1.82 b |
| **NT2 (10- 20 cm)** | 15.24 ± 2.02 a | 19.88 ± 1.03 bc |
| **NT3 (20- 30 cm)** | 15.77 ± 1.77 a | 21.52 ± 2.060 ce |
| **NT4 (30- 50 cm)** | 15.55 ± 0.89 a | 19.16 ±1.52 b |
| **NT5 (50- 70 cm)** | 15.40 ± 1.30 a | 18.44 ± 1.80 b |
| **NT6 (70- 90 cm)** | 14.72 ± 0.84 a | 15.78 ± 1.81 a |
| **P** | 0.143 | $2e^{-16}$ |
| **P** | | *** |

a, b, c, d, e: Means for the same line with different letters are significantly different at the 5% threshold. * : P<0.05; ** : P<0.01; *** : P<0.001

### Water Stable Aggregates (WSA)

Results from the study of the influence of conservative (NT) and conventional (CT) systems on water stability aggregates (WSA) show a significant difference between the two systems. The highest values were obtained under NT system particularly 10-20 cm compared to CT. *(table 4)*. These results are in line with those obtained by (Strudley M.W *et al*, 2008; Alvarez R., Steinbach H.S., 2009; Burtan L. *et al*, 2020).

Thus, in no-tillage soil stability increases, while tillage causes a reduction in soil aggregate size. First, this can be explained by the presence of residues from previous crops that cover the soil and are an element that favours the aggregation of elementary particles in the conservative system. Secondly, in the conventional system, the soil is without plant cover and therefore the formation of structural aggregates is more difficult and the aggregates are reduced in size due to the disturbance of the soil layers.

Table 4

**Effect of tillage on Water stable aggregates (%)**

| Parameters | WSA (%) | WSA (%) |
|---|---|---|
| **CT1 (0- 10 cm)** | 60.72 ± 0.99 a | 86.07 ± 1.21 e |
| **CT2 (10- 20 cm)** | 63.93 ± 1.33 b | 80.89 ± 1.84 d |
| **CT3 (20- 30 cm)** | 69.47 ± 2.19 c | 67.89 ± 1.30 a |
| **CT4 (30- 40 cm)** | 71.85 ± 1.23 cd | 72.59 ± 1.29 b |

| | | |
|---|---|---|
| **NT1 (0- 10 cm)** | 64.62 ± 1.24 b | 88.84 ± 1.19 f |
| **NT2 (10- 20 cm)** | 73.80 ± 1.35 d | 90.16 ± 1.66 f |
| **NT3 (20- 30 cm)** | 68.90 ± 3.93 c | 77.142 ± 2.72 c |
| **NT4 (30- 40 cm)** | 70.13 ± 0.91 c | 73.08 ± 1.59 b |
| **P** | $2e^{-16}$ | $2e^{-16}$ |
| **P** | *** | *** |

a, b, c, d, e: Means for the same line with different letters are significantly different at the 5% threshold. * : P<0.05; ** : P<0.01; *** : P<0.001.

## CONCLUSIONS

This study on the influence of conservation tillage on the main soil physical properties of the winter pea crop in the conditions of Ezareni farm, Iasi, Romania, is part of a research aimed to contribute to a better understanding of the effect of tillage systems on bulk density, moisture and soil aggregate stability in the winter pea crop. In this study the significant effect of the no-tillage system on each of these parameters was observed:

✓ From seeding to harvest, it was observed that the highest bulk density values were obtained with the conservative system. Thus, in this system, the results oscillated in the range of 1.22 g/cm$^3$ and 1.44 g/cm$^3$ at seeding and 1.21 g/cm$^3$ to 1.51 g/cm$^3$ at harvest. Another observation is that the maximum bulk density values were reached at a depth of 10-20 cm at both harvest and seeding and ranged from 1.48 to 1.51 g/cm$^3$.

✓ Soil moisture was influenced by the tillage systems and significantly by the conservation (no-till) system. However, it should be noted that in the first year there was no significant difference between the conservative and conventional systems.

✓ With regard to the water stability of aggregates, another physical soil property studied, a significant influence of the no-till system compared to the conventional system was also observed. The highest values were recorded mainly in the second year of the study, always with the conservative system.

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# MAIN SOIL PARAMETERS GOVERNING HEAVY METAL ADSORPTION AND DESORPTION PROCESSES

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**Abstract**

Heavy metals are distributed within soil profile according to their chemical state, transferred by hydrological flows and taken up by organisms through trophic pathways. Persistent pollutants include Zn, Cu, Pb, Cd and Ni, which cannot be removed or destroyed, leading to bioaccumulation. In soil, the maximum permissible limit for the European Union are 100 mg/kg Cu, 300 mg/kg Zn, 50 mg/kg Ni, 3 mg/kg Cd, 250 mg/kg Pb. Adsorption and desorption take place through the exchange of molecules and ions between the solid phase of heavy metals and the liquid phase of the soil, and are in accordance with the soil components: colloidal clay content, pH, organic matter, amount of carbonates and salts. The paper aim is to characterized two soil profiles up to 110 cm depth, collected from Agromixt Spineni Farm, Iasi which govern heavy metal adsorption and desorption process. The results present a neutral to alkaline (pH up to 8.81) soil pH, a carbonate content between 0.56% - 19.62% and salt content from 52-554 mg/100g soil. The results indicate that heavy metals are deposited and accumulated in the surface soil layers 0-30 cm depth. The solubility of carbonates and the presence of salts in high concentrations can retain copper (3.1 mg/Kg), nickel (1.5 mg/Kg) and lead (6.8 mg/Kg). The increased content of organic matter, weakly acidic or neutral pH values allow an increase in the mobility of zinc (15.5 mg/Kg) and cadmium. (1.0 mg/Kg). The accessibility of heavy metals can be reduced in alkaline media by desorption phenomenon, and their effect on the environment will be low and pose no risk to human health.

**Key words**: soil, heavy metals, adsorption, desorption.

The objective of this work was to follow the adsorption and desorption processes of heavy metals in Agromixt Spineni Iasi Farm, as well as the chemical and physical parameters that influence these phenomena. The category of toxic metals includes a number of chemical elements that are highly toxic to living organisms, such as Ni, Pb, Cd, their toxic effect does not diminish over a long period of time. Depending on the chemical, physical and electrochemical properties of the metals and the organisms subject to contamination, a differentiated toxicity can be established (Duffus *et al*., 2002). The mobility of heavy metals is directly influenced by their "chemical speciation" (free metal ions, dissolved or coprecipitated metal complexes) which directly influences their behaviour and toxicity in the environment (Fairbrother *et al.*, 2007).

Both adsorption and desorption are an exchange of atoms and ions or molecules that takes place between the solid phase of heavy metals in the soil and the soil solution. Adsorption is a process of accumulation of ions at the solid-liquid interface where the colloidal part of the soil participates and desorption takes place when changes occur in an equilibrium situation, occurs at the surface and controls the accessibility and mobility of heavy metals.

An equilibrium is established between the ions in solution and the solid phase, which is constantly changing due to external intervention (base amendment, improper fertilization, acid rain). The most important soil parameters driving the adsorption and desorption processes are: pH and Eh values, organic matter, content of grain size fractions, oxides and hydroxides especially of Al, Mn and Fe, formation of insoluble salts, carbonate content and microorganisms (Kabata-Pendias, 2001).

Zinc is an essential microelement for all plants and has a nutritive effect, but in excessive quantities it becomes toxic. In Romanian soils, zinc content varies between 10 and 300 ppm**.**

Cadmium is a non-essential metallic element, but due to its increased mobility and availability it easily accumulates in plants, which is why it is one of the most dangerous pollutants. In Romania, the maximum limit for Cd in soil is 1.0 mg/kg, which can enter the atmosphere from volcanic eruptions and forest burning, and sewage sludge contains a high load of cadmium.

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Copper is a microelement involved in some sequences of the nitrogen circuit and plays an enzymatic role. Copper retention in soil increases in proportion to the organic matter content and co-precipitates with oxides in the soil material. The normal value of Cu in soil is 20 ppm and the intervention threshold is 100 ppm.

According to the Order 756/1997 issued by the Ministry of Water, Forests and Environmental Protection, the maximum limit for lead content in soil is 100 mg/kg and the average content for plants is between 0.5 and 3 ppm.

Nickel is a transition metal, it associates with Co, Cu, and Pt. According to Order no. 756/1997 issued by the Romanian Ministry of Water, Forests, and Environmental Protection, the maximum permissible limit for the total content of nickel in soil is 50 mg/kg. The risk of soil contamination with heavy metals either directly or indirectly, occurs under conditions of high acidity, when the soil has a low carbonate content. In alkaline soils rich in carbonates and salts, heavy metals are adsorbed and fixed by soil colloids. Some researchers believe that an ion exchange reaction may occur and that calcium carbonate has a buffering effect (Liu *et al*., 2011).

The electrochemical series of metal voltages characterize the behaviour of metals and their salts in aqueous solutions. All metals to the left of hydrogen are able to replace from saline solutions those metals that are in the metal stress series after it, except for alkaline earth metals.

## MATERIAL AND METHOD

In order to study the behaviour of heavy metals Cd, Zn, Pb, Ni, Cu on two profiles from Spineni - Andriseni (cambic cernozite) showing a neutral and alkaline reaction, it was necessary to determine the total and plant-accessible forms. Solubility and mobility of heavy metals is closely related to adsorption, desorption, complexation, oxidation-reduction, precipitation and dissolution processes. (Barman *et al*. 2000, Quartacci *et al.,* 2005)

The whole mechanism of adsorption and desorption of heavy metals is related to soil components, therefore the ability of clay and calcium carbonate to accumulate heavy metals, soil reaction, salt and humus content were evaluated.

In the laboratory the soil samples (dry and wetted) were analyzed potentiometrically on a WTW multi-carrier for pH determination in aqueous extract in soil water ratio of 1: 2.5 The determination of organic carbon and humus content was performed by the Walkley-Black method in the Gogoașă modification by wet mineralization of soil samples and titration with Mohr salt. Organic carbon multiplied by the constant 1.724 leads to the humus value. The determination of grain size fractions was carried out by the classical Kacinski method by differential treatment of samples with or without carbonates. Coarse sand is determined by wet sieving and the pipetting of grain size fractions of fine sand, dust, colloidal clay and physical clay is performed using Kubiena pipette.

The phenomenon of soil enrichment in salts occurs with the help of humid air masses loaded with soluble salts followed by their dissolution, accumulation and transport by water. In order to assess the salinization intensity, it was necessary to obtain an aqueous extract with a soil-water ratio of 1:5 and to determine the content of soluble salts by a conductometric method.

The content of heavy metals in total form was carried out by wet disaggregation with a mixture of acids (nitric, hydrochloric and perchloric) according to the ICPA Bucharest method and the determination of metals by the Flame Atomic Absorption Spectrometer (AAS) 700. The determination of the mobile contents of microelements takes place by an extraction in a solution of EDTA-ammonium acetate.

## RESULTS AND DISCUSSIONS

Research has shown that in soil all heavy metals undergo a rapid initial reaction, followed by slower reactions when the metal is removed from the soluble pool. This process increases the fixed fraction, which cannot be easily changed in the solution phase.

Due to the need to integrate the principles of accumulation, transfer and blocking or removal of heavy metals under certain conditions, the soil taken from Spineni Andriseni Farm was chemically and physically analyzed *(table 1, table 2)*. By comparison the two profiles 8 and 15 showed the adsorption-desorption phenomenon.

Table 1

**Profile characteristics (profile 8)**

| Type | pH | $CaCO_3$ (%) | Salt content (mg/100 g) | Humus (%) | Colloidal Clay (%) |
|---|---|---|---|---|---|
| **A** | 6.85 | - | | 4.22 | 41.7 |
| **A** | 7.05 | - | | 3.73 | 42.4 |
| **A** | 7.20 | - | | 3.05 | 41.8 |
| **AB** | 7.38 | - | | | 40.3 |
| **B** | 7.60 | 0.56 | 52 | | 41.1 |

In order to characterize the state of the soil and to correlate its characteristics with the heavy metal content, it was necessary to determine the concentration of heavy metals in total and accessible form (up to a depth of 60 cm in profile 8 and 80 cm in profile 15). The values obtained for heavy metals in soil were compared with the

standard reference values imposed by the Romanian legislation - Order 756/1997.

Table 2

**Physical and chemical characteristics (profile 15)**

| Type | pH | $CaCO_3$ (%) | Salt content mg/100g | Humus (%) | Colloidal Clay (%) |
|---|---|---|---|---|---|
| A | 7.90 | 5.23 | 220 | 2.80 | 53.3 |
| C | 8.24 | 10.22 | 544 | 0.89 | 52.1 |
| C | 8.61 | 19.62 | 393 | | 51.3 |
| CG | 8.50 | 18.30 | 554 | | 55.4 |
| G | 8.81 | 17.50 | 456 | | 56.2 |

Clay includes aluminosilicates and hydrated metal oxide, exhibits cation exchange and surface characteristics, therefore adsorption of heavy metals occurs, clay being an active adsorption component of soil. Organic matter consists of living organisms, soluble biochemical compounds, and insoluble humic substances. All of these provide sorption sites for metals and participate in the exchange capacity of the soil. The results show that of all the metals analyzed, copper is the most strongly retained on organic matter. (Wu *et al*., 2018). An important parameter that directly influences adsorption and desorption processes or heavy metal precipitation/dissolution phenomena is the soil reaction (*figure 1).* A decrease in the activity of metals in both total and accessible forms is observed with increasing pH values. Under acidic conditions and neutral pH values, heavy metals have a higher activity, which makes them more easily adsorbed by plants.

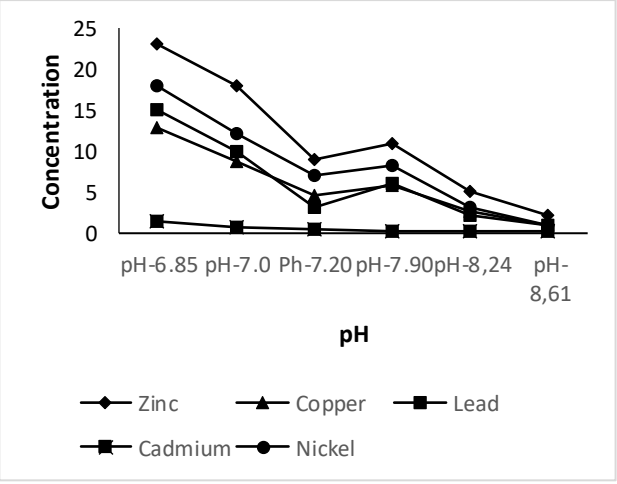


Figure 1 **- Variation diagram of heavy metal concentration (total forms) as a function of soil reaction**

The presence of calcium in the soil can increase the mobility of some metals, triggering a competition for sorption centres between calcium and heavy metal Cations (*figure 2)*. In this situation cation exchange reactions become predominant and occur when there is compatibility with the metal element in the host mineral. In alkaline, $CaCO_3$ - rich soils, heavy metals form insoluble carbonates, hydrated hydroxides or are adsorbed and fixed by soil colloids. It is noted that in the lower carbonate soil the metals were retained by cation exchange reactions, whereas in the higher carbonate soil the heavy metals were retained by specific adsorption on calcium carbonate. In this case desorption occurs, which can significantly reduce exchangeable and carbonate-bound metals. (Liu *et al*., 2021)

The affinity of metals for the soil solid phase is dependent on their concentration, a hypothesis supported by the results obtained and by previous studies (O'Connor *et al*., 1984).

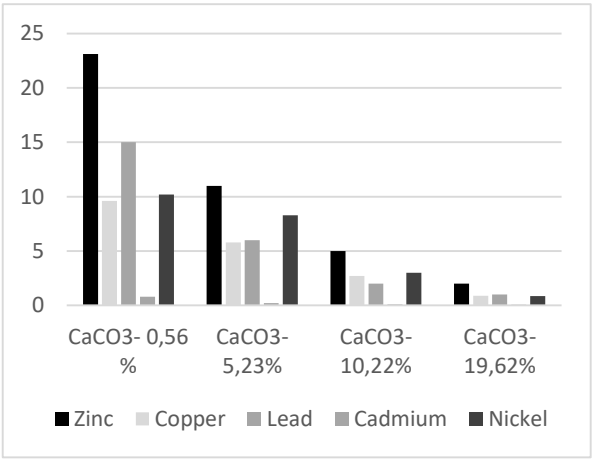


Figure 2 **Exchange reactions between carbonates and heavy metals**

The retention processes of heavy metals in soil involve sorption and precipitation processes. Adsorption is an accumulation of ions at the surface and by precipitation the metals form new compounds. Desorption studies provide information on the strength of the association that is made between a metal and soil and are carried out to determine the reversibility of sorption reactions. The behaviour of metals in soil is determined by sorption-desorption processes in which they participate with other soil components. The total and accessible forms of microelements in the two profiles analyzed are shown in *tables 3* and *4.*

Table 3

**Heavy metals in total (T) and accessible (Ac) (profile 8)**

| Zn mg/Kg | | Cu mg/Kg | | Pb mg/Kg | | Ni mg/Kg | | Cd mg/Kg | |
|---|---|---|---|---|---|---|---|---|---|
| T | Ac | T | Ac | T | Ac | T | Ac | T | Ac |
| **23.1** | 15.5 | 12.8 | 3.1 | 16.0 | 6.8 | 10.1 | 1.5 | 1.3 | 0.5 |
| **18.7** | 8.0 | 8.8 | 1.7 | 10.3 | 4.1 | 7.4 | 0.9 | 0.6 | 0.2 |
| **9.2** | 2.5 | 4.6 | 0.6 | 3.8 | 0.4 | 2.6 | 0.1 | 0.3 | 0.1 |

The mobility of metals is directly influenced by their "chemical speciation" which determines their behaviour and toxicity in the environment (Fairbrother *et al*., 2007). Speciation therefore refers to the occurrence of metals in different chemical forms such as: metal complexes

dissolved in solutions and adsorbed on solid surfaces or metal species that have co-precipitated in their own or other metals' solid phases at much higher concentrations or free metal ions (Qin Yu *et al.*, 2010).

Table 4

**Heavy metals in total (T) and accessible (Ac) (profile 15)**

| Zn mg/Kg | | Cu mg/Kg | | Pb mg/Kg | | Ni mg/Kg | | Cd mg/Kg | |
|---|---|---|---|---|---|---|---|---|---|
| **T** | **Ac** | **T** | **Ac** | **T** | **Ac** | **T** | **Ac** | **T** | **Ac** |
| **11.7** | 4.7 | 5.8 | 1.8 | 6.8 | 2.0 | 7.0 | 0.6 | 0.2 | 0.02 |
| **5.2** | 1.5 | 2.7 | 0.9 | 2.5 | 0.6 | 3.7 | 0.1 | 0.1 | 0.01 |
| **2.1** | 0.9 | 0.9 | 0.2 | 1.4 | 0.07 | 0.9 | 0.02 | - | - |

Speciation of a metal alters both its toxicity and certain chemical processes. The results led to the conclusion that heavy methylated metals were strongly retained in the soil over an increased carbonate content but only 10-15% of the total were converted to mobile forms. In neutral soils, of the total only some of the metal was trapped and the rest became accessible to plants.

The electrochemical voltage series of metals is the descending order of their reactivity and characterizes the behaviour of metals and their salts. It represents a series of voltages for the most important metals and hydrogen.

Mg,Al,Mn,Zn,Cr,Fe,Cd/Ni,Sn,Pb/**H**/Cu,Hg,Ag,Pt

Relative to the H 2 / 2H + system, some substances will behave as oxidizing agents, others as reducing agents. The heavy metals analyzed can replace hydrogen, except for copper. In the case of saline soils, each metal, can displace those metals that are in the metal stress series after hydrogen. When organic ligands are trapped in the soil by complexation, heavy metals tend to lock up.

## CONCLUSIONS

In general, sorption studies are carried out in order to assess the intensity of metal retention phenomena in the soil and on different soil constituents. Sorption occurs at the solid-liquid interface and represents an accumulation of ions. Desorption is an important process that controls to some extent the accessibility and mobility of heavy metals in polluted soils. The affinity of metals for the solid phase of the soil depends on the properties of the metal, its concentration and the nature of the soil surface. Of the cations studied, copper had the lowest mobility and zinc the highest. Soils with higher reaction values and increased carbonate content retained and trapped heavy metals much better than those with neutral pH. It was found that organic matter and clay content are parameters that have a great influence on the adsorption phenomenon. An increase in the mobility of heavy metals through the formation of soluble complex combinations dependent on the soil reaction was observed and it was shown that adsorption intensifies with increasing pH. In the case of saline soils each metal, can replace those metals that are in the metal tension series after hydrogen. If compatibility occurs between the host mineral and the metal element, a uniform substitution takes place which favours cation exchange. The processes of mutual displacement of metals in solutions and their precipitation at the surface are considered as manifestations of transmutation of elements.

The harmful effects of heavy metals depend on their mobility, i.e. their solubility in soil. Therefore, in the case of soils polluted with heavy metals, the first remediation measures will aim to create conditions that allow the heavy metals to pass from the soil solution into stable forms bound to different constituents.

## ACKNOWLEGMENTS

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# THE STUDY OF BREAD VOLUME DETERMINATION USING THE PHOTOMETRIC METHOD

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**Abstract**

The external appearance of the bread is important both for the bread producer and especially for the consumer, it shows that the volume and shape of the bread are the parameters that must be studied with accuracy. Volume changes of bakery products, expansion and contraction occur in the bread making process as a result of transitions and changes that occur over time. The paper aims to determine the volume of three varieties of bread (white bread, black bread, whole meal bread) by the classical (gravimetric) method and the modern photometric method. The results of the determinations show that for white bread a volume difference of 70.34 $cm^3$ minus is obtained with the classic method, for black bread a volume difference of 151.37 $cm^3$ minus with the classic method, and for intermediate bread a difference of 122.93 $cm^3$ in minus with the classic method. From the experimental determinations, a high accuracy is found for the volumes determined by the photometric method compared to the classical method.

**Key words**: bakery, volume, physical parameters

Bread, as a basic product in human nutrition, is studied more and more to improve the physical properties of appearance and shape, nutritional and taste qualities, seeking to increase the yield and make the production, packaging, storage and transportation process more efficient (Serpil S., Servet G.S., 2006). Volume changes of bakery products, expansion and contraction occur in the bread making process as a result of some transitions and changes that occur over time (Mondal A., Datta A.K., 2008) Expansion or increase in volume is a characteristic feature of the baking of leavened bakery and pastry products (bread, cakes). During baking, the thermal expansion of carbon dioxide (produced by leavening agents) and water vapor present inside the porous structure deforms the dough, increasing its volume until starch gelatinization occurs (Lostie M. *et al*, 2002; Wagner M.J. *et al*, 2007). In addition, bread baking appears as a very special case in terms of volume change. During the process, the dough primarily undergoes an increase in volume due to the thermal expansion of carbon dioxide and water vapor (until the dough turns into bread), and then contraction occurs due to the final formation of the crust and setting, where reactions of reticulation (Scanlon M.G., Zghal M.C., 2001; Sommier A. *et al*, 2005; Vanin F.M. *et al*, 2009). While shrinkage is an inverse change observed during bread drying that occurs due to water loss and changes occurring in the porous structure of the bread when the volume of the product decreases (Mayor L., Sereno A.M., 2004). Since the external appearance of the bread is important both for the bread producer and especially for the consumer, it shows that the volume and shape of the bread are the parameters that must be studied with accuracy. This shows the producer new methods of improving the manufacturing technology and new forms of modeling the bread in order to obtain the most consistent volume, and for the consumer the volume is one of the criteria for choosing the respective product (Brennan J.G., 2011). The methods for determining the volume of bakery products are few and with low accuracy, but they have been used until now as well-known and sometimes standardized classical methods, but with the development of the technique, modern methods for determining the volume have appeared, which bring better accuracy and less time to get results. The present study aims to present a new, little-known method for determining the volume of bakery products using photometry. To demonstrate the accuracy of the method, it was compared with the classical method known for determining the volume of bread. present document is arranged so that it can be used as a model.

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## MATERIAL AND METHOD

For the experimental volume determinations, three types of bread were chosen as bakery products. They were made by hand from three types of flour: white flour (*figure 1a*), black flour (*figure 1b*) and whole wheat flour (*figure 1c*). All three varieties of bread had irregular shapes to see if shape is a factor influencing the results of volume determinations. Volume is an important factor in determining the quality of bakery products.

Figure 1 **Bread** a **white,** b **black,** c **whole**

The volume of solids can be determined using several methods: 1. Calculation of regularly shaped objects knowing the characteristic dimensions; 2. Experimental determination by displacing a known volume of liquid, gas or solid; 3. Photometric measurement using image processing. The image processing method has recently been developed to accurately measure volumes of food products with irregular shapes (e.g. braided bread).

The gravimetric method consists in determining the volume of the three types of bread using a laboratory apparatus (*figure 2a*) that reproduces the Fornet "*Bread Volume Meter 13300*" apparatus (*figure 2b*). Both operating on the classic principle of displacing a volume of rapeseed.

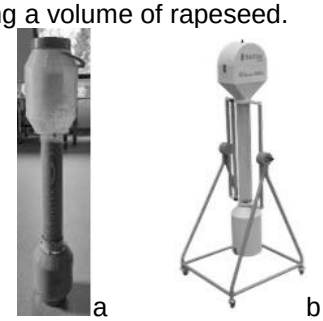

Figure 2 **Apparatus for determining volume** a **laboratory,** b **Bread Volume Meter 13300**

The experimental device for determining the volume of bread by the photometric method (*figure 3*) is composed of a box of parallelepiped shape with white walls, an LED projector with white light mounted on the top and a rotating support at the base. In the center of the rotating stand, the three types of bread were installed with a clamp, on which the bread will be fixed in order to be filmed at a 360-degree rotation for one minute (rotation of the rotating stand 1 rpm). The video was shot with a mobile phone camera (Samsung Galaxy A51) for one minute. The video camera used to record the video samples has a resolution of 48 MP and an aperture of f/2.0, it recorded the video sequences at a resolution of 3840x2160 pixels and 30 frames/s in .mp4 video format.

Figure 3 **The experimental device for photometric method**

For the initial calibration of the object from the obtained movie, two distinct points are marked on the surface of the bread at a measured distance of 5 cm (*figure 4*), this distance is later entered into the specific software for the final scaling of all images, after previously marking in each photo the control points for the accuracy of the final calculation of the volume of the bread.

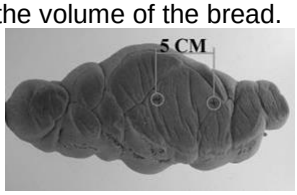


Figure 4 **Initial bread calibration through control points**

To determine the volume of a bakery product with irregular shapes, the video saved in .mp4 format was processed with the help of several software: VLC media player 3.0.8 for video sequencing of the movie, Topaz Gigapixel AI 4.1.1 for increasing the image resolution, NXPowerLite Desktop 8.0.4 which uses an algorithm for reducing the size of images while preserving their quality, Zephyr Aerial 3DF 4.300 for 3D reconstruction of the filmed object, final calibration (*figure 5a*) of the model, correction of the resulting imperfections and volume calculation (*figure 5b*).

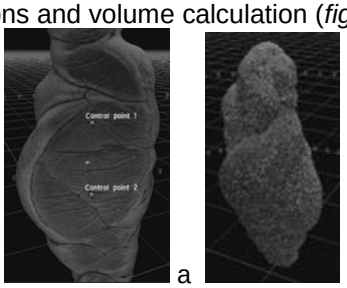


Figure 5 **Bread model** a **final model calibration,** b **3D volume model**

## RESULTS AND DISCUSSIONS

The classic method used to determine the volume of the three types of bread, involves using the principle of displacing a volume of rapeseed. In the first stage of the experiment, the laboratory apparatus in Fig. 2a was calibrated using a non-deformable cylindrical box that has the following measured dimensions: r = 50 mm; h = 156 mm. The calculated volume of the cylindrical box is $V_c$ = 1225.22 $cm^3$, and the average volume obtained

after 3 consecutive measurements by the classic method is $V_m$ = 1220.10 $cm^3$. Through the difference, it follows that by the classical method a minus volume of 5.12 $cm^3$ is obtained.

The same work technique was used to determine the volume of bakery products. The white bread of irregular shape obtained by intertwining two strands of dough was introduced into the laboratory apparatus, after which three volume determinations were made by displacing the volume of rape seeds. The same was done with the irregularly shaped black bread obtained by intertwining three strands of dough, as well as with whole meal bread which had a shape similar to that of a cylinder. The results of the obtained volume are presented according to *figure 6*. This shows that the volume of white bread has the smallest volume of 700 $cm^3$, while the volume of whole meal bread is 860 $cm^3$.

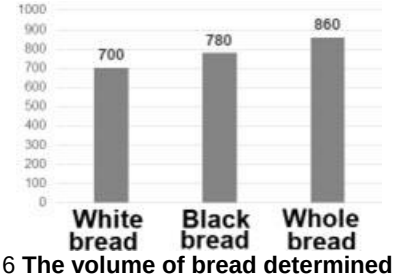


Figure 6 **The volume of bread determined by the classic method ($cm^3$)**

The experimental determination of the volume of the bakery products studied by the photometric method followed as a first step the calibration of the method with the same non-deformable cylindrical box used in the classic method. The calculated volume of the box is $V_c$ = 1225.22 $cm^3$. For the photometric method of determining the volume, the box was measured and marked at the ends to be able to define the control points in the program. With the help of control points, the program scales the box to the actual dimensions, dimensions necessary to accurately determine the volume. The box was filmed from 3 different angles for 60 seconds to obtain a well-defined 3D object, *figure 7*.

Figure 7 **Non-deformable cylindrical box filmed from three different angles**

After processing the images and scaling the 3D object to the actual dimensions of the box r = 50 mm and h = 156 mm, a volume of $V_f$ = 1223.74 $cm^3$ was obtained by photometric processing (*figure 8*). The difference between the calculated volume and the volume obtained by the photometric method was minus 1.48 $cm^3$, i.e. a deviation of 0.12%. From the technique of experimental measurements of volumes, it is known that a deviation below 0.3% is considered a determination with high accuracy.

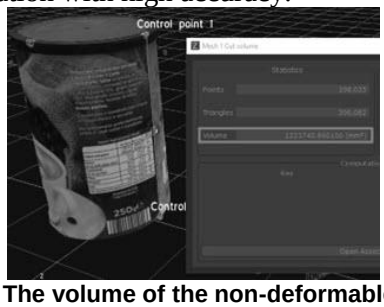


Figure 8 **The volume of the non-deformable box by the photometric method ($mm^3$)**

By the photometric method for determining the volume of bread, the following results were obtained (*figure 9*): White bread 629.66 $cm^3$; Black bread 628.63 $cm^3$; Whole meal bread 736.07 $cm^3$.

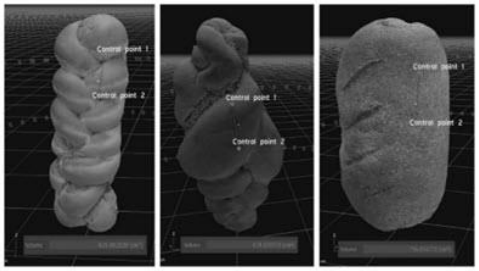

Figure 9 **The volume of bakery products obtained by the photometric method ($cm^3$)**

In summary, the results obtained for the volume of bakery products by the classical method and the photometric method using for comparison and a non-deformable cylinder of known dimensions are shown comparatively according to *figure 10.*

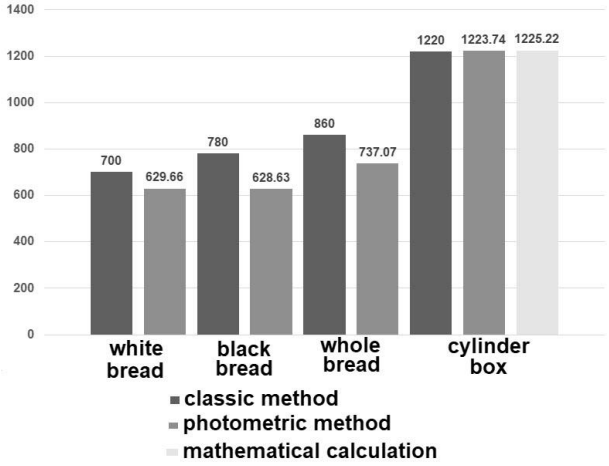


Figure 10 **The volume of bakery products determined by the classical and photometric methods ($cm^3$)**

It can be seen that the volume of bakery products determined by the photometric method is

much more accurate if we refer to the volume of the cylindrical box obtained by the same methods. Although the same bakery products were used for all determinations, the photometric method was proven to be accurate using complex calculation algorithms used in research programs. Of all the determinations made, the biggest difference appears when determining the volume of black bread with a minus of 151.37 $cm^3$, this is due to the irregular shape of the bread. Rapeseed seeds used for volume determination by the classical method do not cover all the gaps resulting from the overturning of the volume determination apparatus. Differences in volume determination appear for all three types of bread between the classical method and the photometric method.

## CONCLUSIONS

Accurate volume measurement of bakery products and especially bread is important for both the bread maker and the consumer. The determination of the volume of three types of bread (white bread, black bread and whole meal bread) was carried out comparatively by two methods. The classical (gravimetric) method of determining the volume of bread was carried out with the help of a device that reproduces the "Bread Volume Meter 13300" Fornet device that uses the principle of displacement of a volume of rapeseed.

The photometric method (image processing) is a modern method for determining the volume of bread built with a device consisting of a white parallelepiped box, a white light LED projector, a rotating support and a video camera for recording images. Through the photometric method using a movie of the bakery product, a 3D volume of each analyzed assortment of bread is obtained through mathematical processing with the help of some software.

The results obtained by the two methods considered, show for a non-deformable cylindrical box with known dimensions a difference between the calculated volume and the volume determined by the photometric method of 0.12%, compared to 0.42% for the classical method. From the experimental technique in volume measurement, a deviation below 0.3% is considered a high accuracy determination. This shows that the photometric method is a much more accurate method compared to the classical method.

The results in the determination of the volume by the two methods for bread show that for white bread a volume difference of 70.34 $cm^3$ minus the classic method is obtained, for black bread a volume difference of 151.37 $cm^3$ minus the classic method, and for the intermediate bread a difference of 122.93 $cm^3$ minus the classic method. All these differences show the precision with which the volume is determined by the photometric method compared to the classical method.

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# RESEARCH ON MINOR RIVERBED MORPHOLOGY IN THE PRESENCE OF ANTHROPOGENIC RISK FACTORS

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**Abstract**

The change in the morphological parameters of the minor bed takes place under the influence of natural and anthropogenic modelling factors. The minor river beds located in the urban areas are intensively affected by the presence of anthropogenic risk factors. The research carried out on the Bahlui River in the area of the city of Iasi highlighted important changes in the minor river bed determined by the regularization works carried out asymmetrically in the river bed. The presence of rainwater drains is an important anthropogenic factor of erosion of the minor bed. In this case, in the minor river bed, there is an important differentiation in the cross section between hydraulic parameters (maximum velocities, roughness, erosion force), a situation that leads to the intense erosion of the right bank.

**Key words**: degradations, erosion, form, riparian zone

Correct exploitation of a river bed for various purposes must not disturb its hydrodynamic balance in longitudinal and transverse profile. The minor riverbed is included in an extensive research program at the current stage regarding the initiation and development of erosion and deposition phenomena, as well as solutions to limit destructive actions.

The minor bed of the rivers is constituted by the land surface with a low elevation where the continuous flow and free surface of the water from the springs to the discharge into the upper water course takes place (Manoliu I.A. *et al*, 1970, Ichim I. *et al*, 1989). The formation flow and transported by the minor bed is a topic of continuous research at the current stage on a national and world level (Manoliu I.A. *et al*, 1970, Ichim I. *et al*, 1989, Oprișan E., Tecuci I., 2014, US Army Corps Eng., 1993).

The research of morphological transformations of natural or regularized riverbeds under the action of natural / anthropogenic risk factors, in the presence or not of some constructions in the bed and the riparian zone is in continuous development in the current period (Ichim I. *et al*, 1989, Avram M., 2020, Bolati I.M. *et al*, 2014, Neuhold C., *et al*, 2009, Rinaldi M., 2003 and others).

The issue of the use and protection of riverbeds together with the riparian area is a continuous concern of the state administration (OM 326, 2007, Order 2115, 2021). The mode of protection of the bed and the riparian zone in Romania is deficient at the current stage and is far below the European level.

The expansion of the areas occupied with various economic and social objectives in the riparian area contributes to the morphological modification of the riverbeds and the disturbance of the aquatic environment. The research of the cooperation of the bed with the new environmental conditions is carried out directly in the field. The data taken from the field can be entered into simulation programs of various bed degradation phenomena (Wang W. *et al*, 2014, Neuhold C., *et al*, 2009, Avram M., 2020, Luca M., Avram M., 2021, Bohorqueza P. Anceyb C., 2015).

The protection of the objectives in the riparian area against the destructive action of the water is carried out through works to regularize the riverbed and protect the banks. These works change the morphology of the river bed in transverse and longitudinal profile, but also disturb the aquatic and riparian ecosystems.

Recent climate changes have modified a number of natural and anthropogenic risk factors that intervene in the functioning of coastal defences (Luca *et al*, 2012). This situation has also determined a change in the concepts of carrying out river bed regularization works (Avram M., 2020, Luca M., Avram M., 2021).

A problem that needs to be solved currently refers to the legal and real estate status of the bed and the riparian zone. Knowing the exact type of

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property on riparian land areas would allow good management of maintenance and environmental protection works.

The aim of the work was to research the morphological transformation of the minor bed of the Bahlui River on the trunk adjacent to the "Era Park" Commercial Complex following the action of natural and anthropogenic risk factors. Also, the influence of the way of operating the rainwater outlets within the commercial complex on the morphology of the bed was analyzed. The research section presents very different regularization works on the two banks.

## MATERIAL AND METHOD

The researches were carried out on the lower course of the Bahlui River, on a section located between the towns of Lecțani and Iaşi (*figure 1*). The research sector is in contact on the right bank with the platform of the "Era Park" Commercial Complex in the outskirts of the city of Iasi (Luca M., 2019).

The Bahlui River has an average slope between the town of Letcani and the city of Iasi of 0.35 m/km. The major river bed on the research sector is wide, but with local narrowing determined by the presence of some economic and social objectives. The minor bed has a cross-sectional shape characteristic of the plateau area; respectively it tends towards a relatively stable parabolic shape. The minor bed shows local erosion phenomena on limited lengths.

The foundation of the river is made up of cohesive rocks, which reduces the erosion of the bed on the watered perimeter (Luca M., 2019).

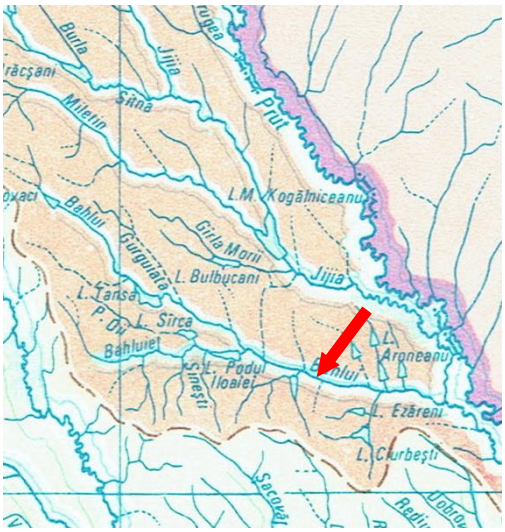


Figure 1 **Hydrographic characteristics in the research area of the Bahlui River (Atlasul geografic al României, 1985)**

The research was carried out on a river section with a semi-regularized bed over a length of 600 m. The regularization works were partially degraded in the last period of time.

The research methodology was differentiated by fields of study: hydrological, hydraulic, resistance and stability of the banks, the influence of natural and anthropogenic factors on the minor bed and the way of producing the erosion phenomena of the bed. The hydrological data (average and maximum flows) are determined in the research site and correlated with those taken from the hydrometric stations on the Bahlui River located in the research area (Podu Iloaei and Iasi).

On the research sector, data were taken on the cooperation of the bed with the outlets of rainwater collected on the platform of the commercial complex. The state of the rainwater drainage works and their contribution to bed erosion in the contact area were also analyzed.

The collected data sets were completed with photo reliefs by study sectors and type of degradations. All technical data were stored in "databases" and processed on the study areas.

## RESULTS AND DISCUSSIONS

A commercial complex consisting of several buildings is located in the research area on the Bahlui River. Rainwater collected from the platform of the complex and the roof of the buildings is transported with a sewage network and stored in two tanks. Rainwater from each reservoir is discharged into the Bahlui River by gravity and pumping (*figures 2*). The discharge mode is used according to the water level in the river bed in the discharge section. The water from the reservoir is taken by a channel located on the slope of the bed and which connects to the bed of the Bahlui river through an outlet (GV2 – GV3) (*figure 2*) (Luca M., 2019).

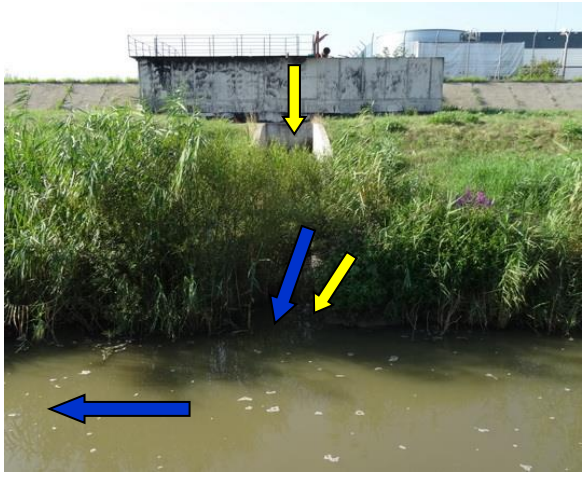

Figure 2 **The location on the bank of the Bahlui river of the rainwater storage and drainage tanks (Luca M., 2019)**

Rainwater from part of the platform of the commercial complex is collected by perimeter

channels. The two canals discharge rainwater into the Bahlui River through two outlets (GV1 and GV2) (Luca M., 2019).

The Bahlui River presents an approximately linear bed on the section at the border of the Commercial Complex, with a section formed that ensure the transit of average flows under normal conditions, but with intense erosion phenomena at maximum flows.

The river section considered in the research has a length of about 600 m and a slope of 0.0015%. The riverbed was partially regularized on the research sector by calibrating and arranging the right bank. The regularization works of the left bank were reduced, and in the current period the bank tends towards the natural state.

The applied regularization works formed a bed with a mixed shape: at the base, the bed has a relatively parabolic shape for low flows; the bed has a trapezoidal-rectangular shape for medium flows (*figure 3*). The width at the bottom is approximately constant along the length.

The riparian area of the right bank is laid out in steps. The slope of the right bank is arranged unevenly along its length (natural and regularized) and has a slope of 1:1. The slope is interrupted by a berm on which the four storage tanks with pumping stations are located. The berm continues with a profiled slope with a 1:1 slope and protected with large concrete slabs according to the norms in force (GE 027, 1997). At the top of the slope are a road and the concrete platform of the shopping complex (*figure 4*).

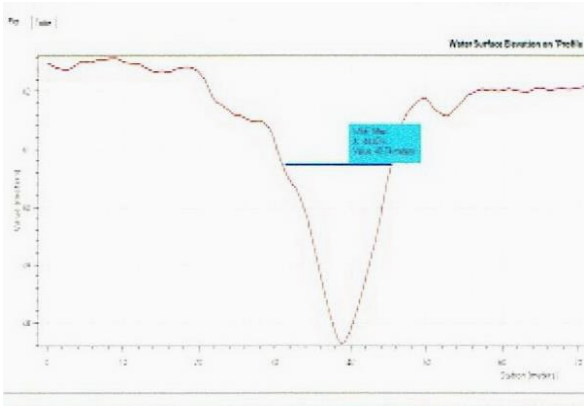

Figure 3 **Simulation of the transverse section through the bed of the Bahlui River in the first third of the research section (Water Basin Administration Prut – Barlad, 2016 - 2021)**

The left slope of the bed has preserved its natural shape, with areas of erosion, and on the non-watered side there is diverse and intensively developed vegetation.

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Figure 4 **General downstream view of the research sector on the Bahlui River (Luca M., 2019)**

The calculation rate of regularization works on the research section is $Q_{1\%}$, and that of verification $Q_{0.1\%}$ (*table 1, figure 4*) (Luca M., 2019, Water Basin Administration Prut –Barlad, 2016 - 2021). The calculations performed on the research section highlighted the values presented in *table 1*. The variation of the flow rate (Q) according to the water depth (h) was calculated and represented graphically.

Table 1

**Flows with calculation probability per sector of research of the Bahlui River (Luca M., 2019)**

| Section | $Q_{5\%}$ ($m^3/s$) | $Q_{1\%}$ ($m^3/s$) | $Q_{0,1\%}$ ($m^3/s$) |
|---|---|---|---|
| **Era Park** | 60 | 150 | 420 |

The change of roughness on the perimeter of the bed through erosion and clogging phenomena, but also through the presence of vegetation determines the continuous change of the water level and the transported flow.

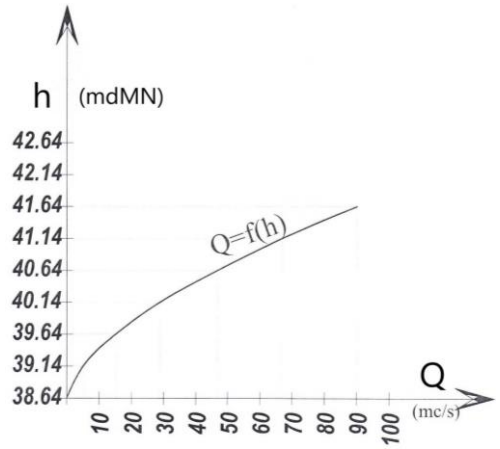


Figure 5 **The limnimetric key of flows on the research sector on the Bahlui River (Luca M., 2019)**

The variation of the water level on the investigated section has an important influence on how the rainwater is discharged from the storage tank. At high water levels in the river, the gravity drainage pipes are blocked, in which case rainwater drainage is used through the use of pumping stations.

The morphology of the minor bed of the Bahlui River on the research section was influenced by the following risk factors:

- natural factors are represented by the floods produced in the last period of time and the rapid variation of the flow in the flow section;

- the significant anthropogenic factors are represented by the bed regularization works, the rapid discharge of rainwater flows, the deficiencies of the drainage construction and the absence of maintenance and remedial works.

In the first research stage, the influence of the regularization works on the hydrological and hydraulic parameters of the minor riverbed was analyzed. The field research revealed the following:

- The regularization works, with the role of stabilizing the right bank and allowing the rapid evacuation of rainwater flows, changed the geometric shape of the minor bed. The minor bed in the initial stage had a mixed shape, where the lower part was an elongated parabola, which continued with a trapezoid (*figure 6*). The regularized minor bed has approximately the same shape, except that the right slope is terraced at a lower slope (*figure 7*).

Figure 6 **General view of the minor bed downstream of the research section (Luca M., 2019)**

Figure 7 **General view of the regularized minor bed on the research section (Luca M., 2019)**

- The left bank of the minor bed is affected by erosion phenomena and clogging in the middle level area. The bank is grassy and covered by a vegetation of shrubs and trees specific to the riparian area (*figure 8*). The way of structuring the left bank influences its roughness coefficient during the transit of average and maximum flows.

Figure 8 **General view of the left bank at the minor bed of the Bahlui River on the research section (Luca M., 2019)**

In the second stage of the research, the influence of rainwater drainage works on the morphology of the minor bed was analyzed. The drainage of rainwater from the site of the commercial complex is carried out through the two outlets and two perimeter channels. The field research revealed the following:

Figure 9 **The structure of the right bank of the Bahlui River bed on the research section and the location of rainwater storage tanks (Luca M., 2019)**

- The shape of the right slope of the bed is fragmented along its length by the presence of the four outlets of rainwater into the river (*figure 9*). The spillways were made with a reduced length, a situation in which the water current evolves on the unprotected slope of the bank. This situation determined the appearance of local erosions of the bank, continued with the degradation of the canal and the outlet (*figure 9*).

- The evacuation of rainwater from perimeter channels 1 and 2 into the Bahlui River is carried out without the existence of an adequate hydrotechnical construction. In this case, the jet formed by the exit of the water from the canal produces an erosion of the right bank (*figure 10*).

Figure 10 **Intense phenomenon of erosion on the right bank of the Bahlui River bed at the outlet of perimeter channel 2 (Luca M., 2019)**

- The bank of the Bahlui river in the GV1 area shows local erosions, which over time advanced the bank and caused its loss of stability. The water from the river eroded the foundation of the drainage channel causing the concrete to crack and parts of the channel to collapse. The discharged water inflow caused the formation of abundant bank and aquatic vegetation, which also obstructs the flow of water in the canal (*figure 11*).

Figure 11 **State of the evacuation sector (GV1) on perimeter channel 1 (Luca M., 2019)**

An active erosion phenomenon is registered in the location of the two bridges bordering the research section. Erosion is intensified by the unsatisfactory structural condition of the perimeter rainwater drainage channels (Luca M., 2019).

From the analysis of the previously presented data, the strong influence of the anthropic factor in the continuous modification of the Bahlui River bed on the section adjacent to the commercial complex is found. The natural river bed was modified into a semi-regularized bed to ensure two requirements:

- flood protection of the location of the commercial complex according to the importance category of the objective and the check flows on the affected bed section;

- taking the rainwater collected from the platform of the commercial complex and discharging it intermittently (gravitationally or by pumping) into the river.

The right bank is degraded locally, especially near the hydrotechnical constructions for the drainage of rainwater, through erosion phenomena and the advance of the river bank. The presence of poorly executed outlets creates eddy zones, a fact that intensifies the phenomenon of erosion of the bank and the outlet channel. The high velocities of rainwater emitted from the tank caused the degradation of the protection of the outlet channel.

On the left bank, natural risk factors predominate, which locally produce erosion, bank collapses and silting. The riverbed of the river maintains its flow transit capacity, but requires annual maintenance.

An indicative element of the need for the application of rehabilitation works is represented by the fact that the four mouths/rainwater drainage channels have not been structurally completed in their entirety up to the minor bed of the Bahlui River. This situation caused the collapse of the GV1 and GV4 concrete channels and the erosion of the bank, but also of the discharge channel near the GV2 and GV3 empties from the rainwater pumping stations (Luca M., 2019).

The minor bed of the Bahlui River and the riparian area in the area adjacent to the commercial complex must be arranged according to OM 326/2007 and Order 2115/2021 for a correct delimitation of the land belonging to the public domain or the private domain. Through the application of the legislative documents, the obligations of those who exploit the land in order to apply the maintenance and rehabilitation works of the minor bed and the riparian area result.

## CONCLUSIONS

The research carried out on a section of the Bahlui River in the area adjacent to a commercial complex highlighted the combined action of natural and anthropogenic factors in the morphological modelling of the bed.

The flow section of the river at low and medium waters has an approximately parabolic shape, and at high waters it tends to a mixed parabolic + double trapezoidal shape, regularized in the area of the right bank (lower slope + berm + upper slope) and with a natural appearance on the bank left.

In the area of the four spillways (GV1 - GV4) there is an active phenomenon of bank erosion determined by the discharge of the rainwater jet. This erosion is corroborated with that produced by the high velocities of the water in the river during the transit of the floods.

In the area of the left bank, alluvial deposits were formed due to the decrease in flow speed influenced by the greatly increased roughness of the bank.

Reducing the phenomena of erosion and clogging of the Bahlui riverbed requires the design and execution of works to rehabilitate and modernize the outlets for rainwater collected from the premises of the commercial complex.

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# FIELD TESTS OF A JOHN DEERE HARVESTER FOR THE PURPOSE OF PRODUCTION MAPS ACHIEVEMENT

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**Abstract**

Errors in the generation of production maps can arise from many causes and are practically inevitable. Even though errors will occur, production data can be improved by following a few best practices. The primary methods for improving harvest data should always be the calibration and proper operation of the combine. In cases where this is not possible or where errors occur, the John Deere Operations Center offers data analysis tools through filtering, post-calibration, and improving the visualization of production maps. These practices will result in obtaining accurate data on which agronomic decisions can be made for subsequent farming years. Mapping productions using the John Deere S780i harvester combine and its associated systems provides the farmer with valuable information about their farm, which can be used to make informed and calculated decisions to increase overall productivity, sustainability, and profitability of the agricultural farm.

**Key words**: production maps, harvest, profitability

Production maps on harvesters are valuable tools in modern agriculture. These maps provide a detailed visual representation of the crop yields and other relevant data as the harvester operates in the field.

Production maps offer a clear and intuitive way to visualize the performance of the harvester in real-time. They show variations in crop yield, moisture levels, and other important parameters across the field.

One of the primary functions of production maps is to monitor and record crop yields. This data can be used to assess the effectiveness of different farming practices and identify areas of the field that may require specific attention.

Production maps are an integral part of precision farming. They enable farmers to make data-driven decisions regarding seeding rates, fertilizer application, and irrigation. This precision can lead to improved resource utilization and reduced waste (Cazacu D., 2021).

Over time, production maps generate a wealth of historical data. Analyzing this data can help farmers identify long-term trends, make predictions, and optimize their farming strategies for maximum efficiency and yield.

If the harvester experiences issues during operation, production maps can help pinpoint the problem areas. This aids in rapid troubleshooting and reduces downtime, ensuring that the harvester remains productive.

Many modern harvesters come equipped with technology that integrates production maps with farm management software. This allows farmers to manage and analyze data from all their equipment in one place.

With accurate and up-to-date production maps, farmers can make informed decisions about post-harvest processing, storage, and sales. They can identify high-performing areas and potentially plan for future crop rotations.

By monitoring crop yields and variations, production maps can also support environmentally responsible farming practices. Farmers can reduce the use of fertilizers and pesticides in areas where they are not needed, minimizing environmental impact (Cazacu D., Roșca R., 2020).

Ultimately, production maps are a valuable tool for optimizing farm profitability. By fine-tuning operations based on the data they provide, farmers can aim for higher yields and better financial outcomes.

Production maps on harvesters have revolutionized modern agriculture by providing real-time data and insights that help farmers make informed decisions, increase efficiency, and optimize their farming practices for greater productivity and sustainability.

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Interpreting data from production maps on agricultural combines is a crucial part of the agricultural analysis process. It involves understanding and extracting useful information from the data collected and graphically represented on production maps (Karampoiki, 2021).

One of the central aspects of data interpretation is analyzing crop yield. Farmers can determine high and low-yield areas, thus identifying the reasons for production variations. For example, low yield may be caused by water stress, poor soils, or plant diseases.

Production maps reveal spatial variations in production. Interpretations can uncover patterns of variation and help farmers understand which geospatial factors, such as topography, soil type, and moisture levels, influence production.

Production maps can help identify potential issues in the field, such as pest infestations or nutrient deficiencies. By identifying these issues, farmers can take more effective corrective measures (Lobachevsky YP, 2016).

Data from production maps can be used to plan agricultural resources such as irrigation, fertilization, and pesticides. This precise planning can reduce resource waste and costs.

Farmers can use data from production maps over multiple agricultural seasons to analyze trends over time. This can help assess the impact of changes in agricultural practices or weather conditions on production.

Based on data interpretation from production maps, farmers can plan future crops and crop rotations to maximize yield and prevent soil depletion (Li, 2022).

Properly interpreting data from production maps can serve as a basis for agronomic decision-making regarding land management. This may include adjusting fertilizer dosages, scheduling irrigation, and implementing crop protection measures (Zhalnin E.V., 2013).

Interpreted data from production maps can be documented and reported, providing farmers with a historical record of their performance and supplying valuable information for agricultural reports or audits.

Properly interpreting data from production maps is essential for modern farmers, as it allows them to make more informed decisions, optimize resources, and increase the efficiency and profitability of their agricultural operations.

## MATERIAL AND METHOD

To achieve the research objectives, we have created production maps for some plots of winter wheat established within a company.

Within the conducted experiments, the following objectives were considered:

- studying the reliability of the software component of the John Deere combine;
- examining the impact of using new technologies on the work process;
- investigating potential ways to utilize the generated production maps.

Based on the harvest maps generated by the combine, we were able to make decisions for the technology to be applied in the next agricultural campaign. Even though we did not have variable-rate seeding or fertilizing equipment at that time, we used the generated map to apply chemical fertilizers differentially in specific zones. Relying on the harvest maps and understanding the reasons for production variations in certain areas of the field, we made decisions to improve soil fertility by applying manure and amendments, initiating the technique of incorporating all plant residues, and reducing the working depth in areas with high organic matter in the upper horizon.

Furthermore, production maps for the following years can be tracked and compared to assess improvements in productivity resulting from the measures taken. This approach allows for a more data-driven and targeted approach to farming practices and can lead to increased agricultural efficiency and yield.

We used a S780i harvester. The John Deere S780i is a high-performance harvester designed for quality harvesting operations across a wide range of agricultural crops. It features advanced technology, such as "Automatic Crop Flow," which utilizes sensors to optimize the harvester's performance and increase efficiency, and the "Command Center" display, providing real-time information to operators about the machine's performance. In addition, the S780i is equipped with a large-capacity grain bin and a powerful engine to handle demanding tasks and provide continuous power in any conditions. It is designed for medium to large farms where advanced farming practices result in high yields and the desire for high-quality, high-yield grain harvesting.

The systems and structural elements that define the combines in the S series, and especially the John Deere S780i, include the following:

- The S780i is powered by a 13.5-liter John Deere PowerTech PSS engine, providing high power and torque for efficient harvesting;
- The S780i is equipped with a 2.75-square-meter threshing drum and a concave or counter-rotating separator system designed to efficiently separate grain from straw;
- The combine features a cleaning system with a surface area of 5.20 square meters and an air volume fan of 740 $m^3$/min, ensuring effective separation of impurities from the harvested crop;
- The Intelligent Power Management (IPM) System allows the combine to automatically adjust the engine power and travel speed based on crop conditions, optimizing efficiency;

- The S780i comes with a CommandCenter Gen 4 display, enabling operators to manage all machine functions, settings, and data from a single location;

- The combine is equipped with John Deere's automated guidance system, allowing the operator to automatically control the combine's path, maintain a consistent speed, and avoid overlap, increasing operational efficiency.

These advanced features and systems contribute to the S780i's ability to provide efficient and effective harvesting for medium to large farms, making it a valuable tool for modern agriculture.

The research was conducted using a harvester speed of 5 to 7 km/h.

## RESULTS AND DISCUSSIONS

Havesting maps generated by John Deere combines are a precision agriculture tool that allows farmers to monitor and analyze crop yields. This tool utilizes GPS technology to create detailed maps of harvested fields and can be used in conjunction with other precision farming equipment, such as liquid or solid fertilizer applicators and precision seeders.

One of the key features of John Deere Harvest maps is their ability to create detailed production maps. These maps can be used to identify areas within a field where yields are low or where there are variations in crop growth. This information can be used to adjust planting or fertilization practices to improve crop yields. Additionally, farmers can use the maps to identify areas where there may be soil or drainage issues affecting crop growth.

Another advantage of harvesting maps is that they allow farmers to easily share data with other team members. Moreover, they can be integrated with other precision farming tools and software, such as precision seeders, to provide detailed and accurate information about crop yields at every point in the field.

Overall, harvesting maps are a valuable tool for modern agriculture, enabling farmers to make data-driven decisions, optimize farming practices, and maximize crop yields.

The dedicated agricultural solution called "Yield Data" allows the analysis of production data and converts it into maps for variable rate application. As mentioned earlier, the system has an integrated feature that provides accurate information. Supported by powerful processing capabilities, Yield Data enables multi-layer analysis and visualization of various production data features, such as moisture content, weight, volume, fuel consumption, speed, and more. A cloud-based platform ensures data storage and transmission across multiple devices, which is vital for multi-year mapping of farm field productions. This facilitates data access and sharing among various stakeholders and supports the long-term analysis and planning of agricultural activities.

The issue arises when combines harvesting the same unit of land or when harvesting the same field using a single combine at different times have not been calibrated and correlated in the ActiveYield system. This can result in significant production differences between the combines. One of the best ways to avoid this problem is to perform calibration before starting work and ensuring data calibration parameters are consistent between the two machines or across different harvest times.

Calibration is a crucial step in ensuring accurate yield data collection. It involves setting up the equipment to precisely measure and record crop yields. When multiple combines are used or harvesting occurs at different times, it's essential to calibrate and correlate their systems to maintain data accuracy and consistency. Proper calibration and data synchronization can help avoid discrepancies and provide reliable production data for better decision-making in agriculture.

We must follow the operating instructions and requirements for the combine and any associated equipment. We must ensure that all equipment is properly calibrated and in optimal working condition before harvesting.

During harvesting, we should make sure the focus is on the operation. Any interferences or deviations from procedures can affect data quality. Monitor the combine and other equipment closely.

We must ensure that all necessary data is recorded correctly during harvesting.

This includes information about the area, crop type, soil type, and more. Accurate and complete data is essential for subsequent interpretation. After harvesting, ensure that the data is transferred and correlated correctly in the Operation Center or the software platform used. Verify that data from combines, GPS, and other equipment is correctly synchronized.

When interpreting data from production maps, we must pay attention to details. Identify variations, patterns, and trends that can provide valuable insights for optimizing farming practices. Recognize that there are potential error factors, such as GPS inaccuracies or terrain variations, that can affect data. Try to quantify and understand these factors in data interpretation.

We must ensure that data is stored in a secure environment and can be accessed for future analyses. Regular backups are essential.

The problems caused by differences in the header's height relative to the ground during

operation can be due to Header Height Switch Issues. Problems with the header height switch, which may fail to detect when the header is raised or lowered, resulting in no production data being recorded. This can lead to missing data or gaps in production data if the issue persists. Commonly, operator error may result from leaving the header in the harvesting position between fields or not raising and lowering the header correctly at field edges or unsown areas. This can lead to missing or inaccurate data. To obtain high-quality harvest maps, the operator should monitor the operating parameters during the harvesting process, and the map generated in the Operation Center. Even if the field does not have a regular shape, the AutoPath system maintains the same working width of the combine, reducing idle pass areas. When the combine harvests an area that doesn't cover the full width of the header with vegetation material, the production yield is reduced, and the obtained data becomes inaccurate. At the end or beginning of a pass, when the combine accelerates or decelerates, the production map is altered, overestimating or underestimating the production. The generated production map on a 7.83 ha area by John Deere S780i harvester is shown in *figure 1*.

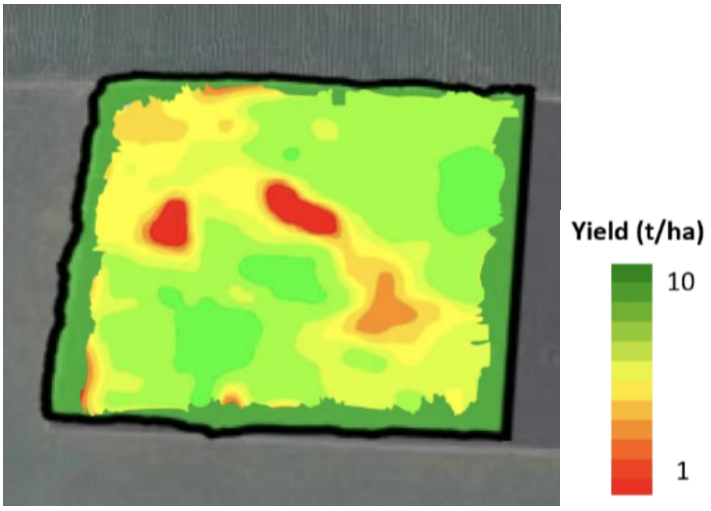


Figure 1 **Production map generated by John Deere S780i harvester for winter wheat**

## CONCLUSIONS

These types of errors can also occur in the middle of a pass if the combine accelerates or decelerates suddenly.

These types of production data issues can be avoided by making gradual adjustments to the combine's speed. In cases where this is not possible, the John Deere Operation Center will eliminate erroneous data during data processing.

In conclusion, mapping productions using the John Deere S780i harvester and its associated systems provides farmers with valuable insights about their farm.

These insights can be used to make well-informed and calculated decisions aimed at enhancing overall productivity, sustainability, and profitability of the agricultural operation. By using production maps and variable rate for sowing, for spreading fertilizers and for phytosanitary treatments, we can reduce very much the costs for each crop.

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# NUMERICAL ANALYSIS OF PORE WATER PRESSURE CHANGES OF AN EARTH DAM AND MONITORING OF VERTICAL DEFORMATIONS. CASE STUDY - PLOPI DAM, IASI COUNTY

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**Abstract**

Numerical analysis of pore water pressure changes and the evolution of vertical deformations are the main aspects of the behaviour monitoring of an earth dam. This study analyzed the main aspects of the behaviour monitoring of the Plopi Dam, built on the Gurguiata River, in the northwest of Iași County, Romania. The main data taken into consideration are the dam body's type and material, the foundation's soils, and the dam's monitoring equipment. For tracking the evolution of the pore water pressure, 12 piezometers are used. The Plopi Dam is equipped with 19 piezometers, of which 9 piezometers (marked with B) show the route of the infiltration curve through the dam body and 10 piezometers (marked with F) have the bottom level in the base of the foundation and record the variation of groundwater levels in the aquifer. The paper presents the pore-pressure ratios in embankment material throughout its existence, mainly in the last 12 years, and the influence of external stresses on the hydrostatic levels. Data obtained for a period of 45 years (1978-2023) from 11 landmarks, placed on the dam canopy, were used in the settlement analysis of the embankment The filling in the dam body is still being consolidated, the settlements are significant, the pore pressures are high, so the monitoring of the dam's behaviour is done continuously so that the dam is safely exploited.

**Key words**: dam, pore pressure, hydrostatic level, settlements, consolidation

Dams are barriers to the river flow that are either constructed or natural. A dam is a hydraulic structure of impervious material built across a flowing stream, river, or water channel to create a reservoir of impounded water for such purposes as water supply, irrigation, hydropower, flood and erosion control, navigation, fishing, recreation, and other uses (Osuagwu J.C. *et al*, 2017).

The high risks associated with earth dams in operation may be mitigated by reliably, extensively, and resolutely checking the embankment and natural reservoir slopes behaviour, through the interpretation of monitored precursors and/or indicators, in order to promptly bring to light the presence of dangerous phenomena. The pore water pressures, the seepage flows, the displacements, and the total stresses are the typically monitored variables to ensure dam safety. Monitoring the surface displacements hence represents an effective tool to check the above mentioned risk phenomena, especially global instabilities within the embankment and reservoir natural slopes (Martire D.D. *et al*, 2014).

Vertical deformations of the embankment dams, if not effectively monitored, could be disastrous for the structural integrity of the dam (Osuagwu J.C. *et al*, 2017). Settlements may lead to dangerously diminished canopy levels, and thus result in dam overtopping, which could result in dam failure and complete washing away of the dam. Deformations of embankment dams have to be measured at least once every year. In this way, any sudden changes in settlement can be detected and a more detailed evaluation can be carried out. The monitoring data must be analyzed and evaluated continuously and presented graphically so that both long-term and short-term tendencies are visualized (Pytharouly S., Stiro S., 2008).

The dam of the Plopi reservoir, located on the Gurguiata River, is an earth dam that provides a global retention to the canopy of 11.293 million cubic meters (Water Basinal Administration Prut – Bârlad, 2022). The dam is made of earth, of homogeneous type, with a maximum height of 10.50 m and a crown length of 330 m and has a 1m high concrete parapet in the upstream part of the

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canopy. Immediately downstream of the Plopi dam, the Huc buffer pond is located. The water level in Huc pond of 75.16 meters above Sea Level is 3.08 m below the established Normal Retention Level in Plopi reservoir (78.24 maSL). In the area of the dam's embankment, the soils consist, from a lithological point of view, of muddy clay soils, weakly consolidated, very compressible, and of very low load-bearing capacity, developed on thicknesses of 2-5 m. In most of the drillings carried out in the dam body, the groundwater was found at depths between 0.5 and 1 m from the surface of the land (*figure 1*).

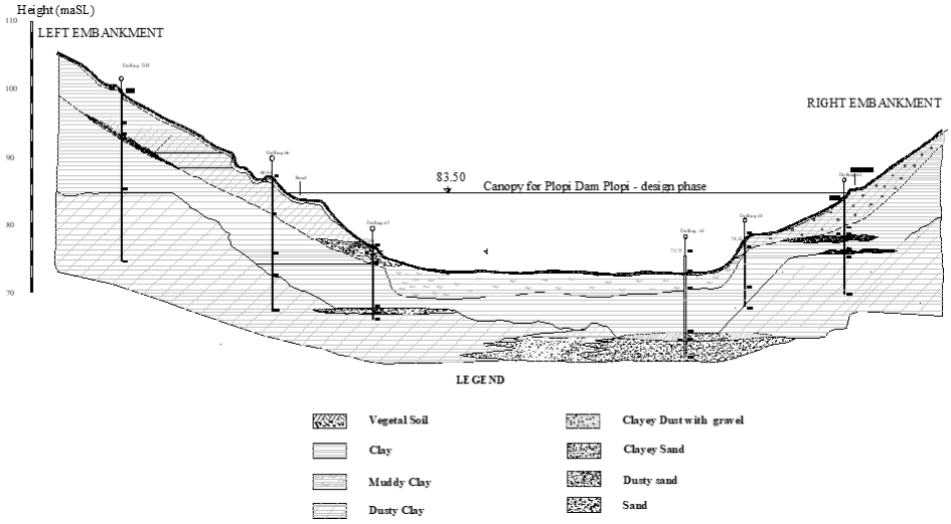


Figure 1 **Longitudinal section through The Plopi Dam foundation ground**

Based on laboratory analyses, from the early stages of design, it was established that the foundation ground, although compressible, still has sufficient load-bearing capacity to support the load transmitted by the weight of the dam. Also, from the early stages of construction of the dam, the layers presented important settlements, longitudinal cracks, tears, and movements of the fillings to the downstream. During construction, higher than expected construction pore pressures were experienced in the dam body. On completion of the embankment construction it was found that pore water pressures in the core were particularly high and that even at the lower elevations there had been very little dissipation of pressure.

The analysis showed that the pore pressures within the dam body are just within acceptable limits, but are dissipating far slower than had originally been envisaged.

The main component of dam behaviour monitoring is represented by the measuring installations for the dam response to stresses.

- For tracking the evolution of vertical deformations are used:
  - 11 vertical axis landmarks on the canopy of the dam
  - 2 fixed landmarks;
  - 2 landmarks on the operation tower;
- For tracking the evolution of body dam seepage there are 19 piezometers (9 piezometers marked with B are for determining the infiltrations through the dam body, 10 piezometers marked with F indicate the level of the water column resulting from the pores of the foundation ground). The piezometers are mainly distributed in 5 major characteristic sections, marked with S I – S V (*figure 2*).

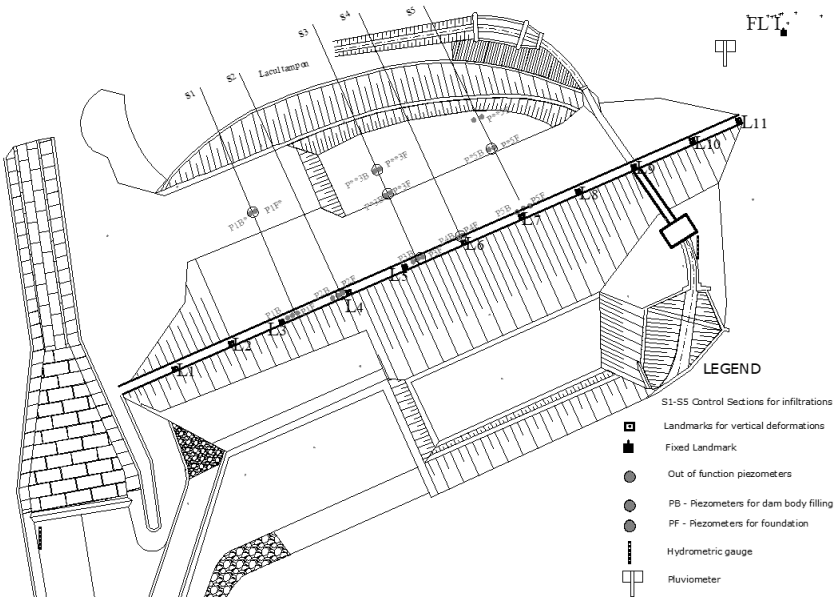


Figure 2 **The Plopi Dam plan view**

## MATERIAL AND METHOD

### 1. Pore pressures

Most methods available to design engineers for predicting the development of pore pressures during and soon after the construction of earth dams use the approach that assumes the equality of pore pressure at a given point with a certain percentage of the over-burden pressure. Additional monitoring of pore pressures can prove necessary, particularly on high concrete dams. Measurement of pore pressures is recorded together with the water level in the reservoir (William P.J., 2004). Geotechnical problems that earth dams could experience during their operational stages are mainly related to slope instability and internal erosion phenomena (Jannati P., Mohammadi M., 2010). Dam loading history should be preventively known; in addition, monitored physical quantities and results from periodical inspections should be suitably collected and interpreted during the different stages of the dam's life (Nayebzadeh R., Mohammadi M., 2000). As consolidation of an embankment occurs, the excess pore pressures are dissipated. The pore pressure ratio is defined as the pare pressure at a given point divided by the overburden pressure above that point (*u/gh*). This ratio is dimensionless.

Bishop has noted that failure is likely to occur in a dam where the average pore pressure ratio throughout the entire cross-section of the embankment exceeds 0.60, *except* in the case of a low dam. For limited water content variation ratio is influenced by fill height which in turn reflects such factors as the length of the drainage path and the degree of saturation (Clough C.W., Snyder J.W., 1966). Infiltration and seepage analyses are important tool to assess the susceptibility of seepage failure in dams and to study the hydraulic conditions for analyzing the stability of dam slopes. Factors that affect the build-up of construction pore pressures are numerous. Placement water content, overburden weight, length of drainage path, rate of construction including construction stoppages, nature of the care material, and presence of drainage features all exert some effect. The seepage through an earth dam generally correlates with the reservoir water level of the dam. The seepage rate through a dam should be measured and used as a basis for a seepage stability evaluation (Lee J.-W. *et al*, 2018). The monitoring of pore pressure as well as water levels of an embankment dam to observe potential seepage problems is important; anomalies that are indicative of internal erosion problems should be detected in advance to prevent catastrophic consequences. Piezometers are the most commonly used instruments to monitor the water level in dams, which can often be used to compute pore water pressures (Crum D., 2011). According to Clough G.W., Snyder J.W. (1966), the research of several scientists, including Hilf J.W., concluded that for a dam that has no upstream sealing or impervious core, but has downstream drain blanket, the maximum pore pressure ratio in a dam body has a value between 0.4 and 0.65 (Clough C.W., Snyder J.W., 1966).

### 2. Vertical deformations

The study reviewed the main features of the embankment and instrumentation devices incorporated into the embankment to accommodate the prediction and analysis of settlement of the fill material and the bedrock in the post-construction era. The scope of this study was limited to the canopy of the dam because landmarks are only placed on the canopy. To calculate the percentage of the total elevation of the dam that settled at a section of the dam, the following equation is used (*eq. 1*):

$$P(\%) = \frac{s}{H_{total}\%} \quad (1)$$

Where, *P(%)* is the percentage of the dam elevation that settled at a section, *s* is the actual settlement at the section and $H_{total}$ is the total dam elevation. A dimensionless parameter known as the *Settlement index* was calculated for each of the eleven sections. Equation 2 is the equation of the settlement index as reported by Pytharouli S. and Stiros S. (2008):

$$S_i = \frac{s}{1000 \cdot H \cdot \log(\frac{t_2}{t_1})} \qquad (2)$$

Where $S_i$ is the settlement index, *s* is the canopy settlement measured in mm between the time period and since the completion of the embankment at a section of the dam meters high - *H* (Charles J.A., 1986). Values greater than 0.02 indicate that mechanisms other than creep or secondary consolidation of the embankment dam material contribute to dam settlements (Tedd P. *et al*, 1997). The settlement index is analogous to the coefficient of secondary consolidation for a clay soil. Pytharouli S. and Stiros S. (2008) identified the mechanisms other than the creep or secondary consolidation affecting crest settlement of the Kremasta Dam in Greece as:

1) Reservoir level fluctuation and

2) Rainfall

So, if the value of Si > 0.02, that means that other than creep or secondary consolidation, the other mechanisms mentioned above are responsible. Limit values are important to give a warning in an emergency situation (Charles J.A., Tedd P., 1991). The average settlement index at each section can be plotted against the time period to obtain a time series consisting of the values of the average settlement index for each of the sections.

## RESULTS AND DISCUSSIONS

The analysis of the behaviour in time of Plopi dam was performed by examining the evolution of the response parameters (piezometer levels, infiltration flows, displacements) to the external stresses. The variations of the water levels in the reservoir, of the precipitations, and of the registered temperatures that acted on the reservoir and in the basin catchment had the main impact on the variation of the water levels in the piezometers and implicitly on the infiltration regime through the dam.

### 1. Pore pressures

For further study of the infiltration regime in the dam, the research focused on the hydrostatic levels in all of the piezometers (Section I-V) for a period of 23 years, with an accent on a shorter period of time, during the years 2012–2023 (*figure 3).*

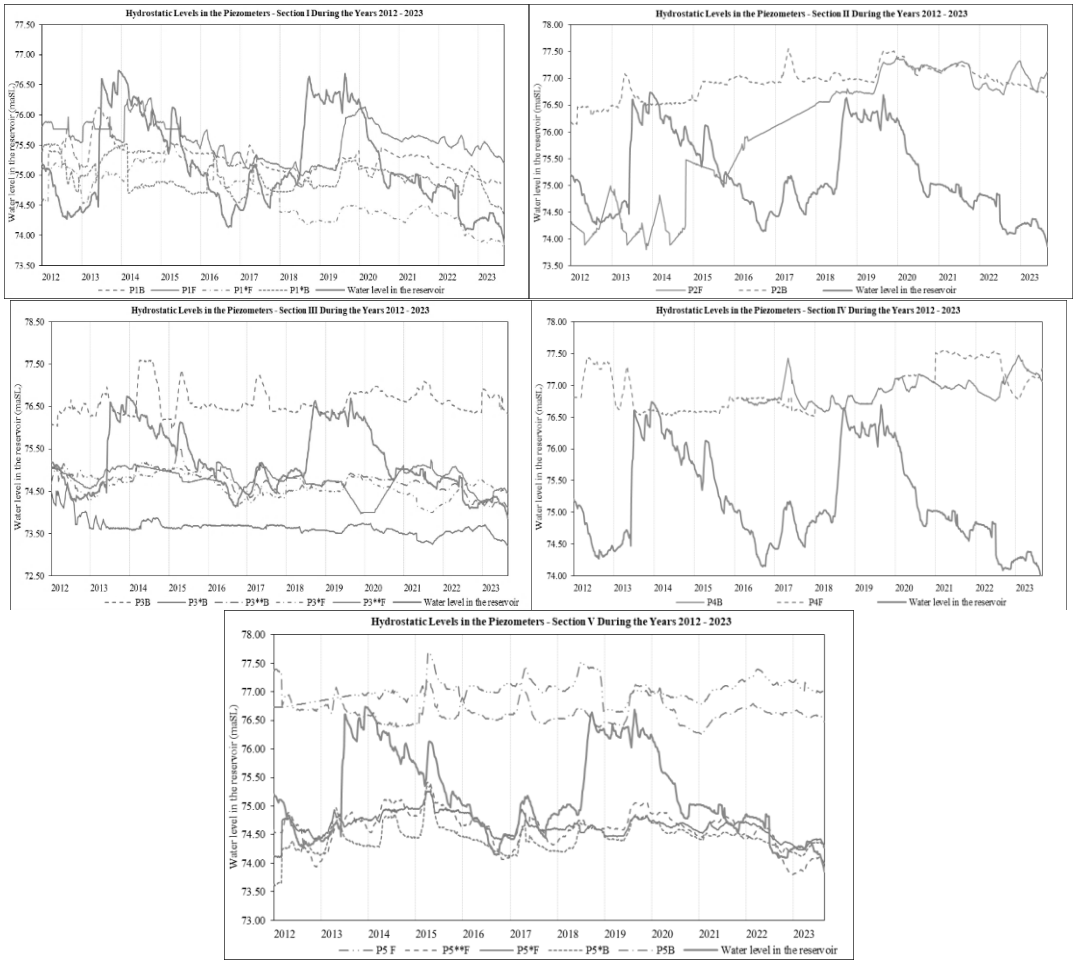


Figure 3 **Hydrostatic Levels in the Piezometers During the Years 2012-2023**

The behaviour monitoring of the dam is a very complex activity, performed by following the indications of specific legislation and technical regulations. Synthetic analysis for the behaviour monitoring of the dam in a given period of time formulates the conclusions regarding the overall exploitation of the dam and suggest the required works of rehabilitation, that may be imposed by the technical status of the hydro-technical construction.

Installed piezometers upstream of the dam body show a higher pressure than the downstream, due to the high saturation state of the phreatic line.

In Romania, the *Reports of Synthetis of Behaviour Monitoring of the Dams* are analyzed according to the specific regulations. In the year 2004, such Report was analyzed by a Comitee and the experts concluded: given that the dam was constructed on muddy clays, and that this is an important risk factor for the stability of the dam, the in-depth judgment of the measurement results of F-type depth piezometers was recommended, paying particular attention to the time variation of the measured values, in correlation with water levels in the reservoir. It was suggested that this follow-up be done on the basis of the *HILF criterion* (the ratio between the water pressure in the pores and the weight of the soil filling at the respective measurement point) by which, at a value of 0.5 it is recommended to enter the *attention phase* (Balan I.E., 2021). Therefore, the pore pressure ratios were calculated for each of the piezometers that indicate the level of the water column resulting from the pores of the foundation ground, and represented in *figure 4*.

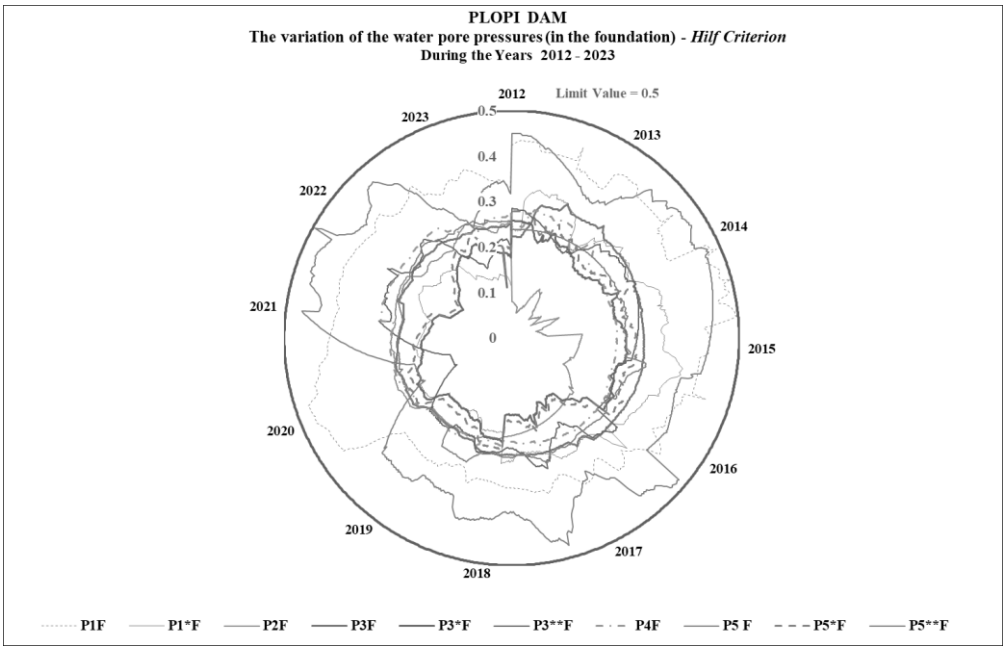


Figure 4 **The Variation of Pore Pressure Ratios during the Years 2012-2023**

The position and height of every tube, and the lower part of the piezometers were taken into account, along with the hydrostatic levels measured by the designated hydrotechnical agent. The calculated pore ratios were all drawn as point lines in one graph shown in Figure 5. It can be concluded that all of these piezometers have pore pressure ratios within the admissible range represented by the value of 0.5 that was set according to the *HILF criterion*. This analysis was only performed for a period of 12 years for which there was available the complete archive of hydrostatic levels. But the incomplete archive that was available for previous periods of time, also showed pore pressure ratios within the admissible range.

**2. Vertical deformations**

For the measurements of the vertical deformations, a Leica Geosystems Total Station was used. The measurements were taken on the vertical axis landmarks placed on the dam canopy, from 1978 to the year 2023. The average annual vertical deformation for the last 23 years is about 11.8 mm/year (*figure 5*).

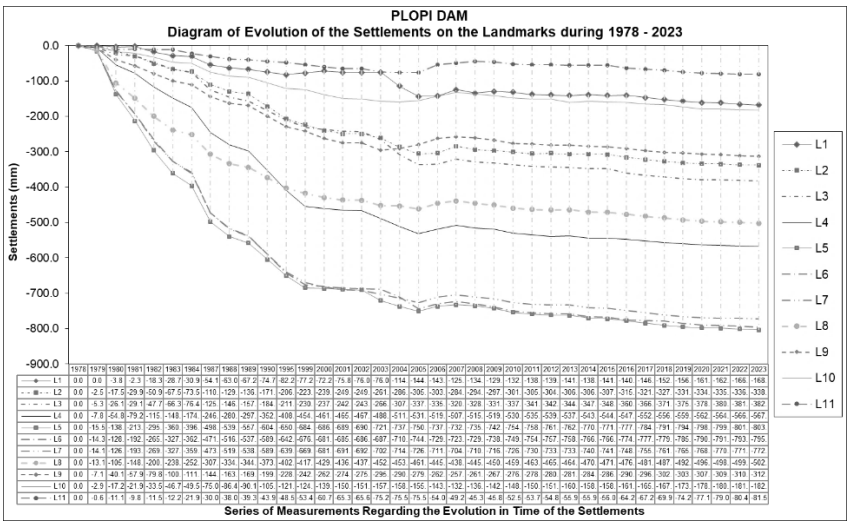


Figure 5 **Evolution of the annual vertical deformations on the L1-L11 landmarks during 1990 – 2023**

The maximum annual settlement, as measured by the landmarks, since the end of construction was about 113.8 mm in the central area of the dam (landmarks L6). The cumulative settlement of the crest, as measured by the landmarks, since the end of construction to the present time is about 803,8 mm, or 7.65% of the maximum embankment height, in the central area of the dam - landmarks L6 (*figure* 6).

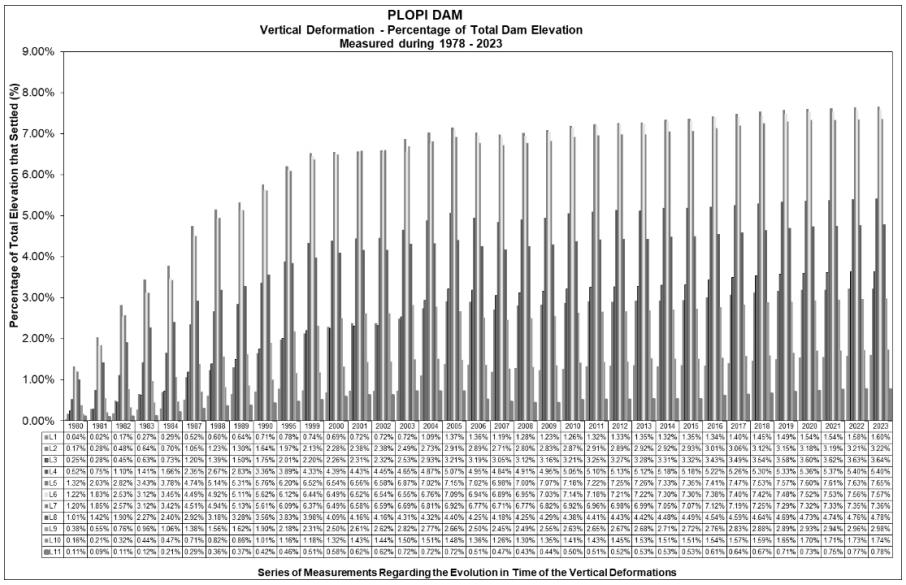


Figure 6 **Evolution of the Settlements on the Landmarks during 1978 – 2020**

The water level in Plopi reservoir hasn't fluctuated considerably throughout the years. Since the final impounding in the year 1984, the water level has mainly been near or below the Normal Retention Level established by design. The daily fluctuation rate has been below 50 cm/ 24 hours, so the water level hasn't influenced considerably the settlements of the dam (*figure 7*).

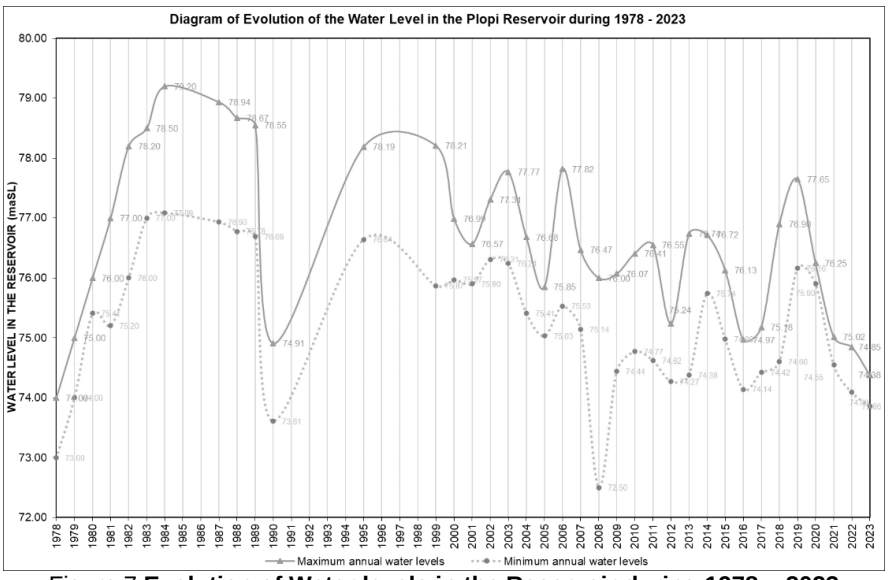


Figure 7 **Evolution of Water levels in the Reservoir during 1978 – 2023**

Settlement indexes $S_i$ were calculated for all of the 11 landmarks, regarding the last 30 years of existence, for separate intervals of time of 5 years (*figure 8*). As seen in *figure 8*, the Settlement Indexes $S_i$ calculated for the deformations measured during 1990 – 2023 are below the value of 0.02 which can indicate the fact that dam settlements are not affected by creep or secondary consolidation of the embankment dam material.

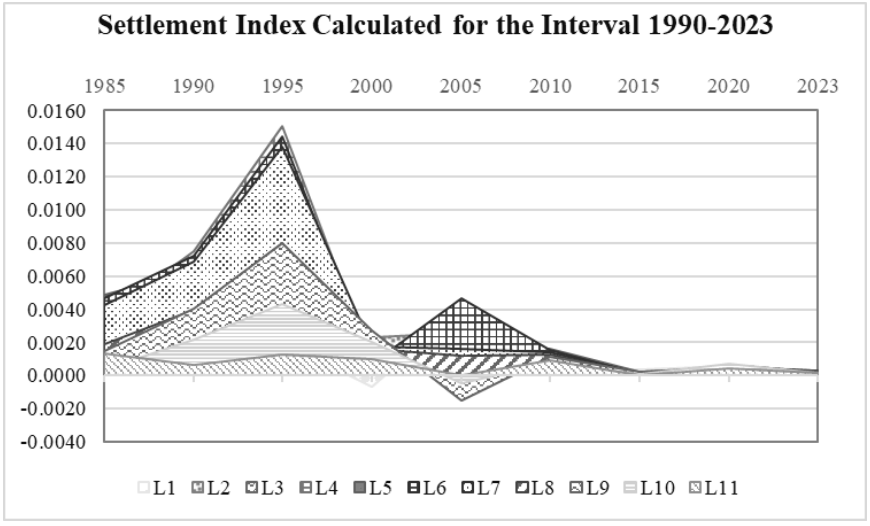


Figure 8 **Settlement Index Calculated for the Measurements on the Landmarks during 1990–2023**

The foundation ground, formed mainly by muddy soils, seems to have insufficient load-bearing capacity to support the load transmitted by the weight of the dam, and the groundwater was found at depths between 0.5 and 1.0 m from the surface of the land. From the early stages of construction of the dam, the layers presented important settlements, longitudinal cracks, tears and movements of the fillings to the downstream. The maximum values of the Settlement Indexes $S_i$ were calculated for the deformations measured during the year 1990. The water level in Plopi reservoir had a significant variation during that year, as there was a difference of 3.6 m compared to the maximum water level values of the previous year. Therefore, it is found that the deformations of the earth fill are affected by the level fluctuations, and the Settlement Indexes $S_i$ are directly influenced by the variation of the water level in the reservoir. The visual observations made by the exploitation personnel have not highlighted visible cracks, or tears in the dam body. It seems that the downstream Huc fishpond has contributed to a state of equilibrium of the dam, so the rate of annual settlement has gradually decreased in the last few years, even if the consolidation process continues.

The special monitoring activity showed that the Plopi dam is characterized by significant vertical deformations of the earth filling (maximum settlement 803.8 mm measured at the L6 landmark positioned in the central part of the dam), high infiltration levels and very high hydrostatic levels of

the aquifer. However, considering the good behaviour of the dam since commission, it is generally agreed that the dam behaves properly, according to the general design predictions.

## CONCLUSIONS

The objective of the study was to evaluate pore water pressure changes in Plopi Dam. The analysis of the behaviour in time the Plopi dam was performed by examining the evolution of the response parameters (piezometer levels, infiltration flows, displacements) to the external stresses. All piezometers had pore pressure ratios within the admissible range represented by the value of 0.5.

*The Synthesis Reports of the Dam Behaviour Monitoring* performed on an annual basis and the *Post-event Reports* elaborated after the occurrence of significant floods, atypical behaviours, incidents or accidents may highlight structural changes in the dam and adjacent hydrotechnical constructions. Subsequently, it is necessary to implement the maintenance, repair or rehabilitation works of the dam. We can conclude that, although the dam body hasn't fully consolidated nowadays, it is in good shape and can still very well ensure the retention of water in Plopi reservoir and can provide the designed flood protection. The intensive behaviour monitoring of the dam is done continuously with an increased degree of attention, according to the regulations for this type of earth dam.

It is necessary to continue the safe operation of the dam in accordance with the *Special Monitoring Project* (Water Basinal Administreation Prut-Bârlad, 2017) and the *Operating Regulations* (Water Basinal Administration Prut-Bârlad, 2016).

## ACKNOWLEGMENTS

There was no external funding for this study. This study presents the results of a research project that took place at the "Gheorghe Asachi" Technical University, Iași and it could not have been made possible without the data provided by the Water Basinal Administration Prut-Bârlad.

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# PARASITIC FUNGI ON ESTIVAL PLANTS FROM THE NE PART OF ROMANIA

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**Abstract**

Diversity is vital for effective ecosystem functioning and represent a part of biodiversity and ecosystem research. Parasitic fungi that grow on plants have reshaped the biosphere and caused the deaths of millions of people since the beginning of agriculture. Nowadays, interest for biodiversity conservation is intensified by concern about the conservation of genetic resources, destruction of forest, extinction of species and the effects of global warming. There are more than 70,000 species of fungi described by mycologists and over 90% of them are classified within Phylum *Basidiomycota* and Phylum *Ascomycota*. Understanding relationships between biodiversity and ecosystem functions is very important in the context of global plant diversity loss. This paper presents some parasitic micromycetes identified on some estival plants from different areas of Iasi County. In our fieldwork made in the spring of 2023 were indentified some parasitic micromycetes to species as: *Corydalis solida* L. Clairv., *Scilla bifolia* L., *Anemone ranunculoides*, *Ranunculus ficaria* L. and *Fritillaria meleagrioides* Patrin ex Schult. & Schult. f.. Identified parasitic micromycetes during the observations that have been made were differentiated according to the disease they cause on plants. Thus, the main diseases identified are represented by: rusts, smuts and downy mildew.

**Key words**: parasitic fungi, biodiversity, conservation.

Global change studies have demonstrated that we must account for the response of pathogens to a changing environment in order to understand host physiological population and community responses (Eviner V.T., Likens G.E., 2008). Mounting evidence shows that biodiversity loss frequently increases disease transmission. conversely, areas of naturally high biodiversity may serve as a source pool for new pathogens. Overall, current evidence indicates that preserving intact ecosystems and their endemic biodiversity should generally reduce the prevalence of infectious diseases (Keesing F. *et al*, 2010)

Throughout time plant pathologists have conventionally been concerned with the control or elimination of plant pathogens from crops, rather than their conservation or studies of their role and significance in natural ecosystems. But with the continuing accelerated loss of habitats and ecosystems world-wide, the increased use of fungicides, pesticides and herbicides in agriculture, and the permittig of genetically modified organisms (GMOs), the threats to pathogen diversity in the wild are immense.

Biodiversity is under threat because steady decline in the numbers of pathogen systematists, brought about by ignorance of the importance of systematics and changes in scientific approach, will undermine the curation of those collections that survive and will lead to significant losses of these irreplaceable resources. Therefore is an urgent need for plant pathologists to address to issues like diversity and significance of plant pathogen populations in natural ecosystems.

## MATERIAL AND METHOD

Observation regarding the presence of the parasitic fungi on estival plants have been conducted according to an itinerary from two different areas of Iași County, durind the months March - April of 2023. A first area in which were made observations is located in the Northern part of the Cotu Morii village, from Popricani commune, Iasi county (Coordonate: 47°18′14″N 27°33′0″E). The other area were situated in Aroneanu commune, also from Iasi county (Coordonate: 47°12'59.5"N 27°37'36.2"E).

Identified host plants were collected and brought to the research laboratory of the Phytopathology discipline, within the “Ion Ionescu de la Brad” Iasi University of Life Sciences (IULS). Micromycetes identification was done based on microscopic preparations and specialized guide book, after that micromycetes were included in Herbarium Mycologicum Moldavicum” C. Sandu

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## RESULTS AND DISCUSSIONS

Environmental conditions of the spring of 2023 were extremely favorable for some parasitic fungi infection on estival plants as: *Corydalis solida* L. Clairv., *Scilla bifolia* L., *Anemone ranunculoides*, *Ranunculus ficaria* L. and *Fritillaria meleagrioides* Patrin ex Schult. & Schult. f. As well as the growth stage of the plants, temperature and humidity have an important role in the onset of infections. The temperatures in the winter of 2023 were quite high, the monthly average exceeded 0 degrees in both January and February. During the observations, the monthly average air temperature (°C) was between 6.71°C in March and 15.79 °C in May (*figure 1*). Precipitation (mm) registred in the spring of 2023 for Iasi were over 75 mm in April (*figure 2*). These weather conditions have led to an increase of some parasitic fungi, such as: *Uromyces ficariae* (Schum) Lév., *Uromyces lilii* (Link.) Fuck., *Puccinia rossiana* (Sacc.) Lagh., *Tranzschelia pruni-spinosae* (Pers.) Dietel, *Ustilago scillae* Ciferri, 1931, *Peronospora corydalis* de Bary or *Peronospora ficariae* (Ness v. Essenb.) Tul. that infected wild estival plants.

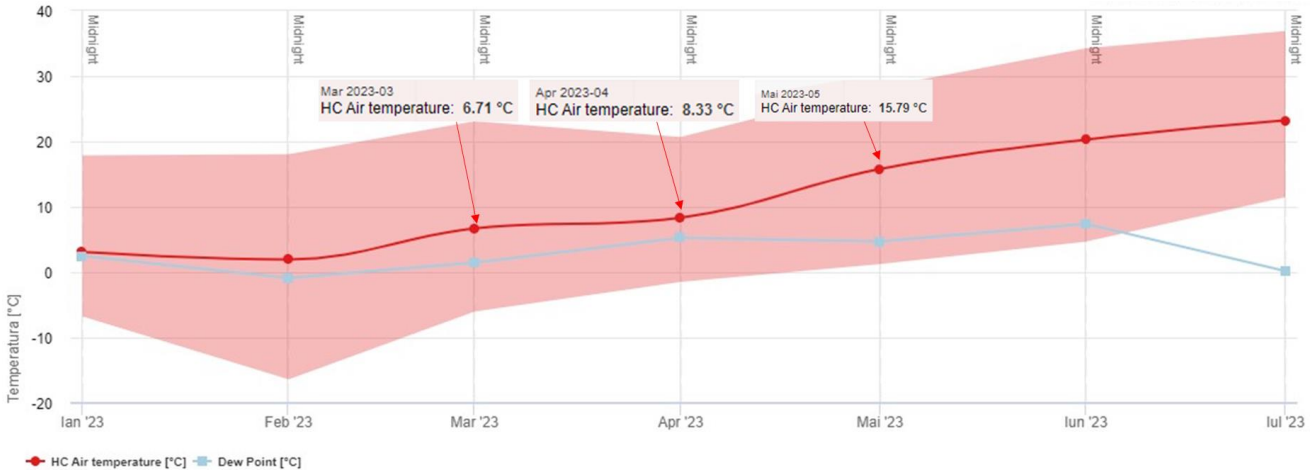


Figure 1 **Average of Air temperature (°C) and Dew Point (°C) registred in the spring of 2023 for Iasi, Romania**

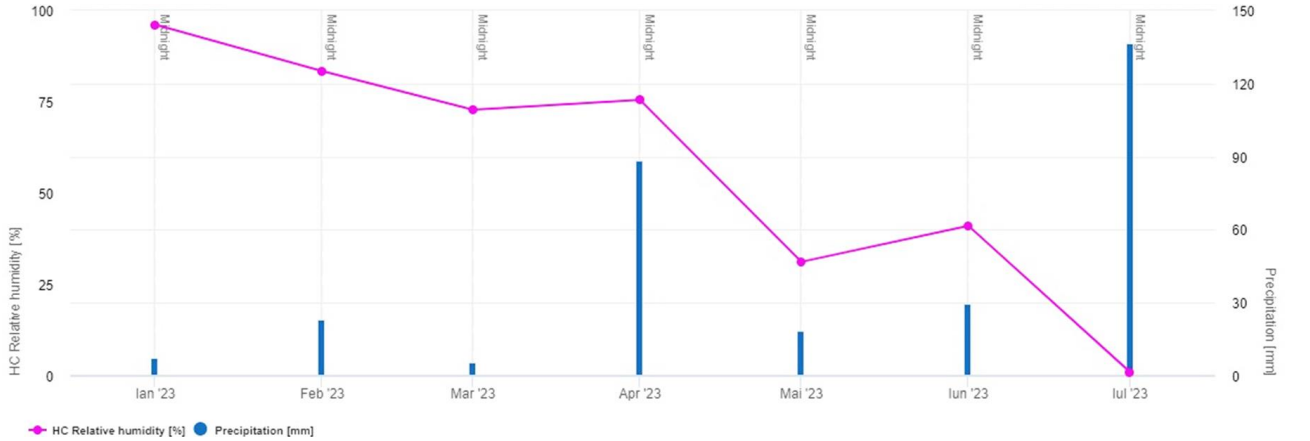


Figure 2 **Average of relative humidity (%) and precipitation (mm) registred in the spring of 2023 for Iasi, Romania**

Species of *Uromyces* occur on a wide variety of plant hosts around the world. The most important ones are caused by *Uromyces* spp. that cause infections and damage to various agricultural crops being recorded also on the host plants belonging to the families *Asteraceae*, *Euphorbiaceae*, *Fabaceae*, *Liliaceae*, *Loranthaceae*, and *Poaceae* (Gautam, A.K. *et al*, 2022).

As rust diseases occur most often in mild, moist conditions, in the spring of 2023 we observed this disease on species as *Anemone ranunculoides* L., *Ranunculus ficaria* L., *Scilla bifolia* L. and *Fritillaria meleagrioides* Patrin ex Schult. & Schult. f.

Human anthropogenic activities also play an important role in the global distribution of these organisms. So far, *Uromyces* species have been found on every land on earth except Antarctica

(Gautam, A.K. *et al*, 2022). One of the little explored groups includes heteroecious grass rusts, having aecial state on species of *Ranunculus* L. and *Ficaria verna* Huds. (Hrabětová, M. *et al*, 2015). On our observations *Ranunculus ficaria* L. was identified *Uromyces ficariae* (Schum) Lév., syn. *Aecidium ficariae* (Schum.) Saccardo, 1884 that produce on leaves yellow aecia in compact groups (*figure 3*), up to 20-50 together with spores about ± 3 µm large, slightly flattened, easily detachable appendages.

Figure 3 **Yellow aecia of *Uromyces ficariae* on *Ranunculus ficaria***

Another species identified was *Uromyces lilii* (Link.) Fuck. on *Fritillaria meleagrioides* Patrin ex Schult. & Schult. f. plant, that is a rare species recently reported in the Romania's vascular flora (Sârbu C. *et al*., 2019). First report of the species *Fritillaria meleagrioides* Patrin ex Schult. & Schult. f., in the northen part of Cotu Morii village (Popricani commune, Iasi) was in the year of 2015 (Oprea A. *et al*, 2015).

Figure 4 **Aecia of *Uromyces lilii* on *Fritillaria meleagrioides***

*Uromyces lilii* (Lk.) Fuck were identified on *Fritillaria meleagrioides* in the last decade of April 2023. Infected plants showed on stem, petiole and leaves pale yellow amphigenous aecia, opening with a central pore (*figure 4*).

Aeciospores are spherical, subspherical, angular spherical or elongated, with a yellow washed almost colorless membrane that presents dense and fine protuberances (*figure 5*).

From our knowledge, *Uromyces lilii* (Link.) Fuck is cited in our contry only on two other different *Fritillaria* species such as *F. tenella* M. Bieb. and *F. meleagris* L. (Florea A.M. *et al*, 2021).

First–largest rust genus is rapresented by *Puccinia*. From this genus we observed in the spring of 2023 the presence of *Puccinia rossiana* (Sacc.) Lagh., Syn. *Puccinia liliacearum* Duby ssp. *rossiana* Sacc. on *Scilla bifolia* L. a herbaceous perennial plant belonging to the genus *Scilla* of the family A*sparagaceae* known as alpine squill or two-leaf squill.

On infected plants teliospores are formed in large groups around the leaf tip, dark brown colored (*figure 6*).

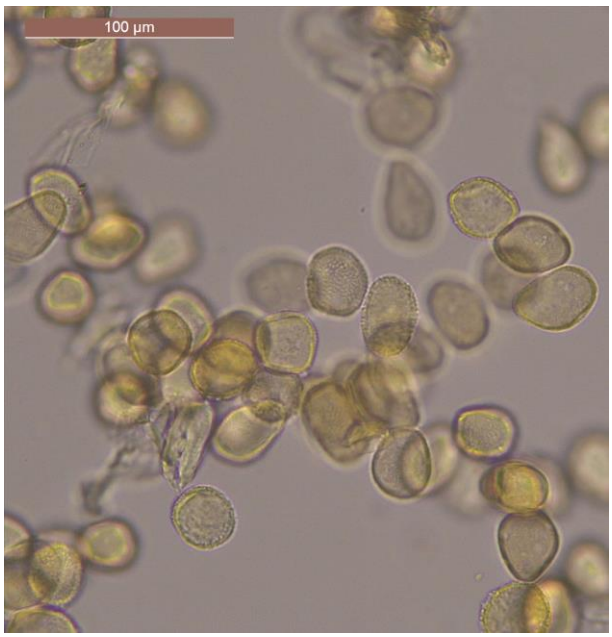


Figure 5 **Aeciospores of *Uromyces lilii***

*Puccinia rossiana* (Sacc.) Lagh forms two-celled teliopores, all with many small but clear pits, also they have pore of the apical cell with an acute papilla (*figure 7*).

Figure 6 **Teliospore pustules of *Puccinia rossiana* on *Scilla bifolia***

Infected plants of *Anemone ranunculoides* L. with the fungus *Tranzschelia pruni-spinosae* (Pers.) Dietel 1922, Syn. *Aecidium puctatum* Pers. appeared paled and with a grow stiffly upwards and narrowed leaves (*figure 8*).

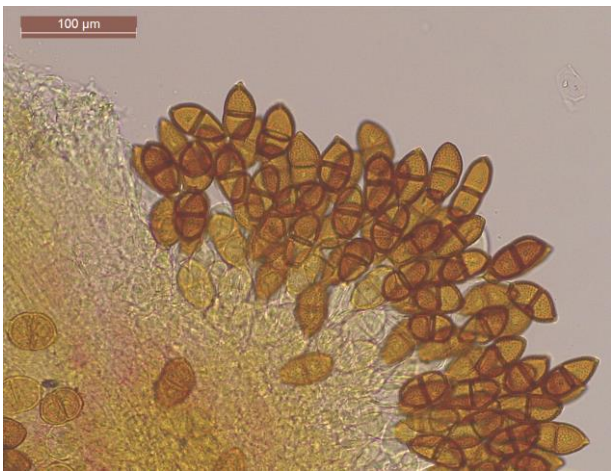


Figure 7 **Teliopores of *Puccinia rossiana***

*Tranzschelia pruni-spinosae* produce leaf rust on plum and the main reason of the incidence is represented by the aecial state of this rust via its aecial host viz. *Anemone ranuncoloides* L., also by increasing of nitrogen and other pollutants as a result of intensive human activity in the forests and climatic change seems to be involved in occurrence of *Tranzschelia pruni-spinosae* (Abbasi, M., 2021).

Figure 8 ***Anemone ranunculoides* infected plants with *Tranzschelia pruni-spinosae***

Smut fungi represent a large group of biotrophic plant pathogens that cause extensive yield loss and are also model organisms for studying plant–pathogen interactions. The vast majority of smut fungi infect angiosperms, and while most prefer annuals, some infect perennial

plants (Van der Linde K., How G.V., 2021). In Central Europe, *Ustilago scillae* Ciferri, 1931, smut fungus is present from the lowland to submontane vegetative belts. It was registred on *Scilla bifolia* L. by Vánky (1985) near Mosonmagyaróvár in Hungary, as well as in Romania and Yugoslavia (Bacigálová K. *et al*, 2005).

From smut group of plant pathogens, in the spring of 2023 we observed the presence of *Ustilago scillae* Ciferri, 1931, syn. *Antherospora scillae* (Ciferri) Bauer, Lutz, Begerow, Piątek & Vánky, 2008 on *Scilla bifolia* L., in a forest area situated in Aroneanu commune, from Iasi county (Coordonate: 47°12'59.5"N 27°37'36.2"E). During in the midle of March, infected plants appeared taller, higher, and bear more flowers with enlarged anthers and covered with silvery epidermis. Anthers and ovaria transformed into a dark olive-brown powdery spore mass (*figure 9*).

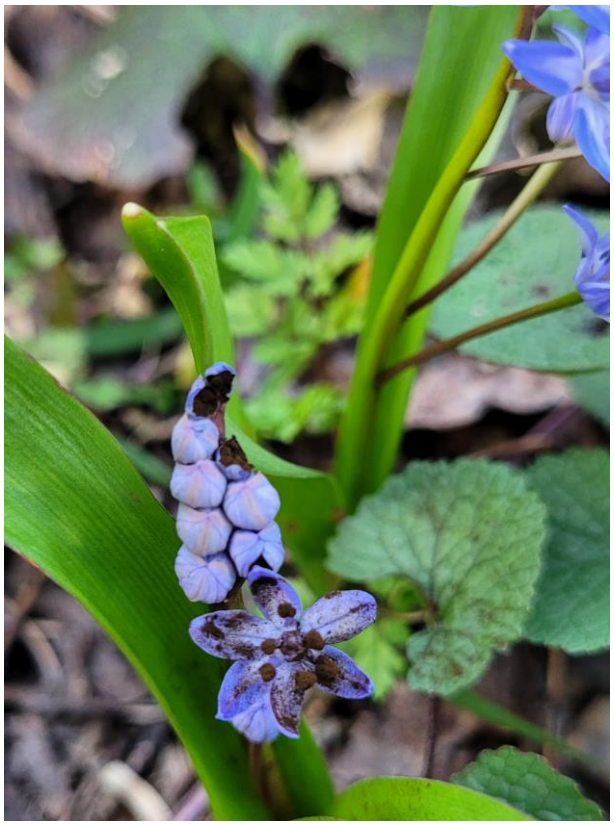

Figure 9 **Spore mass of *Ustilago scillae* on *Scilla bifolia* infected flowers**

*Ustilago scillae* Ciferri spores vary considerably in shape appearing globose, ovoid, elongated and irregular, pyriform, curved or subpolyhedral. Under the microscope the spores are olive-brown, with a thick sporewall, finely and densely verruculose (*figure 10*).

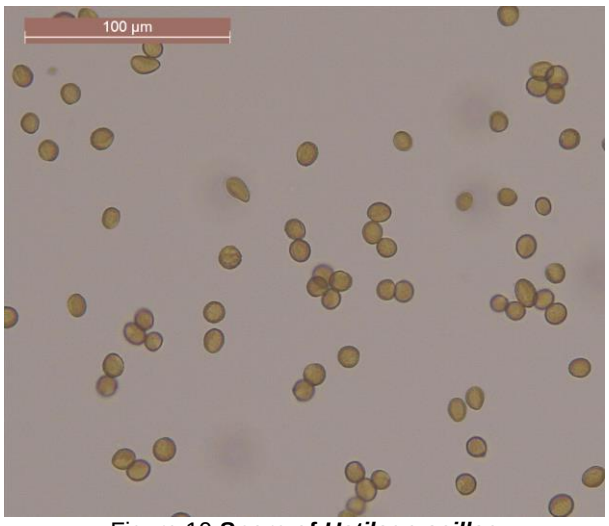


Figure 10 **Spore of *Ustilago scillae***

*Peronospora* is a genus of fungi classified as *Oomycetes*, that parasitisez plants, mainly dicotyledons. This group of parasitic fungi cause diseases called downy mildews and some species are of great economic importance because they cause serious diseases of crops and ornamentals (Thines M. and Choi Y.J., 2015).

We identified *Peronospora corydalis* de Bary on *Corydalis solida* L. Clairv. plants at the beginning of April 2023 in both observation locations. *Corydalis solida*, known as the fumewort, that is a species of flowering plant in the family *Papaveraceae*. On this plants an initial symptom of leaf infection was observed like a yellowing or chlorosis. As the pathogen develops, a purplish down of sporangiophores and sporangia becomes apparent on the lower leaf surface. Systemic infections with *Peronospora corydalis* de Bary. results in stunted growth, producing shorter plants with smaller leaves that are chlorotic and often curled (*figure 11*).

Figure 11 ***Corydalis solida* - healthy leaves (left side) and downy mildew appearance underneath leafs (right side).**

The morphology of sporangiophores, sporangia and oospores from symptomatic plant tissue was examined. Infected fresh leaf material from *Corydalis solida* L. plant was mounted in lactophenol for observing sporangiophores and sporangia.

In the same period and location that was observed downy mildew on *Corydalis solida* L. also we identified *Peronospora ficariae* (Ness v. Essenb.) Tul. on *Ranunculus ficaria* L. plants that are known as lesser celandine or pilewort. Is a low-growing, hairless perennial flowering plant in the buttercup family *Ranunculaceae*.

Symptoms were characteristic of those associted downy mildew on *Coridalis solida* L., leaf infection was observed like smaller leaves that are chlorotic and often curled. Infected fresh leaf material presented only sporangiophores and sporangia, wich were observed on the underside of leaves.

Sporangiophores are erect, hyaline with straight trunck, branches of sporangiophore arise from the main axis in up to seven orders, and ultimate branchlets are straight to curved and have pointed tips. Sporangia are also hyaline, in ovoid to elipsoidal shape.

## CONCLUSIONS

The deficiency of distinguishing morphological characters and poor knowledge of physiological and ecological variability usually leads to a broad concept of the species that can cause important diseases.

Economically important rusts or downy mildew are relatively well known and studied, but not much attention is paid to species infecting wild plants.

Environmental conditions of the spring of 2023 were extremely favorable and lead to occurance of pathogen diversity on wild estival plants. Thus, identified parasitic fungi that infected wild estival plants are: *Uromyces ficariae* (Schum) Lév. on *Ranunculus ficaria* L., *Uromyces lilii* (Link.) Fuck. on *Fritillaria meleagrioides* Patrin ex Schult. & Schult. f., *Puccinia rossiana* (Sacc.) Lagh. on *Scilla bifolia* L., *Tranzschelia pruni-spinosae* (Pers.) Dietel 1922 on *Anemone ranunculoides* L., *Ustilago scillae* Ciferri, 1931 on *Scilla bifolia* L., *Peronospora corydalis* de Bary on *Corydalis solida* L. Clairv. and *Peronospora ficariae* (Ness v. Essenb.) Tul. on *Ranunculus ficaria* L.

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# MORPHOBIOLOGICAL FEATURES AND THE SIGNIFICANCE OF THE SPECIES *PHACELIA TANACETIFOLIA* BENTH. AS HONEY PLANT

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**Abstract**

*Phacelia tanacetifolia* Benth. is a species native to North America and the Andean Mountains in South America. The climatic conditions of the Republic of Moldova are favorable for the growth and development of this species, where it is known as a valuable honey, ornamental and forage species. It is an herbaceous annual, which ends the growing season by producing seeds. The germination capacity of seeds is 77.5±6.18% and the germination energy is 1-3 days. The weight of 1000 seeds is 1.96±0.03 g. The researched species is of interest to beekeepers, being a source of food for honey producing and pollinating insects, available for about 55 days, ensuring high productivity of honey, with long growing season. Insects visit the plants the most frequently between 11:30 and 12:30, since at this time nectar is abundantly produced. It provides food for a wide range of honeybees and pollinators. The entomological monitoring carried out at the "Alexandru Ciubotaru" National Botanical Garden (Institute) revealed the presence of 27 species of insects present on the organs of *P. tanacetifolia* plants, representing 6 orders, 20 families and 24 genera, insects with a diverse trophic spectrum. According to diversity and frequency, species of the genus *Apis* and *Bombus*, the main honey-making and pollinating insects, were more abundantly present.

**Key words**: *Phacelia tanacetifolia*, honey production potential, development.

*Phacelia tanacetifolia* Benth. – annual herbaceous plant, native to North America, which was brought to Europe initially as an ornamental plant, and later was used as a honey plant with nectariferous and polleniferous significance (Williams I., Christian D., 1991). It was subsequently researched by many botanists who highlighted the value of this species as honey plant, which can be also used as fodder with high nutritive value (Hickman J., Wratten S., 1996; Sengonca C., Frings B., 1988; Williams I., Christian D., 1991). In the last decades it has been intensively cultivated as a source of nectar to obtain honey production, for the regeneration processes of degradable land, as green manure and as food supplement in the livestock sector. It can also play the role of a natural remedy in controlling the number of harmful insect species, for the maintenance of natural and anthropogenic coenoses (Hickman J., Wratten S., 1996; Sengonca C., Frings B., 1988; Williams I., Christian D., 1991). In Europe, lacy phacelia, in addition to mustard, oilseed radish, oats, buckwheat and dill, is used for pest control in peach orchards (Brown M., 2002).

From morpho-taxonomic point of view, the species belongs to the order *Solonales*, family Hydrophylaceae Lindl. (Boraginaceae Juss.), genus *Phacelia* Juss., where it includes about 200 species of annuals and perennials (Lakic Z. *et al*, 2018). It grows abundantly regardless of the environmental factors, about 60-96 cm tall, forming main stems on which up to 20-25 lateral shoots develop, which also branch out (Țîței V., Roșca I., 2021). At the top of the shoots, 15-20 compact scorpioid cyme inflorescences are produced, consisting of 4-6 coils (whorls). A coil can have 18-22 flowers (Cîrlig N., Iurcu-Străistaru E., Țîței V., 2021), forming a fan-shaped inflorescence with flowers that open in sequence, facilitating the formation of nectaries – a source of nutrition for honeybees and other species of insects from the useful fauna. The calyx has subequal lobes, linear, 5.0-7.5 mm long and 0.4-0.7 mm wide, consisting of 5 stamens, with 5 blue-violet petals and sepals with bristles. The anthers and style protrude out of the flower (Țîței V., Roșca I., 2021).

In bioecological terms, the flowering period is long. The first flowers appear 40-55 days after sowing, and flowering lasts up to 55-60 days (Cîrlig N. *et al*, 2021), except for the cases of

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abnormally high temperatures and pedological drought during the flowering period. It is a species with high productivity, from which purple pollen is obtained. As for the fruit, it is a small dehiscent capsule with 4 dark brown seeds (granules) reaching 3.5-4.2 mm long and 2.2-3.0 mm wide. The weight of 1000 seeds is 1.9–2.0 g. (Țîței V., Roșca I., 2021).

*P. tanacetifolia* plants develop an extensive and very strong tap root system. This honey plant has a potential productivity of 400-600 kg/ha of honey, in ecological terms, it is an anti-pollution remedy and regulates the number of invasive nematode complexes in the soil, acting as a biological repellent (Țîței V., Roșca I., 2021).

According to the bibliographic data, *P. tanacetifolia* was introduced to Europe in the second half of the 19th century, initially as honey and ornamental plant, and then it was widely explored by beekeepers from the former USSR. Later it has spread to practically all the countries of Eastern and Western Europe. According to Crane et al. 1984, lacy lacy phacelia is among the top twenty honey plant species of major significance worldwide, but mostly in the countries with well-developed beekeeping industry.

Lacy phacelia attracts both honeybees and other complexes of insects feeding on nectar and pollen, the maximum share being attributed to the trophic group - phytophages, followed by omnivorous and zoophagous species (Cîrlig N., Iurcu-Străistaru E., Țîței V., 2021), most of the adult forms feed on the nectar and pollen of flowers, being also the main pollinators of lacy phacelia flowers.

The nectariferous potential of lacy phacelia flowers varies widely depending on the impact of favorable and stressful environmental factors that occur in some vulnerable phases, such as: sowing, the formation of plants, flower buds, affecting the quality of flowers during nectar formation, the maturation of fruits and seeds. The amount of nectar can be in the range of 1.0-4.5 mg/flower, with an average sugar concentration of 28% and honey production – between 500 and 1200 kg/ honey/ ha (Popovic C. *et al*, 2019). For 24 hours in the nectar of a flower, 0.25-0.5 mg of sugar is produced, sometimes up to 2-5 mg (Culacov V., 2007). The honey obtained from lacy phacelia flowers is light beige to cream-white in color, glassy, translucent, with a specific smell and lemony taste, slow crystallization, with an optimal fructose to glucose ratio as compared with honey obtained from the white acacia species (Popovic C. *et al*, 2020).

A special aspect during the growing season of the lacy phacelia is its impact with nectarivorous, pollinophagous, phytophagous insects etc., dependent practically on all nectar-pollinating species, where as a result of the activity of honey producing insects, they are necessary to maintain vitality as well as to obtain bee products, as a biological source with melliferous, curative and fodder potential. Resulting from these significant facts and motivations, we created, for research purposes, a collection of plant species with mainly melliferous potential, but also with other valuable bioecological qualities, within the "Alexandru Ciubotaru" National Botanical Garden (Institute). The collection consists of various native and introduced species from various geographical regions, with different taxonomic affiliations.

The purpose of the research has been to study some morphobiological, ecological and melliferous peculiarities of plants of the species *Lacy phacelia tanacetifolia* and their response to the impact of the climatic conditions of the Republic of Moldova.

## MATERIAL AND METHOD

The research was done on the experimental sector located on the territory of the "Alexandru Ciubotaru" National Botanical Garden (Institute) (NBGI), “Plant Resources” Laboratory. The seeds and plants of the species *Lacy phacelia tanacetifolia* Benth. served as research subjects. The seeds of the autochthonous cultivar 'Melifera' created at the "Alexandru Ciubotaru" National Botanical Garden (Institute) and seed samples of the investigated taxons, obtained by international exchange (France, Russia), including plants available in the collection of honey plants, where there are multiple forms already known for their utility as fodder and energy crops. The research was carried out during the 2020-2022 growing seasons, characterized by temperatures above the norm, periods of insufficient precipitation followed by heavy rains, alternations of diurnal and seasonal temperatures, recorded in the critical spring-summer phases. Sowing was carried out in spring at different dates (19.03; 14.04; 22.04; 11.05), depending on the recorded weather conditions, in well-prepared soil, at a depth of 2 cm. The phenological study carried out according to the appropriate guidelines (Beideman I., 1974). Surveys were done at different phenological stages of plants, from seed germination to maturity. At the same time, comparative observations were made on the emergence of floral shoots, their formation, full flowering, in correlation with the abundance and frequency of honey-making insect species, detected in the same periods, establishing the diversity and trophic spectrum of insects, taxonomic classification, degree of impact, melliferous potential. All the data obtained were recorded in field notes, documented

according to the development stages, substantiated by photos, analyses and visual observations of the collected samples, which were later examined under laboratory conditions and compared with botanical and entomological determination guidelines and other specialized literature (Plavilschikov N., 1997; Bei-Bienco G., 1966).

## RESULTS AND DISCUSSIONS

In the flora of the Republic of Moldova, there are already several species of honey plants that stand out due to their high productivity, but in order to support the integrity and improvement of the resources of honey plants, in certain areas and micro-zones of the country, these species have been cultivated in various sectors, alone or in association with other plants, in meadows, hayfields, with the aim of maximizing honey production, maintenance of the fauna of honey-producing insects, the ecological balance of the soil and the natural environment. Internationally, as well as in the Republic of Moldova, such valuable honey plants are known and largely cultivated: *Helianthus annuus* L.; *Brassica napus oleifera* L.; *Onobrychis viciifolia*; *Melilotus albus* Medik.; *Mellissa officinalis* L., *Echium vulgare* L.; *Nepeta cataria* L. etc., and in the last decade, the species *Lacy phacelia tanacetifolia* Benth. has gained a lot of popularity.

According to the results of the conducted research, this species possesses melliferous, fodder, curative qualities, which are applied in the formation of new honey plant sectors, in agricultural and fodder crop rotation. These reasons lie at the basis of conducting research on this species, which is quite resistant to most environmental stressors and has high estimated productive potential.

This species is attractive to honeybees and other pollinating insects, being visited by them from early morning to late evening, in spring and summer months. Insects visit the plants the most frequently between 11:30 a.m. and 12:30 p.m., since at this time nectar is abundantly produced, besides, the period after light rain is also preferred by pollinating insects.

In morphobiological aspect, the above-mentioned species was researched in terms of its response to the impact of the climatic conditions of the Republic of Moldova, which are characterized by short winter with little snow and hot summer with insignificant amounts of precipitation. Regardless of the action of stressors such as dry weather, high temperatures, short-term rain showers, lacy phacelia plants in the Republic of Moldova show the ability to adapt to weather variability.

The morphological aspect of *P. tanacetifolia* plants is represented by annual growth from seeds planted at different stages, to encompass the entire active period from spring to late summer. Lacy phacelia passes annually and seasonally through the phenological cycle of growth and development consisting of vegetative and generative phases, completing the cycle with the formation of viable fruits and seeds, which are to be used as planting material in the following season. The germination capacity of seeds is 77.5±6.18% and the germination energy is 1-3 days *(figure 1A).* The experiments to determine the germination capacity were set up under laboratory conditions, in Petri dishes, in several repetitions by 100 seeds each. The fruit is a dehiscent capsule with 4 brown seeds. The weight of 1000 seeds is 1.96±0.03 g.

Initially, the young plants have succulent stems *(figure 1B)* and are a little sensitive to climate and soil conditions; they do not tolerate saline soils, but preferentially grow on light soils and on fertile, well-drained ones. The plants growth fast and produce significant amounts of nectar and seeds. Then, once the flower buds are formed, with the transition to full flowering stage, the stem at the base becomes fibrous and lignifies, the process advancing towards the apical area.

A B

Figure 1 **A – seeds of *Lacy phacelia tanacetifolia* in the 3rd day after germination; B – lacy phacelia on the experimental sector of NBGI, vegetative stage/stem development**

*Ph. tanacetifolia*, under the climatic conditions of the Republic of Moldova, develops stems on average 115-120 cm tall, branched, which develop about 20 lateral shoots, tap root and sessile, pinnate leaves, with 7-8 linear or pinnately-lobed leaflets with toothed margin. In the investigated growing seasons of lacy phacelia (from the moment of seedling emergence until seed ripening) plants complete a biological cycle, on average about 80-92 days. The start of the flowering stage depends on the time of sowing;

the floral shoots are formed in sequence, with staggered flowering influenced by environmental factors, this stage usually occurs in May-July. The flowering process lasts about 55 days, due to the successive development of secondary shoots, at the top of which inflorescences are produced; this peculiarity contributes to prolonging this important stage, benefiting to the high value of this species for nectar production in beekeeping. During this period, a plant produces about 15-20 fan-shaped scorpioid inflorescences with 4-6 one-sided coils, which straighten as the flowers mature, wither and the fruits develop. An inflorescence develops 18-22 flowers – blue-violet, sessile, actinomorphic, with a double perianth and long style protruding from the flower. The lifespan of a flower is 1-2 days. Due to the sequenced blooming, on a lacy phacelia plant, there can be inflorescences in different stages of development *(figure 2)* at the same moment – buds in the apical part of the inflorescence, open flowers in the middle area, and fruits at the base.

Figure 2 **Inflorescences in various stages of maturity developed on a shoot**

In the research program, experimental sectors were planted with lacy phacelia seeds at various timing in order to determine the most effective planting timing and flowering period for nectar and pollen production. The seeds were planted into the soil in spring (March-May), at different dates, in correlation with the recorded weather conditions. Seedlings emerged at the soil surface 7-15 days after sowing. The seeds sown in March were the most difficult to germinate (about 20 days) as the soil temperatures were lower, as compared with those sown later. There follows a period when the plants develop their root system and intensively form the green mass, the main shoots appear, from which the lateral ones – of 2$^{nd}$ and 3$^{rd}$ order branch out.

The dates when the phenological stages started, according to the biological cycle, were recorded and analyzed comparatively in correlation with the sowing date, in the research years 2020-2022. Analyzing the results obtained according to the implemented program, the values were indicated in *table 1*, which reflects the date of the start of each phenological stage, from sowing to the ripening of the lacy phacelia seeds. From the obtained data, we can infer that by sowing in different periods, we can plan the needed flowering period, which will coincide with the end of April to end of July, providing the useful entomofauna with food in the required period according to the beekeepers' schedule. In cases if there are seeds left from the previous harvest on the field planted with lacy phacelia, in early spring (mid-March) the seeds germinate as soon as favorable temperatures are recorded, and in May there are already plants in the flowering stage. These periods established according to the calendar program are beneficial to prevent entering the dry and arid periods that usually occur in the second half of summer, which are practically ineffective for the formation of honey production, but significantly accelerate the processes of seed ripening and drying of plants that induce the premature end of the growing season.

Table 1.

**Stages of development of *P. tanacetifolia* plants in the growing seasons 2020-2022.**

| Taxon | Year | Sowing | Seedling emergence | Stage of development | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Budding (full) | Flowering (full) | Fruiting (full) | Seed ripening (full) | Seed harvesting |
| ***Ph. tanacetifolia* 'Melifera'** | 2020 | 11.05 | 18.05 | 22.06 | 26.06 | 09.07 | 28.07 | 02.08 |
| ***Ph. tanacetifolia* 'Melifera'** | 2021 | 22.04 | 04.05 | 10.06 | 21.06 | 28.06 | 20.07 | 26.07 |
| ***Ph. tanacetifolia* 'Melifera'** | 2022 | 19.03 | 13.04 | 20.05 | 01.06 | 20.06 | 26.06 | 08.07 |
| ***Ph. tanacetifolia* (Rusia)** | | 14.04 | 26.04 | 07.06 | 13.06 | 29.06 | 18.07 | 28.07 |
| ***Ph. tanacetifoloa* (Franța)** | | 14.04 | 03.05 | 14.06 | 20.02 | 02.07 | 10.07 | 19.07 |

The species is mentioned in various bibliographic sources and specialized literature as plant with multiple uses: production of honey, fodder and as green fertilizer in agriculture. In ecological terms, this species is relatively resistant to various stressful pedoclimatic conditions. The minimum temperature necessary for seed germination is +5+7 °C, and the optimal

temperature for plant growth is +15..35 C, with moderate humidity (Țîței V., Roșca I., 2021). The biomorphological aspect of lacy phacelia plants is attractive and preferred by pollinating and honey-making insects since the appearance of the first flowers. Honeybees are particularly abundantly present on flowers throughout the day. Lacy phacelia nectar, in addition to being a source of food for honeybee insects, also serves as food for 60 species of parasitoids described and reported by various authors and specialists (Brown M., 2021).

At the same time, on the territory of NBGI, entomological monitoring was also carried out on the experimental sectors planted with lacy phacelia since 2019. The identification of insects begins at the stem elongation stage of plants. Special attention was paid to the entomofauna when the plants entered the generative phase of development (budding-flowering), when a large diversity and varied spectrum of insects was detected and categorized according to trophic specialization, taxonomic affiliation, frequency and abundance. The analyzed samples allowed the revision of the entomological list made previously (Cîrlig N. *et al*, 2021) and its completion with new species of insects. In total, 27 insect species were identified, representing 6 orders, 20 families and 24 genera, insects with a diverse trophic spectrum and enormous significance in the flower pollination process.

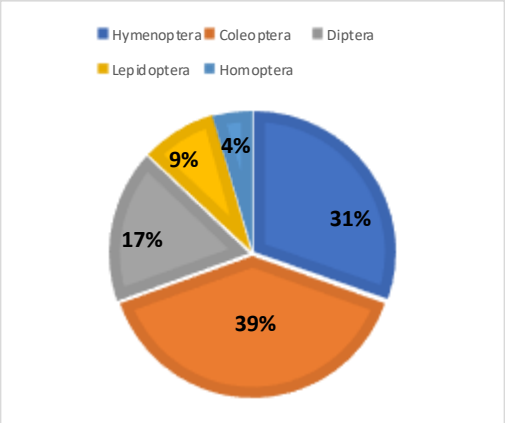
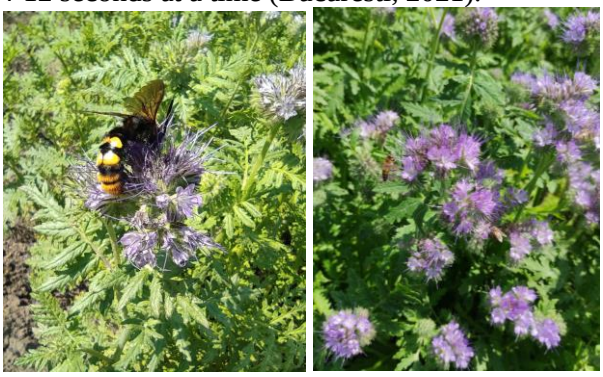



Figure 4 **Comparative share of insect species according to orders**

The comparative analysis of the number of insects, demonstrated that the maximum share belongs to the order Coleoptera with 38% of the total number of determined species, followed by the order Hymenoptera with 31 %, Diptera – 17 %, Lepidoptera – 9 % and the order Homoptera – 4 %, represented by *Cercopis arcuata* (Fieber, 1844).

Lacy phacelia flowers are also attractive to giant insect species. One of them is *Megascolia maculata*, the mammoth wasp, a vulnerable species, included in the Red Book of the Republic of Moldova *(figure 3).* It feeds on lacy phacelia nectar and pollen and plays an important role in the pollination process.

Studying the activity of insects on lacy phacelia plants in NBGI, it was found that a honey bee collects pollen/nectar from an inflorescence for 4-12 seconds at a time (Bucuresti, 2021).

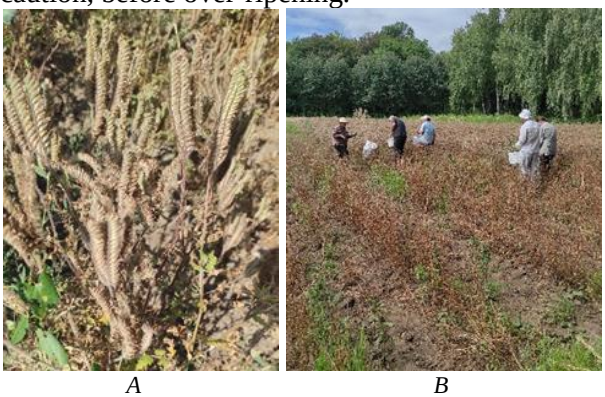


A B

Figure 3 **A – a representative of the species *Megascolia maculata* (Drury, 1773) feeding on lacy phacelia flowers; B – lacy phacelia in the full flowering stage**

About 5-9 insects belonging to 2-4 species were detected on one plant at the same time. The species of the genera *Apis* L., *Bombus* Latr., *Coccinella* L. have the maximum frequency during the day, but also during the entire flowering period of the plants.

At the same time, the study was continued on the formation, ripening and harvesting of seeds, which coincide with the last days of June - July - beginning of August, shown in figure 5. Seeds are harvested manually, preferably in the first half of the day, after which, there are several stages of seed processing/cleaning. The productive potential of lacy phacelia plants under the climatic conditions of the Republic of Moldova is about 53 t/ha of fresh mass, 260-300 l/kg biogas and the calorific value is 18.1-18.4 MJ/kg, being used as agricultural residues after harvesting the seeds (Țîței V., Roșca I., 2021). The seeds fall off easily, for this reason harvesting must be done with caution, before over-ripening.



*A* *B*

Figure 5 ***Ph. tanacetifolia* at the end of the growing season; A – full fruiting stage/beginning of seed ripening; B – manual harvesting of lacy phacelia seeds at NBGI**

## CONCLUSIONS

The conducted research highlighted the valuable biological features of the species *Lacy phacelia tanacetifolia* Benth., a high-potential honey plant, as an important contribution to the enrichment of the range of honey plants. Under the climatic conditions of the Republic of Moldova, the flowering period of the plants lasts for about 55 days, a plant producing on average 15-20 fan-shaped inflorescences with 4-6 coils. In an inflorescence, there are 18-22 blue-lilac flowers, with double perianth and long style protruding from the flower. The lifespan of a flower is 1-2 days. Due to the structure of the inflorescences and the sequenced flowering, lacy phacelia plants provide insects with food over a long time. Planting lacy phacelia seeds at different timing can provide honeybees with food for the period needed by beekeepers, thus controlling the production of bee products. Lacy phacelia flowers are a source of nectar and pollen for a wide range of honeybees and pollinators. The list of insects detected and determined on *P. tanacetifolia* plants from the "Alexandru Ciubotaru" National Botanical Garden (Institute) has been revised and supplemented, currently including 27 species of insects classified into 6 orders, 20 families and 24 genera. The identified species are recognized as valuable honeybees and important pollinators.

## ACKNOWLEGMENTS

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# RESEARCH ON THE BEHAVIOUR OF SHORE DEFENCES TO HYDRODYNAMIC EROSION

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**Abstract**

Shore defences located in beds formed in weakly cohesive rocks are affected by the phenomenon of hydrodynamic erosion in a differentiated way. Research carried out over a period of about 20 years on a section of the Moldova River highlighted the behaviour of "heavy - concrete slabs" and "light – geo-bags" bank defences. The climate changes of the last period of time, which influenced the hydrological regime of the river, determined a rapid degradation of the shore protection made of concrete slabs. The replacement of the shore defence made of concrete slabs with a structure made of geo-bags filled with ballast influenced the behaviour of the shore to hydrodynamic erosion. At the same time, the shore protection made with geo-bags filled with ballast stabilized with cement allowed a better cooperation with the foundation ground consisting of weakly cohesive rocks. The defence made of geo-bags has a larger area and perimeter at the same cross-section of the river. This situation causes the reduction of the velocities and the frictional effort at the wall, respectively the reduction of the erosion effort.

**Key words**: geo-bags, river beds, type of degradation, unstable banks

The riparian zone of the watercourses is intensively populated or occupied with social and economic objectives. The protection of the population and the objectives of interest from the destructive actions of water is achieved by regularizing the riverbeds and protecting the banks. These works modify the river bed in transverse and longitudinal profile, but disturb the aquatic and riparian ecosystems. A factor for changing the riverbed morphology is represented by hydrodynamic erosion (Luca M. *et al*, 2012). This phenomenon acts extremely aggressively on coastal defences. The choice of defence solutions must respect the technical and economic requirements, as well as the ecological ones.

A detailed analysis of morphological changes in riverbeds is presented by Ichim I. *et al* (1989) on various fields of research, namely hydrological, hydraulic, geotechnical, etc. A documentary study carried out by Avram M. (2020) highlighted the presence of an important number of articles published in the last 10 - 20 years and which deal with riverbed erosion under morphological aspects, the differential determination of roughness on the perimeter of the bed, the type of bed degradation and its rehabilitation methods, the characteristics regarding the transport of alluvium, the behaviour of coastal defence constructions and others.

The use of adequate bibliographic material allowed the authors to obtain a representative volume of data on the phenomenon of river bed erosion both in cross section and in longitudinal profile (Dapporto S. *et al*, 2003, Neuhold C. *et al*, 2009, Rinaldi M., 2003, Thorne C., 1982, Wang W. *et al*, 2014).

The phenomenon of erosion of the bed and implicitly the degradation of hydrotechnical and civil constructions located in it is a complex problem of international study, and the treatment also reaches the governmental level (US Army, 1993, Government of India, 2012).

Recent climate changes have modified a number of natural and anthropogenic risk factors that intervene in the functioning of coastal defences (Luca M. *et al*, 2012). This situation has also determined a change in the concepts of creating bank defences on rivers in contact with human communities or economic objectives (Avram, 2020, Luca M and Luca Al. L., 2012).

The study of the phenomenon of hydrodynamic erosion of shore defences is carried out by direct methods (topographical measurements in the field) and by simulating the phenomena of erosion - sedimentation using physical and numerical models. Among the frequently used hydraulic-mathematical models can be listed: Maik 21, Hec-Ras, Mohid and others

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(Avram M., 2020, Bohorqueza P., Anceyb C., 2015, Creţu Gh., Badaluţă C., 2006). Most of the simulation models have a disadvantage due to the relativity of the data and the presentation of a single erosion depth on the simulated river section. The research carried out in this field shows the importance of the phenomenon in various economic, social and environmental protection fields.

## MATERIAL AND METHOD

The researches were carried out on the lower course of the Moldova River (*figure 1*), in a large area near the town of Soci, Miroslovesti commune, Iaşi county. The Moldova River is part of the hydrographic basin of the Siret River. The BH surface of the Moldova River is 4326 km$^2$, and the length is 205 km. The longitudinal profile of the river is balanced and has an average slope of 1% (Atlas of Water Cadastre in Romania).

The foundation of the river is made up of weakly cohesive rocks, a fact that favours bed erosion on the watered perimeter (Luca M., 2006, Luca M., 2012, Luca M. *et al*, 2019). The river bed in the research area consists of alternating layers of ballast, dusty sand and clayey sand (Luca M., 2006).


Figure 1 **Geo-physical characteristics in the research area of the Moldova River (The geographical Atlas of Romania, 1985)**

The research was carried out on a regularized river section with a length of 380 m and equipped with a bank defence made of geo-bags. The bed in the research area presents two arms separated by an island (*figure 2*). The arrangement of the river section was imposed by the presence of three pipes with large diameters (800 – 1000 mm), which cross the river bed transversely.

The research methodology was differentiated by fields of study: hydrological, hydraulic, technological, resistance and stability of coastal defence works, analysis of structural and functional states of constructions. The hydrological data (average and maximum flows) are determined in the research site and correlated with those taken from the Pildești Hydrometric Station on the Moldova River located downstream of the research area.

Figure 2 **General downstream view of the Moldova River bed with the two arms and the separation island (Luca M., 2006)**

The structural state of the coastal defence was analyzed from its commissioning (year 2015) until 2023, by performing systematic analyzes at time intervals and after floods. The researched coastal defence was divided into study sectors with lengths of 50 - 60 m.

On each analysis sector, data were taken on the state of the defence work regarding the following: type of degradations, number of degradations, geometric characteristics of the degradations, the evolution of the degradations over time, the influence on the stability of the work, the possibility of remediation.

The technical data were supplemented with photo reliefs by sector and type of degradation. All technical data were stored in "databases" and processed on the study areas.

## RESULTS AND DISCUSSIONS

In the research area on the Moldova River, a hydrotechnical construction is located with the function of undercutting three intake pipes for the transport of drinking water. The pipelines are part of the Regional Water Supply System of Iasi County. The first pipe has a diameter of 800 mm and the next two 1000 mm. The last two pipelines were executed in the years 1969 – 1971, when the river bed was regularized and a shore defence with concrete slabs was created (Luca M., 2012). The shore defence protected the shore in good condition until 2004 (*figure 3*).

Between 2005 and 2012, a series of high-flow floods occurred on the Moldova River (Romanescu G., Stoleru C., 2008; Luca M., 2012), which partially degraded the hydrotechnical construction of the undercrossing (Luca M., 2006; Luca M., 2012). At the same time, the hydrodynamic erosion determined the lowering of the bottom of the bed and the total uncovering of the first adduction pipe and partially of

the second pipe. Degradation of the stability of the first supply pipe led to its total failure.


Figure 3 **The structural state of the left bank defence on the Moldova River in 2005 (Luca M., 2006)**

The phenomenon of hydrodynamic erosion, intensified by a series of natural factors (the high frequency of floods with high flows in the last 50 years), but also anthropogenic ones (limitation of maintenance works, intensive exploitation of ballast at the upstream limit of the sub-crossing area), determined the partial degradation (*figure 4*) and then the total degradation of the shore defence in the period 2005 - 2012 (*figure 5*).

Figure 4 **The structural state of the shore defence made of concrete slabs in 2008 (Luca M., 2012)**

The technical expertise carried out in 2012 highlighted the state of degradation of the undercrossing construction, as well as the shore defence and indicated its rehabilitation (Luca M., 2012). The rehabilitation project was carried out in 2012 and applied in 2015 (Luca M., Luca Al. L., 2012).

Taking into account the extremely aggressive action of the phenomenon of hydrodynamic erosion, the foundation of the bed consisting of weakly cohesive rocks and the location of the undercrossing in a natural site, a shore protection made of elastic constructive elements was designed, which is adaptable to the conditions of the site. In this case, the shore defence was designed from elastic structures like geo-bags over a length of 520 m.

Figure 5 **The total degradation of the defences on the left bank of the Moldova River, year 2012 (Luca M., 2012)**

Shore defences made of geo-bags are used in many countries (Government of India, 2012), especially where local materials (ballast) can be used. Geo-bags are used to protect the banks of rivers or lakes, at the slopes of embankments (Găzdaru A. *et al*, 1999).

The analyzed geo-bags are made with bags made of polyester fabric and filled with ballast. Geo-bags have a rectangular shape with a length of 1.50 m, a width of 0.80 m and a height of 30...40 cm. Geo-bags used in coastal defence are of two types, where the differentiation is given by their position in the site:

- type I is represented by geo-bags filled with ballast, and in shore defence they occupy the position of contact with the shore;
- type II is represented by geo-bags filled with ballast and cement addition, and in the shore defence it occupies the water contact position.

The geometric dimensions of the shore defence were determined by dimensioning calculations and verification of the groupings of forces existing in the site in accordance with the norms in force (Luca, 2012, GE 027-97) (*figure 6*). The shore defence made of geo-bags adapts very well to unstable foundations and achieves favourable conditions for the aquatic ecosystem.

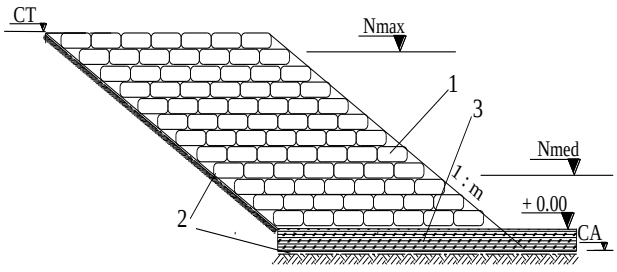


Figure 6 **The structure of shore defence with geo-bags: 1 – geo-textile bags, 2 - geogrid, 3 - fascia mattress (Luca et Luca, 2012).**

Shore defence was carried out in battlements placed in the area of each shore. The installation

conditions were difficult, being determined by the presence of water originating from infiltration from outside the cofferdam. Geo-bag protection on the Moldova River was carried out in 2015 by using ballast as a filling material. The ballast was taken from the location area (*figure 7*).

Figure 7 **Execution stage of the shore defense with geo-bags on the Moldova river (photo Luca M., 2015)**

Starting in 2016, a systematic monitoring of the behaviour of the shore defence made of geo-bags was carried out, immediately after its entry into operation. The monitoring was carried out in several research directions. A direction of research was the behaviour of the coastal defence structure to hydrodynamic action. Data on the behaviour of these structures to the multiple actions of water are relatively scarce in the specialized literature.

The analysis carried out in the field between 2016 and 2023 and the data processing indicated the following results:

- the shore defence behaved differently along the length and height of the shore, generally during the seven years of operation; no important structural degradation generated by the water and especially the flood of 2016 can be highlighted (*figure 8*);

Figure 8 **General view of the left bank defense after the 2016 flood (photo author, 2016)**

Figure 9 **General view of the left bank defence after the 2018 flood (photo Luca M., 2015)**

- a series of geo-bags placed in contact with water showed degradation in the form of tearing of the material; the tears are in the direction of flow and have lengths of 4-10 cm with widths of 2-5 cm through which part of the material was expelled (*figure 10*); the number and size of material breaks is greater for geo-bags located towards the base of the shore defence; the number of material breaks is random on the research sectors, being influenced by the quality of the material filling the geo-bags, as a result of the absence or decrease of the amount of cement;

Figure 10 **Degradation of geo-bags through cracks and tearing of material 2018 (photo author, 2020)**

- the flood of 2018 caused significant damage to a series of geo-bags placed in contact with the water, by tearing the fabric in small lengths, but with an intense process of expelling the material from the inside (*figure 11*); the Moldova River transports coarse alluvium by creeping during floods, a situation that produces such degradations:

The hydrodynamic erosion of the water, as well as the action of the floaters, manifested itself extremely aggressively through the floods that

spread on the Moldova River in 2016 ($Q_{max}$ = 444 $m^3/s$) and 2018 ($Q_{max} \approx 1200$ $m^3/s$).

The analysis carried out in the field after the transit of the floods did not highlight the movement of geo-bags on the vertical and horizontal shore defence.

Figure 11 **Degradation of geo-bags in contact with water in the flood of 2018 (photo author, 2018)**

The shore defence made of geo-bags has been degraded at the top by anthropogenic actions. Some activities such as fishing (*figure 11*) and grazing by herds of sheep and goats have contributed to the degradation by tearing and breaking of the geo-bags (*figure 12, figure 13*).

Figure 12 **Degradation of the geo-bags located at the top of the coastal defence by anthropogenic actions (photo author, 2022)**

A good performance presents the shore defense from geo-bags to ensure the environmental requirements demanded by the aquatic and riparian environment. The mounting structure of geo-bags allows the deposition of alluvial material following floods or level variations, an aspect that favors the growth of aquatic and riparian vegetation (*figure 14*).

Figure 13 **Degradation of the geo-bags located at the top of the coastal defence by the movement of sheep flocks (photo author, 2020)**

Figure 14 **Coastal defence area with the presence of vegetation and alluvium deposits (photo author, 2019)**

The elastic shore protection modifies the hydraulic regime in the flow section through the presence of macro-roughnesses determined by the shape and method of mounting the geo-bags. The geo-bags bank protection compared to the one with concrete slabs has the following particularities: a dappled wetted perimeter, a much greater roughness and an accentuated fragmentation of the current in the bank area. All this leads to a decrease in the velocity and implicitly in the tangential effort at the wall, so that the hydrodynamic erosion force is substantially reduced (Luca M. *et al*, 2012).

The research carried out in the field highlights the need to apply annual maintenance works to restore the integrity of geo-bags degraded by natural and human action. Also, after the passage of a high-flow flood, a check of the structural condition must be carried out to highlight degradations.

## CONCLUSIONS

The research carried out on the bank defence carried out on the Moldova river highlighted a differentiated behaviour of geo-bags in the hydrodynamic action depending on the location (river bed / bank), their arrangement in the horizontal plane and the structure of the filling (ballast with / without addition of cement).

Geo-bags located below the average water level show degradations resulting from the action of submerged materials, which produced cracks and breaks limited in size to the geo-textile material.

Geo-bags located in the area of water level variation show degradations in the form of longitudinal breaks limited to the length of the fabric, where the filling material was partially washed away, a situation that determined the change in the geometric shape.

Geo-bags located in the area of high levels are degraded the most by human actions in the riparian zone, but also by the movement of animals.

Compliance with the filling recipes and the way of placement in the shore defence structure are basic conditions in ensuring the resistance over time of geo-bags.

The elastic shore protection works made of geo-bags are modelled very well in the erodible riverbeds, effectively taking over the subsidence and displacements in a three-dimensional plane and contribute to the protection of the aquatic environment.

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# SOME AGROBIOLOGICAL PECULIARITIES AND THE ECONOMICAL VALUE OF CHIA *SALVIA HISPANICA* L. IN MOLDOVA

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**Abstract**

The aim of this study was to evaluate some agrobiological peculiarities, the quality of the harvested fresh mass and prepared haylage of the genotypes of chia – *Salvia hispanica*, cultivated under the conditions of the Republic of Moldova. The studied genotypes of chia were characterized by optimal growth and development rates. It was determined that the nutrients of the dry matter of *Salvia hispanica* whole plants cut in the flowering period were: 87-107 g/kg CP, 77-83 g/kg ash, 347-377g/kg CF, 348-362 g/kg ADF, 517-533 g/kg NDF, 62-65g/kg ADL, 283-331 g/kg Cel, 166-174g/kg HC, 107-123 g/kg TSS, with nutritive value: 60.70-61.79% DMD, RFV= 106-111, 12.00-12.19 MJ/kg DE, 9.85-10.1 MJ/kg ME, 5.87-6.03 MJ/kg NEl. The fermentation quality and the nutritive value of the haylage prepared from chia plants were characterized by the following indices: pH= 4.81, 18.5 g/kg lactic acid, 2.3 g/kg acetic acid, 0.3 g/kg butyric acid, 100 g/kg CP, 80g/kg ash, 400 g/kg CF, 419 g/kg ADF, 593 g/kg NDF, 72 g/kg ADL, 347 g/kg Cel, 199 g/kg HC, with nutritive value: 56.3% DMD, RFV= 88, 11.21 MJ/kg DE, 9.20 MJ/kg ME, 5.22MJ/kg NEl. The studied fresh and ensiled substrates from *Salvia hispanica* have C/N=29.2-36.8 and the biochemical methane potential reaches 285-298 l/kg ODM. Chia – *Salvia hispanica* – can serve as multi-purpose crops for forage production and feedstock for renewable energy production.

**Key words**: agrobiological peculiarities, biochemical composition, biomethane potential, green mass, haylage, nutritive value, *Salvia hispanica*

The incorporation of neglected and underused crops, as well as the domestication of new species would promote agricultural diversity and would provide a solution to many of the problems associated with food security, nutrition, healthcare, medicine and industrial needs.

*Salvia* L. is the largest genus of the *Lamiaceae* family, *Nepetoideae* subfamily, *Mentheae* tribe, *Salviinae* subtribe, and has about 1000 species that are widely distributed in different areas of the world, including South Africa, Central America, North America, South America and Southeast Asia. *Salvia hispanica* L., known as chia, is native to Central America, the mountainous areas of western and central Mexico, as well as Guatemala, and is a multifunctional plant whose culinary use may be traced back as far as 2500 B.C. Domesticated in Mesoamerica around 2600 B.C., it was a staple food in Mexico between 1500 and 900 B.C. (Pozo S., 2010). It was re-discovered due to its high content of nutraceuticals and therefore the potential for a functional food and a feed capable of increasing the nutritional value of milk and meat products (Ayerza R., Coates W., 2006; Bochicchio R. *et al*, 2015; Porras-Loaiza P. *et al*, 2014; Jamshidi A.M. *et al*, 2019; Noori A., Zebarjadi A., 2022; Rahal E.K. *et al*, 2023). The use of chia for human consumption has been approved by the European Parliament and the European Council (European Commission, 2009; 2020).

*Salvia hispanica* is an annual herb, developing numerous fibrous roots, forming a dense roots mass under favorable conditions. Stems – erect, simple or sparingly branched, quadrangular, deeply sulcate between the prominent, rounded angles, appressed pubescent with whitish hairs, up 200 cm tall. Leaves petiolate, lamina 1.4–11.5 × 0.5–6 cm, ovate to ovate-elliptic, apex acute to shortly acuminate, base cuneate to rounded, margin serrate, adaxially pubescent, abaxially densely pubescent with whitish hairs; petioles 0–3.5 cm, diminishing in length upwards, pubescent. Inflorescence – dense racemes, (1–) 5–17 cm long and 1.3–2 cm wide, terminal on the main stem and on branches from

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the uppermost leaf axils, usually solitary but occasionally with a pair of racemes arising at the base of the main raceme, the flowers grouped in 6–12-flowered verticillasters, imbricate except the lowermost, up to 5 mm apart; peduncles 1.5–11.5 cm long, pubescent; bracts 5–8 × 3–5 mm, narrowly to broadly deltoid, acuminate and mucronate, entire, pubescent, 3-veined, persistent; calyx about 5 mm long in the flowering stage, accrescent to 11 mm in the fruiting stage, rather bulbous (ventricose) at the base, pubescent with sessile glands near base, 2-lipped, the upper lip with 3 prominent veins and sometimes two additional less prominent veins, teeth mucronate, the lower lip 2-lobed; corolla 2-lipped, blue with small white 'honey guide' on lower lip, 7–8 mm long, tube 4–5 mm long, ± enclosed by the calyx, white, ±glabrous, the upper lip about 3 mm long, blue, hooded, densely pubescent, the lower lip 4–5 mm long, shallowly 3-lobed, pubescent on the exterior, papillae absent from the interior; stamens included in the upper lip, anthers about 1.25 mm long, yellowish; style – whitish, glabrous, glandular at base, shortly exserted, bilobed, the arms blue, upper arm reflexed, twice as long as the lower arm. Nutlets ovoid, 1.4–2 mm long, smooth, glabrous, pale grey-brown mottled with white or (rarely) entirely white, becoming mucilaginous when wetted (Wood *et al*, 2022) The seed color varies from black, grey and black spotted to white, and the shape is oval with size ranging from 1 to 2 mm (Wood J.R.I. *et al*, 2022). The weight of 1 000 seeds varies between 0.94 and 1.29 g (Rahal E.K. *et al*, 2023). Chia seed is composed of protein (18.5–22.3%), fibres (20.1–36.1%) and fats (21.5–32.7%) with highest proportion of omega-3 and omega-6 fatty acids, minerals, vitamins, also contains a high amount of antioxidants, phenolic compounds varied from 0.53 to 0.71 mg/g GAE (Porras-Loaiza P. *et al*, 2014).

The chia plants grows well in sandy-loam and loam soils with good drainage. They thrive mainly in acidic soils and grow best at a pH of 6.5–8.5. Temperatures between 11 °C and 36 °C are ideal for seed growth, but they cannot stand frost and freeze in all development stages. Chia is a drought-resistant plant, so it can grow in semiarid and arid environments. It is a short-day plant sensitive to photoperiod. These plants need a lot of sunlight and do not bear fruit in the shade, are sensitive to salt stress, and salinity can considerably diminish the yield of seed oil. Chia is resistant to pests and diseases, has low requirements of water and fertilizer supply, and is a good candidate for organic production (Bochicchio R. *et al*, 2015).

Recently, phytomass production as a potential forage source has gained a lot of attention, opening new possibilities for the introduction of chia, *Salvia hispanica*, in forage production systems (Peiretti P.G., Gai F., 2009; Peiretti P.G., 2010; Amato M. *et al,* 2015; Bilalis D. *et al*, 2016; Jamshidi A.M. *et al*, 2019; Rossi R. *et al*, 2020; Bhardwaj H.L., 2021; Chernov R.V. *et al*, 2022; Filik G. *et al*, 2022).

Under the conditions of the Republic of Moldova, the *Salvia hispanica* genotypes have optimal growth and development rates, the duration of the growing season being 122-126 days, finishing with the ripening of seeds, the weight of 1000 seeds was 1.2-1.4 g and the potential yield reached 2030 kg/ha seeds (Chisnicean L., 2017).

The aim of this study was to evaluate some agrobiological peculiarities, the quality of the harvested fresh mass and prepared haylage of the 2 genotypes of chia, *Salvia hispanica*, cultivated under the conditions of the Republic of Moldova.

## MATERIALS AND METHODS

The *Salvia hispanica* genotype with gray seeds and the genotype with white seeds, grown in monoculture in the experimental sector of the Institute of Genetics, Physiology and Plant Protection of Republic of Moldova, served as research subjects, Common sainfoin – *Onobrychis vicifolia* was used as control variant. The chia seeds were sown in middle May, at a depth of 1.5-2.0 cm and a distance between rows of 70 cm, with soil compaction before and after sowing, the sowing density was 0.6 g germinable seeds per $m^2$. The surveyed area of the plot constituted 10 $m^2$.

The green mass samples were collected in the early flowering stage. The haylage was produced from wilted green mass, cut into small pieces, compressed in well-sealed glass containers, stored at ambient temperature (18-20 °C) for 45 days, to allow complete fermentation to occur. Following the 45-day fermentation period, each glass container was opened and the content was visually examined, the colour and the aroma were recorded. The dry matter content was detected by drying samples up to constant weight at 105°C. For biochemical analysis, the plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm. The pH of the haylage was measured immediately after removal from the containers. The prepared hay was dried directly in the field. The fresh mass 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), minerals, total soluble sugars (TSS), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), dry matter digestibility (DMD), organic matter digestibility (DMD), 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), cellulose (Cel), digestible energy (DE), the metabolizable energy (ME), the net energy for lactation (NEl) 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 C.M. *et al* (1979). The biochemical methane potential was calculated according to Dandikas V. *et al* (2015).

## RESULTS AND DISCUSSIONS

Some agrobiological peculiarities and the structure of the green mass of the studied *Salvia hispanica* genotypes are presented in Table 1. At the time when the green mass was harvested, the plants of the gray seed genotype reached 200 cm in height, but the white seed genotype – 210 cm. The yield of the white seed genotype reached 6.56 kg/m$^2$ green mass or 1.48 kg/m$^2$ dry matter, but the yield of the gray seed genotype was 6.02 kg/m$^2$ or 1.29 kg/m$^2$ dry matter, respectively. The harvested green mass of *Salvia hispanica* white seed genotype was characterized by a higher content of dry matter (22.56 %) and leaves (49.55%).

Animals need for growth, development, reproduction and realization of some products, numerous nutrients they receive from feed. Analyzing the results of the determination of the biochemical composition of dry substances of chia whole plants (*table 2*) we would like to mention that the nutrient content was 8.7-10.7 % CP, 34.7-37.7% CF, 34.8-36.2% ADF, 51.7-53.3 % NDF, 6.2-6.5% ADL, 28.3-33.1 % Cel, 16.6-17.4% HC, 10.7-12.3 TSS % and 7.7-8.3 % ash. A higher content of crude protein, total soluble sugars, hemicellulose, ash and lower content of structural carbohydrates was found in the fodder from the chia genotype with white seeds. The content of organic matter and its biochemical composition influence the nutritional and energy value of fodder. Thus, the natural fodder from the white seed genotype of chia is of high quality, reaching 61.8 % DMD, RFV=111 with 12.19 MJ/kg DE, 10.01 MJ/kg ME and 6.03 MJ/kg NEl, but – from the gray seed genotype of chia – 50.7 % DMD and RFV=106 with 12.00 MJ/kg DE, 9.85 MJ/kg ME and 5.87 MJ/kg NEl. We would like to mention that the level of crude protein and energy value of green mass fodder from *Salvia hispanica* was significantly lower as compared with *Onobrychis vicifolia* green mass.

Some authors mentioned various findings about the yield and nutrient quality of the green mass from *Salvia hispanica*. According to Peiretti P.G., Gai F. (2009), the protein content in *Salvia hispanica* plants varied from 188 g/kg DM at early vegetative stage to 76 g/kg DM during shoot stage. Peiretti P.G. (2010) mentioned that chia herbage contained 84.0-224.0 g/kg DM with 8.60-21.30% WSC. Ouzounidou G. *et al* (2015) reported that chia leaves contained 16.54-22.70% proteins, 7.40-11.20 % carbohydrates and 1.85-3.85% fat. Bilalis D. *et al* (2016) reported that biomass yield ranged between 4.48 and 15.36 t/ha dry mass, depending on the sowing rate and fertilization. Rossi R. *et al* (2020) found that the total plant yield of chia in Italy varied from 5.64 to 9.0 t/ha and crude protein in chia plants varied from 18% in early vegetative to 8% in early flowering stages, respectively. Bhardwaj H.L. (2021) reported that the 60-day old whole *Salvia hispanica* plants, in the vegetative phase, of black-seeded genotype contained 21.40% protein, 2.24% crude fat, 23.8% ADF, 31.4 % NDF, 0.32% phosphorus, 2.03% calcium, and white-seeded genotype 21.1% protein, 2.64% crude fat, 18.6% ADF, 27.2% NDF, 0.36% phosphorus, 2.10% calcium. Chernov R.V. *et al* (2022) revealed that the biochemical composition of the dry matter in the green mass of *Salvia hispanica* was 17.4% CP, 4.8% EE, 21.9% CF, 10.1% ash and the nutritive value 10.21 MJ/kg ME and 0.84 fodder units/kg. Kazydub N.G *et al* (2022) reported that the sucrose content in chia leaves varied over the years between 6.2% and 14.3%.

Table 1

**Some agrobiological peculiarities and the structure of the yield of *Salvia hispanica***

| Plant species | Plant height, cm | Stem, g | | Leaf + flower, g | | Productivity, kg/m$^2$ | |
|---|---|---|---|---|---|---|---|
| | | fresh mass | dry matter | fresh mass | dry matter | fresh mass | dry matter |
| *Salvia hispanica* genotype gray seeds | 200.00 | 238.00 | 51.89 | 151.00 | 31.55 | 6.02 | 1.29 |
| *Salvia hispanica* genotype white seeds | 210.00 | 273.40 | 49.21 | 158.80 | 48.34 | 6.56 | 1.48 |
| *Onobrychis viciifolia control* | 99.20 | 10.10 | 2.50 | 12.50 | 2.90 | 4.23 | 1.01 |

Table 2

**The biochemical composition and the feed value of green mass of *Salvia hispanica***

| Indices | *Salvia hispanica* | | *Onobrychis viciifolia* |
|---|---|---|---|
| | genotype white seeds | genotype gray seeds | control |
| Crude protein, g/kg DM | 107 | 87 | 177 |
| Crude fibre, g/kg DM | 347 | 347 | 293 |
| Ash, g/kg DM | 80 | 77 | 96 |
| Acid detergent fibre, g/kg DM | 348 | 362 | 309 |
| Neutral detergent fibre, g/kg DM | 517 | 533 | 447 |
| Acid detergent lignin, g/kg DM | 65 | 62 | 49 |
| Total soluble sugars, g/kg DM | 123 | 107 | 114 |
| Cellulose, g/kg DM | 283 | 300 | 260 |
| Hemicellulose, g/kg DM | 199 | 166 | 138 |
| Digestible dry matter, g/kg DM | 618 | 607 | 648 |
| Relative feed value | 111 | 106 | 135 |
| Metabolizable energy, MJ/kg DM | 12.19 | 12.00 | 12.73 |
| Net energy for lactation, MJ/kg DM | 10.01 | 9.85 | 10.45 |
| Digestible energy, MJ/kg DM | 6.03 | 5.87 | 6.48 |

Table 3

**The biochemical composition and the nutritive value of the fermented fodder from *Salvia hispanica***

| Indices | *Salvia hispanica* genotype white seeds | *Onobrychis viciifolia* control |
|---|---|---|
| pH index | 4.81 | 4.68 |
| Organic acids, g/kg DM | 21.10 | 23.40 |
| Free acetic acid, g/kg DM | 0.90 | 1.10 |
| Free butyric acid, g/kg DM | 0 | 0 |
| Free lactic acid, g/kg DM | 4.50 | 4.40 |
| Fixed acetic acid, g/kg DM | 1.40 | 2.20 |
| Fixed butyric acid, g/kg DM | 0.30 | 0 |
| Fixed lactic acid, g/kg DM | 14.0 | 15.70 |
| Total acetic acid, g/kg DM | 2.30 | 3.30 |
| Total butyric acid, g/kg DM | 0.30 | 0 |
| Total lactic acid, g/kg DM | 18.50 | 20.10 |
| Acetic acid, % of organic acids | 10.90 | 14.10 |
| Butyric acid, % of organic acids | 1.42 | 0 |
| Lactic acid, % of organic acids | 87.68 | 85.90 |
| Crude protein, g/kg DM | 100 | 142 |
| Crude fibre, g/kg DM | 400 | 312 |
| Ash, g/kg DM | 83 | 118 |
| Acid detergent fibre, g/kg DM | 419 | 317 |
| Neutral detergent fibre, g/kg DM | 593 | 470 |
| Acid detergent lignin, g/kg DM | 72 | 40 |
| Total soluble sugars, g/kg DM | - | 135 |
| Cellulose, g/kg DM | 347 | 277 |
| Hemicellulose, g/kg DM | 174 | 153 |
| Digestible dry matter, g/kg DM | 563 | 642 |
| Digestible energy, MJ/kg DM | 11.21 | 12.63 |
| Metabolizable energy, MJ/kg DM | 9.20 | 10.37 |
| Net energy for lactation, MJ/kg DM | 5.22 | 6.38 |
| Relative feed value | 88 | 127 |

Table 4

**The biochemical composition and the biomethane production potential of *Salvia hispanica* substrates**

| Indices | *Salvia hispanica* genotype white seeds | | *Salvia hispanica* genotype gray seeds | *Onobrychis viciifolia* | |
|---|---|---|---|---|---|
| | green mass | haylage | green mass | green mass | haylage |
| Crude protein, g/kg DM | 107.00 | 100.00 | 87.00 | 177.00 | 142.00 |
| Nitrogen, g/kg DM | 17.12 | 16.00 | 13.92 | 28.30 | 22.70 |
| Carbon, g/kg DM | 511.11 | 509.44 | 512.78 | 502.20 | 490.00 |
| Ratio carbon/nitrogen | 29.20 | 31.80 | 36.80 | 17.7 | 21.6 |
| Acid detergent lignin, g/kg DM | 65.00 | 72.00 | 62.00 | 49.0 | 40.0 |
| Hemicellulose, g/kg DM | 199.00 | 174.00 | 166.00 | 138.0 | 153.0 |
| Biomethane potential, L/kg VS | 298 | 285 | 298 | 335 | 343 |

It is known that the preserved forage has substantial effects on the nutritive value, which has a positive effect on the health of farm animals, particularly in autumn and winter. During the sensorial assessment, it was found that, the haylage from *Salvia hispanica* white seed genotype had light olive leaves and yellow stems with peculiar smell, while the haylage prepared from *Onobrychis viciifolia* consisted of yellowish-green leaves and yellow-green stems and it had a pleasant smell like pickled vegetables. The texture of the plant mass stored as haylages was preserved well, without mold and mucus. The results regarding the quality of the prepared haylages are shown in Table 3. It has been determined that the pH values depended on the species, thus, *Salvia hispanica* haylage had pH=4.81, but *Onobrychis viciifolia* haylage – pH=4.68. The content of organic acids in *Salvia hispanica* haylage was lower in comparison with *Onobrychis viciifolia* haylage. Most organic acids were in fixed form, butyric acid was detected in minor quantities. According to the Moldavian standard SM 108, the ratio of acetic acid and lactic acid of the studied fermented fodder corresponds to the 1-st class quality. It was found that during the process of ensiling, the concentrations of crude protein in *Salvia hispanica* haylage decreased, but the level of minerals, structural carbohydrates and acid detergent lignin increased in comparison with the harvested green mass. In *Salvia hispanica* haylage, the amount of crude protein and energy concentrations were reduced as compared with *Onobrychis viciifolia* haylage.

According to Peiretti P.G. (2010) the chia silage from fresh mass contained 228.0 g/kg DM with pH=5.1, 0.14 g/kg methanol, 1.4 g/kg ethanol, 0.66 g/kg acetic acid, 0.67 g/kg propionic acid, 0.35 g/kg butyric acid, 1.30 g/kg isobutyric acid, lactic acid was not detected, 12.9 g/kg total nitrogen, 18.10MJ/kg GE, but – from wilted plants: 285.0 – 531.0 g/kg DM with pH= 5.3-5.4, 0.05-0.12 g/kg methanol, 0.5-2.1 g/kg ethanol, 0.26-0.39 g/kg acetic acid, 0.01-0.82 g/kg propionic acid, butyric acid, 0.1-0.5 g/kg isobutyric acid, butyric and lactic acids – not detected, 12.9-13.1 g/kg total nitrogen, 17.50-18.80 MJ/kg GE. Filik G. *et al* (2022) reported that quality of chia plant silage was: pH=4.17, 4.32+0.01 CP %, 5.40±2.36% EE, 51.74±0.92% NFE, 42.69±2.89 g/kg NFC, 65.95±3.99 ADF%, 36.73±0.69 NDF%, 26.72±0.05 ADL%, 27.68±1.28 % HC, 10.88±0.18% ash, RFV=95.23±9.68, RFQ=140.71±2.40, 2.34±0.01 Mcal/kg DE, 1.92±0.01 Mcal/kg ME, 1.18±0.00 Mcal/kg NEl.

Biogas is a product of anaerobic digestion of organic products. The methane produced from plant mass has a great importance and can successfully replace natural gas to obtain electric power and heat, and the material remaining after anaerobic digestion of substrates is called digestate and consists of liquid phase (fugate) and solid phase (called digestate). Digestate and fugate are believed to be good fertilizers in organic farming. The results regarding the quality of the phytomass substrates and the potential for obtaining biomethane are shown in Table 4. We found that in the investigated *Salvia hispanica* substrates, according to the C/N ratio, which constituted 29.2-36.8, the amount of acid detergent lignin (62-72 g/kg) and hemicellulose (166-199 g/kg) met the established standards; the biochemical methane potential of studied substrates varied from 285 to 343 l/kg ODM. The lowest results were achieved in chia haylage substrate, with rather high concentration of acid detergent lignin. A high biochemical methane potential was also characteristic of *Onobrychis viciifolia* substrates.

## CONCLUSIONS

The dry matter of *Salvia hispanica* whole plants cut contained: 87-107 g/kg CP, 77-83 g/kg ash, 347-377g/kg CF, 348-362g/kg ADF, 517-533 g/kg NDF, 62-65g/kg ADL, 283-331 g/kg Cel, 166-174 g/kg HC, 107-123 g/kg TSS, with nutritive value 60.70-61.79% DMD, RFV= 106-111, 12.00-12.19 MJ/kg DE, 9.85-10.1 MJ/kg ME, 5.87-6.03 MJ/kg NEl.

The white seed genotype of *Salvia hispanica* was characterized by higher yield, nutritional and energy value of fodder.

The quality indices of *Salvia hispanica* haylage: pH= 4.81, 18.5g/kg lactic acid, 2.3 g/kg acetic acid, 0.3 g/kg butyric acid, 100 g/kg CP, 80g/kg ash, 400 g/kg CF, 419 g/kg ADF, 593 g/kg NDF, 72 g/kg ADL, 347 g/kg Cel, 199 g/kg HC, with nutritive value 56.3% DMD, RFV=88, 11.21 MJ/kg DE, 9.20 MJ/kg ME, 5.22 MJ/kg NEl.

The fresh and ensiled substrates chia from have C/N=29.2-36.8 and the biochemical methane potential reaches 285-298 l/kg ODM.

The *Salvia hispanica* is characterized by optimal productivity and the harvested green mass may be used as forage for farm animals as natural fodder and haylage, and also may be used as substrates for renewable energy production.

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*development of agriculture and guarantees high quality raw material predestined to the perfumery, cosmetic, pharmaceutical and food industry" and 20.80009.5107.12 "Strengthening the "food-animal-production" chain by using new feed resources, innovative sanitation methods and schemes"*

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# PRELIMINARY RESULTS REGARDING THE ATTACK PRODUCED BY THE SPECIES *DIABROTICA VIRGIFERA VIRGIFERA* LE CONTE ON MAIZE, DEPENDING ON THE CHEMICAL TREATMENT APPLIED TO THE SOIL, IN THE CONDITIONS OF CENTRAL MOLDOVA

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**Abstract**

The species *Diabrotica virgifera virgifera* Le Conte (western corn rootworm) belongs to the order Coleoptera, family Chrysomelidae. It originates from North America and entered in Europe in 1992, in Yugoslavia. It entered in Romania in 1996, when three specimens were recorded in Nădlac (Arad county), and since then the range of the pest has continuously expanded (Manole, 2017). To reduce the spread of this pest, it is recommended to avoid monoculture and practice rotation. It can reduce the root attack to a minimum level, the larvae not being able to survive in soils cultivated with other plants (Ciobanu, 2009). The most common strategies used to protect maize roots against the pest *Diabrotica virgifera virgifera* are the application of an insecticide to the soil at seeding and the use of an insecticide in the seed treatment (Sutter et al., 1990). The average number of larvae per plant varied from 1 to 5, the fewest larvae being recorded in the variant where the granular insecticide Force G was applied to the soil at a dose of 15 kg/ha. The frequency of swan neck ranged from 0% to 58%. The attack produced by adults on the leaf recorded frequencies between 32% and 66%, and on maize silk, the attack was 100%. The average number of adults per plant was between 9 and 10.

**Key words**: maize, soil treatment, larvae, *Diabrotica*, attack

The species *Diabrotica virgifera virgifera* Le Conte (western corn rootworm) belongs to the order Coleoptera, family Chrysomelidae. It originates from North America and entered in Europe in 1992, in Yugoslavia. It entered in Romania in 1996, when three specimens were recorded in Nădlac (Arad county), and since then the range of the pest has continuously expanded (Manole T., 2017). By 2011, the pest was already reported in 22 countries in Europe. The speed of spread is approximately 25-50 km/year, but with the help of the wind it can move up to 300 km/year (Bacal S. *et al*, 2020).

Between 1997 and 2009, pheromonal traps were installed at A.R.D.S. Secuieni to monitor the appearance of the pest in maize crops in the Central area of Moldova, but it was not reported. The pest was identified in maize crops in the Central area of Moldova starting in 2015, the flight intensifying from one year to the next (Trotuș E. *et al*, 2020).

The insect attacks both in the larval and adult stages. Larval feeding reduces the ability of plants to absorb water and nutrients by disrupting the structure and function of the root system, leading to significant yield losses (Ferracini C. *et al*, 2021). The adults feed on leaves, silk, pollen, but also on the grains from the top of the cobs which are in the milk phase.

It was observed that large areas cultivated with maize in monoculture for several years in a row contributed to the huge multiplication and spread of the species *Diabrotica virgifera virgifera* Le Conte.

To reduce the spread of this pest, it is recommended to avoid monoculture and practice rotation. It can reduce the root attack to a minimum level, the larvae not being able to survive in soils cultivated with other plants (Ciobanu C. *et al*, 2009).

The most common strategies used to protect maize roots against the pest *Diabrotica virgifera virgifera* are the application of an insecticide to the soil at seeding and the use of an insecticide in the seed treatment (Sutter G.R. *et al*, 1990).

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In the last few years, the use of maize seeds treated with neonicotinoid insecticides has been banned in several European countries, for this reason insecticides applied to the soil are increasingly being used (Blandino M. *et al*, 2017).

In the present work, preliminary results are presented regarding the reduction of the attack of the species *Diabrotica virgifera virgifera* Le Conte in the year 2023, in the conditions of the Central area of Moldova, through the chemical treatment applied to the soil.

## MATERIAL AND METHOD

In the spring of 2023, a maize experience was placed in the experimental field of the Plant Protection laboratory within A.R.D.S. Secuieni, consisting in a number of four variants, placed according to the method of randomized blocks, in three repetitions, where was followed the influence of the applied chemical treatment on the soil on reducing the attack produced by the species *Diabrotica virgifera virgifera* Le Conte.

In parallel, was also monitored the influence of insecticides on the attack produced by *Tanymecus dilaticollis*.

The Agricultural Research - Development Station Secuieni is located in the SE part of Neamţ county, being located on the geographical coordinates of 26051'00" east longitude and 46051'15" north latitude. The placement of the experience was carried out on a typical cambic phaeosium type soil, with pH in water 6.29, nitrogen index 2.1, mobile P2O5 39 ppm, mobile K2O 161 ppm.

The predecessor plant was maize. Soil work, fertilization, seedbed preparation and crop maintenance were carried out according to the maize cultivation technology for the specific conditions in Central Moldova (Trotuș E. *et al,* 2020).

Sowing was done on 05.05.2023, the hybrid used was Turda Star. The emergence of the plants was recorded on 22.05.2023.

Were tested three granular products with insecticidal action, applied to the soil at the same time as sowing: Force G (tefluthrin 15 g/kg) – 15 kg/ha, Picador (cypermethrin 1.6 g/kg) – 12 kg/ha and Trika expert (lambda cyhalothrin 4 g/kg) – 15 kg/ha. The area of each experimental variant was 22.4 square meters.

To determine the attack produced by the *Tanymecus dilaticollis* species, were made observations on 25 plants, in three repetitions, giving marks on a scale of 0-6. Based on the marks given, the frequency, intensity and degree of attack was established. Pest density was determined using the 25/25 frame.

To identify the larvae of *Diabrotica virgifera virgifera* in the soil, were made determinations consisting of the analysis of 10 maize plants in three repetitions and the counting of the larvae on the root.

To determine the attack of adults of *Diabrotica virgifera virgifera* on leaves and silk, were made observations on 25 plants, in three repetitions, and was determined the frequency of attack. The determination of the number of adults per plant was achieved by visual assessment of 25 plants in three repetitions.

The experimental data obtained were analyzed by appropriate statistical methods using the difference test (DL) (DL < 0.01 %– ***/OOO – highly significant positive/highly significant negative; DL < 1% —**/– ** / OO – distinctly significant positive/distinctly significant negative; DL < 5 %—*/ – */O – significant positive/significant negative).

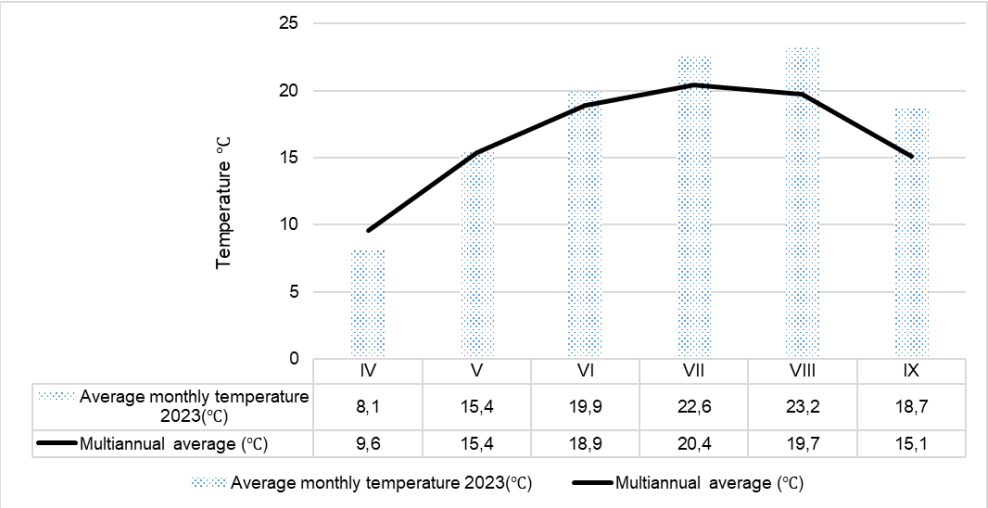
Climatic conditions: The temperatures recorded during the maize vegetation period characterized it as warm.

As for spring, the month of March was characterized by high average temperatures, the deviation being 3.2°C above the multiannual average of 2.8°C. The average temperature in April (8.1°C) was below the multiannual average (9.5°C), the month being characterized as cool. At the beginning of April, there was snow and blizzard. And the first decade of May was cooler, the other decades being close to the multi-year average. These conditions delayed the sowing of maize which was carried out in early May, with the plants emerging in the second half of May (figure 1). The summer months are hot (June) and very hot (July and August) with average monthly temperatures between 1°C and 3.5°C above the multiannual average.

In terms of precipitation, the monthly deviations from the multiannual amount were between -54.9 mm (June) and 24 mm (April). An atypical phenomenon recorded in April was the layer of snow that fell in the first decade, which made it difficult to prepare the land for sowing and sowing, the moment being shifted to the beginning of May. The precipitation in April, in the form of snow and rain, exceeded the multiannual average by 24 mm, thus reducing the water deficit. The last spring month, May, was dry, recording 21 mm of the 64.3 mm, the highest amount of precipitation being recorded in the first decade of the month. The month of June was very dry, the deviation from the multiannual amount being -54.9 mm. The month of July was characterized as being less dry, the deviation being -12.4 mm, and the storms recorded highlighted the attack produced by the larvae of the species *Diabrotica virgifera virgifera* (swan neck symptom). The precipitation that fell at the beginning and end of August led to the reduction of the water deficit, the monthly amount of precipitation (77 mm) exceeding the multiannual average by 19 mm (Figure 2).

Meteorological data comes from the unit's own VANTAGE PRO 2 weather station located in

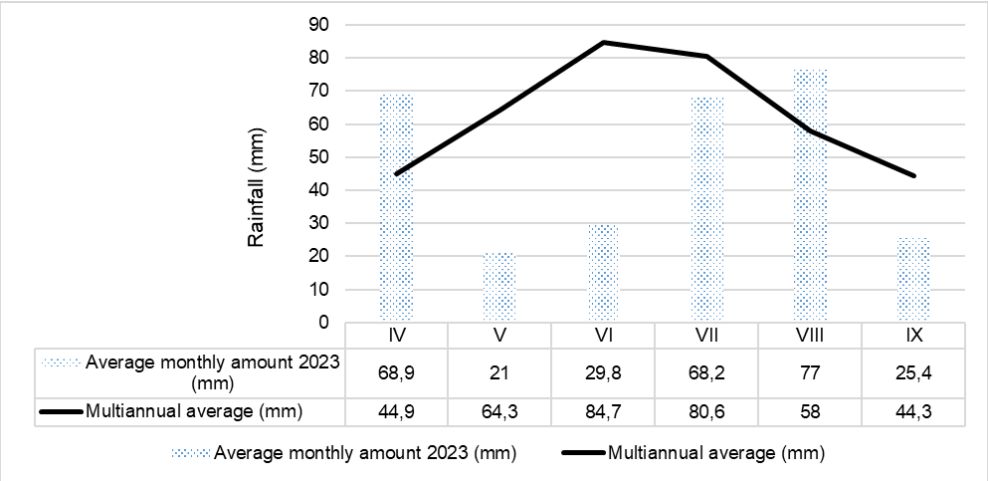
the experimental field, the station being automated with data recording and computer storage.




| | IV | V | VI | VII | VIII | IX |
|---|---|---|---|---|---|---|
| Average monthly temperature 2023(°C) | 8,1 | 15,4 | 19,9 | 22,6 | 23,2 | 18,7 |
| Multiannual average (°C) | 9,6 | 15,4 | 18,9 | 20,4 | 19,7 | 15,1 |

Average monthly temperature 2023(°C) — Multiannual average (°C)

Figure 1 **Temperatures recorded during the maize vegetation period, 2023**




| | IV | V | VI | VII | VIII | IX |
|---|---|---|---|---|---|---|
| Average monthly amount 2023 (mm) | 68,9 | 21 | 29,8 | 68,2 | 77 | 25,4 |
| Multiannual average (mm) | 44,9 | 64,3 | 84,7 | 80,6 | 58 | 44,3 |

Average monthly amount 2023 (mm) — Multiannual average (mm)

Figure 2 **Precipitation recorded during the maize vegetation period, 2023**

## RESULTS AND DISCUSSIONS

Due to the low temperatures and precipitation in the form of snow recorded in April, the sowing of maize was possible in the first decade of May. In the first phases of vegetation, maize suffers attacks from the complex of soil pests, made up of the species *Tanymecus dilaticollis* and *Agriotes* spp. (Trotuș E. *et al*, 2021). In the conditions of the spring of 2023, the emergence, spread and attack of adults of *Tanymecus dilaticollis* in the maize crop were found. This fact led to the uneven emergence of plants, the density at emergence being on average 46205 plants/hectare. In the absence of chemical treatment of the seed against soil pests, the frequency of the attack produced by the *Tanymecus dilaticollis* species was 100%, and the degree of attack was between 85.3% (V2 – Force G) and 96% (V1 - control) (Figure 3). The density of the *Tanymecus dilaticollis* pest was between 6 exp/m$^2$ and 15 exp/m$^2$ (Figure 4). The first larvae of the species *Diabrotica virgifera virgifera* were identified in maize crops in the first decade of June.

During the dynamic determinations, it was found that the average number of larvae per plant varied from 1 to 3 (20.06.2023), respectively from 2 to 5 (26.06.2023), with the fewest larvae recorded in the variant where the Force G granulated insecticide was applied to the soil in a dose of 15 kg/ha (Figure 5).

At a first determination of the swan neck frequency, it recorded values between 0 and 10%. But after a strong wind in the first decade of July, the swan neck symptom in the attacked plants was much more pronounced, the frequency of the attack registering values between 0% (V2 – Force G) and 58% (V1 – control) (Figure 6).

Also, was followed the attack produced by the adults of the species *Diabrotica virgifera virgifera* Le Conte on leaves and silks and it was

found that they were present in the crop and attacked the maize plants.

The attack produced by adults on the leaf recorded frequencies between 32%, as recorded in the variant where was applied to the soil the granular insecticide Force G (tefluthrin), and 66%, as recorded in the control variant. On maize silk, the attack was 100%, in some plants consuming the silk entirely (Figure 7). The average number of adults per plant was between 9 and 10.

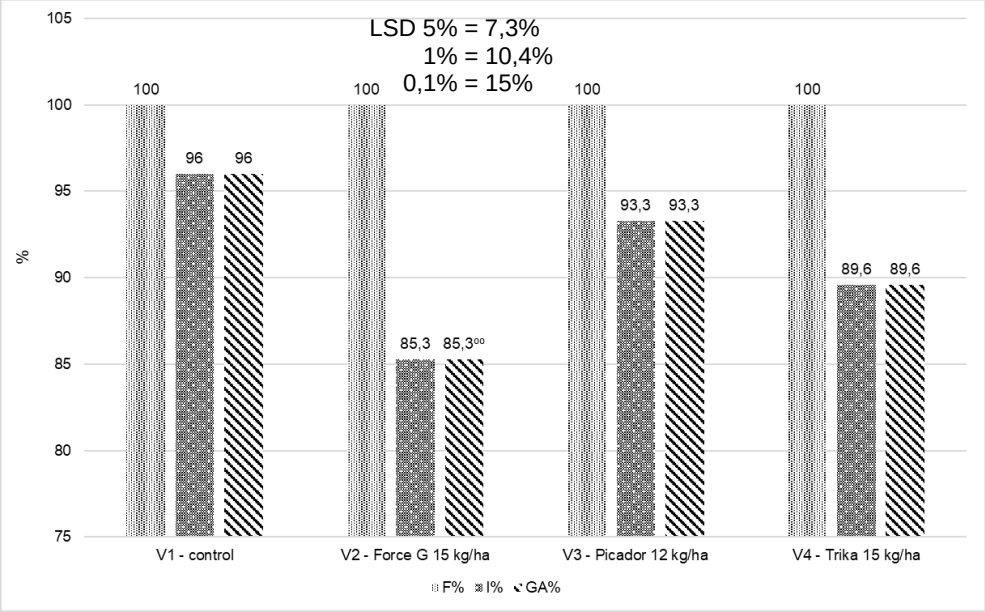



Figure 3 **The attack produced by the species *Tanymecus dilaticollis* on maize plants, 2023**

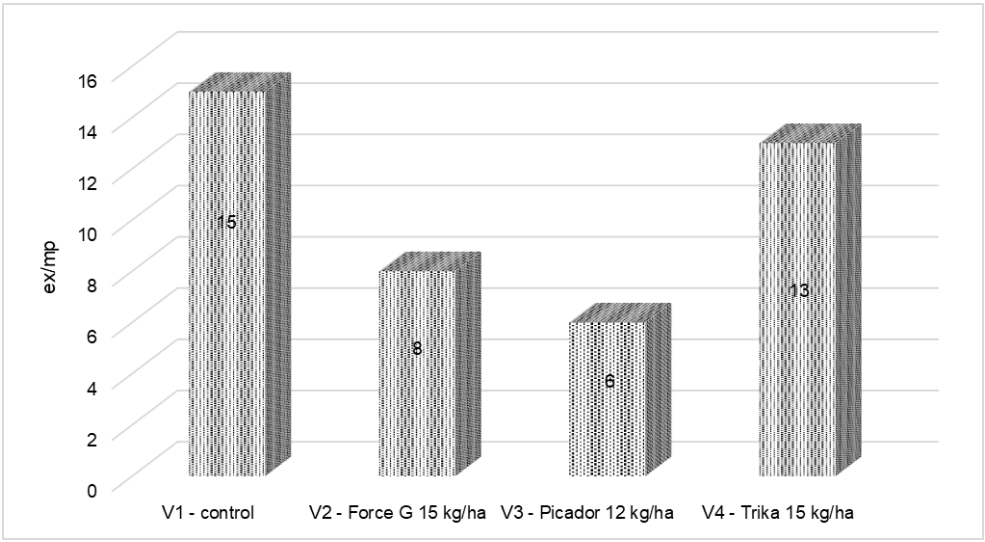



Figure 4 **The density of the *Tanymecus dilaticollis* species/m$^2$, 2023**

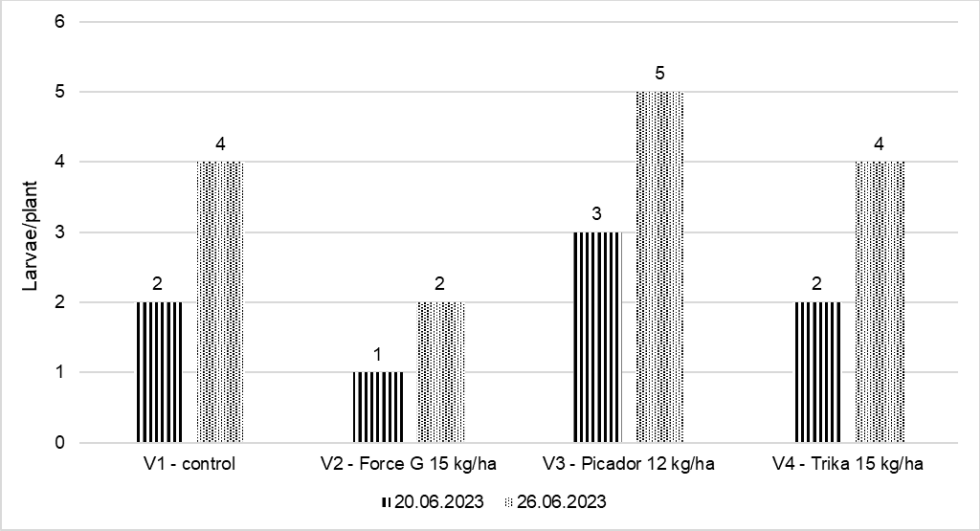



Figure 5 **Number of larvae of *Diabrotica virgifera virgifera* Le Conte per plant, 2023**

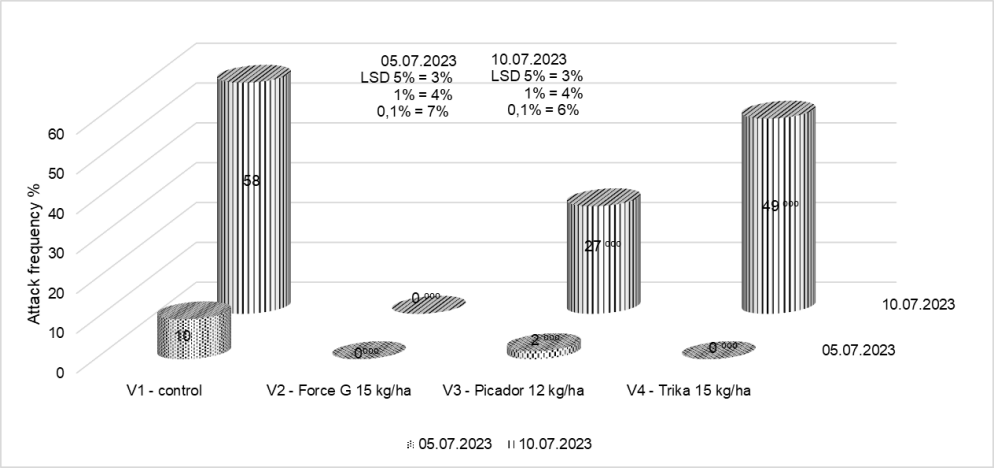



Figure 6 **Frequency of plants with "swan neck" symptom, 2023**

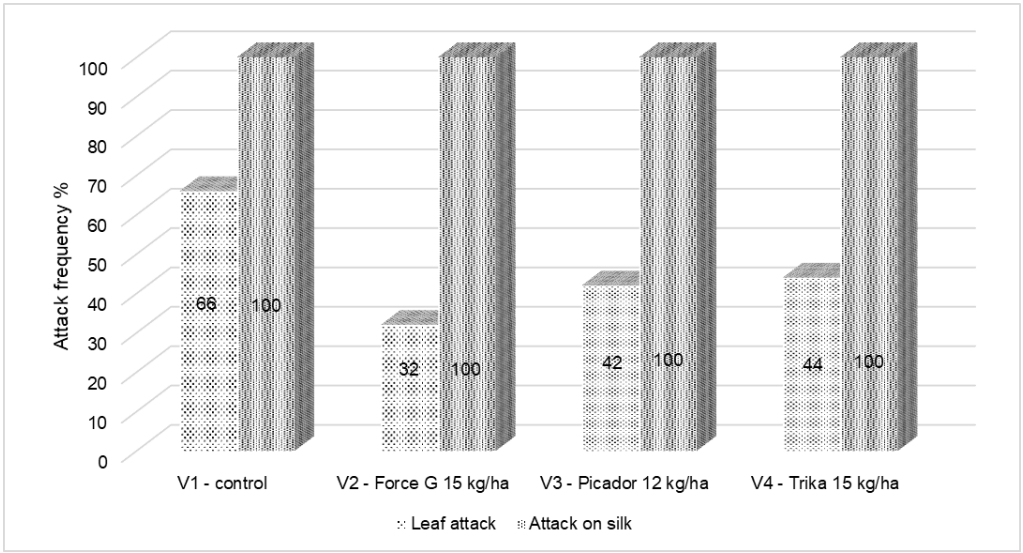



Figure 7 **Frequency of attack by adults of the species *Diabrotica virgifera virgifera* Le Conte on leaf and silk, 2023**

## CONCLUSIONS

In 2023, the species *Diabrotica virgifera virgifera* produced attack in both the larval and adult stages. The larvae attacked the roots of the maize plants, causing stem bending, and the adults attacked the leaves, silk and pollen.

The average number of larvae per plant was between 1 and 5, with the lowest number of larvae recorded in the variant where Force G insecticide was applied to the soil.

Attack by adults on leaf was as high as 66%, while attack on maize silk recorded a frequency of 100%. The average number of adults per plant was between 9 and 10.

From the observations made in 2023, the best results were obtained in the variant where the granular insecticide Force G (tefluthrin 15 g/kg) was applied to the soil in a dose of 15 kg/ha.

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# IDENTIFICATION OF SUNFLOWER GENOTYPES TOLERANT AT DROUGHT

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**Abstract**

Sunflower represents the main oil crop in Romania and is considered a moderately crop drought tolerant but in years with low precipitations seed yield is affected. In recent years, the temperature increased by 2-3 degrees Celsius compared to multi-year average over 60 years. In south-eastern of Romania (agricultural area Fundulea), in 2020, the average annual temperature was 13.5°C, in 2021, it was 12.1°C and in 2022, it was 13.3°C compared with multi-year average over 60 years who was 10.9°C. Rainfalls decreased in last four years from value of 303.3 mm, multi-year average over 60 years total from months April to August in South eastern of Romania, to a total of 180 mm in years 2020 and 2022, 269 mm in year 2021 and a total of 200 mm in 2023. In conditions of water stress and global warming we must identify sunflower genotypes with tolerance at drought and heat. In this paper we present results regarding resistance at water stress through observations development root system of sunflower genotypes under artificial condition in greenhouse and behavior in non-irrigated field in Calarasi county (Fundulea area) and Braila, in year 2023. Sunflower genotypes, S 23-5, S 23-6 and S 23-7 with very developed root system in water stress conditions was obtained thought interspecific hybridization with wild annual specie *Helianthus argophyllus* and with wild perennial species *Helianthus tuberosus* and *Helianthus maximiliani*.

**Key words**: sunflower, drought, *Helianthus argophyllus*, root system, water stress

Seed yield and oil content of sunflower genotypes, are affected in agricultural years with low precipitations, especially in phenophase of vegetation of seed filing. Sunflower plants suffer when is water missing and temperature of air is very high and negative effects are reduction of fertility through viability of pollen is decreasing followed by low seed yield (Port A. *et al*, 2023; Clapco S. *et al*, 2018). In conditions of water stress, sunflower has morpho-physiological and biochemical mechanisms that allow maintaining high hydric potential in the plant and saving water (Andrade A. *et al*, 2021; Ahmad H.M. *et al*, 2020; Boero A. *et al*, 2023). To avoid drought in the sunflower growth stage, we must obtain very early hybrids and select genotypes with the phenomenon stay green (Hilli H.J., Immadi S.U., 2021).

## MATERIAL AND METHOD

Sunflower genotypes studied for tolerance to drought was obtained through by interspecific hybridization between inbred sunflower lines and wild sunflower species in various generations of selection (*table 1*).

Table 1

**Sunflower genotypes studied for tolerance to drought**

| Sunflower genotype | Interspecific hybridization with annual/ perennial wild species | The selection generation | Branching/ No branching |
|---|---|---|---|
| S 23-1 | 1C x *Helianthus argophyllus* | F3 | Branching |
| S 23-2 | 2C x *Helianthus maximiliani* | F4 | Branching |
| S 23-3 | 3B x *Helianthus maximiliani* | F3 | No branching |
| S 23-4 | 4B x *Helianthus argophyllus* | F3 | No branching |
| S 23-5 | 5C x *Helianthus argophyllus* | F3 | Branching |
| S 23-6 | 6C x *Helianthus tuberosus* | F3 | Branching |
| S 23-7 | 7C x *Helianthus maximiliani* | F3 | Branching |
| S 23-8 | 8B x *Helianthus debilis* | F8 | No branching |

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| | | | |
|---|---|---|---|
| S 23-11 | 11C x *Helianthus mollis* | F8 | Branching |
| S 23-13 | 13C x *Helianthus petiolaris x Helianthus praecox* | F8 | Branching |
| S 23-14 | 14C x *Helianthus petiolaris x Helianthus debilis* | F8 | Branching |
| S 23-15 | 15C x *Helianthus praecox* | F8 | Branching |
| S 23-16 | 16C x *Helianthus praecox* | F7 | Branching |
| S 23-17 | 17C x *Helianthus debilis* | F8 | Branching |
| S 23-18 | 18C x *Helianthus maximiliani* | F8 | Branching |
| S 23-19 | 19C x *Helianthus mollis* | F8 | Branching |
| S 23-20 | 20B x *Helianthus neglectus* | F7 | No branching |
| S 23-21 | 21Bx *Helianthus argophyllus* | F12 | No branching |
| S 23-22 | 22B x *Helianthus argophyllus* | F12 | No branching |
| S 23-23 | 23C x *Helianthus argophyllus* | F12 | Branching |
| S 23-25 | 25C x *Helianthus argophyllus* | F12 | Branching |

B = maintainer of fertility sunflower line
C = restorer of fertility sunflower line

These sunflower genotypes were sown on date February 1, 2023, in Fundulea, in artificial condition, in green house, in 4 buckets of 10 liter with soil, one watered every two days (the irrigated control) and three, every 4 days (*figure 1a*). We make notations about root development and height plant compared with check irrigated normal.

In natural condition, in no irrigated field was sowing in Fundulea, on April 28, 2023 and in Braila, on May 1, 2023 for testing to drought (*figure 1b, 1c*).

a. b. c.

Figure 1a **Sunflower genotypes in artificial conditions, in the greenhouse, in Fundulea, in 2023;** 1b **Sunflower genotypes tested for drought, in natural conditions, in no irrigated field, in Fundulea;** 1c **Sunflower genotypes tested for drought, in natural conditions, in no irrigated field in Braila, in 2023**

## RESULTS AND DISCUSSIONS

Observations were made regarding tolerance and drought resistance through numbers from 1 (drought-sensitive genotypes) to 5 (drought-tolerant genotypes) after analyzing the development of the root system and after analyzing the height of the plants in comparison with the normal irrigated control (*table 2* and *figure 2*).The most tolerance at drought in artificial conditions, in greenhouse, was observed at genotypes S23 -5, obtained from interspecific hybridization between restorer inbred line 5C and annual wild species *Helianthus argophyllus*, at S23-6, obtained from interspecific hybridization between restorer inbred line 6C and perennial wild species *Helianthus tuberosus*, at restorer inbred line S23-7, obtained from interspecific hybridization between restorer inbred line 7C and perennial wild species *Helianthus maximiliani*.

In the flowering vegetation stage, in Fundulea, in 2023, in month July, average monthly temperature of air, was 26.1 degrees Celsius and in Braila was 24.7 degrees Celsius, compared with multi-year average over 60 years (Braila) who was 22.7 degrees Celsius. Absolute monthly maximum temperatures registered in Fundulea, in 2023, in month July, was 39.6$^{o}$ Celsius and in Braila, was 31.8 $^{o}$ Celsius.

Table 2

**Notes about resistance/tolerance to drought, in artificial conditions, in greenhouse,in Fundulea, in year 2023**

| Sunflower genotype | Interspecific hybridization with annual/ perennial wild species | Resistance\tolerance at drought |
|---|---|---|
| **S 23- 1** | 1C x *Helianthus argophyllus* | 1 |
| **S23- 2** | 2C x *Helianthus maximiliani* | 3 |
| **S23- 3** | 3B x *Helianthus maximiliani* | 3 |
| **S23 -4** | 4B x *Helianthus argophyllus* | 1 |
| **S23 -5** | 5C x *Helianthus argophyllus* | 4.5 |
| **S23-6** | 6C x *Helianthus tuberosus* | 4.5 |
| **S23-7** | 7C x *Helianthus maximiliani* | 4.5 |
| **S23-8** | 8B x *Helianthus debilis* | 2.5 |
| **S23-11** | 11C x *Helianthus mollis* | 2.5 |
| **S23-13** | 13C x *Helianthus petiolaris x Helianthus praecox* | 1 |
| **S23-14** | 14C x *Helianthus petiolaris x Helianthus debilis* | 3.5 |
| **S23-15** | 15C x *Helianthus praecox* | 2.5 |
| **S23-16** | 16C x *Helianthus praecox* | 3.5 |
| **S23-17** | 17C x *Helianthus debilis* | 3 |
| **S23-18** | 18C x *Helianthus maximiliani* | 3 |
| **S23-19** | 19C x *Helianthus mollis* | 3 |
| **S23-20** | 20B x *Helianthus neglectus* | 4 |
| **S23-21** | 21Bx *Helianthus argophyllus* | 1 |
| **S23-22** | 22B x *Helianthus argophyllus* | 3.5 |
| **S23-23** | 23C x *Helianthus argophyllus* | 3.5 |
| **S23-25** | 25C x *Helianthus argophyllus* | 1.5 |

1= sensible; 5=resistant/tolerant

Figure 2 **The root system at sunflower genotypes S23-5, tolerant to drought and S23-8 sensible to drought**

In stage of vegetation of seed filing, in Fundulea, in 2023, in month August, the average month temperature of air, was 26.1 degrees Celsius and in Braila was 24.7 degrees Celsius, compared with multi-year average over 60 years (Braila) who was 22.3 degrees Celsius. We observe an increase of at least 2 degrees Celsius compared to the multi-annual average for 60 years (Fundulea) in the months of June, July and August (*table 3* and *figure 3*).

Maximum absolute monthly temperatures recorded in Fundulea, in 2023, in month August, was 39.6º Celsius and in Braila, was 32.4 º Celsius. In stage of vegetation of seed filing, in Fundulea, in 2023, in month August, the average monthly rainfalls, was 6.6 mm and in Braila was 55.1 mm, compared with multi-year average over 60 years (Fundulea) who was 49.7 mm (*table 4* and *figure 4*).

Table 3

**Average monthly air temperature, registered in Braila and Fundulea, in year 2023**

| Month | Fundulea 2023 | Braila 2023 | Average of 60 years (Fundulea) |
|---|---|---|---|
| January | 4.9ºC | 4.4 ºC | -2.4 ºC |
| February | 3.3 ºC | 1.4 ºC | -0.4 ºC |
| March | 8.2 ºC | 7.9 ºC | 4.9 ºC |
| April | 10.8 ºC | 10.4 ºC | 11.3 ºC |
| May | 16.9 ºC | 16.6 ºC | 17 ºC |
| June | 22.3 ºC | 21.6 ºC | 20.8 ºC |
| July | 26.1 ºC | 24.7 ºC | 22.7 ºC |
| August | 26.1 ºC | 24.7 ºC | 22.3 ºC |

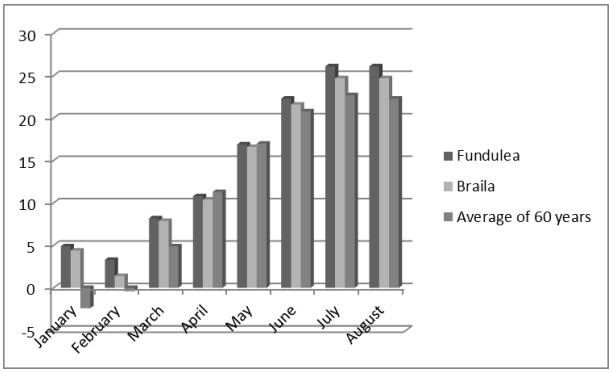



Figure 3 **Average monthly temperatures registered in Fundulea and Braila, in year 2023**

Table 4

**Average monthly rainfalls, registered in Braila and Fundulea, in year 2023**

| Month | Fundulea 2023 | Braila 2023 | Average of 60 years (Fundulea) |
|---|---|---|---|
| January | 64.2 mm | 64.2 mm | 35.1 mm |
| February | 5.8 mm | 7.1 mm | 32 mm |
| March | 10 mm | 13.2 mm | 37.4 mm |
| April | 77.2 mm | 65.5 mm | 45.1 mm |
| May | 32.2 mm | 39.6 mm | 62.5 mm |
| June | 40.2 mm | 25.6 mm | 74.9 mm |
| July | 43.8 mm | 105.9 mm | 71.1 mm |
| August | 6.6 mm | 55.1 mm | 49.7 mm |
| Total rainfalls | 280 mm | 376.2 mm | 407.8 mm |

In Fundulea and Braila, in year 2023, the best average seed yield/sunflower plant was at sunflower genotypes S 23-8, obtained from interspecific hybridization between maintainer inbred line 8B and annual wild species *Helianthus debilis* and S 23-22, obtained from interspecific hybridization between maintainer inbred line 22B and annual wild species x *Helianthus argophyllus* (*table 5* and *figure 5a*).

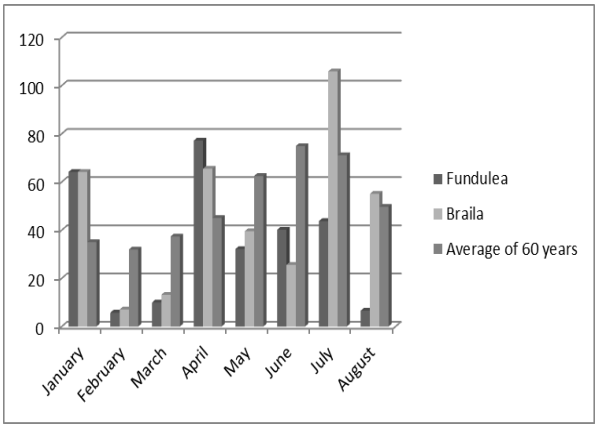



Figure 4 **Average monthly rainfalls registered in Braila and Fundulea, in year 2023**

Table 5

**Average seed yield of sunflower genotypes, registered in Braila and Fundulea, in year 2023**

| Sunflower genotype | Interspecific hybridization with annual/ perennial wild species | Average seed yield /sunflower plant (grams) Fundulea, 2023 | Average seed yield /sunflower plant (grams) Braila, 2023 |
|---|---|---|---|
| S 23-1 | 1C x *Helianthus argophyllus* | 9.77 | 9.16 |
| S 23-2 | 2C x *Helianthus maximiliani* | 12.71 | 10.71 |
| S 23-3 | 3B x *Helianthus maximiliani* | 10.87 | 8 |
| S 23-4 | 4B x *Helianthus argophyllus* | 5.55 | 15 |
| S 23-5 | 5C x *Helianthus argophyllus* | 21.46 | 12.9 |
| S 23-6 | 6C x *Helianthus tuberosus* | 20.24 | 15.29 |
| S 23-7 | 7C x *Helianthus maximiliani* | 4.75 | 11.85 |
| S 23-8 | 8B x *Helianthus debilis* | 55.19 | 61.73 |
| S 23-11 | 11C x *Helianthus mollis* | 34.44 | 20 |
| S 23-13 | 13C x *Helianthus petiolaris x Helianthus praecox* | 10.12 | 7.5 |
| S 23-14 | 14C x *Helianthus petiolaris x Helianthus debilis* | 25.64 | 18.75 |
| S 23-15 | 15C x *Helianthus praecox* | 10.18 | 16.47 |
| S 23-16 | 16C x *Helianthus praecox* | 4.64 | 5.6 |
| S 23-17 | 17C x *Helianthus debilis* | 23.39 | 27.5 |
| S 23-18 | 18C x *Helianthus maximiliani* | 5.53 | 7.69 |
| S 23-19 | 19C x *Helianthus mollis* | 18.44 | 18 |
| S 23-20 | 20B x *Helianthus neglectus* | 32.42 | 21.66 |
| S 23-21 | 21Bx *Helianthus argophyllus* | 22.94 | 29.6 |
| S 23-22 | 22B x *Helianthus argophyllus* | 43.68 | 53.75 |
| S 23-23 | 23C x *Helianthus argophyllus* | 11.83 | 10 |
| S 23-25 | 25C x *Helianthus argophyllus* | 14.57 | 9.09 |

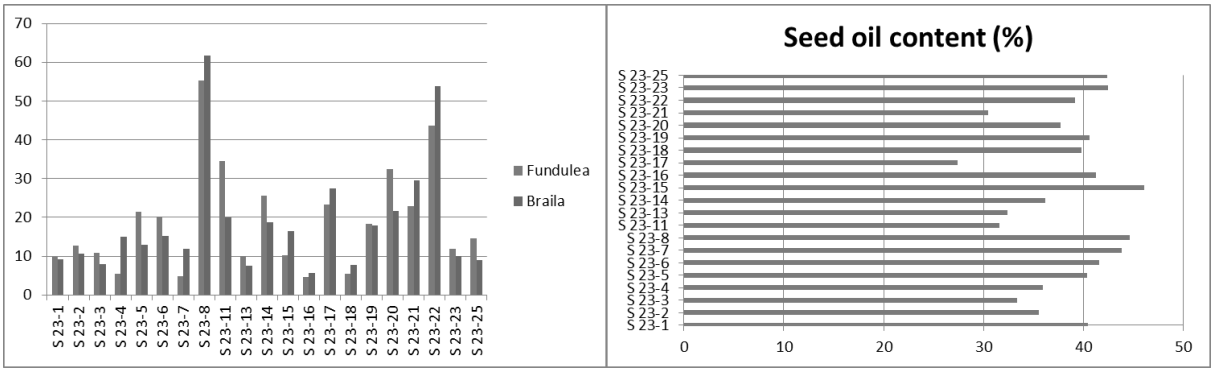
Seed oil contents was determined with magnetic resonance device Oxford MQC+5, only from genotypes tested for drought in Fundulea, in year 2023 and was between 27.39% at S23-17 and 46.1% at S23-15 (*table 6* and *figure 5b*).

Table 6

**Seed oil content of sunflower genotypes, registered in Fundulea, in year 2023**

| Sunflower genotype | Interspecific hybridization with annual/ perennial wild species | Seed oil content (%) |
|---|---|---|
| S 23-1 | 1C x *Helianthus argophyllus* | 40.44 |
| S 23-2 | 2C x *Helianthus maximiliani* | 35.53 |
| S 23-3 | 3B x *Helianthus maximiliani* | 33.39 |
| S 23-4 | 4B x *Helianthus argophyllus* | 35.94 |
| S 23-5 | 5C x *Helianthus argophyllus* | 40.38 |
| S 23-6 | 6C x *Helianthus tuberosus* | 41.55 |
| S 23-7 | 7C x *Helianthus maximiliani* | 43.86 |
| S 23-8 | 8B x *Helianthus debilis* | 44.63 |

| | | |
|---|---|---|
| S 23-11 | 11C x *Helianthus mollis* | 31.56 |
| S 23-13 | 13C x *Helianthus petiolaris x Helianthus praecox* | 32.37 |
| S 23-14 | 14C x *Helianthus petiolaris x Helianthus debilis* | 36.16 |
| S 23-15 | 15C x *Helianthus praecox* | 46.1 |
| S 23-16 | 16C x *Helianthus praecox* | 41.26 |
| S 23-17 | 17C x *Helianthus debilis* | 27.39 |
| S 23-18 | 18C x *Helianthus maximiliani* | 39.82 |
| S 23-19 | 19C x *Helianthus mollis* | 40.62 |
| S 23-20 | 20B x *Helianthus neglectus* | 37.74 |
| S 23-21 | 21Bx *Helianthus argophyllus* | 30.44 |
| S 23-22 | 22B x *Helianthus argophyllus* | 39.18 |
| S 23-23 | 23C x *Helianthus argophyllus* | 42.49 |
| S 23-25 | 25C x *Helianthus argophyllus* | 42.36 |




a. b.

Figure 5a **Average seed yield of sunflower genotypes, in Braila and Fundulea, in year 2023;** 5b **Seed oil content (%) of sunflower genotypes registered in Fundulea, in year 2023**

In Fundulea, in year 2023, Thousand kernel weight (TKW) was between 20.32 grams at sunflower genotype S 23-1 and 66.56 grams at sunflower genotype S 23-8 (*table 7* and *figure 6*).

Table 7

**Thousand Kernel Weight (TKW)of sunflower genotypes, in Fundulea and Braila, in year 2023**

| Sunflower genotype | Fundulea 2023 (TKW- grams) | Braila 2023 (TKW-grams) |
|---|---|---|
| S 23-1 | 20.32 | 13.75 |
| S 23-2 | 26.36 | 15 |
| S 23-3 | 58.8 | 13.6 |
| S 23-4 | 59.31 | 29.82 |
| S 23-5 | 30.08 | 15.4 |
| S 23-6 | 32.96 | 15.47 |
| S 23-7 | 24.75 | 13.79 |
| S 23-8 | 66.56 | 32.94 |
| S 23-11 | 32.16 | 30.52 |
| S 23-13 | 34.92 | 15.8 |
| S 23-14 | 40.08 | 21.26 |
| S 23-15 | 16.32 | 16.77 |
| S 23-16 | 16.45 | 15.72 |
| S 23-17 | 35.61 | 35.42 |
| S 23-18 | 26.02 | 10.99 |
| S 23-19 | 47.28 | 20.95 |
| S 23-20 | 22.88 | 22.64 |
| S 23-21 | 55.2 | 28.35 |
| S 23-22 | 63.56 | 21.48 |
| S 23-23 | 25.04 | 19.94 |
| S 23-25 | 22.04 | 13.2 |

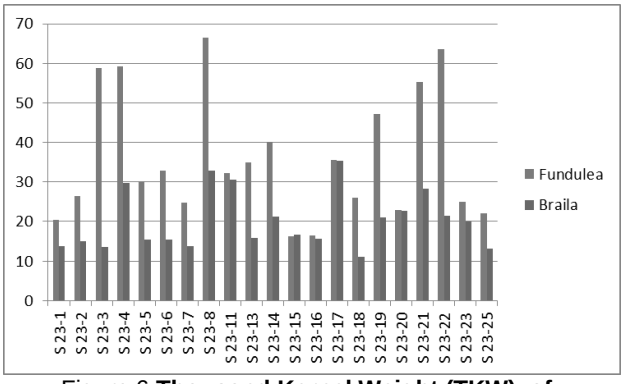



Figure 6 **Thousand Kernel Weight (TKW) of sunflower genotypes in Fundulea and Braila in year 2023**

## CONCLUSIONS

Results from artificial conditions in greenhouse, regarding tolerance at drought not was the same like in natural conditions, in field. Average seed yield of sunflower genotypes, registered in Braila was different from Fundulea, in year 2023, when some inbreed line has a better yield in one location and other has a better yield in other location. Sunflower genotype S23-8 had a average seed yield of 55.19 grams/sunflower plant in Fundulea, in 2023 and 61.73 grams/sunflower plant, in Braila. One thousand seed weight (g) of sunflower genotypes tested for tolerance at

drought, had higher values in Fundulea than in Braila, in year 2023.

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# RESEARCH FOR COMPETITION RELATIONS BETWEEN THE *Festuca arundinacea* Schreb. and *Trifolium pratense* L. SPECIES CULTIVATED IN SIMPLE MIXTURES

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**Abstract**

Purpose of research carried out in 2022 at Ezăreni Farm (47°05'-47°10 ' North latitude and 27°28'-27°33' Eastern longitude) belonging to the Iași University of Life Sciences, was to analyze the competitiveness of the species *Festuca arundinacea* Schreb. and *Trifolium pratense* L., grown alone or in simple mixtures, under different conditions of fertilization with complex mineral fertilizers, under the climatic conditions from Moldavian Forrest Steppe. The studied factors were: species or mixture of grasses and perennial legumes, with 5 graduations, respectively $a_1$ - *Festuca arundinacea* Schreb. (100%); $a_2$ - *Festuca arundinacea* Schreb. (75%) and *Trifolium pratense* L. (25%); $a_3$ - *Festuca arundinacea* Schreb. (50%) and *Trifolium pratense* L. (50%); $a_4$ - *Festuca arundinacea* Schreb. (25%) and *Trifolium pratense* L. (75%); $a_5$ - *Trifolium pratense* L. (100% ) and fertilization with mineral fertilizers, with 5 graduations, respectively $b_1$ - unfertilized, $b_2$ - $N_{50}P_{50}$; $b_3$ - $N_{75}P_{75}$; $b_4$ - $N_{100}$; $b_5$ - $N_{150}P_{150}$. In the second year of vegetation, the RYT index (Relative Yield Total) recorded values >1, except for variants fertilized with $N_{150}P_{150}$, showing that the *Festuca arundinacea* Schreb. and *Trifolium pratense* L. species competes for the same vegetation factors, and the CR index for the *Festuca arundinacea* Schreb. species was higher than in the case of the *Trifolium pratense* L. species only at a percentage of participation in the mixture of 75%, under fertilization conditions, in which case the species was more competitive.

**Key words**: mixture percentage, fertilization, RYT (Relative Yield Total), CR (Competition Rate)

The formation of mixtures of perennial grasses and leguminous species for feed must be conditioned by the biological properties of the species, in accordance with the use and duration of existence of temporary grasslands (Marușca T., 2001, Belesky D.P. *et al*., 2002). The ability of each species to compete will also be considered when compiling mixtures of perennial grasses and leguminous species. This is a species trait, but it is greatly influenced and to a large extent by pedoclimatic conditions and the exploitation mode (Skinner R.H. *et al*., 2006, Lazaridou M., 2008, Stanciu A.Ș. *et al*., 2015).

Fertilization on temporary meadows influences their production capacity, obtaining a feed of better quality. It also leads to a change in the structure of vegetation, the species of grasses being, as a rule, advantageous and there is also a change in the properties of the soil and the activity of microorganisms in the soil (Kleczek C., 1991).

Through research carried out in the 2021-2022 agricultural year, at Ezăreni Farm belonging to the Iaşi University of Life Sciences, was to analyze the competitiveness of the species *Festuca arundinacea* Schreb. and *Trifolium pratense* L., grown alone or in simple mixtures, under different conditions of fertilization with complex mineral fertilizers.

## MATERIAL AND METHOD

Purpose of research was to analyze the competitiveness of the species *Festuca arundinacea* Schreb. and *Trifolium pratense* L., grown alone or in simple mixtures, under different conditions of fertilization with complex mineral fertilizers, under the climatic conditions from Moldavian Forrest Steppe.

To achieve the proposed goal, in the spring of 2021, an experience was established in the experimental field of Ezăreni Farm (47°05'-47°10 ' North latitude and 27°28'-27°33' Eastern longitude) belonging to the Iaşi University of Life Sciences, by subdivided plots method with two factors (5 × 5 type), in 3 replications, having the dimensions of a plot of 4 x 3 m (12 $m^2$), and the harvestable area of 6 $m^2$, the total area of experience being 940 $m^2$ (47 × 20 m).

The factors studied were: A - species or mixtures

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of perennial grasses and leguminous species, with 5 graduations: $a_1$ - *Festuca arundinacea* Schreb (100%); $a_2$ - *Festuca arundinacea* Schreb (75%) and *Trifolium pratense* L. (25%); $a_3$ - *Festuca arundinacea* Schreb (50%) and *Trifolium pratense* L. (50%); $a_4$ - *Festuca arundinacea* Schreb (25%) and *Trifolium pratense* L. (75%); $a_5$ - *Trifolium pratense* L. (100%) and B - fertilization with mineral fertilizers, with 5 graduations: $b_1$ - unfertilized; $b_2$ - $N_{50}P_{50}$; $b_3$ - $N_{75}P_{75}$; $b_4$ - $N_{100}P_{100}$; $b_5$ - $N_{150}P_{150}$. in the first year of vegetation, the fertilizers were applied before sowing, and from the second year of vegetation, in early spring.

The biological material studied was represented by *Festuca arundinacea* Schreb (tall fescue) - Vio Jucu variety created at the U.A.S.V.M. Cluj-Napoca in 2012 and *Trifolium pratense* L. (red clover) - David Liv variety created at the Livada Agricultural Research and Development Station in 2015.

The amount of green mass per hectare was determined by weighing the production obtained from each cut on the harvest area of 6 $m^2$ and then reported to hectare.

The dry matter content (D.M.) of each repetition was determined by drying on the oven at 105 ° C for 3 hours; standard - SR ISO 6496/2001.

A sample of 2 kg green mass was taken from the green mass sample which was gravimetrically analyzed on the two component species.

The calculation of the RYT index (Relative Yield Total), which characterizes the species used in the mixture with respect to the ecological resources used, one in relation to the other and was done with the relationship (Zuo Y. *et al*., 2010):

RYT = YAB/YAA + YBA/YBB, where:

YAB = D.M. production for A species, in mixture culture;

YAA = D.M. production for A species, in pure culture;

YBA = D.M. production for B species, in mixture culture;

YBB = D.M. production for A species, in pure culture.

Depending on the result obtained, the following three situations can be encountered:

- when RYT > 1, species occupy different eco-niches;
- when RYT = 1, species use common resources;
- when RYT < 1, species are in antagonism relatio.

The calculation of the CR index (Competition Rate), which characterizes the species used in the mixture, in terms of mutual competitiveness and has been calculated with the relationship (Zuo Y. şi colab., 2010):

CR = (YAB/YAA x ZAB)/(YBA/YBB x ZBA) , werw:

YAB = D.M. production for A species, in mixture culture;

YAA = D.M. production for A species, in pure culture;

ZAB = proportion of species A and B in mixture;

YBA = D.M. production for B species, in mixture culture;

YBB D.M. production for B species, in pure culture;

ZBA = proportion of species B and A in mixture;

Depending on the result obtained, the following three situations can be encountered:

- when CR > 1, species A is more competitive than species B;
- when CR = 1, the species are just as competitive;
- when CR < 1, species A is less competitive than species B.

The results were statistically interpreted by analyzing the variance and calculating the least significant differences.

The study area is characterized by continental temperate climatic conditions. The 2021-2022 agricultural year it was less favorable year for mixtures of perennial grasses and leguminous species, with periods of water stress, especially in May, June and July.

## RESULTS AND DISCUSSIONS

The analysis of the influence of the interaction between the mixture used and the fertilization with nitrogen and phosphorus-based mineral fertilizers, on the interspecific relations in the second year of vegetation, showed that the RYT index (*figure 1*) recorded values were > 1, except for variants fertilized with $N_{150}P_{150}$. This result showed that the *Festuca arundinacea* Schreb. and *Trifolium pratense* L. competes for the same vegetation factors. In the second year of vegetation the plants are at a high productive potential, they have the aerial part, but especially the more debated underground part. Thus, competition is manifested for vegetation factors in the case of unfertilized or fertilized variants with $N_{50}P_{50}$, $N_{75}P_{75}$ and $N_{100}P_{100}$ doses, while in variants fertilized with $N_{150}P_{150}$ the values of the RYT index were > 1.

In the second year of vegetation, the CR index for the *Festuca arundinacea* Schreb. species was higher than for the *Trifolium pratense* L. species only at a mixed participation rate of 75%, under fertilization conditions, in which case the species was more competitive. The variants in which the *Festuca arundinacea* Schreb. species recorded small and very small values of the CR index were those in which the participation percentage was 50% and 25% respectively. In this case the species is considered weakly competitive (*table 1*).

The values of the CR index for the *Trifolium pratense* L. species were lower, in the case of

variants in which it had a participation of 25%, which shows a lower competitiveness, compared to the *Festuca arundinacea* Schreb. species. The variants in which the *Trifolium pratense* L. species registered large and very high values of the CR index were those in which the participation percentage was 50% and 75% respectively. In this case the species is considered very competitive.

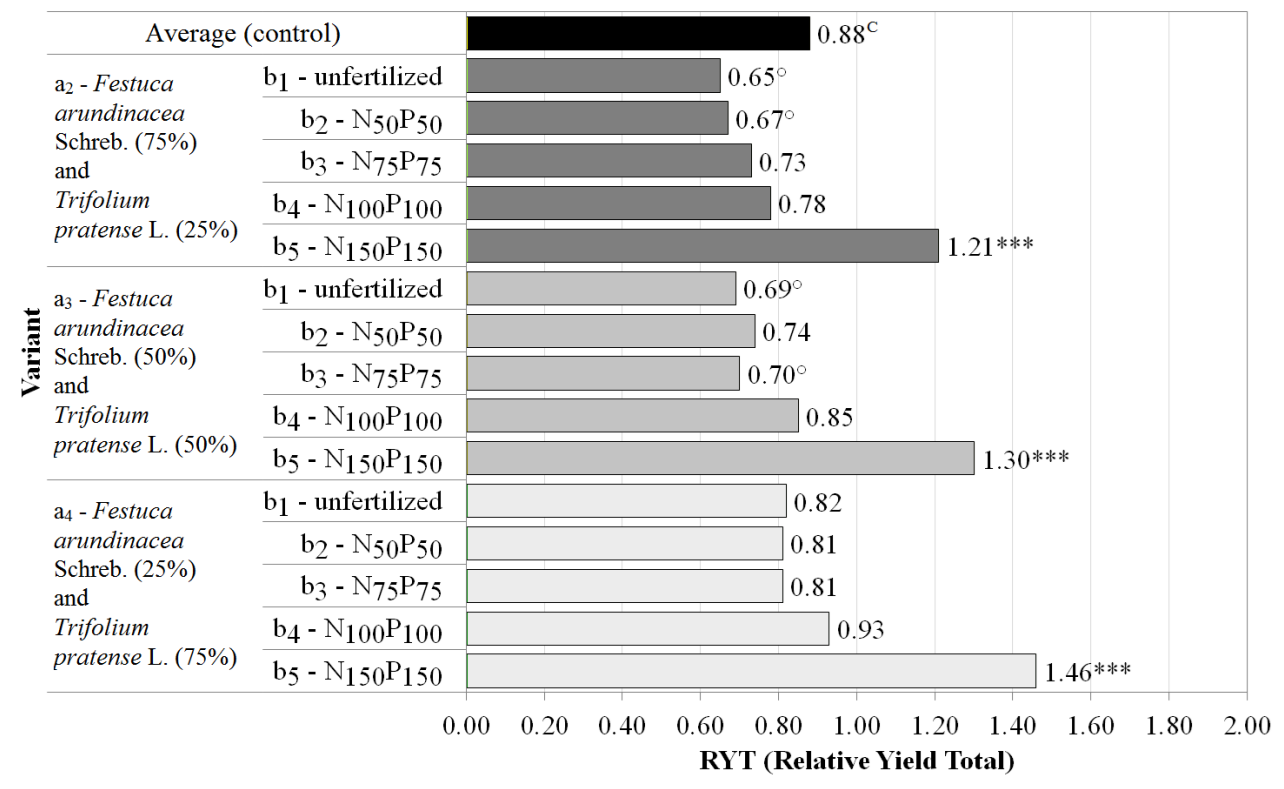


Figure 1 **The influence of the interaction between the culture system used and fertilization on the RYT index (Relative Yield Total), at the first cut, in the second year of vegetation**

Table 1

**The influence of the interaction between the culture system used and fertilization on the CR index (Competition Rate), at the first cut, in the second year of vegetation**

| Variant | | RYT | CR *F.a.** | CR *T.p.** |
|---|---|---|---|---|
| **Average (control)** | | 0.88$^{Mt}$ | 0.95$^{Mt}$ | 15.99$^{Mt}$ |
| $a_2$ - *Festuca arundinacea* Schreb. (75%) and *Trifolium pratense* L. (25%) | $b_1$ - unfertilized | 0.65º | 2.42 | 0.41$^{ooo}$ |
| | $b_2$ - $N_{50}P_{50}$ | 0.67º | 1.91 | 0.52$^{ooo}$ |
| | $b_3$ - $N_{75}P_{75}$ | 0.73 | 1.82 | 0.55$^{ooo}$ |
| | $b_4$ - $N_{100}P_{100}$ | 0.78 | 2.81** | 0.36$^{ooo}$ |
| | $b_5$ - $N_{150}P_{150}$ | 1.21*** | 3.86*** | 0.26$^{ooo}$ |
| $a_3$ - *Festuca arundinacea* Schreb. (50%) and *Trifolium pratense* L. (50%) | $b_1$ - unfertilized | 0.69º | 0.23 | 4.33$^{ooo}$ |
| | $b_2$ - $N_{50}P_{50}$ | 0.74 | 0.22 | 4.57$^{ooo}$ |
| | $b_3$ - $N_{75}P_{75}$ | 0.70º | 0.24 | 4.20$^{ooo}$ |
| | $b_4$ - $N_{100}P_{100}$ | 0.85 | 0.27 | 3.72$^{ooo}$ |
| | $b_5$ - $N_{150}P_{150}$ | 1.30*** | 0.31 | 3.21$^{ooo}$ |
| $a_4$ - *Festuca arundinacea* Schreb. (25%) and *Trifolium pratense* L. (75%) | $b_1$ - unfertilized | 0.82 | 0.04 | 26.32*** |
| | $b_2$ - $N_{50}P_{50}$ | 0.81 | 0.03 | 39.63*** |
| | $b_3$ - $N_{75}P_{75}$ | 0.81 | 0.02 | 42.98*** |
| | $b_4$ - $N_{100}P_{100}$ | 0.93 | 0.03 | 39.26*** |
| | $b_5$ - $N_{150}P_{150}$ | 1.46*** | 0.01 | 69.54*** |
| | *LSD* 0.5 | *0.17* | *1.24* | *1.40* |
| | *0.1* | *0.25* | *1.68* | *1.90* |
| | *0.01* | *0.33* | *2.28* | *2.55* |

**: F.a. = Festuca arundinacea, T.p. = Trifolium pratense*

Intraspecific competition is a consequence of the mass effect and occurs between individuals

of the same species, for space, minerals, light and water. The mass effect of intraspecific competition is regulated by homeostatic mechanisms or by human intervention, indicating that individuals can maintain a stronger species. On the one hand, interspecific competition determines which species and how many species can coexist within the same community; on the other hand, interspecific competition affects population dynamics, changes the structure of the species and the structure of the community (Rotar I., 1993).

## CONCLUSIONS

Analysis of the influence of the interaction between the mixture used and fertilization with nitrogen and phosphorus-based mineral fertilizers, on the interspecific relations in the second year of vegetation, showed that the RYT index recorded values > 1, except for variants fertilized with $N_{150}P_{150}$, showing that the *Festuca arundinacea* Schreb. and *Trifolium pratense* L. species compete for the same vegetation factors.

In the second year of vegetation, the CR index for the *Festuca arundinacea* Schreb. species was higher than for the *Trifolium pratense* L. species only at a mixed participation rate of 75%, under fertilization conditions, in which case the species was more competitive.

The variants in which the *Festuca arundinacea* Schreb. species recorded small and very small values of the CR index were those in which the participation percentage was 50% and 25%, respectively, in which case the species is considered weakly competitive. These aspects were highlighted inversely-proportional in the case of the *Trifolium pratense* L. species.

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# INVASIVE NEMATOFAUNA AFFECTING PLUM UNDER THE ENVIRONMENTAL CONDITIONS OF THE REPUBLIC OF MOLDOVA

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**Abstract**

Parasitic nematodes and virus diseases individually can cause serious losses in plum production, however in combination, they can be very destructive to crops in the long-term. The result of the conducted investigation revealed that abundance of nematodes in the Northern area of the Republic of Moldova ranged from 50 to 250 specimens per 100 g of sampled soil. In the Central area, the values varied more comparative to Northern part, ranging from 80 to 300 specimens per 100 g of soil, due to temperature variations and humidity. Altogether, 32 species of free living and plant parasitic nematodes from fruit trees crops were revealed. The most frequent proved to be species from the orders *Thylenchida* and *Dorylaimida*, the genera *Pratylenchus*, *Rotylenchus*, *Ditylenchus* and *Criconemoides*. In addition, the species *Xiphinema index*, *X. brevicole*, *X. vuitennezi* and *Longidorus elongates* were identified as vectors of viral pathogens. According to the trophic specialization, 5 groups of nematodes were detected, the most abundant being the group of plant parasitic nematodes (18 species), which seriously affect absorbing bristles, followed by specialized endo- and ecto-parasitic adaptations and vectors of viral pathogens. These investigations present a major significance for developing netological management programs in plum orchards.

**Key words**: nematodes, plum crops, biological control, abundance, diversity, trophic specialization

Plum cultivation is a centuries-old tradition, surpassed only by apple, and the production of plum fruits obtained in the Republic of Moldova constitutes over 35%, with an important share of export, approximately 30%. (Balan V., 2010; Istrati L, 2018; Juraveli A., Terentii P., Cozmic R, 2020). Current plum plantations of intensive and super-intensive type are a capital investment, which can be exploited for 20-25 years, and the efficiency of exploitation depends on the correctness of the establishment of orchards, and the appropriate implementation of plant care technologies in the first 3-4 years after planting (Chira L., Honza D., 2010; Coroid A., 2020; www. statistic.md. 2021-2022).

Another significant priority in plum cultivation is the study of the evolutionary parasitic and vectorial impact of complexes of harmful organisms, associated with invasive nematode complexes that form trophic parasitic associations, with impact aggravated by adverse factors, triggered by the perennial monoculture of fruit trees (Bădărău S., 2012; Nesterov P., 1988; Stegărescu O., 1997; Poiras L., 2012).

According to the activity program, elaborated on the basis of the research project, our work is integrated in the strategic objectives of the development of fruit growing in the Republic of Moldova. This, research is conducted annually, on the biological control of invasive and vector nematode communities and on the parasitic and functional impact and the structure of populations in young and productive plum orchards, nurseries for the production of planting material, including other related species such as peach, apricot, cherry etc. Based on the current situation and the research program mentioned, the goal of our research has been: the phytosanitary helminthological control of some associations of parasitic nematodes and vectors of pathogenic viruses from the class *Secerneatae*, order *Thylenchida* and *Dorylaimida*, in plum, cultivated in intensive systems of orchards and nurseries, planted with various modern varieties, with diverse spectrum of precocity, in the Republic of Moldova. The main objective of our research has been to study, analyze and compare the diversity of the most dangerous complex of invasive nematodes and vectors of pathogenic viruses from the genera *Pratylenchus*, *Rotylenchus*,

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*Ditylenchus* and *Criconemoides*, families *Xiphinematidae, Trihodoridae, Longidoridae,* in plum, compared by zones, sectors and age, in the context of changes in climatic factors.

## MATERIAL AND METHOD

Research was conducted in intensive plum orchards and nurseries for reproducing planting material from 6 administrative districts, on areas of over 500 hectares in the North, Center and South-East area of the Republic of Moldova. More than 300 samples of soil, productive trees, young trees and shoots attacked by viruses were collected in the field and laboratory and young saplings – from various orchard systems and nurseries. Samples were taken at a depth of 30-60 cm, in intervals of 15-20 days, covering the main phenological stages during the entire growing season. The surveys to establish the helminthic and viral parasitic impact were carried out by visual and optical observations to identify local damage, the severity of symptoms of specific viral diseases of trees. The results of the surveys are interpreted by the abundance and frequency indices of the species (individuals/100 $cm^3$ soil) and by the ratio of ecological-trophic specialization depending on the investigated plantations, environmental conditions, variety, soil, cultivation systems etc., indicated in pictures 1-6.

The analyzes of the nematofaunistic material collected were carried out in the Laboratory of Parasitology and Helminthology of the Institute of Zoology, by the *Baermann funnel* method, flotation-settling and fixation, to establish the numbers of individuals, the taxonomic identification in terms of genus and species, the trophic specialization and other biological peculiarities (Кириянова Е., 1971; Dekker M., 1972; Stegărescu O., 1972; Brown D., 1989; Siddiqi D., 2000; Baldwin J., 2004).

Figure 1-3 **Phytosanitary monitoring in productive plum orchards, Criuleni district, June, 2022**

Figure 4-6 **Plant nurseries investigated at the time of extracting and sorting the fruit tree saplings, Zaim village, Căușeni district, November, 2022**

The values of the damage indices of the degree of parasitic impact were established, such as: the values of the numerical density (*N.d.*) of the individuals, the intensity of the development of viral diseases (*I%*), estimation scales used in carrying out surveys of phytosanitary records, according to "Methodical guidelines for testing chemical and biological plant protection products against pests, diseases and weeds in the Republic of Moldova", 2022. In the laboratory, we used the available equipment to perform helminthological analyses, as well as auxiliary laboratory equipment. After the analyses on taxonomic determination, we frequently consulted the current bibliographic sources on helminthology, virology, as methodical support in the Laboratory of Parasitology and Helminthology: Деккер Х., 1972; Santos M. and col., 1997; Нестеров П., 1976-1988; Stegărescu O., 1970-1997; Aurel Maxim and col. 1994-2017; Decramer W., 2006; Poiras L. and col. 2004-2019.

## RESULTS AND DISCUSSIONS

Damage and losses of fruit production despite favorable conditions for both trees and

harmful organisms, as parasitic agents and vectors, require maximum attention and particular research in productive, young orchards and nurseries for the production of seedlings. A lot of damage is also caused by the presence of invasive nematode complexes and vectors of pathogenic viruses, which also periodically trigger helminthoses with specific and non-specific pathogenic effects on the fruit material, associated with viral diseases.

Parasitic nematodes and vectors of pathogenic phytoviruses are among the most damaging and at the same time little studied as interactive pathogens, due to the complexity and specificity of the pathological process caused by them. In the case of infestation of plants with invasive nematodes and phytoviruses, trophic relationships are established between their genomes depending on the type of host's reaction to the respective pathogen. The negative economic impact caused by these pathogens varies between 10-70%, and sometimes it can reach 100%, where practically the level of infestation and inoculation is far above the economic threshold of damage (Haririson B., 1967; CalaseanI., Rapcea M., 2004; Decramer W., 2006; Poiras L. and col., 2013).

The research carried out initially resulted in the determination of the numerical abundance, recorded in average values per areas, according to the samples collected in the plum orchards indicated in Table 1. As a result of the comparative analyses, the numerical density of nematode populations (N.d.) was determined in average values per zones, from 19 to 130 individuals per 100 g soil. It was found out that the abundance was by 15-20% higher in the sectors of the North zone, being influenced by variations in temperature, humidity and more fertile soil, than in the Center and South-East zones. In the sectors of young orchards and nurseries of fruit trees, the numerical density was estimated in lower average values (7-15%) as compared with already productive orchards, aged 8-10 years, conditions caused by the formation of denser nematode complexes, depending on the environmental factors and the trophic reserve accumulated in the fruit monoculture. As compared with the North zone, the orchards in the Center and South-East zones were characterized by lower numerical density and low-to-moderate infestation (1-2 points, according to the estimation scale), which is also partly due to the more rigorous observance of technological management and the appropriate application of effective integrated protection measures.

Table 1

**The numerical density of the parasitic nematode complexes, detected in plum orchards, in comparison, by new exploitation systems, 2021-2022**

| Zone, investigated district | Productive orchards, 8-10 years, (individuals/100cm$^3$ /soil) | Young orchards, 3-5 years, (individuals/100 cm$^3$ soil) | Nurseries 1-2 years, (individuals/100cm$^3$ soil) |
|---|---|---|---|
| **North: Briceni d.** | 35-110 | 45-105 | 36-95 |
| **Soroca d.,** | 68-130 | 54-115 | 58-100 |
| **Center: Criuleni d.,** | 45-83 | 28-75 | 27-73 |
| **Nisporeni d.,** | 33-74 | 19-58 | 23-62 |
| **South-East: Căuşeni d., Zaim v.** | 57-98 | 32-74 | 42-80 |
| **Ştefan-Vodă d.** | 28-79 | 21-67 | 31-74 |
| **Average per zones:** | 28-130 | 19-115 | 23-100 |

Based on the previous and current investigations, according to the obtained results, we estimate that the complexes of invasive and vector nematodes detected in fruit orchards, including plum, restructured in new systems belong to the order *Tylenchida* and *Dorylaimida* (Dekker M., 1972, Siddiqi D., 2000). According to the establishment of the taxonomic aspects and the trophic group, the analyzed nematode samples found in plum orchards, are reported as ectoparasitic forms and vectors of pathogenic phytoviruses (Stegărescu O., 1972; Nesterov P.,1987; Poiras L., 2012).

The results of the comparative analysis on the abundance, frequency and diversity of detected species, which form invasive and vectorial complexes of pathogenic viruses belong only to the *Dorylaimida* order, detected in various areas, orchards and nurseries, which are presented in tables 2 and 3, where 15 more species are more dangerous and practically infests all fruit tree species, including plum, according to the results obtained by us, as well as those mentioned in the bibliographic sources. The abundance and frequency of species is obviously higher, by 30-40%, in the sectors of the Center and South-East zones, the reasons being, however, the higher average temperatures and the more humid microclimate, which are advantageous factors for

the proliferation and formation of specialized complexes.

The analyses of nematode species, as vectors of viruses in fruit crops, as well as different forms of relationships between viruses and nematodes, indicated their interspecificity, which determines the invasions that annually cause various forms of mixed parasitism (helminthic-viral), even from the first half of the growing season, favored also by the influence of environmental factors. The detected species infest and parasitize more actively on young trees recently planted in nurseries and orchards, they are polyphagous-oligophagous, thermo-hydrophilic pests of agro-economic importance, according to references noted and published a lot in the last decades by various specialists in nematology and virology, as well as in our own current research.

Table 2

**Analysis of the comparative spatial frequency and structure of the complexes of parasitic nematodes and vectors of pathogenic viruses from the order *Dorylaimida* spp., detected in various fruit-growing areas, restructured productive orchards and nurseries**

| Nematode species | North zone | | Center zone | | South-East zone | |
|---|---|---|---|---|---|---|
| | Productive orchards | Nurseries | Productive orchards | Nurseries | Productive orchards | Nurseries |
| **I.Gen. *Xiphinema,* Cobb, 1913,** ***1.X. index,* Thorne et al., 1950** | ++ | + | ++ | + | ++ | + |
| ***2.X. brevicolle,* Lordello et da Costra, 1961** | ++ | + | + | + | + | + |
| ***3.X. rivesi, Dalmasso, 1969*** | + | - | + | - | + | - |
| ***4.X. vuittenezi,* Luc et Lima,1964** | + | - | ++ | + | ++ | + |
| ***5. X. pachtaicum,*Tulganov,1938** | ++ | + | + | + | - | - |
| ***6.X. diversicaudatum,*Thor. 1939** | + | + | ++ | ++ | + | + |
| ***7.X. americanum,* Cobb,1913** | - | - | + | + | + | + |
| ***8.X. italiae,* Meyl, 1953** | - | - | + | + | + | + |
| ***9.X.turcicum,* Dalmasso, 1963** | - | - | + | - | + | + |
| **II.Gen.*Longidorus, Micole.1922*** ***10.L.elongatus,* de Man, 1876** | - | - | - | - | + | + |
| ***11.L. macrosoma,* Hooper, 1961, Andrassy, 1956** | + | - | + | + | + | - |
| ***12.L.eunymus,* Hooper, 1974** | - | - | - | - | + | + |
| **III.G. *Trichodorus,* Cobb, 1931,** ***13.T. primitivus,* de Man, 1880** | - | - | + | - | + | + |
| ***14.T. simili,* Seinhorst, 1963** | - | - | + | + | + | + |
| ***15.T.cylindricus,* Hoof, 1962** | - | - | + | - | + | + |
| **Total species – 15 species** | 7 | 4 | 13 | 10 | 14 | 12 |

Legend: - no individuals **(0 points) ;** + from 50 to 100 individuals **(1 point);** ++ from 100 to 150 individuals **(2 points);** +++ from 150 to 200 individuals **(3 points);** ++++ over 200 individuals **(4 points).**

We would like to mention that the species of nematodes estimated in table 2, belong to the genera: *Xiphinema spp.*, *Longidorus spp.*, *Trichodorus spp.*, they are ectoparasites and specialized vectors of various forms of pathogenic viruses, which participate in triggering specific viral diseases in fruit tree plantations. They maintain particles of infectious viruses in their body from 8 weeks to 2 years (for example, tomato ring spot virus, detected on the nematode *Xiphinema riversi* (Bitterlin și Gonsales, 1986; Cerneț A., Poiras L. *et al* 2006-2016). Pathogenic viruses also detected in the Republic of Moldova in fruit-bearing species, transmissible through 15 species of nematodes according to the classification of Cadman (1986), are divided into 9 large groups, but only 2 groups are significant for fruit-growing in our area, according to table 3, which are involved in the transmission of the specific virus, equally contagious, disastrous in the process of pathogenesis and absolutely incurable. The specific symptoms also reported in plum, during the growing season, are: deformations, mosaic appearance, chlorosis, ring spots, deformations, dwarfing and proliferations. *Table 3* estimates the main species of viruses detected in the Republic of Moldova and transmitted by some species of nematode vectors to fruit trees, also detected on plum trees, associated with arthropod complexes. They are responsible for triggering severe viral diseases, as confirmed by the authors who detected the vector aspects of the nematode species and the pathogenic virus found in fruit trees in the Republic of Moldova (Verderevckaia *et al.* 1986; Aurel M. *et al*. 2002-2007; Cerneț A. *et al.*, 1995-2020; Poiras L. *et al*. 2006-2016).

The research results obtained from the phytosanitary surveys in intensive plum orchards

and nurseries revealed the degree of helminthic disease and the presence of viruses in the trees, especially on the roots, shoots and young leaves of plum, in average values of 15-30 % frequency (*F.%)* and 10-20% intensity (*I%*), detected practically in all the investigated agrocenoses.

Table 3

**Nematode vectors of pathogenic viruses invasive to fruit trees, including plums, detected in the Republic of Moldova.**

| Virus | Vector – Nematodes | Authors of vector nematodes and phytoviruses |
|---|---|---|
| *1. Cherry leaf roll*<br>*2. Prunus necrotic rihg spot*<br>*3. Prunus dwarf virus*<br>*4. Arabis mozaic*<br>*5. Raspberry ring spot*<br>*6.Tomato ring spot*<br>*7.Tomato black ring*<br>*8. Cherry mottle leaf*<br>*9. Cherry necrotic rusty mottle,*<br>*10. Cherry virus A*<br>*11.Cherry leaf roll virus*<br>*12.Prunus latent virus*<br>*13. Apple cholorotic*<br>*14.Perach mosaic leaf spot*<br>*15.Grapevine fanleaf virus*<br>*16.Strawberry latent ringspot*<br>*17.Peach rosette mosaic virus*<br>*18.Mulberry ringspot virus*<br>19.*Carnation ringspot virus*<br>20.*Cherry rosette disease virus,*<br>**(Nepovirus**). | ***1. Xiphinema index***<br>***2. X. riversi***<br>***3. X. brevicole***<br>***4. X. diversicaudatum***<br>***5. X.vuittenezei***<br>***6. X. italia***<br>***7. X. pachtaicum***<br>***8. X. americanum***<br>***9. X. turcicum***<br>***10. Longidorus macrosoma***<br>***11. L. elongatus***<br>***12. L. eunymus*** | Fritzsche 1972<br>Fritzsche & Kegler, 1964<br>Flegg, 1969<br>Fritzsche & Kegler, 1968<br>Bloom et al., 1972<br>Cameron et al.,1977<br>Harrison et al., 1971<br>Harrison & Cadman,1959<br>Thorne & Allen,1950<br>Dalmasso, 1963-1070<br>Stegărescu, 1966-1979;<br>Fritzeche & Sehmelzer, 1967<br>Sol & Seinhorst, 1961<br>Harrison & colab.,1955<br>Verderevskaia et al.,1970<br>Nesterov et al.,1976<br>Brown et al., 1995<br>Taylor & Braun, 1997<br>Maxim A. et al.,1994-2010 Isac et al., 1998<br>Poiras et al., 2007-2016 |
| *21. Tobacco rattle virus-TVR*<br>*22. Pea early-browning- PEBV*<br>*23.Pepper ring spot-virus-PRSV,* **(Tobravirus**). | ***13.Trihodorus primitivus***<br>***14.T. similis***<br>***15.T. cylindricus*** | Cobb. 1931, Thorne, 1931<br>Kramer & Coistra, 1964<br>Cadman, 1963, Hoof, H. A. Van 1963, Seinhorst, 1963<br>Harrison et al.,1974 |

Some ectoparasite-vector species of nepoviruses were detected more frequently, such as: *Xiphinema index, X. diversicaudatum, X. brevicole, X. vuitennezi, X. riversi, Longidorus elongatus* and specific viral diseases were identified in plum trees: *Prunus necrotic ring spot, Prunus dwarf virus, Arabis mozaic, Cherry leaf roll virus, Prunus latent virus, Prunus rosette disease virus,* listed in table 3. These nemato-virological investigations have theoretical-applicative significance and act as long-term bio-indicators in the evaluation of the phytosanitary status in fruit orchards, including plum, in the prevention and adjustment of some methods of controlling the nemato-parasitic and patho-viral impact.

## CONCLUSIONS

As a result of the research carried out in the new type of intensive plum orchards, in production associations in the North, Center, South-East zones of the Republic of Moldova, on areas of more than 300 hectares, over 250 soil samples and plant organs affected by helminthes and viruses were collected and analyzed, determining the parasitic and viral impact, the numerical density values and the severity of damage, abundance, frequency and diversity of nematodes and viruses.

The abundance of the populations was from 20 to 130 individuals/100 g/soil and young roots in orchards and nurseries, analyzed comparatively in the investigated sectors and zones, where the abundance was by 15-20% higher in the sectors of the North zone than in the Center and South-East zones. However, in young orchards and nurseries, the numerical density was estimated in relatively lower average values (7-15%) as compared with productive orchards, but the frequency of species was by 30- 40% higher in the sectors of the Center and South-East zones than in the North.

There was a significant diversity, of 15 species, of invasive nematodes and vectors of pathogenic viruses of the order *Dorylaimida*, genera *Xiphinema* spp., and *Longidorus* spp., species*: Xiphinema index X. vuitennezi, X. riversi, X. brevicolle, X. diversicaudatum Longidorus elongatus,* with poly-oligophagous ectoparasitic trophic specialization, responsible for the transmission of 20 species of pathogenic viruses, causing symptomatic diseases in plum, detected practically in all investigated zones and sectors.

The results of the phytosanitary surveys conducted to establish the helminthological and viral impact of parasites, revealed nematological diseases on young roots in values of 5-20% and 7 specific viruses detected especially on young saplings, leaves and shoots of plum in values of 15- 30% frequency (F.%) and 10-20% intensity (I%). Viral diseases detected more frequently in current plum orchards are caused by the following specific forms of viruses: *Plum pox virus, Prunus necrotic ring spot, Prunus dwarf virus, Arabis mozaic virus, Prunus latent virus, Prunus rosette disease virus*.

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# CONTRIBUTIONS REGARDING SOME SOIL YEAST STRAINS FROM VINEYARD IAȘI, ROMANIA

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**Abstract**

The aim of this research was to isolating and selection soil yeast strains from the indigenous flora of Iasi vineyard. In the research activity, were chosen four grape varieties Fetească Neagră, Fetească Albă, Busuioacă de Bohotin and Fetească Neagră from the ampelographic collection of "Vasile Adamachi" Farm, from viticultural center Copou. The experimental protocol provided for the sampling of soil samples in two different phenophases of plant development: the end of flowering and ripeness stage. The soil samples were taken at 3 depths: 5-10 cm, 10-15 cm, 15-20 cm. Isolation of yeast strains and obtaining pure cultures was accomplished by successive replicates using the GPCA solid medium seeding technique. Thus, 18 yeast strains were selected from the soil and analyzed morphologically regarding the shape, profile, color and surface of the colony.

Key words: isolation, soil yeast strains, vineyard Iași

The soil microbiota exhibits fluctuations directly proportional to climate changes and the dynamics of vine phenophases.

The analysis of the phenological stages of grapevine is also of particular importance in viticulture technology as it provides information on the application of treatments to combat diseases and pests, the application of the fertilization and also the optimal execution of the work required in the grapevine.

Yeasts are defined as unicellular fungi belonging to the suborders Ascomycotina and Deuteromycotina that reproduce mainly vegetatively by budding or fusion (Kurtzman C.P. and Fell J.W., 1998). Currently there are approximately 1500 yeast species identified and their size can vary depending on the species.

From an oenological point of view, non-sporogenic yeasts such as Kloeckera, Candida, Torulospora, Kluyveromyces start and lead the process of alcoholic fermentation until close to the alcoholic threshold of 5-7% vol. alc., at which point Saccharomyces species become dominant, thus taking over directing the fermentation. (Fleet G.H., Heard G.M., 1993; Romancino D. *et al*, 2008; Sabate J. *et al*, 1998; Demuyter C. *et al*, 2004).

Oenological indigenous yeast strains with particular characteristics are representative for a certain vineyard and their presence can increase the wines typicality (Barrera Cardenas S.M., 2011).

## MATERIAL AND METHOD

The aim of this research was to isolating and selection soil yeast strains from the indigenous flora correlated with the pedoclimatic conditions of the vineyard Iasi.

Were chosen four grape varieties Fetească Neagră, Fetească Albă, Busuioacă de Bohotin and Fetească Neagră from the ampelographic collection of "Vasile Adamachi" Farm, from viticultural center Copou.

The sampling process was carried out in the summer of 2021 and 2022 from vineyard Copou, in two different phenophases: the end of flowering and ripeness stage.

For each sampling depth for each variety, 3 partial samples of the same weight (approximately 100g) were made. The sampling points of the partial samples were made by sampling by the zig-zag method. The partial samples were mixed to obtain the average sample. The soil sampling scheme excluded marginal areas of plots/rows.

In order to inoculate, was used the method of successive dilutions. Seedings were performed using dilutions diluțiile10-3, 10-4, 10-5 and 10-6 in various replicates. It was used as a solid culture medium: Glucose peptone chloramphenicol agar (GPCA) containing: 2% yeast extract, 5% peptone, 1% potassium phosphate, 0,05% chloramphenicol, 20% dextrose, 15% agar.

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The Petri plates were incubated at 28°C for 4 days and after this period the number of colony-forming units (CFU) was calculated by the quantification method. The method involves the actual counting of the colonies developed on the Petri plates counted and morphologically examined

## RESULTS AND DISCUSSIONS

In *figure 1* is presented the yeast load in the soil at the end of flowering and at full maturity in the two years of the study.

Comparing the samples from the two phenophases of sampling, a constant increase in the number of colonies in the superficial layer of the soil (5-10 cm deep) can be observed up to a maximum value reached at the ripeness stage of the grapes. This trend was evident both in the first year of study and in the second year.

Regard to the yeast load of the lower layers of the soil, a constancy of the number of CFU between the end of flowering and the full maturity of the grapes was highlighted.

In the phenophase of ripeness in the superficial layers of the soil, the maximum UFC threshold was reached by the Fetească Neagră Colecția variety, with an average of approx. 16.5 x 106 UFC/g soil, closely followed by the Fetească Neagră variety with an average of 15 x 106 UFC/g soil. The Fetească Albă and Busuioacă de Bohotin varieties at ripeness grapes stage recorded an average of 14.5 x 106 CFU/g soil in the superficial layer.

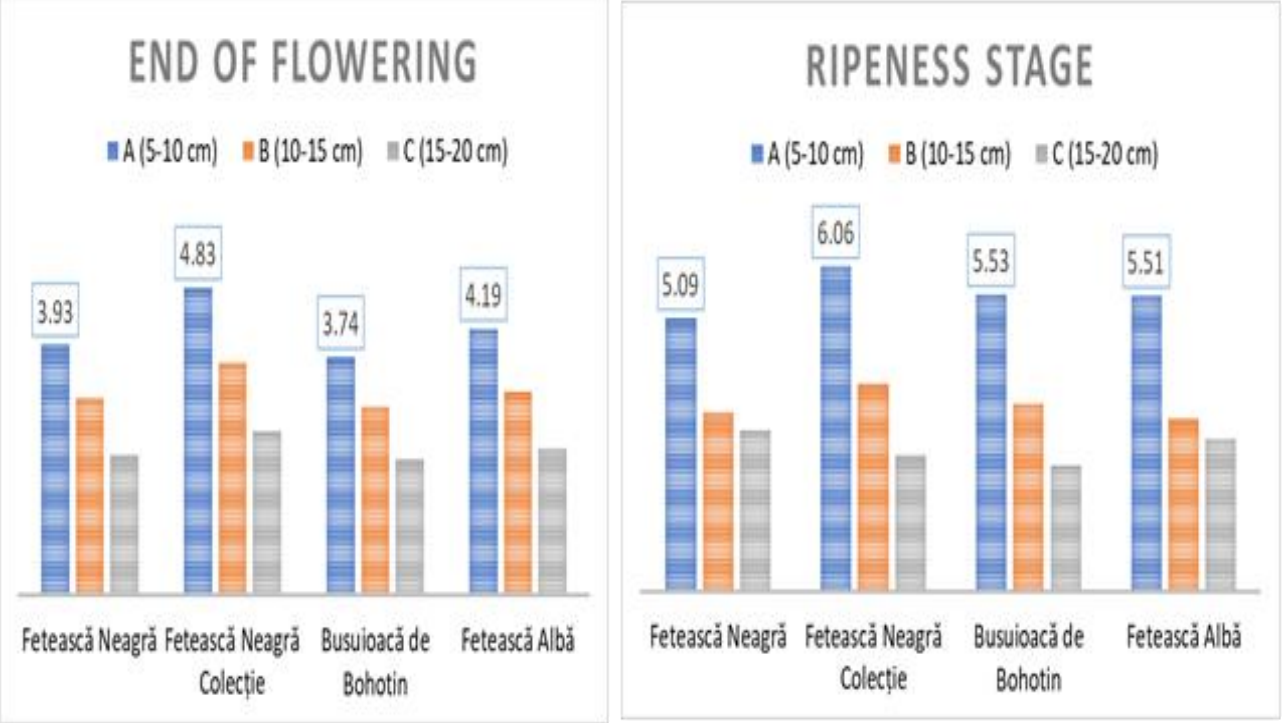


Figure 1 **Microbial load of the studied colony**

Therefore, 15 yeast strains colonies were morphological analyzed, establishing the specific characteristics (shape, profile, surface and color). The colonies of the isolated strains showed convex and flat profile, shiny, opaque surface and in some cases mucoid appearance, colors ranging from white to gray and red (*tabel 1*).

Table 1

**Colony characteristics**

| Yeast strain code | Isolation source | Colony form | Colony profile | Colony surface | Colony color |
|---|---|---|---|---|---|
| **A5** | Fetească Albă | circular | convex | opaque | white |
| **B6** | Busuioacă de Bohotin | circular | convex | opaque | white |
| **N7** | Fetească Neagră | circular | convex | opaque-mucoid | gray-white |
| **C8** | Fetească Neagră Colecție | circular | convex | opaque-mucoid | white |
| **B9** | Busuioacă de Bohotin | circular | convex | shiny | white |
| **B10** | Busuioacă de Bohotin | circular | convex | opaque-mucoid | white |
| **B11** | Busuioacă de Bohotin | circular | convex | opaque-mucoid | white |
| **B12** | Busuioacă de Bohotin | circular | convex | opaque | gray-white |
| **C14** | Fetească Neagră Colecție | circular | convex | opaque | white |
| **C15** | Fetească Neagră Colecție | circular | convex | opaque-mucoid | yellowish |
| **C20** | Fetească Neagră Colecție | circular | convex | shiny | gray-white |

| | | | | | |
|---|---|---|---|---|---|
| **N21** | Fetească Neagră | circular | convex | opaque | white |
| **A22** | Fetească Albă | circular | convex | opaque | white |
| **A23** | Fetească Albă | circular | convex | opaque | white |
| **A24** | Fetească Albă | circular | convex | opaque | white |
| **C29** | Fetească Neagră Colecție | circular | convex | shiny | red |
| **A32** | Fetească Albă | circular | flat | opaque | white-light pink |
| **B34** | Busuioacă de Bohotin | circular | convex | shiny | white |

Thus 4 yeast strains presented shiny surface, 9 yeast strains opaque surface and 5 yeast strains opaque-mucoid surface.

Analyzing the yeast strains according to the sampling depth, the yeasts taken from the depth of 5-10 cm showed white-gray coloration, variable surface from shiny to opaque and convex profile.

In the case of the strains taken from the lower depths of the soil, respectively 10-15 cm and 15-20 cm, their morphological characteristics showed circular shape, white color and opaque-mucoid surface.

According to the presented results, it can be concluded that the yeast microbiota in the superficial layers of the soil shows high diversity compared to the lower layers.

## CONCLUSIONS

Various strains of yeast were isolated from the soil of the Iași vineyard and analyzed quantitatively and morphologically. Following successive replications and obtaining pure cultures, 18 strains were isolated that presented complex morphological characteristics.

In the vertical division of the yeasts in the soil, it is found that the number of microorganisms shows a downward slope proportional to the sampling depth, thus it can be deduced that the constant decrease in the activity of the microbiota in the lower layers is characterized by the lack of oxygen, the decrease in the amount of nutrients.

Based on morphological characteristics, the analyzed yeast strains showed a complex diversity in terms of colony color, structure and surface.

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# EFFECTS OF FOLIAR FERTILIZATION ON ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF TWO VARIETIES OF FENNEL

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**Abstract**

Volatile oils extracted from various species of aromatic and medicinal plants establish a certain category of raw materials of high interest both in food industry and in cosmetics or perfumery. The chemical compounds from essential oils composition are much more appreciated, compared to synthetic substances, whereas natural extractions are associated with a much lower number of risk factors for population health. This research is topical at international level, because there is a lack of scientific data that would highlight as clearly as possible the influence of some factors on the quality of the essential oil obtained from aromatic and medicinal plants. The aim of this research was to determine the influence of foliar fertilizers on essential oil composition and antioxidant activities of two varieties of *Foeniculum vulgare* Mill., var. *vulgare* and var. *dulce*. The field research was established in USV Iasi Research Station – Ezăreni Farm, on May 2021, using a randomized block design with three replications. The data obtained in this research showed a significant influence of foliar treatments on the major components of the essential oil of bitter fennel and sweet fennel seeds. The main components of the oil extracted from fennel seeds were: anethole, fenchone, estragole, anisaldehyde and α-pinene. Also, by using complex foliar treatments (macronutrients, micronutrients and various amino acids) the specific synthesis processes of secondary metabolites (phenolic components, flavonoids) can be positively influenced.

**Key words**: chemical composition, sweet fennel, bitter fennel, foliar fertilization, antioxidant activity

*Foeniculum vulgare* Mill. is an aromatic and medicinal plant species, being part of *Apiaceae (Umbelliferae)* botanical family. Since ancient times, this plant has been used as medicine, and can be used as a remedy for a wide range of medical conditions.

Volatile oils extracted from various species of aromatic and medicinal plants form a certain category of raw materials of high interest both in food industry and in cosmetics. The chemical compounds from essential oils composition are much more appreciated, compared to synthetic substances, whereas natural extractions are associated with a much lower number of risk factors for population health.

## MATERIALS AND METHOD

**Plant material**

Two fennel varieties, var. *vulgare* and var. *dulce*, used in this experiment were obtained from the University of Life Sciences „Ion Ionescu de la Brad", Iași and from National Agricultural Research and Development Institute Fundulea.

**Field experiment**

The field research was located in USV Iasi Research Station – Ezăreni Farm. A randomized block design with three replications was used in the experiment. The field experiment was established in May 2021 using 2 months old fennel seedlings. The harvesting was on October 2021, when plants reached ripe stage – the primary umbels were fully matured and the seeds were brownish. The row spacing was 50 cm, and 30 cm between plants on the row.

**Foliar fertilization**

The research included the usage of three foliar fertilizers, with different chemical composition: AA+M+µ (NPK, micronutrients and amino acids), M+µ (NPK and micronutrients), AA (Organic foliar fertilizer with animal hydrolyzed proteins - 20 amino acids). The treatments were applied one time, at the same development stage - fulfillment of inflorescence rachis.

**Essential oil extraction**

After harvesting, the fennel seeds were separated from the umbels and then were shade-exsiccated for 14 days. Dried seeds (500 g/sample) were distilled for 3 hours using the hydro distillation method in a Clevenger type apparatus, according to European Pharmacopeia (European Pharmacopeia, 2005). The collected volatile oil was then dried using anhydrous sodium sulfate and kept at 4°C, prior to chromatographic analysis.

**Volatile oil GC-MS analysis**

Chemical composition of essential oils was analyzed using gas chromatographic – mass spectrometry method. The GC-MS analysis was carried out using GC-MS Triple Quad (Agilent) apparatus, with helium 5.0 as carrier gas. The oils were diluted in methanol

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of analytical purity, and then 1 µl of each sample were injected into the system. The column temperature was first programmed at 60°C for 2 minutes, then gradually raised to 240°C at 10°C/min and kept for 10 minutes. The essential oil chemical components were identified by comparison with those saved in NIST MS-08 database.

**Seed extracts preparation**

Prior the antioxidant analysis, determination of polyphenols and flavonoids, the seeds were ground and then the extracts (5 g/ sample) were prepared by incubation with 95 mL ethanol (80%). After 24 hours, the seeds extracts were filtered. The analyzes were carried out in triplicate using a UV/Vis spectrophotometer DLAB PS-V1100.

**Antioxidant activity**

In order to determine the antioxidant activity, 0,1 mL of seed extract was incubated with 2.9 mL of alcoholic solution od DPPH (60 µM) for 180 minutes. The absorbance of the samples (optical density OD) was read at 515 nm.

The DPPH radical scavenging activity was expressed as ratio (% inhibition) to the maximum absorbance of the DPPH solution.

**Total phenolic content (TPC)**

The quantification of total polyphenols was performed using Folin-Ciocalteu method (Lobiuc A., *et al*, 2017). The absorbance was read at 760 nm using the spectrophotometer, with the control sample consisting of the same reagents and the seed extract being replaced by an equal volume of solvent. The results were expressed as mg gallic acid (GAE) $g^{-1}$ sample.

**Total flavonoid content (TFC)**

Total flavonoid content analysis was performed using aluminium chloride method (Lobiuc A., *et al*., 2017). The seed extract was mixed with $NaNO_2$ (5%) and $AlCl_3$ (10%). Subsequently incubation, the absorbance was read at 510 nm. The results were reported as quercetin (QE) $g^{-1}$ seed sample.

**Statistical analysis**

All results were referred as mean of three replications. Data were analyzed by analysis of variance (ANOVA) and in order to assess the difference between treatment means, the Tukey test was performed. Statistical analysis was achieved using IBM SPSS 28.0.

## RESULTS AND DISCUSSIONS

### Climate conditions

In 2021, the highest temperature value was registered in July, 22.2℃, and in February was reported the lowest value, -0.8℃. The values were related to the multiannual average, with slight differences.

The year 2021 was defined by a higher rainfall regime (with an annual sum of 691.8 mm) compared with the multiannual average sum for Iași region (517.8 mm). The climatic conditions for year 2021 are shown in *table 1*.

Table 1

**Climatic conditions for 2021 in Iași Region**

| Month | Average Precipitations (mm) | Average Air Temperature (℃) |
|---|---|---|
| **January** | 26.8 | -0.7 |
| **February** | 22.8 | -0.8 |
| **March** | 65.5 | 3.1 |
| **April** | 56.4 | 7.8 |
| **May** | 86 | 14.9 |
| **June** | 107.4 | 19.8 |
| **July** | 80.3 | 22.2 |
| **August** | 155.6 | 20.9 |
| **September** | 12.4 | 14.4 |

### Influence of foliar fertilizers on the chemical composition of fennel essential oil

The chemical composition of essential oils extracted from the two fennel varieties (var. *dulce* and var. *vulgare*) are presented on *table 2*. The chemical elements are indexed according to their elution time from chromatographic column.

Following the GC-MS analysis, 15 chemical components were identified in the volatile oil extracted from sweet fennel and bitter fennel fruits.

The main components of the oil extracted from *Foeniculum vulgare* var. *vulgare* were: anethole (70.49% - 76.21%), fencone (12.03% - 14.25%), estragole (3.45% - 4.19%), anisaldehyde (1.05% - 5.51%) and α-pinene (2.08% - 3%).

The major components of the volatile oil of *Foeniculum vulgare* var. *dulce* were: anethole (64.89% - 74.65%), fencone (12.43% - 15.34%), estragole (5.69% - 7.79%) and α-pinene (2.14% - 5.61%).

The data obtained in the research showed a significant influence of foliar treatments on the major components of the essential oil of bitter fennel and sweet fennel seeds.

**Influence of foliar treatments on antioxidant activity**

Alcoholic extracts from fennel seeds contain high amounts of phenolic components, most of them also having the ability to reduce free radicals. The main such compounds are caffeoylquinic acid, rosmarinic acid, 1,5 dicaffeoylquinic acid,

hyperoside. Hydroxycinnamic acid derivatives and glycosides have also been identified. (Parejo I.V. *et al*, 2004)

From the results presented on *figure 1*, it is positive that foliar fertilization has a significant influence on the free radical scavenging activity of fennel seeds extracts. *Foeniculum vulgare* var. *vulgare* established a lower antioxidant activity, compared to *Foeniculum vulgare* var. *dulce* where the data recorded higher values (over 50%).

A clear influence of foliar treatments can also be observed, in the case of sweet fennel, all values being improved by using different nutrients. Statistically significant differences were obtained with fertilizers AA+M+µ (22%) and M+µ (21%).

In bitter fennel plants, the usage of foliar fertilizers based on AA+M+µ and M+µ, contributed to an increase in antioxidant activity, while the value obtained in case of the treatment with amino acids presented a lower value and similar to blank value (35.62 % inhibition against DPPH solution).

Table 2

**Main fennel essential oil compounds (%)**

Means with the same lower-case letter are not significantly different at P < 0.05 according to Tukey's test

| Fennel variety | var. *vulgare* | | | | *var. dulce* | | | |
|---|---|---|---|---|---|---|---|---|
| **Compound** | **blank** | **AA** | **AA+M+µ** | **M+µ** | **blank** | **AA** | **AA+M+µ** | M+µ |
| **1R-α-pinene** | 2.71$^{a}$ | 2.08$^{a}$ | 2.62$^{a}$ | 3.00$^{a}$ | 3.93$^{b}$ | 5.61$^{c}$ | 2.14$^{a}$ | 4.31$^{b}$ |
| **camphene** | 0.12$^{ab}$ | 0.05$^{a}$ | 0.07$^{a}$ | 0.07$^{a}$ | 0.07$^{a}$ | 0.11$^{ab}$ | 0.05$^{a}$ | 0.10$^{ab}$ |
| **β-pinene** | 0.22$^{a}$ | 0.2$^{a}$ | 0.25$^{a}$ | 0.28$^{a}$ | 0.33$^{a}$ | 0.46$^{ab}$ | 0.23$^{a}$ | 0.36$^{a}$ |
| **β-terpinene** | 0.09$^{a}$ | 0.07$^{a}$ | 0.06$^{a}$ | 0.09$^{a}$ | 0.09$^{a}$ | 0.13$^{a}$ | 0.09$^{a}$ | 0.12$^{a}$ |
| **α-phellandrene** | 0.05$^{a}$ | 0.30$^{b}$ | 0.71$^{c}$ | 0.18$^{b}$ | 1.74$^{a}$ | 2.23$^{b}$ | 1.40$^{a}$ | 1.74$^{a}$ |
| **D-limonene** | 1.12$^{b}$ | 0.61$^{a}$ | 0.77$^{a}$ | 0.74$^{a}$ | 0.86$^{ab}$ | 1.10$^{b}$ | 0.68$^{a}$ | 1.10$^{b}$ |
| **β-phellandrene** | 0.43$^{a}$ | 0.37$^{a}$ | 0.31$^{a}$ | 0.35$^{a}$ | 0.50$^{a}$ | 0.64$^{a}$ | 0.53$^{a}$ | 0.66$^{a}$ |
| **γ-terpinene** | 0.62$^{b}$ | 0.15$^{a}$ | 0.25$^{a}$ | 0.16$^{a}$ | 0.58$^{a}$ | 0.70$^{ab}$ | 0.45$^{a}$ | 0.66$^{ab}$ |
| **β- cymene** | 0.27$^{a}$ | 0.69$^{ab}$ | 0.58$^{ab}$ | 0.95$^{b}$ | 0.79$^{a}$ | 0.81$^{a}$ | 0.64$^{a}$ | 0.78$^{a}$ |
| **L-fenchone** | 14.25$^{b}$ | 12.03$^{a}$ | 12.92$^{a}$ | 13.68$^{ab}$ | 12.43$^{a}$ | 15.02$^{b}$ | 12.82$^{a}$ | 15.34$^{b}$ |
| **alcanfor** | 0.29$^{a}$ | 0.25$^{a}$ | 0.26$^{a}$ | 0.29$^{a}$ | 0.24$^{a}$ | 0.29$^{a}$ | 0.26$^{a}$ | 0.31$^{a}$ |
| **4-terpineol** | 0.07$^{a}$ | 0.07$^{a}$ | 0.06$^{a}$ | 0.06$^{a}$ | 0.04$^{a}$ | 0.08$^{a}$ | 0.07$^{a}$ | 0.09$^{a}$ |
| **estragole** | 3.65$^{a}$ | 4.19$^{ab}$ | 3.56$^{a}$ | 4.14$^{ab}$ | 6.03$^{ab}$ | 7.79$^{b}$ | 5.69$^{a}$ | 7.38$^{b}$ |
| **anethole** | 75.46$^{b}$ | 74.80$^{b}$ | 76.21$^{b}$ | 70.49$^{a}$ | 72.14$^{b}$ | 64.89$^{a}$ | 74.65$^{b}$ | 66.81$^{a}$ |
| **p-anisaldehyde** | 1.34$^{a}$ | 4.15$^{b}$ | 1.38$^{a}$ | 5.51$^{b}$ | 0.22$^{a}$ | 0.15$^{a}$ | 0.29$^{a}$ | 0.23$^{a}$ |
| **Total identified** | **99.90** | **99.81** | **99.91** | **99.99** | **99.99** | **99.98** | **99.99** | **99.99** |

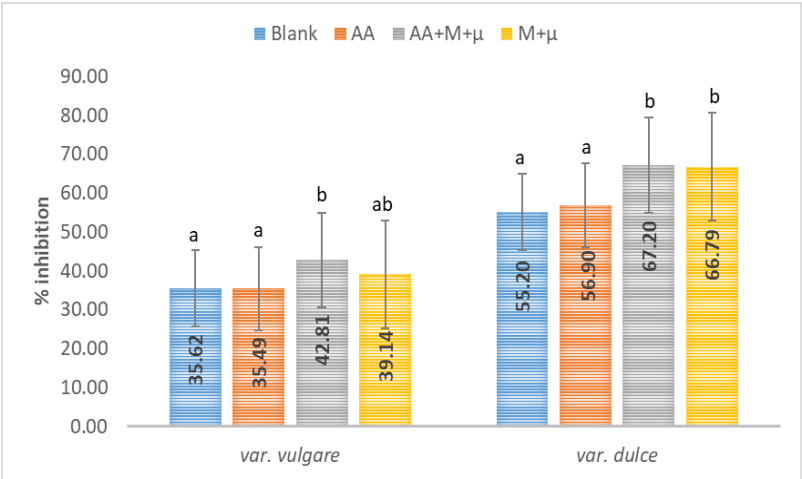


Figure 1 **Antioxidant activity of ethanolic extracts from fennel seeds**

Means with the same lower-case letter are not significantly different at P < 0.05 according to Tukey's test

**Influence of foliar fertilizers on total phenolic content (TPC)**

Phenolic compounds from plant substrates are secondary metabolites that have different roles, such as protection against the actions of biological factors, structural and signaling roles between organism cells. The synthesis processes of these chemical substances can be modified under the action of the compounds present in the structure of the fertilizers (Veberic R., 2016).

Fertilization has a stimulating effect on the content of polyphenolic compounds in case of *Foeniculum vulgare* Mill. This improvement of components with antioxidant actions may be due to the influence of fertilizers in achieving the biosynthesis of flavonoids and polyphenols through the acetate and shikimic acid pathway. (Sousa C. *et al*, 2008)

The synthesis of phenolic components in both fennel varieties was significantly influenced by using foliar treatments (*figure 2*). The data in case of var. *vulgare* were increased with all three fertilizers, the results showed statistically significant differences for treatment with AA+M+µ (10.71 mg gallic acid/g extract) with an increase of 27.5%, compared to blank, where phenolic content was 8.40 mg GAE. Also, at var. *dulce*, all treatments induced an important improvement in the content of phenolic components of the ethanolic extracts, the differences compared to the control being statistically significant, P <0.05.

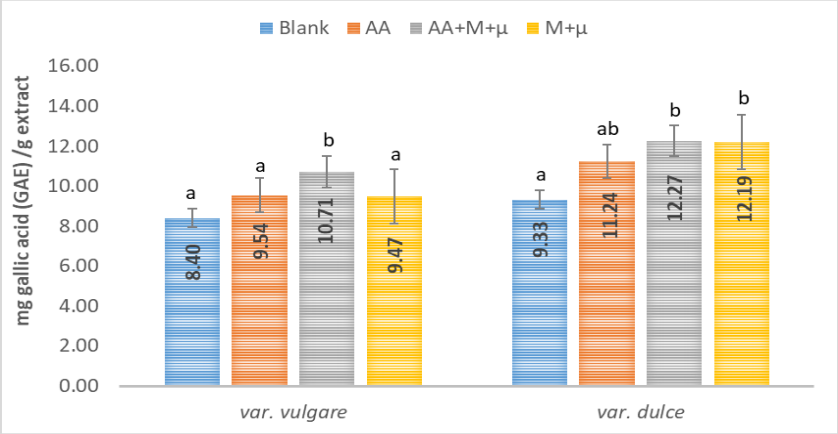


Figure 2 **Total phenolic content of ethanolic extracts from fennel seeds**
Means with the same lower-case letter are not significantly different at P < 0.05 according to Tukey's test

**Influence of foliar treatments on total flavonoid content (TFC)**

From data showed in *figure 3*, the total flavonoid content of the ethanolic extracts of var. *vulgare* was lower (14.76 – 18.62 mg quercetin/g extract) in comparison with var. *dulce*, where the flavonoid compounds registered values between 19.41 mg QE and 23.68 mg quercetin/g extract.

The application of foliar fertilizers significantly influenced the synthesis of flavonoids in bitter fennel plants, the differences being statistically significant (P<0.05) in case of the treatments with amino acids (AA) and complex fertilizer (amino acids, macroelements and microelements - AA+M+µ).

For sweet fennel plants, the most important effect was identified with AA+M+µ fertilization, with a positive difference of 21%, compared to the reference plants. Higher values were also achieved when fertilizing with macronutrients and micronutrients, with a total flavonoid content of 21.53 mg quercetin/g extract.

By using some additions such as amino acids and other stimulants, the ability to synthesize beneficial substances by the plants, as well as the physiological activities, can be improved. It can also increase the rate of some metabolic transformations, including the synthesis processes of secondary metabolites. However, nitrogen fertilization has as main result the reduction of phenolic content in plant substrates. This is explained by the competition that occurs between the synthesis reactions of secondary components and the basic processes for obtaining the proteins and essential amino acids in growth and development of plants (Koricheva J., 1998).

So, the increase of the concentration of polyphenolic compounds, as well as the antioxidant activity of ethanolic extracts of both fennel varieties, by using a treatment with AA+M+µ, is due to the combined action of the amino acids and especially of the microelements, and to a minor extent of macronutrients nitrogen, phosphorus and potassium.

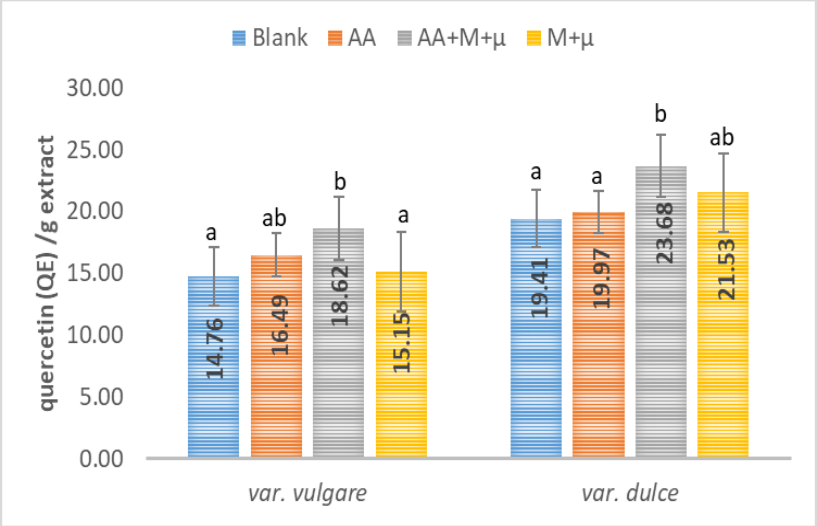


Figure 3 **Total flavonoid content of ethanolic extracts from fennel seeds**
Means with the same lower-case letter are not significantly different at P < 0.05 according to Tukey's test

The results of this research can also be correlated with those obtained in other studies.

Damjanović A.M. *et al*, (2005) determined the main chemical compounds in the composition of fennel essential oil, from Montenegro: trans-anethole (62.0%), fencone (20.3%), estragole (4.90%) and limonene (3.15%).

Anwar F. *et al*, (2009) determined the main components of fennel essential oil: trans-anethole (69.87%), fencone (10.23%) and estragole (5.45%), with a total of 23 chemical compounds identified.

Olgun Ç. *et al*, in 2017, identified, as the main component in the volatile extract of *Foeniculum vulgare* Mill., anethole in proportion of 80%.

Certain phenylpropanoic compounds were also identified in the composition of the volatile oil: p-methoxy-phenyl-acetone, anisic acid, anisic ketone, dihydroanethole, anisic aldehyde and p-methoxy-1-phenyl-1-propanol (Anka Z.M. *et al*, 2019).

## CONCLUSIONS

Based on the data showed in this research, it can be concluded that all antioxidant activity values were improved by using the foliar treatments. Also, the synthesis of phenolic and flavonoids components in plants from both fennel varieties was significantly influenced by the application of foliar fertilizers.

Furthermore, the chemical composition of the essential oil of sweet and bitter fennel seeds is substantially influenced through use of foliar nutrients, due to the combined action of the amino acids and especially of the microelements.

## ACKNOWLEGMENTS

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# RESEARCH ON THE INFLUENCE OF THE PRECEDING CROP ON THE YIELDS OF THE TRITICALE CROP UNDER THE CONDITIONS OF A.R.D.S. SECUIENI

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**Abstract**

One of the important factors contributing to increased yield is represented by crop rotation, which by alternation can reduce or prevent infestation with weeds, pathogens, plant and soil pests, but can improve the texture and structure of the soil so that the next crop can capitalize these benefits. Considering that the grain yield of straw cereals is influenced by the number of ears per surface unit, the number of grains per ear but also by the individual weight of the grain, this study was carried to determine which preceding crop favorably influences the yiled of grains to the triticale crop. The experience was placed in the experimental field at A.R.D.S. Secuieni, and followed the influence of three preceding plants: soybeans, sunflower and corn on grain yield at two triticale varieties: Haiduc and Utrifun. Following research, the best average yields were obtained when the triticale was cultivated after corn and soybeans. Of the two varieties of triticale, Utrifun stood out for its superior yields, with average values between 6213 kg· $ha^{-1}$ and 8277 kg· $ha^{-1}$.

**Key words**: triticale, yields, precedind crop

The cultivation of triticale is a requirement of the current agriculture, which consists of the exploitation of less productive surfaces for wheat and corn, acidic surfaces, affected by drought, waterlogging, and poor in nutrients. Although the main destination of triticale grains is use in animal nutrition, laboratory tests have indicated that it can also be used in baking (in human nutrition), by applying a special technology (Ittu *et al*, 1986, 1988, 2001, 2004).

After a decade of genetic manipulation and breeding, triticale stands out as a crop with high biomass and productivity potential, generally exceeding that of wheat. Its high productivity is most likely derived from high carbon assimilation rates related to stomatal physiology and probably a low respiration rate. Being a derivative of rye, triticale has always been considered to be relatively resistant to abiotic stress. The last review of triticale's adaptation to abiotic stress, published by Jessop (1996), highlighted its general and specific ability to harsh growth and development conditions.

The multi-year results of the field experiments are influenced by the different conditions of the rainfall and thermal regime, as well as by the physical and chemical characteristics of the soil. Climate changes in the last period of time have accentuated these extreme variations, with serious consequences on agricultural production (Săulescu *et al*, 2006).

The triticale species is characterized by the ability to withstand unfavorable biotic and abiotic environmental factors and, as a consequence, by the ability to achieve good yields in marginal regions (Martinek *et al*, 2008).

Triticale (*Triticosecale* Wittmack.) is a potential cereal to provide better yield under wet stress conditions. It performed better than wheat in terms of yield, plant height, and other characteristics with a very high level of resistance to wheat diseases. All this evidence demonstrates that triticale can compete with long-duration cereal crops such as wheat in many situations, including drought. The yield could be increased by selecting plants with taller plant height and more spikelets (Zaheer, 1991).

The potential advantage of triticale over wheat in obtaining biomass and high grain yields was confirmed in a study in Spain (Estrada-Campuzano *et al*, 2012).

It has been reported that triticale can perform better than wheat on poor-quality soil (Kavanagh, 2015, Ayalew *et al*, 2018). To date,

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triticale is mainly grown as a fodder grain, cover crop, and for biogas production (Randhawa *et al*, 2015).

Although triticale contains gluten, it may play a role in the growing health food market due to its health benefits with its good balance of essential amino acids, minerals, and vitamins (Glamoclija *et al*, 2018, Zhu, 2018).

The advantages that this species confers are the ability to achieve high grains yield and biomass in a wide variety of pedoclimatic conditions and with crops technologies with lower inputs compared to other winter cereals. These advantages have made triticale a crop increasingly appreciated by farmers, currently in continuous expansion (Ittu *et al*, 2007).

## MATERIAL AND METHOD

The research was carried out during 2022/2023 in the experimental field at A.R.D.S. Secuieni (Agricultural Research and Development Station Secuieni), having the coordinates of 26°5' east longitude and 46°5' north latitude. The soil on which the research was done is a typical cambic chernozeum (phaeozeum), characterized as being well supplied in phosphorus (P2O5 - 39 ppm), calcium and magnesium, medium supplied in active humus (2.3%) and nitrogen and poorly supplied in potassium (K2O - 161 ppm) with a slightly acidic pH of 6.29% (Leonte et. al., 2021).

The purpose of the experience was to establish the influence of the preceding plant on yield and yield indices. To achieve the proposed goal, two varieties of Romanian autumn triticale (Haiduc and Utrifun) cultivated after three different predecessor plants as a botanical family (soybean, corn, and sunflower) were tested.

The cultivation technology, due to the pedological drought that has been manifesting in the last years in the Central area of Moldova, consisted of performing the basic work of the land with the heavy disc, followed by the leveling and shredding of the soil through passes with the combinator. No basic fertilization was applied, and sowing took place in the third decade of October (24.10.2022).

In the last years, climatic conditions led to the accentuation of the pedological drought in the area of influence of the unit, and the period of growth and development of crop plants was characterized as being dry and hot.

The thermal regime recorded during the vegetation period of winter cereals was predominantly warm and very warm.

On average, the temperatures recorded between October 1, 2022 and July 31, 2023 were 2.0ºC higher than the multiannual average (7.3ºC). This deviation is due to the high temperatures in the autumn and winter months, atypical of the area of influence of the unit (*table 1*).

Table 1

**Temperatures (ºC) and multiannual average (1962-2022) recorded at A.R.D.S. Secuieni, in the 2022/2023 year**

| Monthly | X | XI | XII | I | II | III | IV | V | VI | VII | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2022/2023 | 11.5 | 5.0 | 0.8 | 2.4 | 1.0 | 6.0 | 8.1 | 15.4 | 19.9 | 22.6 | 9.3 |
| Multiannual average | 9.2 | 3.6 | -1.5 | -3.7 | -1.9 | 2.8 | 9.6 | 15.4 | 18.9 | 20.4 | 7.3 |
| Deviation | 2.3 | 1.4 | 2.3 | 6.1 | 2.9 | 3.2 | -1.5 | 0.0 | 1.0 | 2.2 | 2.0 |

The conditions for the growth and development of crop plants were negatively influenced by the large rainfall deficit recorded between October 1, 2022 and July 31, 2023, a period characterized as being very dry. The amount of recorded rainfall was 301.9 mm, which is -127.7 mm less than the multiannual amount for the same period, which is 429.6 mm (Table 2).

During the triticale vegetation period, only in April, there was a positive deviation of 24.0 mm compared to the multiannual average (44.9 mm). This deviation was due to the snow cover recorded in the first decade of April (*table 2*).

Table 2

**Rainfall (mm) and multiannual average (1962-2022) recorded at A.R.D.S. Secuieni in the 2022/2023 year**

| Monthly | X | XI | XII | I | II | III | IV | V | VI | VII | Sum |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2022/2023 | 19.8 | 41.8 | 19.0 | 13.2 | 12.6 | 7.6 | 68.9 | 21.0 | 29.8 | 68.2 | 301.9 |
| Multiannual average | 36.9 | 27.7 | 25.4 | 19.6 | 19.2 | 26.3 | 44.9 | 64.3 | 84.7 | 80.6 | 429.6 |
| Deviation | -17.1 | 14.1 | -6.4 | -6.4 | -6.6 | -18.7 | 24.0 | -43.3 | -54.9 | -12.4 | -127.7 |

## RESULTS AND DISCUSSIONS

One of the most important indices in obtaining high yields is represented by the number of ears per surface unit (Skuodiene R., Nekrošiene R, 2009).

According to Bîlteanu Ghe., (1998), the premise of a high grain yield based on a large number of ears per surface unit must be obtained from more plants, less twinned than, from fewer plants but strongly twinned.

Similar studies on triticale crop were carried out at Moara Domneasca Farm by Dumbrava, M., *et al*, (2016), characterizing the species as being much more adaptable to climatic conditions than wheat and corn, exploiting soil resources differently depending on the predecessor plant.

Taking into account this index, namely the number of ears per square meter, the triticale varieties reacted differently depending on the preceding plant.

The number of ears/square meter was influenced by the preceding plant but also by the variety. The highest density was recorded in the varieties sown after corn and soybeans, with an average of 605 ears/square meter for the Utrifun variety. Also, the lowest density, of only 481 spikes/square meter, was also obtained by the

Utrifun variety, when it was cultivated after the sunflower (*figure 1*).

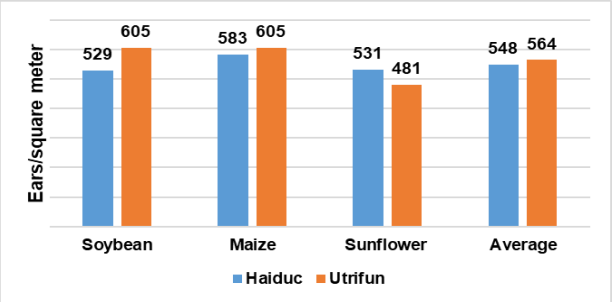


Figure 1 **The number of ears/square meter recorded in two studied triticale varieties**

The grain/ear weight was higher in the case of the varieties sown with the Utrifun variety regardless of the previous crop after which it was grown. The triticale variety Utrifun recorded the highest average value, of 1.45 g/ear in the variant sown after soybeans, and the lowest value was recorded in the case of the variant sown after sunflower, of 1.22 g/ear (*figure 2*).

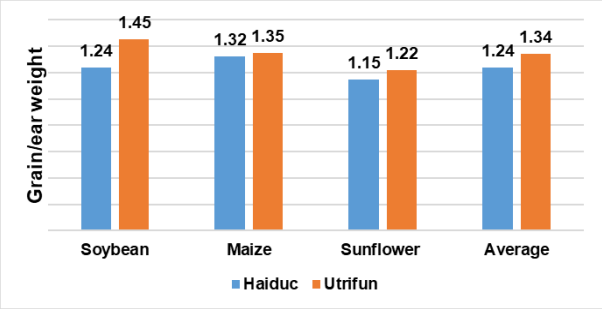


Figure 2 **Grain/ear weight recorded in two studied triticale varieties**

On average, the Utrifun variety obtained higher values in terms of the number of grains per ear. The Haiduc variety registered a number of 37.1 grains/ear in the case of the variant sown after sunflower and the lowest value, of only 33 grains/ear, was recorded in the case of the variant sown after soybeans (*figure 3*).

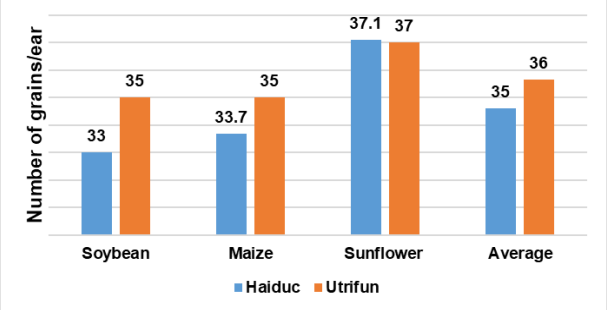


Figure 3 **Number of grains/ear recorded in two studied triticale varieties**

The mass of a thousand grains (TGW) is an important index in the analysis of cereal grains, its values being influenced by the applied technology, variety, and also by climatic conditions.

In the research from A.R.D.S. Secuieni, the TGW had values between 36.5 g (Haiduc) and 40.5 g (Utrifun) (*figure 4*).

The highest values of thousand grain weight (TGW) were obtained in the variants sown after maize in both autumn triticale varieties. The lowest values were obtained in the variants sown after sunflower (*figure 4*).

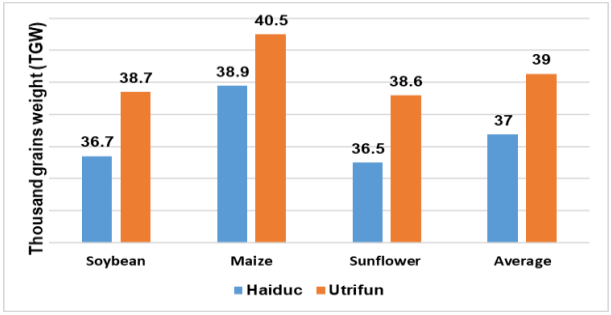


Figure 4 **Average values of thousand grains weight (TGW) obtained by two varieties of triticale**

The hectoliter mass of cereals is currently a very important indicator for the milling industry because in the milling units, the total extraction of flour is established according to the value of this indicator. Although the triticale crop is not yet known for its use in baking, it is still used to obtain flour pasta and biscuits.

In the bakery industry, it is known that wheat intended for obtaining flour must have a hectoliter mass of at least 78 kg/hl. In the specialized literature, it is known that the hectoliter mass of triticale has values between 70-76 kg/hl, but as can be seen in *figure 5*, by using a favorable precursor plant this index can reach higher values, as in the case of the Utrifun variety grown after soybeans.

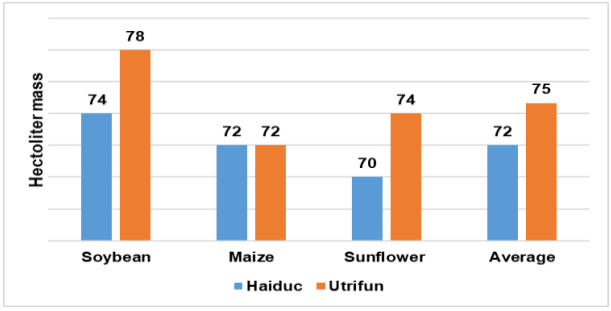


Figure 5 **Hectoliter mass recorded in two varieties of triticale**

The grain yield is influenced by the crop technology applied, the variety but also by the climate and soil conditions.

The yields obtained in the 2022/2023 year at A.R.D.S. Secuieni had values between 5463 kg $ha^{-1}$ and 8277 kg $ha^{-1}$ (*figure 6*).

The highest grain yields were obtained, on average, in the case of variants sown after corn. The highest grain yield was obtained by the Utrifun variety, of 8277 kg ha$^{-1}$ in the case of the variant sown after soybeans, and the lowest production was achieved in the variants sown after sunflower of 6213 kg ha$^{-1}$ (*figure 6*).

The Haiduc variety obtained lower average yields than the Utrifun variety, of 6258 kg ha$^{-1}$. The highest average grain yield of the Haiduc variety was obtained in the varieties sown after corn (7263 kg ha$^{-1}$) and the lowest average yield was obtained in the case of the varieties sown after sunflower (5463 kg ha$^{-1}$) (*figure 6*).

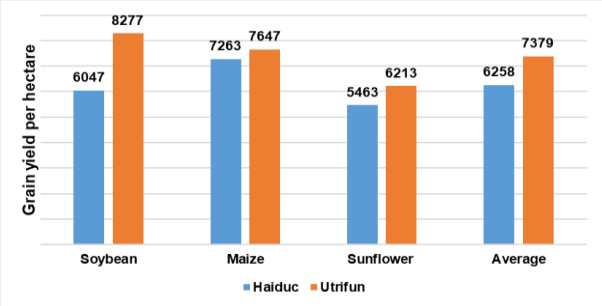


Figure 6 **Grain yield per hectare obtained according to the predecessors plant**

## CONCLUSIONS

Following the results obtained, we can draw the following conclusions:

- the 2022/2023 year was characterized as very hot and dry during the vegetation period of winter cereals, and the snow in the first part of April negatively influenced the yields obtained;
- grain yield is influenced by the variety, and the climatic conditions, but also to a large extent by the preceding plant;
- the Utrifun triticale variety has the ability to obtain higher grain yields regardless of the preceding plant;
- soybean or corn crops can be used as a precursor plant to obtain higher yields;
- the lowest grain yields were obtained in the variants sown after sunflower (5463 kg ha$^{-1}$), therefore crop rotation must be taken into account.

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# THE STUDY OF THE BEHAVIOR OF SOME CORN HYBRIDS FOR THE OPTIMIZATION OF THE CULTIVARS STRUCTURE UNDER IRRIGATION CONDITIONS IN IASI COUNTY

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**Abstract**

Commercial corn hybrids have a very high yield potential, with valuable features of adaptation to more or less favorable growing conditions. In the particular conditions of technology applied in a certain farm and in particular climatic conditions of the crop year, the production and the efficiency can approach more or less the yield potential of these hybrids, through their comparative testing being able to obtain information to determine the best assortment. Evaluation of the results obtained following the testing of three semi-early corn hybrids, from three different companies, at SC Semtop Group in Iași county, at sowing plots of 80,000 and 70,000 germinable grains/m$^2$, respectively, and under conditions of optimal assurance of the necessary irrigation water allowed to highlight the hybrids that behave best in the respective conditions.

**Key words**: assortment optimization, commercial hybrids, maize

Through its multiple ways of use, and through its high productivity (Wang J., Hu X., 2021) corn has become an indispensable crop for man. Although from 1960 until now, breeders have succeeded in significantly improving the productive and adaptive potential of maize hybrids (Nanda D., 2020), high and economic yields at the farm level directly depend on the appropriate selection of cultivars in relation to with environmental conditions and the available level of cultivation technology (Butts-Wilmsmeyer C. *et al*, 2019). As at the global level, agricultural crops in Romania are also facing increasingly obvious climate changes, with a negative impact on yield (Haș V. *et al*, 2021). Testing the behavior of cultivars in real on farm conditions can make the choosing the most suitable assortment easier. The results of such tests carried out by Haș V *et al* (2021) at Turda, over a period of four years (2017-2020) highlights the fact that, for certain areas in Romania, including the north-eastern area of the country, semi-early hybrids, selected for lower sensitivity to high temperatures and drought during grain filling will determine their ability to overcome problems due to climate change. Also, the yield per area unit depends to a very high extent on the sowing area and the effective harvesting area (da Silva E.E. *et al*, 2021), a fact for which the most accurate information can beeobtained through direct testing in the individual conditions of the farm.

## MATERIAL AND METHOD

The biological material tested was represented by three semi-early maize hybrids, from the FAO 350-390 group, respectively DKC 4943 (Dekalb), Kashmir (KWS) and Rulexx (RAGT), which were sown on a 5-ha plot each, in the last decade of April 2021, for all three the same culture technology was applied, the only difference being the sowing rate. DKC 4943 and Kashmir hybrids were sown at the rate of 80,000 germinating grains/ha, and Rulexx hybrid at 70,000 germinating grains/ha. The previous crop was barley, the autumn fertilization was carried out by applying 200 kg/ha complex fertilizer NPK 16:16:16, the autumn plowing was carried out at a depth of 35 cm, the preparation of the germinal bed was carried out in the third decade of April, with the combiner at a depth of 7-8 cm, after which sowing was carried out simultaneously with the application of 120 kg/ha complex fertilizer NPK 20-20-0+0.20% Zn. For weeds control, pre-emergence herbicide Adengo (0.4 l/ha) and post-emergence herbicide Laudis (2.25 l/ha) were applied, in the latter case a foliar fertilizer was also added (Foliar Extra, 1.5 l/ha). A few weeks after weeding, a mechanical grid was made, together with the applying of 150 kg/ha of ammonium

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nitrate. Irrigation, by aspersion, consisted in the application of three waterings with a rate of 30 l/m$^2$, after sowing, at the start and at the end of flowering. During the vegetation period and at harvest, determinations were made on ten plants for the evaluation of several parameters. Were determined: the height of the plants; the degree of development of the female inflorescence, the width and length of the cobs, the number of rows of grains per cob and the number of grains per row, as well as the productivity of the tested hybrids, expressed by the average weight of a cob, the weight of grains/cob, grain yield and average production (t/ha). At harvest, the plant density was determined, expressed by the number of plants/ha, compared to the seeding density, to evaluate its influence on the average production of the hybrids.

The obtained data were used to calculate the average values ($\bar{X}$), the amplitude of variation (A) and the coefficients of variability (s%) for each character and each hybrid separately.

## RESULTS AND DISCUSSIONS

The average height of the plants (*table 1*) varied very little from one hybrid to another and, although in all three hybrids small values of the coefficient of variability resulted, in the hybrid DKC 4943 it was at least double (9.91 %) compared to the values of the other two hybrids. This aspect resulted as a result of the large amplitude of plant height variation (of 1.00 m) in the mentioned hybrid.

The average cob length (*table 2*) was between 18.99 cm in the Kashmir hybrid and 20.91 cm in the DKC 4943 hybrid, for the latter also resulting in the largest amplitude of variation, of 12.1 cm, as well as the highest coefficient of variability, of 15.84%. The lowest value of the amplitude of variation (2.9 cm) and the coefficient of variability (4.27 %) were recorded in the case of the Kasmir hybrid.

Cob diameter (*table 3*) ranged from 5.11 cm in the DKC 4943 hybrid to 4.58 cm in the Kashmir hybrid, and the highest uniformity was recorded in the Rulexx hybrid. For all three hybrids, the variability of this character was small.

The average number of grain rows per cob (*table 4*) was between 16.2 in the Rulexx hybrid and 17.0 in the Kashmir hybrid, for the latter the smallest amplitude of variation was also determined, of four grain rows, of the other two hybrids, with an amplitude of variation of six grain rows. Character variability was medium till low for all three hybrids.

Regarding the number of grains per row (*table 5*), the Rulexx hybrid recorded the highest value, 40.3 grains, with the other two hybrids being almost identical. The lowest values of the amplitude of variation and the coefficient of variability led to the highlighting of the Kashmir hybrid as the most stable from this point of view.

The highest value of the average weight of a cob (*table 6*), of 180.02 g, was determined in the Rulexx hybrid, compared to the Kashmir hybrid, with the lowest value, of 146.05 g. However, for the last hybrid, the amplitude of variation and the coefficient of variability highlight the highest stability of this character. The three hybrids behave according to a similar pattern also regarding the weight of grains on the cob (*table 7*).

The grain yield, calculated as the ratio between the cob weight and the weight of grains per cob (*table 8*), places the Kashmir hybrid in first position, closely followed by the DKC 4943 hybrid.

The ranking of the three hybrids according to the yield (*table 9*) places the hybrids DKC 4943 (with 11.25 t/ha) and Kashmir (with 10.79 t/ha) on the first two places, in direct correlation with the tenth of high sowing, of 80,000 germinating grains/ha, in third place being the Rulexx hybrid, where the maximum recommended sowing rate was a maximum of 70,000 germinating grains/ha.

Table 1

**Variability of plant height**

| Hybrids | DKC 4943 | KASHMIR | RULEXX | Mean (control) |
|---|---|---|---|---|
| Mean ($\bar{x}$)± S.E.(m) | 3.055 ± 0.10 | 2.888 ± 0.04 | 2.959 ± 0.04 | 2.97 |
| The amplitude of variation (m) | 1.00 | 0.46 | 0.39 | 0.62 |
| The coefficient of variability (s%) | 9.91 | 4.83 | 3.83 | 6.19 |

Table 2

**Variability of cob lenght**

| Hybrids | DKC 4943 | KASHMIR | RULEXX | Mean (control) |
|---|---|---|---|---|
| Mean ($\bar{x}$)± S.E.(cm) | 20.91 ± 1.05 | 18.99 ± 0.26 | 20.90 ± 0.68 | 20.27 |
| The amplitude of variation (cm) | 12.10 | 2.90 | 6.40 | 7.13 |
| The coefficient of variability (s%) | 15.84 | 4.27 | 10.27 | 10.12 |

Table 3

**Variability of cob diameter**

| Hybrids | DKC 4943 | KASHMIR | RULEXX | Mean (control) |
|---|---|---|---|---|
| Mean (x̄)± S.E.(cm) | 5.11 ± 0.13 | 4.58 ± 0.11 | 4.81 ± 0.05 | 4.83 |
| The amplitude of variation (cm) | 1.50 | 1.10 | 0.50 | 1.03 |
| The coefficient of variability (s%) | 8.27 | 7.34 | 3.46 | 6.36 |

Table 4

**Variability of the number of rows of grains on the cob**

| Hybrids | DKC 4943 | KASHMIR | RULEXX | Mean (control) |
|---|---|---|---|---|
| Mean (x̄)± S.E.(nr.) | 16.80 ± 0.53 | 17.00 ± 0.54 | 16.20 ± 0.63 | 16.67 |
| The amplitude of variation (nr.) | 6.00 | 4.00 | 6.00 | 5.33 |
| The coefficient of variability (s%) | 10.04 | 10.00 | 12.28 | 10.77 |

Table 5

**Variability of the number of grains per row**

| Hybrids | DKC 4943 | KASHMIR | RULEXX | Mean (control) |
|---|---|---|---|---|
| Mean (x̄)± S.E.(nr.) | 37.60 ± 1.74 | 37.30 ± 0.70 | 40.30 ± 1.61 | 38.40 |
| The amplitude of variation (nr.) | 16.00 | 6 | 17 | 13.00 |
| The coefficient of variability (s%) | 14.63 | 5.93 | 12.60 | 11.06 |

Table 6

**Variability of cobs weight**

| Hybrids | DKC 4943 | KASHMIR | RULEXX | Mean (control) |
|---|---|---|---|---|
| Mean (x̄)± S.E.(g) | 161.03 ± 9.71 | 146.05 ± 3.42 | 180.02 ± 7.86 | 162.37 |
| The amplitude of variation (g) | 105.78 | 32.14 | 73.86 | 70.59 |
| The coefficient of variability (s%) | 19.08 | 7.41 | 13.81 | 13.43 |

Table 7

**Variability of grain weight per cob**

| Hybrids | DKC 4943 | KASHMIR | RULEXX | Mean (control) |
|---|---|---|---|---|
| Mean (x̄)± S.E.(g) | 138.20 ± 8.13 | 125.67 ± 2.68 | 151.09 ± 6.87 | 138.32 |
| The amplitude of variation (g) | 87.18 | 23.93 | 61.5 | 57.54 |
| The coefficient of variability (s%) | 18.61 | 6.73 | 14.37 | 13.24 |

Table 8

**Variability of shelling percentage - (grain weight / ear weight) x 100**

| Hybrids | DKC 4943 | KASHMIR | RULEXX | Mean (control) |
|---|---|---|---|---|
| Mean (x̄)± S.E.(%) | 85.89 ± 0.35 | 86.09 ± 0.40 | 83.87 ±0.34 | 85.28 |
| The amplitude of variation (%) | 3.63 | 3.96 | 3.4 | 3.66 |
| The coefficient of variability (s%) | 1.28 | 1.47 | 1.28 | 1.34 |

Table 9

**The influence of the hybrid and the sowing thickness on corn yield**

| Hybrids | Plants per hectare | | | | Yield | |
|---|---|---|---|---|---|---|
| | At sowing | At harvesting | The difference compared to the control, at harvest | | t/ha | % |
| | No. | No. | No. | % | | |
| DKC 4943 | 80000 | 77456 | 3062 | 104.12 | 11.25 | 104.85 |
| KASHMIR | 80000 | 76987 | 2593 | 103.49 | 10.79 | 100.56 |
| RULEXX | 70000 | 68739 | -5655 | 92.40 | 10.16 | 94.69 |
| Mean (control) | 76667 | 74349 | - | 100.00 | 10.73 | 100.00 |

## CONCLUSIONS

In the specific conditions of the farm, the highest productions were obtained in the hybrids DKC 4943 and Kashmir, in direct correlation with the sowing density.

Under identical technology conditions, at a 12.5% lower seeding rate used in the Rulexx hybrid, compared to a seeding rate of 80,000 germinating grains/ha used in the other two hybrids, the yield differences were 1,09 t/ha (compared to the DKC 4943 hybrid), respectively 0.63 t/ha (compared to the Kashmir hybrid).

From the analysis of the production yield, the same ranking is kept, so that, for the maximum utilization of the specific soil and technology conditions, it is recommended to cultivate those hybrids with a high potential to exploit the existing intensification opportunities at the farm level.

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# RESEARCH RESULTS OF BM 86 AS A FOLIAR FERTILIZER FOR GRAPEVINES UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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**Abstract**

Environmental contamination, weather anomalies, nutrient imbalance, drought as well as long-term cultivation of multiannual crops are factors that condition intensive soil depletion. Considerable losses in plant productivity and loss of product quality can be observed. The article presents the results of research on the influence of the foliar fertilizer BM 86 of the French company "Arysta Life Science SAS" on the quantity and quality of grapes. Foliar fertilizer BM 86 tested in the conditions of the Republic of Moldova demonstrated its effectiveness in the growth and development of vines manifested by increased yield and quality of berries compared to the standard (Cosir, 2.0 l/ha). Based on the testing results it was recommended to include BM 86 fertilizer in the dosage of 3.0 l/ha in three foliar treatments of vines in the "State Register of Products for Phytosanitary Use and Fertilizers of the Republic of Moldova."

**Key words**: vine, foliar fertilizer, test, productivity, quality

Viticulture is one of the priority areas of the economy in the Republic of Moldova. According to IOVW data, the area of vineyards in the Republic of Moldova is 140 thousand ha, which represents 1.9% of the total area of vineyards in the world. The majority of plantations are represented by technical varieties used for wine and juice production. The contribution of the Republic of Moldova was 2 million hectolitres of the 279 million hectolitres of wine produced in the world in 2018. In general, most varieties grown in the country have a genetic potential determined by a fairly high productivity. However, the realization of this potential is slowed down by two factors: monoculture and periodic thermal stresses.

It is already known that the realization of the yield potential of vines can be increased by applying foliar fertilizers which are applied directly to the leaves of the vine and take effect quickly and safely. They can be used as a treatment for vines, depending on the needs of the plant and its deficiencies. Foliar fertilizer treatments on vines can be applied to all varieties of vines. They are sprayed on the leaves and solve specific problems. In order to produce tasty and healthy grapes, the best quality foliar fertilizers are chosen to ensure the success of any vine production.

In this context, in accordance with the Test Work Programme, drawn up annually by the "State Centre for Approval and Homologation of Plant Protection Products and Fertilizers", the French preparation BM 86 from Arysta Life Science SAS was tested for the first time.

BM 86 contains microelements (B, Fe, Mn, Zn). The role of these microelements in plant metabolism is quite well known and conditions the accumulation of vegetative mass, the size of the harvest and the quality of the production obtained. Foliar fertilization with secondary macroelements that are also present in this preparation (Ca, Mg, S), which vines need several tens of kilograms per year, comes as a complement to basic fertilization in periods of nutritional disorders of plants. Foliar fertilization is unavoidable for the main macro-elements (NPK). Foliar fertilization during the growing season is therefore part of sustainable agriculture as the main measure to improve plant nutrition under ecological protection conditions.

The aim of our research is to test different doses of foliar fertilizer at critical stages of vine development, and to assess the effectiveness on yield and quality of production obtained under the conditions of the Republic of Moldova.

## MATERIAL AND METHOD

The testing work of BM 86 preparation was carried out on the experimental lot located in the

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vineyard of Pinot Blanc variety in Codru commune, Chisinau municipality, Technological-Experimental Station (STE) "Codru" of the Scientific-Practical Institute of Horticulture and Food Technologies (*figure 1*).

Figure 1 **Spatial location of the experimental plot**

To determine the biological activity of the preparation BM 86 on Pinot Blanc vines, an experiment was set up in the field according to the scheme:

1.Control (unfertilised);
2.Standard Cosir, 2 litres/ha;
3.BM-86, 2 litres/ha;
4.BM-86, 3 litres/ha.

Three sprays were made according to the programme (*figure 2,3,4*).

Three sprays were performed according to the schedule:

1 - In the vegetation restart phase (26 April);
2 - during the bud swelling phase (12 May);
3 - in the after-flowering phase (1 July).

Cosir standard contains nutrients (NPK): 3.0 - 33.7 - 24.3 + Cu (0.02%) + B, Mn, Zn (0.1%) + Mo (0.001%). 40 : 25 + 2MgO + 2B + TE).

The tested fertilizer BM 86 contains NPK - 9 - 53 - 9 + Fe, B, Zn (0,1 %) + Cu, Mn (0,05 %) + Mo (0,005 %), therefore the tested preparation contains macroelements (N-241 g/l + MgO - 61,6 g/l + SO3 -123,3 mg/l) and microelements (B-26,0 g/l + Mo-0,25 g/l + algae extract *Ascophylum nodosum* Ga-142-257 g/l). The preparations were applied foliar in three replicates of 10 bushes. The area of nutrition is 1.50 x 2.75 m, per hectare - 2400 grapevine bushes.

Figure 2 **First treatment in the early vegetation phase**

Figure 3 **Second treatment at bud swelling**

Figure 4 **Third treat blooming phase**

## RESULTS AND DISCUSSIONS

In vine cultivation, fertilization is intended to ensure an increased grape harvest without lowering the quality parameters and reducing the plants' resistance to diseases, pests or other natural factors. In order to achieve these goals on the vineyard, it is mandatory to have a real knowledge of the soil condition, what nutrients are accessible to the plants and what is the annual food consumption according to the planned production. In this context agrochemical mapping was carried out. Soil samples were taken and laboratory analyses were carried out. Field and laboratory work were carried out according to standards and methodical recommendations.

According to the data obtained (*table 1*) the soil of the experimental field is characterized with an optimal level of nutrient supply.

Table 1

**Agrochemical indices of leached chernozem (TES"Codru"). Testing the preparation BM 86 as a foliar fertilizer**

| Experience variant | Humus, % | Phosphorus | Potassium | Nitrification capacity, N-$NO_3$ |
|---|---|---|---|---|
| | | mg/g sol | | |
| 1.Control (unfertilized); | 3.62 | 2.40 | 24.10 | 2.50 |
| 2.Standard Cosir, 2 liters/ha; | 3.57 | 2.54 | 24.10 | 1.85 |
| 3.BM-86, 2 liters/ha; | 3.41 | 3.00 | 25.31 | 1.85 |
| 4.BM-86, 3 liters/ha. | 3.77 | 3.88 | 28.92 | 2.32 |
| Media | 3.59 | 2.96 | 25.61 | 2.13 |

The soil resource assessment confirms the natural potential of the experimental land, which has a below average productivity capacity. This can be increased by applying mineral and organic fertilisers. From the calculations and measurements carried out during the test it was established that the application of BM 86 fertilizer has a beneficial influence on the growth and development of vines by increasing yield (*table 2)* and berry quality (*table 3*).

Table 2

**Influence of fertilizer BM – 86 on the harvest of Pinot Blanc grapes obtained on leached chernozem, t/ha. TES "Codru", 2021**

| Experience variant | Grape harvest, t/ha | Harvest surplus | |
|---|---|---|---|
| | | t/ha | % |
| 1.Control (unfertilized); | 12.0 | - | - |
| 2.Standard Cosir, 2 liters/ha; | 13.7 | 1.7 | 14 |
| 3.BM-86, 2 liters/ha; | 14.4 | 2.4 | 20 |
| 4.BM-86, 3 liters/ha. | 16.1 | 4.1 | 34 |
| DL 0.5% | 1.2 | 0.5 | 5.0 |
| Sx, % | 6.0 | 6.0 | 6.0 |

Table 3

**The influence of fertilizer on the quality of grapes of the Pinot Blanc Variety obtained on leached chernozem. TES "Codru", 2021**

| Index and units of measurement | Experience variant | | | |
|---|---|---|---|---|
| | Control (unfertilized) | Standard Cosir, 2 liters/ha | BM-86, 2 liters/ha | BM-86, 3 liters/ha |
| Average harvest per bush, kg | 5.0 | 5.7 | 6.0 | 6.7 |
| Number of grapes per Bush, pcs | 37 | 42 | 47 | 53 |
| Grape weight, g | 135 | 135 | 128 | 126 |
| Weight per 100 grapes, g | 158 | 162 | 180 | 170 |
| Volume per 100 grapes, $cm^3$ | 140 | 147 | 165 | 162 |
| Sugar level, % | 22.0 | 22.6 | 23.4 | 23.9 |
| Total acidity, ‰ | 7.1 | 6.8 | 6.0 | 5.4 |
| Vitamina C, mg/l | 17.1 | 19.0 | 19.9 | 21.0 |

Note: The analyses were carried out in the Laboratory "Agrochemistry" (IPASP "Nicolae Dimo") and the Laboratory "Wine Technology, BDO" and "Hard Drinks" (IȘPHTA).

The production per hectare in the variants treated with bm 86 fertilizer was 14-16 t/ha, which was significantly higher than that obtained in the standard and unfertilized control variants (13.7-12.0 t/ha). The yield of pinot blanc grapes in the variants fertilised with bm 86 was 2.4 - 4.1 t/ha, (20 - 34 %).

The application of bm 86 fertilizer led to improved grape production quality. The average yield per bush is 6.0 - 6.7 kg for the bm 86 treated variety with a grape number between 47 and 53, contributing to a grape weight of 126 - 128 grams. At the same time, the volume of 100 grapes is 170 - 180 grams. The quality of grape production is improved: the sugar content is increased and the total acidity is reduced, while the vitamin c content is increased. It is known that acetylsalicylic acid protects the organic substance from oxidation, which ultimately acts beneficially on the organoleptic priorities of sparkling wines. The sugar in the must of the variants treated with the preparation bm 86 is at an increased level and the total acidity is medium. The must sugar content in the reference variants is - 22,0 %, total acidity - 7,1 ‰ and vitamin c content - 17,1 mg/l.

When applying the foliar preparation bm 86 at doses of 2-3 litres per hectare compared to the control and cosir, 2 litres per hectare (standard) the sugar content increased respectively by 1.4-1.9 %, vitamin c by 1.9-3.9 mg/l and the total acidity content decreased by 0.3-0.7 ‰. A more significant yield increase compared to the unfertilised control was observed in the variant treated with the preparation bm 86, at the dose of 3 litres per hectare - 4.1 t/ha (34 %). The results of the mathematical calculations showed that the grape yield increases from the preparations tested were distinctly significant.

## CONCLUSIONS

Foliar application of BM 86 fertilizer at a rate of 3 litres per hectare to Pinot Blanc vines had a beneficial influence on grape productivity and quality. Sugar content increased by 1.9% and vitamin C by 3.9 mg/l. The total acidity content decreased by 0.7 ‰.

The increase in grape production compared to the reference variety was 4.1 t/ha (34 %) and 1.7 t/ha (14 %) compared to the Cosir standard.

The French preparation BM 86 can be used as a foliar fertilizer on vines at the rate of 3 litres per hectare in three treatments and it is recommended to include it in the "State Register of Products for Phytosanitary Use and Fertilizers of the Republic of Moldova".

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# THE INFLUENCE OF TREATMENTS WITH VARIOUS PHYTOSANITARY PRODUCTS (FUNGICIDES) ON THE ATTACK OF SOME PHYTOPATHOGENIC FUNGI ON WHEAT HARVEST – GLOSA VARIETY - IN 2022 PEDOCLIMATIC CONDITIONS OF THE EASTERN BARAGAN

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**Abstract**

This study aims at monitoring the dynamics of the occurrence and evolution of the attack of some pathogens to Glosa Romanian wheat variety, among which we mention: *Puccinia recondita* f. sp. *tritici* (sin. *Puccinia triticina*) which produces wheat's brown rust and *Septoria* sp. which produces wheat's brown leaf spotting (septoriosis). Also, the influence of applying these fungicides on the harvest, as compared to the untreated control variant, has been monitored. One experiment with 5 variants (4 variants with phytosanitary treatment, plus one control variant not treated) was taken into consideration for this study, for which the following phytosanitary products were used, as follows: NATIVO PRO 325 SL (prothioconazole 175 g/l + trifloxystrobin 150 g/l) and EVALIA (azoxystrobin 250 g/l). The treatment variants were the following: V1 – NATIVO PRO 0.6 l/ha, 1 treatment applied on 20$^{th}$ April + 1 treatment applied on 23$^{rd}$ May, V2 – EVALIA 1.00 l/ha, 1 treatment applied on 20$^{th}$ April + 1 treatment applied on 23$^{rd}$ May, V3 – NATIVO PRO 0.7 l/ha, 1 treatment applied on 13$^{th}$ May; V4 - EVALIA 1.00 l/ha, 1 treatment applied on 13$^{th}$ May and V5 – Untreated Control Variant. The experiment was placed in Latin square, the 5 variants being placed in 5 repetitions. The year 2022 was very dry, especially in March, April and May. The experiment received two waterings with norms of 800 m$^2$/ha/watering in the spring of the year 2022. These waterings led to good wheat yields. We emphasize that the experiment also received, in the autumn of the year 2021, a sprout watering with a norm of 400 m$^2$/ha. Even though three waterings were applied, the attacks of the pathogens that frequently cause foliar diseases in wheat were very low, even in the untreated control variant. This led to very uniform yields in the variants studied. Basically, there were no significant yield differences between the untreated control variant and the variants that were treated with fungicides. The yields of the variants were as follows: V1 – 9.914 to/ha, V2 – 10.668 to/ha, V3 – 10.376 to/ha, V4 – 9.649 to/ha, V5 (untreated control variant) – 10.200 to/ha.

**Key words**: *Puccinia* spp, *Septoria* spp, latin square

The wheat, *Triticum aestivum*, is attacked by many pathogenic agents, such as: mildew - *Blumeria graminis* f. sp. *tritici*, brown rust - *Puccinia recondita* f. sp. *tritici*, brown leaf spotting - *Septoria tritici*, *Septoria nodorum*, stem's fusariosis and ear's rot *Gibberella zeae*, *Gibberella avenacea* (Iacob Viorica, Hatman, M., Ulea, E., Puiu, I. 1998). The first half of the year 2022 was very unfavorable to wheat, in what concerns the climatic conditions. The year 2022 was one of the driest years in the last 10 years in the Baragan Plain area. During this period, rainfall in totally insufficient quantities was recorded. Average temperatures were higher than normal for this date. In January, the average temperature was 2.6ºC and the rainfall totaled only 5.2 l/m². In February, the average temperature was 5.1ºC and the rainfall totaled 10.5 l / m². In March, the average temperature was 4.1ºC, the rainfall totaled only 8.5 l/m² and the average relative humidity of the air was only 54%. In April, the average temperature recorded was 12.7ºC, the rainfall totaled 30 l/m² and the average relative humidity of the air was only 50.7%. In May, the average temperature was 19.7ºC, the rainfall totaled 21 l/m$^2$ and the average relative humidity of the air was 47%. In June, the average temperature recorded was 25.3ºC, the rainfall totaled 14 l/m$^2$ and the average relative humidity of the air was low - 47.3%. Under these circumstances, the fungus *Puccinia recondita f. sp. tritici* that produces brown rust in wheat (Velichi E., 2012) made its presence known to a very small extent in 2022, compared to previous years. We emphasize that this phytopathogenic fungus has made its appearance even in very dry years - it is true that in the form of very weak attacks. These weather conditions, totally unfavorable for the first half of 2022, have led to the effective absence of attacks by pathogens that are usually present in this area.

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Among them we mention: *Blumeria graminis f. sp. tritici*, which produces wheat mildew, S*eptoria sp.* which produces, in wheat, diseases known as septoriosis, *Gibberella* spp. which produces in wheat diseases known as fusariosis (*Fusarium* sp.).

We emphasize that the experiment was carried out under irrigation conditions, benefiting from three waterings. The first watering was applied in autumn with a norm of 400 $m^2$/ha. The other two waterings were applied in spring, with norms of 800 $m^2$/ha/watering. The two waterings in the spring of 2022 were applied as follows: the first watering in the last week of April, the second in mid-May, exactly in the phases of maximum need of water for wheat. The three waterings applied during the growing season had a decisive role in obtaining very good wheat yields in the location where the experiment was established.

Table 1

**The results of the experiment (the first treatment applied at straw's extension, the second at kernel's filling) with fungicide products (4 variants of treatment + 1 untreated control variant) in what concerns the attack (D.A.%) of *Puccinia recondita f. sp. tritici* fungus ("flag" leaf and the next leaf). The observations had been made on 9th June 2022**

| | "Flag" leaf | | | Second leaf | | |
|---|---|---|---|---|---|---|
| Variant | D.A.% | Difference as compared to the control variant | Significance | D.A.% | Difference as compared to the control variant | Significance |
| **V1 -** NATIVO PRO 0.6 l/ha 1 treatment applied on 20th April + 1 treatment applied on 23rd May | 0.06 | 11.38 | ** | 9.52 | 20.9 | ** |
| **V2** - EVALIA 1.00 l/ha 1 treatment applied on 20th April + 1 treatment applied on 23rd May | 0.18 | 11.26 | ** | 8.66 | 21.76 | ** |
| **V3** - NATIVO PRO 0.7 l/ha 1 treatment applied on 13th May | 0.12 | 11.32 | ** | 6.88 | 23.54 | ** |
| **V4** - EVALIA 1.00 l/ha 1 treatment applied on 13th May | 0.12 | 11.32 | ** | 9.28 | 21.14 | ** |
| **V5** - Untreated control variant | 11.44 | | | 30.42 | | |

LD D.A. % for "flag" leaf
LD 5%= 0.954%
LD 1%= 1.314%

LD D.A. % for the second leaf
LD 5%=7.78%
LD 1%=10.72%

Table 2

**The results of the experiment (the first treatment applied at straw's extension, the second at kernel' filling) with fungicide products (4 variants of treatment + 1 untreated control variant) in what concerns the yield (t/ha) obtained at the treated variants, as compared to the untreated control variant**

| Variant | Yield (t/ha) | Difference as compared to the control variant (t/ha) | Significance |
|---|---|---|---|
| **V1 -** NATIVO PRO 0.6 l/ha 1 treatment applied on 20th April + 1 treatment applied on 23rd May | 9.914 | -0.286 | Not significant |
| **V2** - EVALIA 1.00 l/ha 1 treatment applied on 20th April + 1 treatment applied on 23rd May | 10.668 | 0.468 | Not significant |
| **V3** - NATIVO PRO 0.7 l/ha 1 treatment applied on 13th May | 10.376 | 0.176 | Not significant |
| **V4** - EVALIA 1.00 l/ha 1 treatment applied on 13th May | 9.649 | -0.551 | Not significant |
| **V5** - Untreated control variant | 10.200 | - | |

LD 5% = 0.806 to/ha
LD 1% = 1.050 to/ha


Figure 1 - **Aspects from the experimental field (original)-the plants are in the ear's release phase**


Figure 2 **- Aspects from the experimental field (original)-the plants are in full maturity phase**

## MATERIAL AND METHOD

One experiment with 5 study variants each had been conceived for performing the observations. This experiment comprised 4 phytosanitary treatment variants (fungicide products containing various active substances) and one untreated control variant. The variants of the experiment were the following:

- V1 - NATIVO PRO 0.6 l/ha 1 treatment applied on 20th April + 1 treatment applied on 23rd May
- V2 - EVALIA 1.00 l/ha 1 treatment applied on 20th April + 1 treatment applied on 23rd May
- V3 - NATIVO PRO 0.7 l/ha 1 treatment applied on 13th May
- V4 - EVALIA 1.00 l/ha 1 treatment applied on 13th May
- V5 - Untreated control variant

The experiment was placed in Latin square; the 5 variants were placed in 5 repetitions. Each experimental plot had an area of 15 $m^2$ (5 x 3m). The total number of experimental plots was 25. The surface of an experimental variant was of 15 $m^2$ x 5 repetitions = 75 $m^2$. The total area of the experiment was of 75 $m^2$ x 5 = 375 $m^2$. The treatments had been performed manually, with a manual sprayer type of equipment. Weed control was achieved with the help of Mustang herbicide (6.25 g/l florasulam + 300 g/l 2,4-D EHE acid) at a dosage of 0.5 l / ha applied, separately, with the vermorel. The experiment has shown the effectiveness of these plant protection products in relation to their price. The efficiency and, respectively, the profitability of applying a single treatment to a product with a fungicidal effect or two phytosanitary treatments with a product with a fungicidal effect were also monitored during wheat's vegetation period. It was considered that the spring and the first summer month of 2022 were particularly dry.

The assessment of the attack's frequency (F %), of attack's intensity (I%) and respectively of the degree of attack (D.A.%) was done separately, on each and every experimental plot, being analyzed 10 plants / experimental plot. The degree of affectation (attack intensity, I%) of the last two leaves was assessed, especially of the "flag" leaf which has the greatest contribution to the ear's

production at strawy cereals. The phytosanitary analyses on the plants' samples had been done with the help of the stereo-microscope and of the optic microscope at the laboratory of Braila's Phytosanitary Office – National Phytosanitary Authority, institution subordinated to the Ministry of Agriculture and Rural Development. These analyses have revealed in the analyzed samples the presence of *Puccinia recondite f. sp.tritici* fungus which produces wheat's brown rust. Other pathogenic agents specific to wheat were not signaled in the climatic conditions of the first half of the year 2022.

For assessing the production of each variant under study, samples of kernels from each experimental plot, 5 samples each / plot, had been analyzed by spot check. Each sample contained 10 plants, so 50 plants had been taken from each experimental plot, for which the yield was weighted manually. The delimitation of each sample was done with a metric frame with an area of 0.25 $m^2$ (0.5/0.5m). The average of the samples from the experimental plots was used for calculating the yield of each experimental parcel. The statistic interpretation was executed with the help of limit differences (LD %) (Săulescu N., 1967).

The used variety, Glosa, is a Romanian variety created by the Fundulea National Agricultural Research & Development Institute. Glosa variety is an early variety. It has good resistance to falling, resistance to wintering, drought and heat and it has a good resistance at sprouting into ear. It has average resistance to brown rust and is resistant to mildew and to the actual strains of yellow rust (Fundulea Seeds Company, 2021).

The assessment of pest attack can be done with the help of the following values (Methods of Prognosis and Warning 1980):

- Frequency of attack (F %);
- Intensity of attack (I %);
- Degree of attack (D.A %).

- The frequency of attack represents the relative value of the number of plants or organs of the plant under attack (n) reported to the number of observed plants or organs (N). The value of the frequency is established by direct observation on a number of plants or organs, according to the case and to the conditions, existing different methods of sample taking and for performing the observations. In the case of our observations, for the foliar diseases, the number of attacked plant organs out of the total of observed plant organs (leaves) was taken into consideration, being thus established the attack's frequency expressed in percentages %. In the case of blight, it is used the number of wheat's attacked ears, as reported to the total number of observed ears. The frequency is calculated with the formula F%= nx100/N.

- The intensity of the attack represents the degree or percentage whereby a plant or a plant's organ is attacked and how much from the surface of the plant or of the organ analyzed (leaf, fruit) is covered by the disease under study.

- The assessment of the surface under attack is done with the naked eye or with the magnifying glass, assessing the percentage occupied by spots or burns caused by the pathogenic agent. The affectation percentages can be noted or grades can be given for each plant or organ attacked by the disease and/or by the pest. The usage of grades can make easier data summarization in a great extent. It can be used a scale with 6 degrees of intensity, as follows:

- Grade 0 no attack
- Grade 1 attack between 1 and 3%
- Grade 2 attack between 3 and 10%
- Grade 3 attack between 11 and 25%
- Grade 4 attack between 26 and 50%
- Grade 5 attack between 51 and 75%
- Grade 6 attack between 76 and 100%

After data's summarization, the attack's intensity is determined by the formula:

$$I\% = \frac{\Sigma (i\ xf)}{n}$$

Where:

I% – attack's intensity (in %);

i – intensity according to the grade given to the organ or plant under attack;

f – number of cases (plants, organs) attacked;

n – number of plants attacked.

Grades from 1 to 6, separately, to the "flag" leaf and to the next leaf situated beneath it, had been awarded in our experiment.

The degree of attack is the expression of the extension of the severity of the attack onto the crop or onto the total number of plants on which we perform the observations. The following relation gives the value expression of D.A.:

$$D.A\ (\%). = \frac{F \times I}{100}$$

In most cases, there is a negative correlation between the degree of attack of a pathogenic agent or pest and the quantitative and/or qualitative level of production

## RESULTS AND DISCUSSIONS

The agricultural year 2021–2022 was a totally unfavorable year for the occurrence of the attack of the wheat-specific pathogen complex compared to previous years. In January and February, including the spring of 2022, very little rainfall was recorded and average temperatures were very high. For example, in January, the rainfall totaled only 5.2 l/m² and the average temperature was 2.6ºC, which is very high. In February, the average temperature recorded was 5.1ºC and the rainfall totaled only 10.5 l / m². In

March, the average temperature recorded was 4.1ºC, the rainfall totaled only 8.5 l / m². In April, the average temperature recorded was 12.7ºC, the rainfall totaled 30 l/m². In May, the average temperature was 19.7ºC and the rainfall totaled 21 l/m$^2$. June was very dry compared to previous years (only 14 l/m$^2$). It should also be noted that the average air humidity had low values in the spring of 2022 and in June, that is: March 54%, April 50.7%, May 47%, June 47.3%. Relatively little rainfall fell in the fall of 2021. However, the plants sprouted on time, as the crop also benefited from a sprouting watering with a norm of 400 m$^2$.

Regarding the dynamics of the occurrence of pathogen attacks in wheat, we mention that the pathogen that appeared in the 2022 experiment was the *Puccinia recondita f. sp. tritici* fungus that produces brown rust in wheat, at degree of attack (D.A%) values much lower compared to the previous year.

If we analyze the data of *table 1*, we notice that the degree of attack of the pathogens monitored, on each treatment variant, was as follows:

-V1 NATIVO PRO 0.6 l/ha 1 treatment on 20$^{th}$ April + 1 treatment on 23$^{rd}$ May resulted in a degree of attack (D.A.%) of the *Puccinia recondita f sp. tritici* fungus of 0.06 % in the "flag" leaf, and of 9.52 % in the second leaf, thus lower by 11.38 % and by 20.9% respectively, compared to the untreated control variant (V5).

-V2 EVALIA 1.00 l/ha +1 treatment on 20$^{th}$ April + 1 treatment on 23$^{rd}$ May caused a degree of attack (D.A.%) of the *Puccinia recondita f sp. tritici* fungus of 0.18% in the "flag" leaf, and of 8.66% in the second leaf, thus lower by 11.26% and by 21.76% respectively, compared to the untreated control variant (V5).

-V3 NATIVO PRO 0.7 l/ha 1 treatment on 13$^{th}$ May caused a degree of attack (D.A.%) of the *Puccinia recondita f sp.tritici* fungus of 0.12% in the "flag" leaf, and of 6.88% in the second leaf, thus lower by 11.32% and by 23.54% respectively, compared to the untreated control variant (V5).

-V4 EVALIA 1.00 l/ha 1 treatment on 13$^{th}$ May determined a degree of attack (D.A.%) of the *Puccinia recondita f sp. tritici fungus* of 0.12% in the "flag" leaf and of 9.28% in the second leaf, i.e. lower by 11.32% and by 21.14% respectively, compared to the untreated control variant (V5).

-V5 Untreated control variant showed a degree of attack (D.A.%) of the *Puccinia recondita f. sp.tritici* fungus of 11.44% in the "flag" leaf, and 30.42% in the second leaf

All differences regarding the degree of attack (D.A.%) are statistically assured according to Table 1.

By analyzing *Table 2*, we can also see the yield differences compared to the untreated control variant, V5, as follows:

-V1 achieved a yield of 9.914 to/ha, thus 0.286 to/ha lower than that of the control variant.

-V2 achieved a yield of 10.668 to/ha, thus 0.468 to/ha higher than that of the control variant.

-V3 achieved a yield of 10.376 to/ha, so 0.176 to/ha higher than the control variant.

-V4 achieved a yield of 9.649 to/ha, so 0.551 to/ha lower than the control variant.

-V5 The untreated control variant achieved a yield of 10,200 to/ha

The yield increases of the treated variants, versus the untreated control variant (V5), do not provide statistical assurance.

## CONCLUSIONS

The observations made in the summer of 2022 on the experiment with Glosa Romanian wheat-variety led to the following conclusions and recommendations:

1. The attacks of some pathogens were much lower than in previous years. Of these, only *Puccinia recondita f.sp. tritici*, which produces, in wheat, the disease known as brown rust, appeared. The values of the degrees of attack of this phytopathogenic fungus, both in the „flag" leaf and in the second leaf were much lower compared to previous years.

2. For a good protection of the wheat crop, when using the Romanian variety Glosa, we recommend performing, especially in years with dry spring, a single treatment with fungicidal products.

3. We emphasize that, in the years with dry spring, the attack of pathogens that produce foliar diseases in wheat, causes very low attack degrees even when the crop is irrigated. In this case, the experiment received 3 waterings. The first was applied in autumn, for a good sprouting.

4. The prices of products (2023) with fungicidal effect used in 2022 are as follows:

- NATIVO PRO 35 SL 230 lei/l (46.62 €) 0.7 l/ha was applied to V3, i.e., 161 lei/ha (32.63 €/ha);
- EVALIA-270 lei/l (54.73 €) 1.0 l/ha was applied to V4, i.e., 270 lei/ha (54.73 €/ha).
- For the V1 variant, 2 treatments with NATIVO PRO 35 SL were applied (0.6 l / ha / treatment), i.e., a total of 1.2 l of phytosanitary product / ha - the cost was 270 le / ha (54.73 €).
- For the V2 variant, 2 treatments with EVALIA were applied (1.00 l / ha / treatment), i.e., a total of 2 l phytosanitary product / ha - the cost was 540 lei / ha (109.46 €).

The differences in yield, expressed in value (lei) compared to the untreated control variant (V5), were as follows:

-V1 was - 0.286 to/ha, amounting to 343 lei/ha (69.52 €);

-V2 was + 0.468 to/ha, amounting to 561.6 lei/ha (113.83 €);

-V3 was +0.176 to/ha, amounting to 211.2 lei/ha (42.81€);

-V4 was -0.551 to/ha, amounting to 661.2 lei/ha (134.02 €);

-V5 - The yield of the untreated control variant was 10,200 to/ha.

5. From the analysis of economic profitability, it seems, that in the climatic conditions of 2022, which was very dry and hot in the first half, the most profitable turned out to be V5– the untreated control variant. However, we do not recommend, even in very dry and hot years, the cultivation of wheat without applying any treatment, during vegetation, with a phytosanitary product with fungicidal action.

6. In years with dry and hot winters and springs, we recommend a single treatment with fungicidal products for wheat. During these years, cheaper products with fungicidal effect, approved in Romania, can be used, such as those based only on tebuconazole: ARMADA (250 g / l tebuconazole) at a dosage of 0.5 l / ha, ORIUS 25 EW (SALVATOR 25 EW – a second trade name) 0.5 l / ha according to the Pest-Expert website of the National Phytosanitary Authority, structure subordinated to the Ministry of Agriculture and Rural Development of Romania.

The export-quoted wheat price was 1.2 lei/ton (247.25 €/to) in Romania on 22nd July 2023, according to the Agri Portal website.

The leu/€ exchange rate, for the first 6 months of 2023, was 4.9335 lei/1 €, according to the website of the National Bank of Romania.

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# RESERCH ON THE INFLUENCE OF TEHNOLOGICAL FACTORS ON SEED PRODUCTION AT THE *BROMUS INERMIS* LEYSS. IN THE THIRD YEAR OF VEGETATION

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**Abstract**

Seed production is undoubtedly of particular importance for the overseeding or reseeding of permanent grasslands and the establishment of temporary meadows, by providing the necessary seed material. The research conducted during the period 2022-2023, at the Research and Development Station for Meadows, Vaslui (46°40' - 36°10' north latitude and 27°44' - 20°40' east longitude) pursued the influence of fertilization and the distance between rows on seed production (kg/ha) for smooth brome (*Bromus inermis* Leyss.). The organized experience was trifactorial, 2×3×5 type, it was placed according to the method of subdivided plots, with the plot harvestable area of 20 $m^2$ (2 m × 10 m), in three replications, and the studied factors were: A - variety ($a_1$ - Mihaela, $a_2$ - Iulia Safir), B - the distance between rows with three graduations ($b_1$ - 25 cm, $b_2$ - 37.5 cm and $b_3$ - 50 cm) and C - fertilization with five graduations ($c_1$ - unfertilized, $c_2$ - $N_{50}P_{50}$, $c_3$ - $N_{50}P_{50}K_{50}$, $c_4$ - $N_{75}P_{75}K_{75}$ and $c_5$ - $N_{100}P_{100}K_{100}$). Following the study, it was found that by applying mineral fertilized with $N_{75}P_{75}K_{75}$ and by sowing at 25 cm distances between rows seed production was higher.

**Key words**: variety, distance between rows, fertilization

In the context of climate change, drought is becoming the most significant and acute problem affecting crop growth, survival and persistence in many parts of the world, especially in arid and semi-arid regions (Mollasadeghi V. *et al*, 2011; Hussain S.S. *et al*, 2018). The development of drought-tolerant varieties is an essential objective of plant improvement programs. It is expected to be a key component in climate change mitigation, loss minimization and production stability strategies (Gustafan D.L., 2011).

Field management practices, including sowing, fertilization, irrigation and weed control etc., are important factors in improving seed yield. Research on perennial grasses has shown that agronomic practices, such as plant density, fertilization and residue management, have influenced the level of yield and the quality of seeds (Khan S. *et al*, 2017).

Permanent pastures, in Romania represent 33% of the total agricultural area (4.9 million ha) and they are an important forage resource but inappropriate management systems in the past have led to their present state of degradation (Vîntu V. *et al*, 2011; Samuil *et al*, 2012).

Rational use of fertilizers can produce substantial increases of the production and biodiversity and fodder quality improvement (Vîntu V. *et al*, 2008).

## MATERIAL AND METHOD

The purpose and objectives of the research conducted during the period of 2022-2023, at the Research and Development Station for Meadows, Vaslui (46°40' - 36°10' N latitude and 27°44' - 20°40' E longitude), were represented by the analysis of the influence of row spacing and fertilization on seed production ($kg \cdot ha^{-1}$), at the smooth brome (*Bromus inermis* Leyss.), in the third year of vegetation.

To achieve the proposed purpose, a trifactorial experience was organized, 2×3×5 type, placed according to the method of subdivided plots, with the plot harvestable area of 20 $m^2$ (2m x 10m), in three replications.

The studied factors were*:*

***A - variety*** with two graduations ($a_1$ - Mihaela, $a_2$ - Iulia Safir),

***B - the distance between rows*** with three graduations ($b_1$ - 25 cm, $b_2$ - 37.5 cm and $b_3$ - 50 cm),

***C - fertilization*** with five graduations ($c_1$ - unfertilized, $c_2$ - $N_{50}P_{50}$, $c_3$ - $N_{50}P_{50}K_{50}$, $c_4$ - $N_{75}P_{75}K_{75}$ and $c_5$ - $N_{100}P_{100}K_{100}$).

The biological material used is represented by the varieties Mihaela and Iulia Safir, both

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varieties were created at the Research and Development Station for Meadows, Vaslui (Silistru D., 2010; Silistru D., 2011).

The fertilizers were applied early in the spring, at the start of plant vegetation.

Seed production (kg·ha$^{-1}$) was determined by weighing the manually harvested seeds on each variant, then reporting to the area unit.

The agricultural year 2022-2023 was a dry year (*figure 1*), the amount of precipitation was 443.6 mm, 90.2 mm lower than the annual average (533.2 mm), affecting the growth and development of plants.

Although this agricultural year has been more rainy, the rainfall deficit of the previous year was felt this year as well. In terms of temperatures, it was a very hot year, the monthly average being 2.2 $^{o}$C higher than the multi-annual average. During the growing season the precipitation deficit and very high temperatures (May-June) had less favorable effects on the growth and development of smooth brome plants (*figure 2*).

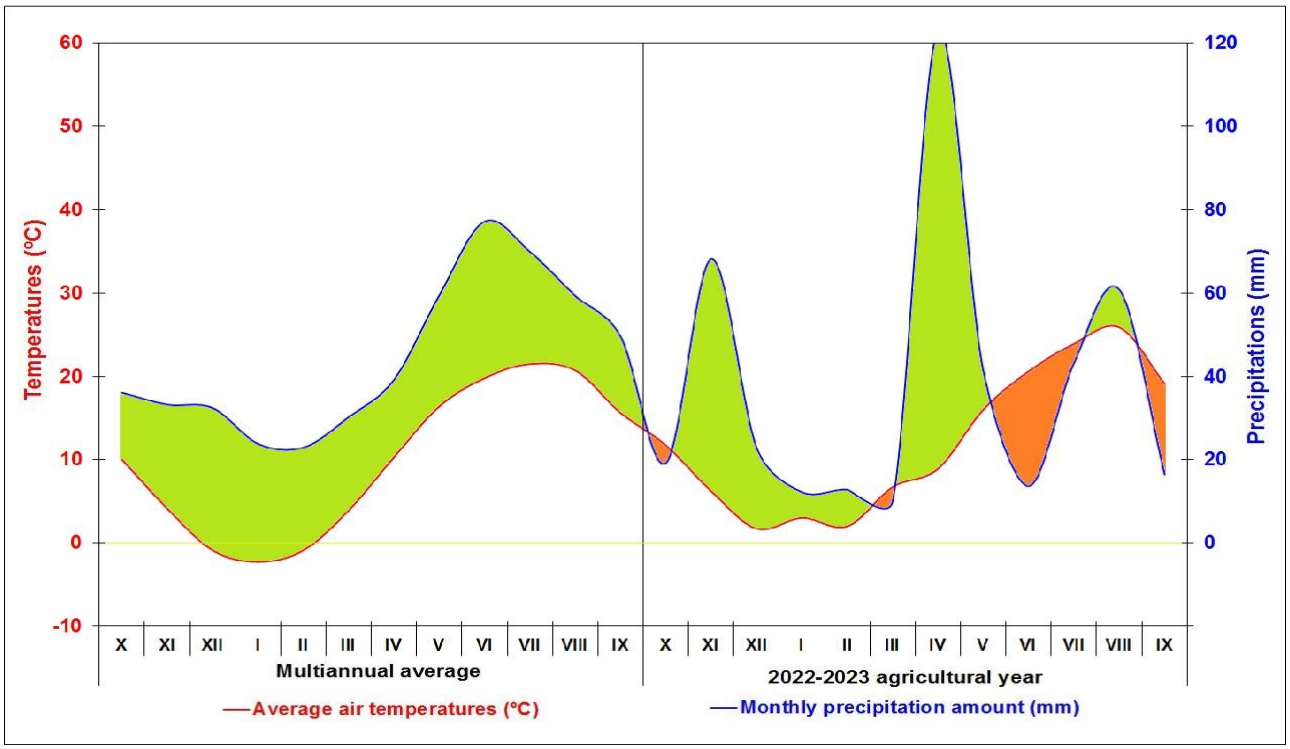


Figure 1 **Climadiagram of the 2022-2023 agricultural year**

Figure 2 - **Aspects of the experimental field**

The results were statistically interpreted by analyzing the variance and calculating the least significant differences.

## RESULTS AND DISCUSSIONS

Smooth brome (*Bromus inermis* Leyss.) is a perennial grass for meadows with high feed value and high productivity (Liu G.X. *et al*, 2014). It is characterized by high tolerance to drought and low temperatures and average resistance to soil salinity, this species being introduced for the restoration of degraded meadows and the establishment of pastures (Liu G. *et al*, 2008; Antonova E.V. *et al*, 2015).

Analyzing the interaction between the cultivated variety, the distance between the rows and the fertilization on the seed production at the smooth brome, in the third year of vegetation, at cycle I of vegetation (*table 1.*) values between 171 kg·ha$^{-1}$ at the $a_2b_3c_5$ variant (variety Iulia Safir, sown at 50 cm between rows, fertilized with $N_{100}P_{100}K_{100}$) and 363 kg·ha$^{-1}$ at the $a_2b_1c_4$ variant (variety Iulia Safir, sown 25 cm between rows, fertilized with $N_{75}P_{75}K_{75}$).

From the point of view of statistical insurance, positive differences were obtained in both varieties, while in variants sown at 25 cm between rows a very significant statistical difference was obtained in variants $a_1b_1c_4$, $a_2b_1c_1$-$c_5$.

The variety Iulia Safir obtained a larger amount of seeds (kg·ha$^{-1}$) on average by 0.10 kg·ha$^{-1}$ more (*figure 3.*) compared to the control variety (Mihaela). By sowing at shorter distances between rows, the highest values were obtained, by sowing at 25 cm between rows an average of 313 kg·ha$^{-1}$ was obtained, when increasing the sowing distance the production obtained was decreasing.

After the application of mineral fertilizers the values had a growth tendency, thus in the variant fertilized with $N_{75}P_{75}K_{75}$, the largest quantity of seed, 268 kg·ha$^{-1}$, was obtained, and from the point of view of statistical insurance, the same variant obtained a very significant statistical difference from the control variant.

Sowing at 25 cm between rows and by administering mineral fertilizers in the pedoclimatic conditions of the agricultural year 2022-2023, led to the obtaining of larger quantities of seeds (kg·ha$^{-1}$).

Table 1

**The influence of the distance between the rows and the fertilization on the seed production**

| Variant | | | Seed production (kg·ha$^{-1}$) | Diferences (kg·ha$^{-1}$) | Diferences (%) | Statistical significance |
|---|---|---|---|---|---|---|
| $a_1$ - Mihaela (control) | $b_1$ - 25 cm (control) | $c_1$ - unfertilized (control) | 259 | control | 100 | control |
| | | $c_2$ - $N_{50}P_{50}$ | 277 | 18 | 106,9 | |
| | | $c_3$ - $N_{50}P_{50}K_{50}$ | 277 | 18 | 106,9 | |
| | | $c_4$ - $N_{75}P_{75}K_{75}$ | 315 | 56 | 121,6 | *** |
| | | $c_5$ - $N_{100}P_{100}K_{100}$ | 288 | 29 | 111,2 | * |
| | $b_2$ - 37,5 cm | $c_1$ – unfertilized | 225 | -34 | 86,9 | oo |
| | | $c_2$ - $N_{50}P_{50}$ | 230 | -29 | 88,8 | o |
| | | $c_3$ - $N_{50}P_{50}K_{50}$ | 247 | -12 | 95,4 | |
| | | $c_4$ - $N_{75}P_{75}K_{75}$ | 255 | -4 | 98,5 | |
| | | $c_5$ - $N_{100}P_{100}K_{100}$ | 232 | -27 | 89,6 | oo |
| | $b_3$ - 50 cm | $c_1$ – unfertilized | 173 | -86 | 66,8 | ooo |
| | | $c_2$ - $N_{50}P_{50}$ | 192 | -67 | 74,1 | ooo |
| | | $c_3$ - $N_{50}P_{50}K_{50}$ | 207 | -52 | 79,9 | ooo |
| | | $c_4$ - $N_{75}P_{75}K_{75}$ | 217 | -42 | 83,8 | ooo |
| | | $c_5$ - $N_{100}P_{100}K_{100}$ | 205 | -54 | 79,2 | ooo |
| $a_2$ - Iulia Safir | $b_1$ - 25 cm | $c_1$ – unfertilized | 328 | 69 | 126,6 | *** |
| | | $c_2$ - $N_{50}P_{50}$ | 344 | 85 | 132,8 | *** |
| | | $c_3$ - $N_{50}P_{50}K_{50}$ | 333 | 74 | 128,6 | *** |
| | | $c_4$ - $N_{75}P_{75}K_{75}$ | 363 | 104 | 140,2 | *** |
| | | $c_5$ - $N_{100}P_{100}K_{100}$ | 341 | 82 | 131,7 | *** |
| | $b_2$ - 37,5 cm | $c_1$ - unfertilized | 208 | -51 | 80,3 | ooo |
| | | $c_2$ - $N_{50}P_{50}$ | 213 | -46 | 82,2 | ooo |
| | | $c_3$ - $N_{50}P_{50}K_{50}$ | 227 | -32 | 87,6 | oo |
| | | $c_4$ - $N_{75}P_{75}K_{75}$ | 258 | -1 | 99,6 | |
| | | $c_5$ - $N_{100}P_{100}K_{100}$ | 233 | -26 | 90,0 | o |
| | $b_3$ - 50 cm | $c_1$ - unfertilized | 180 | -79 | 69,5 | ooo |
| | | $c_2$ - $N_{50}P_{50}$ | 177 | -82 | 68,3 | ooo |
| | | $c_3$ - $N_{50}P_{50}K_{50}$ | 176 | -83 | 68,0 | ooo |
| | | $c_4$ - $N_{75}P_{75}K_{75}$ | 201 | -58 | 77,6 | ooo |
| | | $c_5$ - $N_{100}P_{100}K_{100}$ | 171 | -88 | 66,0 | ooo |
| | | *LSD* | *5%* | 22 | | |
| | | | *1%* | 30 | | |
| | | | *0,1%* | 38 | | |

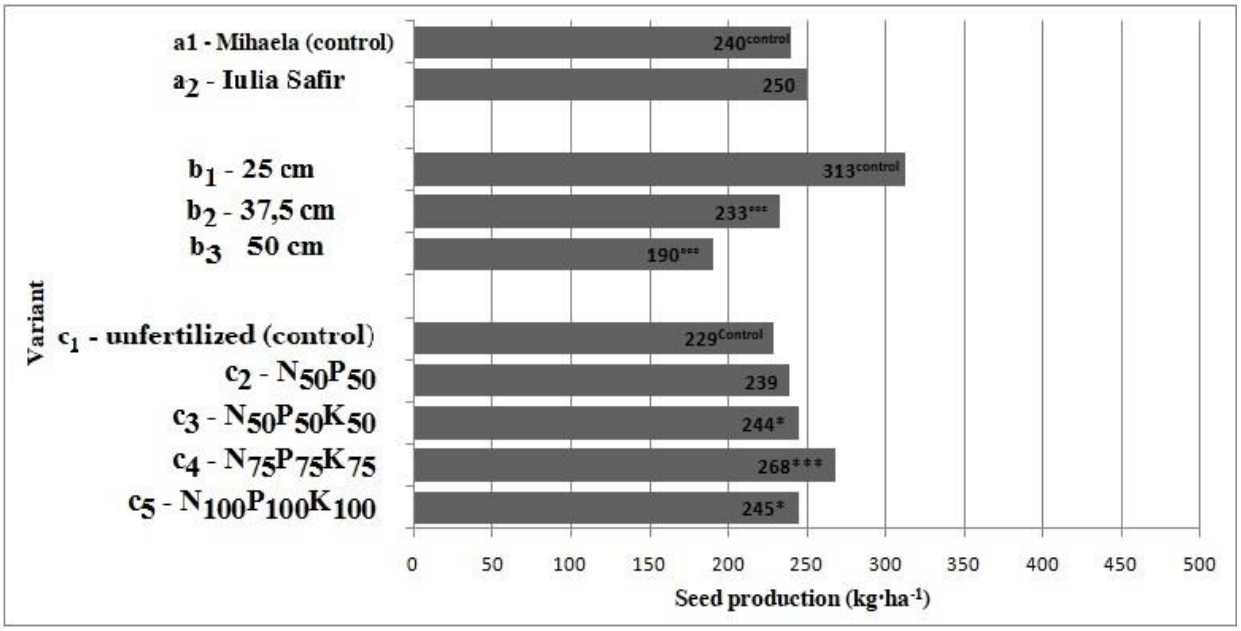


Figure 3 **Separate influence of the distance between the rows and the fertilization on the seed production**

## CONCLUSIONS

From the analysis of the results obtained it was differentiated that each of the studied factors influenced the quantity of seed obtained but in the climatic conditions specific to the agricultural year 2022-2023, the results obtained were greatly influenced by the atmospheric drought felt from the previous year.

The variety Iulia Safir obtained a larger amount of seeds (kg·ha$^{-1}$) on average by 0.10 (kg·ha$^{-1}$) more compared to the Mihaela variety.

By sowing at a distance of 25 cm between rows larger quantities of seed were obtained compared to the variants that were sown at larger distances.

By administering $N_{75}P_{75}K_{75}$ the largest quantities of seed were obtained (kg·ha$^{-1}$).

By sowing at 25 cm between rows, by administering mineral fertilizers with $N_{75}P_{75}K_{75}$ and using the variety Iulia Safir, the largest quantities of seeds (kg·ha$^{-1}$) were obtained.

## ACKNOWLEGMENTS

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# THE ECONOMIC EFFICIENCY OF THE IRRIGATION DEVELOPMENT FROM SC LIVADA MERE DE ITESTI BACĂU

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**Abstract**

The evaluation of the investment regarding the arrangement of the irrigation system within SC Livada mere de Iteşti SRL is made by calculating the economic efficiency. This is a fairly modern concept, that helps to substantiate decisions within the firm, in order to use resources as favorably as possible. The decision to invest in a complex set of measures, meant to bring production increases, as is the case with the irrigation system, was taken after an analysis of the evolution of the economic situation of the company. Any irrigation method used in a crop or plantation has the role of reducing the risks of the agricultural business. Therefore, especially in times of drought, crop yields and quality increase, creating additional incomes and, implicitly, profit. The economic efficiency assessment was calculated for the 2020 and 2021 crop years, because the plantation is young and has entered fruition in year 2 of planting. Important production increases were obtained, obtaining a 5% higher profit rate in year 4 of planting, compared to an non-irrigated orchard, which is in full fruit.

**Key words**: irrigation system, fruit plantation, economic efficiency

The apple tree is a crop with high water requirements, demanding 70-75% of the field capacity.The insufficient amount of water in the soil has a negative effect on the fruit plantation, especially in the first years after planting: the growth of the shoots is reduced, the development of the leaves is affected, the roots do not develop enough, and the entry on the fruiting is delayed. (Cimpoieş Gh., 2012)

The most widespread method of irrigation in superintensive apple orchards is local drip irrigation. It consists in the localized wetting of the soil area, in which most of the active roots of the trees are extended, by slowly administering the water in the given area, based on the physiological requirements of the trees. (Grădinariu G., 2002)

This method allows saving water and fuel, over 50% compared to other methods, saving energy when pumping, labor force at service, allows practicing on any type of relief and, at the same time, mineral fertilizers (fertigation) can be given through this installation. In the soil, favorable conditions are created for the development of the root system, the leaves and air from the plantation are not moistened, it is suitable for programming the optimal irrigation regime with the help of the computer (Popa S., 2017).

At the same time, the number of maintenance works is reduced because this irrigation system does not foster the formation of crusts on the soil surface nor the cryptogamic diseases (Yildirim F. *et al*, 2016). Due to the fact that the weeds develop quite difficult, the number of hoeing is reduced.

## MATERIAL AND METHOD

Applying a project for the reconversion of the apple orchard from Bereşti-Bistriţa commune, SC Livada de meri Iteşti SRL managed to make an extensive investment, which includes: the replacement of the 30-year-old plantation, which is in an advanced stage of physiological degradation, presenting 35% gaps, the development of a drip irrigation system, the construction of a concrete platform, the purchase of machinery and agricultural installations. (Cantoriu P., Tudorache A., 2016). The implementation period of the project is 3 years, and the trees begin to fructify in year 2 after planting.The economic and financial analysis was carried out taking into account the investment expenses with the irrigation system, as well as the operating expenses.

Data from the company's accounting records were used to calculate revenues and expenses.

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Table 1

**Investment expenditure on the development of the irrigation system**

| Item No. | Name | Value (lei) |
|---|---|---|
| 1 | Expenditure on the irrigation tank | 348835 |
| 2 | Expenditure related to the irrigation system | 310980 |
| 3 | Expenditure on water supply | 160409 |
| 4 | Expenditure related to design and engineering, consultancy and technical assistance | 41217 |
| 5 | Other expenditure | 2002 |
| Total expenditure | | 863443 |

Table 2

**Production costs for apple cultivation (lei/ha)**

| Item no. | Name of the work | Irrigated culture Cost (lei/ha) | | Non-irrigated culture Cost (lei/ha) | |
|---|---|---|---|---|---|
| | | 2020 | 2021 | 2020 | 2021 |
| 1 | Tree maintenance works | 4120 | 4200 | 4120 | 4200 |
| 2 | Basic fertilization works | 102 | 105 | 102 | 105 |
| 3 | Large screed per row | 350 | 355 | 350 | 355 |
| 4 | Irrigation of the crop | 1200 | 1200 | 0 | 0 |
| 5 | Maintenance of the drip irrigation installation | 928 | 952 | 0 | 0 |
| 6 | Application of foliar fertilizers | 265 | 272 | 265 | 272 |
| 7 | Fruit harvesting | 2850 | 2920 | 2630 | 2700 |
| 8 | Phytosanitary treatments | 1820 | 1860 | 1820 | 1860 |
| 9 | Other maintenance work | 550 | 560 | 550 | 560 |
| 10 | Materials | 2300 | 2360 | 2300 | 2360 |
| 11 | Wage expenses | 45120 | 45120 | 43260 | 43260 |
| | Total | 59605 | 59904 | 55397 | 55672 |

Table 3

**Average yields obtained (t/ha)**

| Year | Average production obtained in the irrigated system (t/ha) | Average production obtained in the non-irrigated system (t/ha) |
|---|---|---|
| 2020 | 26.8 | 27.5 |
| 2021 | 31.4 | 28.1 |

Table 4

**Average income from the sale of fruits (lei/ha) and registered profit (lei/ha)**

| Year | Irrigated plantation | | Non-irrigated plantation | |
|---|---|---|---|---|
| | Income (lei/ha) | Profit (lei/ha) | Income (lei/ha) | Profit (lei/ha) |
| 2020 | 67000 | 7096 | 68750 | 13353 |
| 2021 | 78500 | 18596 | 70250 | 14578 |

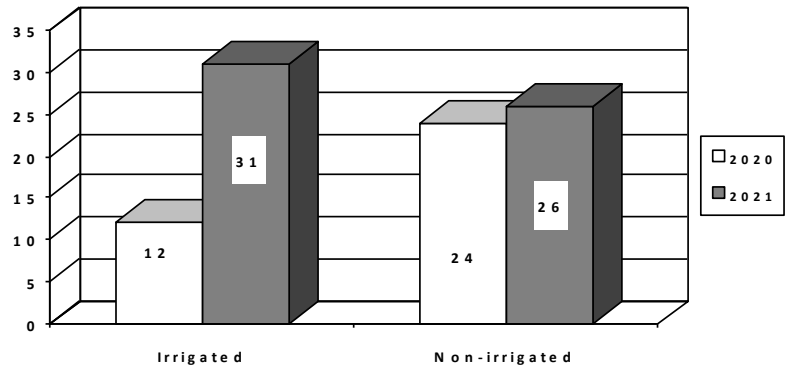


Figure 1. **Profit rate (%)**

## RESULTS AND DISCUSSIONS

Analyzing the consumption of apples, both nationally as well as internationally, it is noticed that Romanians have the lowest consumption of apples among the European states, of only 220 g / person / day. WHO recommends a consumption of at least 440 g fruits and vegetables per person /

day, for a healthy intake of nutrients to the human body.

The agricultural market comprises both producers and those who sell the products – either fresh or industrialised. The apple can be consumed both fresh and industrialized, in the form of juice, nectar, jams, but also used in pastries and confectionery. The demand for apples on the market will always be constant (MADR, 2014).

By ensuring the optimal water intake, due to the installation of the irrigation system, the productions will be of quality and in a sufficient quantity to cover the local market, as well as to provide raw materials to the processing companies in the area.

The market and marketing strategies are related to distribution, so that by correctly establishing the criteria for the qualification of the distributors, the discount policy and the types of distribution channels, the company will be able to efficiently and profitably capitalize on its apple harvests. (Mihăescu G., 2007).

The managerial policies of SC Livada mere de Iteşti SRL are based on practicing an average price, compared to those practiced by other local producers, for similar products.

Setting the price at 2.5 lei/kg took into account the commercial value of apples from the young orchard, more qualitative than those from the previous orchard (which is in physiological decline).

The total value of the investment is 3851.915 thousand lei, including all expenses generated by the activities carried out during implementation, according to the general estimate (Cantoriu P. *et al*, 2016).

The investment costs related to the development of the irrigation system are shown in *table 1*.

For the tank and the water supply there were expenses generated by the constructions, in addition to the expenses for materials and installation.

The irrigation system only generated expenses for materials and installation.

The watering norm for the drip irrigation system (sqm) is lower than the norms applied to the other watering methods, because it is calculated only for wetting an area smaller than the volume occupied by the entire crop.

In the case of drip irrigation, it is optimal to wet the soil in a proportion of 20% of the volume of soil that reaches to the roots of plants, in sub-humid areas (Yildirim F. *et al*, 2016).

In the years of production 2020 and 2021, 2 irrigations were applied annually, with a norm of 4 liters/tree, the first in the period 15-25 July, the second in the period 5-15 September, which meant a consumption on the entire plantation of 446 cubic meters.

The water and electricity costs necessary for the operation of the irrigation system are 1200 lei/ha, annually.

The maintenance costs of the installation, which amounted to 928 lei/ha in 2020 and 952 lei/ha in 2021, add to the mentionned costs.

The personnel expenses, both permanent and seasonal, calculated for 1 ha were 45120 lei, in both years under analysis.

The works for the maintenance of the crop as well as the cost of materials have been calculated per unit of area, for each year, and are presented in *table 2*.

Irrigated production was compared with the non-irrigated production in the same local producer organisation, where there are the same environmental conditions, the same cropping system (superintensive) and the same varieties grown (*table 3*).

The only difference was that the non-irrigated plantation, the reference one, is in full fruit, being at the 9$^{th}$ and, respectively, the 10$^{th}$ year of fruiting.

The years 2020 and 2021 meant the 3$^{rd}$ and 4$^{th}$ years of crop of the young, irrigated orchard, so the trees did not reach the maximum fruiting potential, and therefore, the average yields obtained are lower.

However, very large differences in production are recorded from one year to the next. This is due to the fact that some varieties later came into fruiting, only in the 4$^{th}$ year after planting (2021), with a rather modest fruiting.

Compared to the non-irrigated tree plantation, but which is already in the stage of maximum productivity, the young, irrigated orchard has achieved higher yields.

The proceeds from the sale of the fruit and the profits made are shown in *table 4*.

The rate of profit of irrigated and non-irrigated crops is shown in *figure 1*.

In 2020, being the third year of cultivation, the average yield was quite low, so that the profitability of the crop was also modest.

In the following year, higher production was achieved, the profitability of the irrigated plantation already exceeds the profitability of the non-irrigated plantation.

## CONCLUSIONS

The demand for apple fruits is constantly increasing in Romania, with the development of

small fruit and vegetable processing enterprises, the apple being a basic raw material.

Preferred in consumption both fresh and processed, the nutritional and curative principles of the apple are known for a long time.

Lately, the apple has become a raw material for confectionery and pastry products, so that the market has diversified.

The expenses for the investment in the development of the irrigation system are of 863443 lei.

Production costs were higher in the second year under review, mainly due to higher input costs, with average production almost 5 t/ha higher compared to the previous year.

In the first years of fruiting the yields are small, the full capacity being reached only from the 6$^{th}$ year after planting.

However, revenues from the sale of production are starting to cover the budget deficit caused by the investment with the irrigation system and the planting of the new orchard.

Compared to the non-irigated apple plantation, where the trees are at the maximum fruiting potency, the young plantation, thanks to the drip irrigation system, suceeds to obtain higher yields in 2021.

Thus, the rate of profit of the young apple orchard exceeded the rate of profit of a plantation in full fruit, but not irrigated.

The increase in production due to irrigation is very high and the revenues from the sale of production are increasing. This is the premise for a faster coverage of the investment made.

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# THE PYTOMASS QUALITY OF CHICKPEA, *CICER ARIETINUM* L., UNDER THE CONDITIONS OF MOLDOVA

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**Abstract**

We investigated the quality indices of the phytomass from chickpea, *Cicer arietinum*, the local cultivar *'Ichel'*, grown in monoculture in an experimental field of the National Botanical Garden (Institute), Chişinău, Republic of Moldova It was determined that the nutrient content of the dry matter of chickpea whole plants cut in the flowering - early pod stage included: 19.31% CP, 4.23% EE, 22.62% CF, 43.57% NFE, 10.26 % ash, 1.45% Ca, 0.32% P with 18.46 MJ/kg GE, 9.83 MJ/kg ME, 5.74 MJ/kg NEl. The fermentation quality and nutritive value of silage and haylage prepared from chickpea plants were characterized by the following indices: pH= 4.40-4.47, 27.6-34.5 g/kg lactic acid, 2.5-2.8 g/kg acetic acid, 0.3-0.4 g/kg butyric acid, 19.64-20.61% CP, 25.67-6.15% EE, 24.57-25.32 % CF, 36.77-39.20% NFE 10.92-11.15 % ash, 1.39-1.41% Ca, 0.35-0.39 % P, 18.75-18.90 MJ/kg GE, 9.81-9.81 MJ/kg ME, 5.47-5.55 MJ/kg NEl. The nutrient content and energy value of the prepared hay was: 19.77% CP, 2.64% EE, 27.01% CF, 39.21% NFE, 11.37% ash, 1.46% Ca, 0.31% P, 18.07 MJ/kg GE, 8.81 MJ/kg ME, 4.98 MJ/kg NEl. It has been determined that the studied fresh and ensiled substrates have C/N=15-16 and the biochemical methane potential reaches 312-322 l/kg ODM. The local chickpea cultivar *'Ichel'* can be used as an alternative forage source for farm animals or as co-substrate in biogas generators for renewable energy production.

**Key words**: biochemical composition, biomethane potential, *Cicer arietinum*, green mass, hay, haylage, nutritive value, silage

Feed quality is a crucial factor for better health and performance of livestock. Forage crops play a significant role in agriculture and the animal food supply chain. Climate change not only dramatically affects forage yields but also alters the nutritive value of forage.

*Fabaceae* species are grown across the world as pulse crops, forage and fodder for animals. As many as 60 different legume crops have been cultivated as sources of forage and feed for animals. The agricultural policy of the EU encouraging the increase of the area cultivated with legumes has a dual purpose, on the one hand, environmental, on the other, providing more protein from own sources in animal nutrition. Forage legumes are an important source of feed for livestock and possess the potential to provide a sustainable solution for food and protein security. Some of the annual grain legumes can be used, not only for the grain itself, but the whole plant as a source of green forage or hay, including the straws after grain threshing. The efficient use of the biological potential of the *Fabaceae* plants that are adapted to the local climatic conditions becomes more and more relevant (Kulkarni K.P. *et al*, 2018).

*Cicer arietinum* L., commonly known as chickpea, belongs to family *Fabaceae*, and is native to the south-eastern area of Turkey and adjoining Syria. This is one of the earliest cultivated legumes in the world, currently grown on an area of 17.8 million hectares (Maphosa L. *et al*, 2020). *Cicer arietinum* is an herbaceous annual plant; the stem is erect, branched, viscous, hairy, terete, green and solid. The leaves are petiolate, compound, and uni-imparipinnate (pseudoimparipinnate), the rachis is 3-7 cm long, with 10-15 leaflets that are 8-17 mm long and 5-14 mm wide, opposite or alternate with a terminal leaflet. The solitary flowers are borne in an axillary raceme. Sometimes, there are 2 or 3 flowers on the same node. The pod is about 2 cm long and usually contains two seeds. Chickpea is cultivated mainly in arid and semi-arid areas in more than 50 countries across the Mediterranean Basin, Central Asia, East Africa, Europe, Australia, and North and South America. (Maessen L.J.G., 1972; Balashov V.V. *et al*, 2012; Voshedsky N.N. *et al*,

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2020; Vargas-Blandino D., Cárdenas-Travieso R.M., 2021).

Makenova S.K. (2005) remarked that chickpea crops in the southern forest-steppe of the Omsk region, Russia, made it possible to obtain an average of 21.2 t/ha of green mass, 4.66 t/ha of fodder units and 0.91 t/ha of crude protein. Kertikov T., Kertikova D. (2016) found that the chickpea plants contained 40.8 % stems, 50.7 % leaves and 6.7 % inflorescences, the productivity was 21 t/ha fresh mass, 4.58 t/ha dry matter and 774 kg/ha crude protein.

In our region, chickpea has also been researched, thus, new productive cultivars have been created and cultivated (Arseni A.A, 1974; Celac V., Machedon M., 2010).

The objective of this research was to evaluate the quality indices of green phytomass, hay, haylage and silage prepared from chickpea, *Cicer arietinum*, and the prospects of its use as forage for farm animals or as substrates for biomethane production.

## MATERIALS AND METHODS

The local cultivar *'Ichel'* of chickpea, *Cicer arietinum*, created at the Institute of Genetics, Physiology and Plant Protection by professor Valentin Celac, and grown in monoculture on the experimental sector 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. Alfalfa - *Medicago sativa*, sand sainfoin - *Onobrychis arenaria,* common sainfoin - *Onobrychis vicifolia,* and bird's-foot-trefoil - *Lotus corniculatus* were used as control variants. The chickpea samples were collected in the flowering - early pod stage, and alfalfa, sand sainfoin and bird's-foot-trefoil, the first cut, in early flowering periods. The prepared hay was dried directly in the field. The chickpea silage was prepared from directly harvested green mass, but haylage was produced from wilted green mass, cut into small pieces. The chopped green mass or wilted green mass was compressed in well-sealed glass containers, stored at ambient temperature (18-20 C) for 45 days, to allow complete fermentation to occur. Following the 45-day fermentation period, each glass container was opened and the content was visually examined, the colour and the aroma were recorded. The dry matter content was detected by drying samples up to constant weight at 105°C. For biochemical analysis, the plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm. The pH of the samples of silage and haylages was measured immediately after removal from the containers. At the same time, samples were taken to determine the content of organic acids (lactic, acetic and butyric) in free and fixed state. The evaluation of chemical composition: crude protein (CP), crude fat (EE), crude cellulose (CF), nitrogen-free extract (NFE), ash, calcium (Ca), phosphorus (P), content of organic acids in silage were carried out in the Laboratory of Nutrition and Forage Technology of the Scientific-Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine, in accordance with the methodological indications. The gross energy (GE), metabolizable energy (ME), net energy for lactation (NEl) were calculated according to standard procedures.

The carbon content of the substrates was determined using an empirical equation according to Badger C.M. *et al*, (1979). The biochemical methane potential was calculated according to Baserga U. (1998) corrected with the digestibility index of nutrients.

## RESULTS AND DISCUSSIONS

Forages are a major source of nutrients for herbivores. Sometimes the balance of nutrients or the presence of some constituents in the forage will have positive or negative effects on animal health and productivity. The biochemical composition nutritive and energy value of the green mass from chickpea, *Cicer arietinum*, is presented in *table 1*. We would like to mention that chickpea green fodder was characterised by higher content of crude protein, crude fats, but low level of crude cellulose, as compared with the green forage produced from the traditional forage legume crops – alfalfa, sainfoin and bird's-foot-trefoil. The concentration of nitrogen free extract in chickpea green fodder is optimal (43.57 %), about the same level as in sainfoin, but much higher as compared with alfalfa and bird's-foot-trefoil forage. The content of mineral substances in chickpea green reaches 10.26 %, exceeding essentially sainfoin and alfalfa fodders. Besides, the amount of phosphorus and calcium is much higher than in the traditional forage legume crops. The chickpea fodder is characterized by very high digestibility, which has a positive effect on the concentration of metabolizable energy (9.83 MJ/kg) and net energy for lactation (5.74 MJ/kg).

In the specialized literature, there is little information regarding the nutrient content of green mass from the genus *Cicer*. Larin I.V. *et al* (1952), reported that the harvested *Cicer macracanthum* plants contained in dry matter 14.8 % crude protein, 3.5 % crude fats, 21.6 % crude cellulose and 53.8 % nitrogen free extract. Maessen L.J.G.V (1972) remarked that *Cicer arietinum* forage contained 10.8-11.3 % crude protein, 2.1-2.2 % crude fats, 27.2-33.1 % crude cellulose, 444.9-48.0 % nitrogen free extract, 9.1-11.4 % ash. Kirilov A. *et al* (2016) compared the quality of green mass of

whole plants of perennial and annual legumes harvested in the flowering-pod formation stage, and reported that the chemical composition of *Cicer arietinum* was 14.06 % crude protein, 3.44 % crude fats, 27.14 % crude cellulose, 44.04 % nitrogen free extract and 11.32 % ash; *Onobrychis viciifolia*, in turn, contained 17.53 % crude protein, 3.12 % crude fats, 20.08 % crude cellulose, 51.17 % nitrogen free extract and 8.1 % ash; *Medicago sativa*: 17.36 % crude protein, 2.32 % crude fats, 27.84 % crude cellulose, 42.63 % nitrogen free extract and 9.85 % ash; *Lotus corniculatus*: 17.14 % crude protein, 3.14 % crude fats, 25.63 % crude cellulose, 45.32 % nitrogen free extract and 8.77 % ash; *Pisum sativum* 13.04 % crude protein, 2.14 % crude fats, 25.06 % crude cellulose, 58.30 % nitrogen free extract and 8.01 % ash; *Glycine max* 13.13 % crude protein, 2.48 % crude fats, 29.87 % crude cellulose, 45.50 % nitrogen free extract and 9.02 % ash. Nasiyev B. N. *et al* (2017) mentioned that the protein content of fodder units for green forage at chickpea reached 213.4 g, in barley green forage – 99.94 g, but the combination of barley and chickpea green forage contained 197.71 g, respectively. Tedeeva V.V. (2018) reported that chickpea leaves contained 2.16-3.48 % N, 0.31-0.49 % $P_2O5$, 2.09-2.36 % $K_2O$ and chickpea stems – 1.57-2.40 % N, 0.28-0.36 % $P_2O_5$, 1.71-2.01 % $K_2O$, respectively. Semina A. Yu., Telic K.M., (2020) evaluating the quality of 15 collection samples of *Cicer arietinum* of various ecological and geographical origin, mentioned that the protein content in the green mass varied from 10.64 % to 15.06 %. Voshedsky N.N. *et al* (2020) found that the chemical composition of plants of the chickpea cultivar 'Donplaza' harvested in the flowering period was 2.41-4.19 % N, 0.84-1.24 % $P_2O_5$, 3.22-4.12 % $K_2O$.

Hay is the oldest, and still the most important conserved fodder, despite its dependence on suitable weather at harvest time. Hay is an essential part of livestock diet, providing them, during winter, with the necessary protein, fibers and other nutrients they need to maintain good health and be productive. The results regarding the forage quality of hay prepared from the studied forage legume species are shown in *table 2*. We would like to mention that the hay prepared from *Cicer arietinum* is characterized by high content of crude protein (19.77%) and minerals (11.37%), optimal nitrogen free extract content (43.57%), but lower content of crude cellulose. The concentration of crude fats in chickpea hay is optimal (2.64%) about the same level as bird's-foot-trefoil hay. It was found that the levels of phosphorus and calcium in chickpea hay were significantly higher as compared with sand sainfoin and bird's-foot-trefoil hays.

Table 1

**The biochemical composition and the fodder value of the green mass from the studied leguminous species**

| Indices | *Cicer arietinum* | *Medicago sativa* | *Onobrychis arenaria* | *Onobrychis vicifolia* | *Lotus corniculatus* |
|---|---|---|---|---|---|
| Crude protein, % DM | 19.31 | 16.28 | 16.96 | 15.29 | 16.35 |
| Crude fats, % DM | 4.23 | 2.75 | 3.18 | 3.03 | 3.91 |
| Crude cellulose, % DM | 22.62 | 33.25 | 27.72 | 29.03 | 35.70 |
| Nitrogen free extract, % DM | 43.57 | 39.58 | 43.74 | 45.58 | 33.83 |
| Ash, % DM | 10.26 | 8.22 | 7.32 | 6.97 | 10.21 |
| Calcium, % DM | 1.45 | 1.43 | 0.73 | 0.82 | 1.06 |
| Phosphorus, % | 0.32 | 0.22 | 0.25 | 0.22 | 0.26 |
| Gross energy, MJ/ kg | 18.46 | 18.59 | 18.54 | 18.68 | 18.57 |
| Metabolizable energy, MJ/ kg | 9.83 | 8.25 | 9.11 | 9.07 | 8.13 |
| Net energy for lactation, MJ/ kg | 5.74 | 4.56 | 5.15 | 5.12 | 4.40 |

Table 2

**The biochemical composition and the fodder value of the hay from the studied leguminous species**

| Indices | *Cicer arietinum* | *Onobrychis arenaria* | *Lotus corniculatus* |
|---|---|---|---|
| Crude protein, % DM | 19.77 | 16.38 | 18.19 |
| Crude fats, % DM | 2.64 | 1.70 | 2.56 |
| Crude cellulose, % DM | 27.01 | 36.67 | 32.42 |
| Nitrogen free extract, % DM | 39.21 | 37.71 | 36.33 |
| Ash, % DM | 11.37 | 7.55 | 10.49 |
| Calcium, % DM | 1.46 | 0.77 | 1.16 |
| Phosphorus, % | 0.31 | 0.23 | 0.29 |
| Gross energy, MJ/ kg | 18.07 | 18.56 | 18.25 |
| Metabolizable energy, MJ/ kg | 8.1 | 7.50 | 8.11 |
| Net energy for lactation, MJ/ kg | 4.98 | 4.12 | 4.49 |

Table 3

**The fermentation quality and biochemical composition of the investigated ensiled forage**

| Indices | *Cicer arietinum* | | *Onobrychis arenaria* haylage | *Lotus corniculatus* haylage |
|---|---|---|---|---|
| | *silage* | haylage | | |
| pH index | 4.50 | 4.47 | 5.16 | 4.70 |
| Total organic acids, g/kg | 45.3 | 49.8 | 60.5 | 37.1 |
| Free acetic acid, g/kg | 2.1 | 2.2 | 0.6 | 1.7 |
| Free butyric acid, g/kg | 0.0 | 0.0 | 0.0 | 0.2 |
| Free lactic acid, g/kg | 10.8 | 10.3 | 4.1 | 7.6 |
| Fixed acetic acid, g/kg | 2.2 | 2.5 | 2.4 | 4.0 |
| Fixed butyric acid, g/kg | 0.4 | 0.3 | 0.4 | 0.0 |
| Fixed lactic acid, g/kg | 29.8 | 34.5 | 53.0 | 23.6 |
| Total acetic acid, g/kg | 4.3 | 4.7 | 3.0 | 5.7 |
| Total butyric acid, g/kg | 0.4 | 0.3 | 0.4 | 0.2 |
| Total lactic acid, g/kg | 40.6 | 44.8 | 57.1 | 31.2 |
| Acetic acid, % total acids | 9.49 | 9.43 | 4.96 | 15.36 |
| Butyric acid, % total acids | 0.89 | 0.62 | 0.66 | 0.54 |
| Lactic acid, % total acids | 89.63 | 89.95 | 94.38 | 84.10 |
| Crude protein, % DM | 19.64 | 20.61 | 16.49 | 17.09 |
| Crude fats, % DM | 5.67 | 6.15 | 2.61 | 4.16 |
| Crude cellulose, % DM | 24.57 | 25.37 | 30.03 | 33.22 |
| Nitrogen free extract, % DM | 39.20 | 36.77 | 42.89 | 35.75 |
| Ash, % DM | 10.92 | 11.15 | 7.99 | 9.78 |
| Calcium, % DM | 1.41 | 1.39 | 0.99 | 1.00 |
| Phosphorus, % | 0.35 | 0.39 | 0.30 | 0.27 |
| Gross energy, MJ/ kg | 18.75 | 18.90 | 18.58 | 18.68 |
| Metabolizable energy, MJ/ kg | 9.81 | 9.70 | 8.69 | 8.55 |
| Net energy for lactation, MJ/ kg | 5.55 | 5.47 | 4.89 | 4.64 |

Table 4

**Biochemical methane production potential of green and ensiled mass substrates from leguminous species**

| Indices | *Cicer arietinum* | | | *Medicago sativa* | *Onobrychis arenaria* | | *Onobrychis vicifolia* | *Lotus corniculatus* | |
|---|---|---|---|---|---|---|---|---|---|
| | green mass | silage | haylage | green mass | green mass | haylage | green mass | green mass | haylage |
| Organic dry matter, g/kg | 897.4 | 890.8 | 888.5 | 917.8 | 926.8 | 920.1 | 930.3 | 897.9 | 902.2 |
| Digestible matter, g/kg | 640.8 | 631.3 | 628.0 | 580.9 | 603.4 | 603.4 | 609.5 | 621.6 | 625.9 |
| Digestible proteins, g/kg | 142.9 | 145.3 | 152.5 | 122.1 | 127.2 | 123.6 | 114.6 | 119.4 | 124.8 |
| Digestible fats, g/kg | 24.1 | 32.3 | 35.1 | 13.7 | 17.5 | 14.4 | 16.7 | 23.1 | 24.5 |
| Digestible carbohydrates, g/kg | 473.8 | 453.7 | 440.4 | 445.1 | 458.7 | 465.4 | 478.8 | 479.1 | 476.6 |
| Carbon, g/kg | 498.6 | 497.9 | 493.6 | 509.9 | 514.9 | 511.2 | 516.8 | 498.8 | 501.2 |
| Nitrogen, g/kg | 30.9 | 31.4 | 33.0 | 26.0 | 27.1 | 26.4 | 24.5 | 26.2 | 27.3 |
| Ratio carbon/nitrogen | 16.2 | 15.8 | 15.0 | 19.6 | 19.0 | 19.4 | 21.1 | 19.0 | 18.4 |
| Biochemical methane potential, L/kg DM | 289 | 278 | 279 | 247 | 258 | 257 | 260 | 267 | 270 |
| Biochemical methane potential, L/kg OM | 322 | 312 | 314 | 269 | 278 | 279 | 279 | 297 | 299 |

The energy supply of the chickpea hay reached 8.81MJ/kg metabolizable energy and 4.98 MJ/kg net energy for lactation, exceeding the hay produced from sand sainfoin and bird's-foot-trefoil.

According to Karpova O.S. *et al* (1964), the concentration of nutrients in chickpea hay was 21.9 % crude protein, 2.4 % crude fats, 34.2 % crude cellulose, 29.4 % nitrogen free extract. Maessen L.J.G.V (1972) found that the chemical composition of *Cicer arietinum* hay was 12.9 % crude protein, 1.5 % crude fats, 36.3 % crude cellulose, 38.1 % nitrogen free extract and 11.2 % ash. Sainz-Ramírez A. *et al* (2022) reported that the dry matter content, the chemical composition and nutritive value sunflower-chickpea hay consisted of 694.07 g/kg DM, 17.34 % crude protein, 17.12 % crude fats, 42.52 % NDF, 27.03 % ADF, 11.00 % ash and 67.84 % IVDOM, but alfalfa hay contained 880.14 g/kg DM, 18.05 % crude protein, 2.25 % crude fats, 36.06 % NDF, 28.05 % ADF, 10.00 % ash and 67.04 % IVDOM, respectively.

Ensiling, a fermentation process, is now a major conservation method for large-scale enterprises. The production of fermented fodder,

silage and haylage, minimizes the risk associated with field losses, which can be incurred under rainy conditions during hay making. Besides, fermented fodders are an important source of nutrients for the dairy production sector in the autumn - middle spring period. When opening the glass containers with silage and haylage from *Cicer arietinum*, there was no gas or juice leakage from the preserved mass. The chickpea fermented mass had homogeneous, agreeable olive colour with pleasant smell, similar to the smell of green pea, the texture was preserved, in comparison with the initial green mass, without mould and mucus. The haylage prepared from *Lotus corniculatus* consists of green leaves and yellowish-green stems, has a pleasant smell of pickled vegetables; the texture of the plants stored as haylage was preserved well, without mold and mucus. The *Onobrychis arenaria* haylage had yellowish-green leaves and yellow-green stems with pleasant smell like pickled vegetables. The results regarding the quality of the fermented fodder from studied forage legume species are illustrated in *table 3*. It was determined that the pH values of the fermented fodder depended on the species, thus, chickpea silage had pH=4.4 and haylage pH=4.47, lower than sand sainfoin and bird's-foot-trefoil haylages. The concentration of organic acids in the chickpea ensiled forage is high in comparison with sainfoin haylage, but lower than bird's-foot-trefoil haylage. Most organic acids in the investigated fermented fodders were in fixed form. According to the Moldavian standard SM 108, the ratio of acetic acid and lactic acid of the studied fermented fodders correspond to the first class quality. In chickpea fermented fodders, butyric acid was detected in fixed form, in very small quantity (0.3-0.4 g/kg). Analyzing the biochemical composition of fermented fodders, it has been determined that the concentrations of nutrients in the dry matter varied. We would like to mention that chickpea fermented forage contained high content of crude protein (19.64-20.61%) and crude fats (5.67-6.15%), but low concentration of crude cellulose (24.57-25.37%) which had a positive effect on the energy supply 9.70-9.81MJ/kg metabolizable energy and 5.47-5.55 MJ/kg net energy for lactation, being higher than in the haylage produced from sand sainfoin and bird's-foot-trefoil. The concentration of phosphorus and calcium in chickpea fermented forage was significantly higher as compared with sand sainfoin and bird's-foot-trefoil haylages. We would like to mention that chickpea haylage contains higher amounts of crude protein, crude fats, crude cellulose, ash and phosphorus, but lower amount of nitrogen free extract, as compared with the chickpea silage.

Yücel C. *et al* (2020) found that chickpea silage had pH=5.66 and its dry mater contained 12.4% CP, 39.3% ADF, 42.5% NDF, 58.3% OMD and RFV=131.

Renewable bioenergy is an interesting alternative to meet the world's energy needs without extra economic burden and any significant environmental impacts. Biogas is a product of anaerobic fermentation of organic products. Among the fuels from plant biomass, biogas has a great importance and can successfully replace fossil fuels to obtain electric power and heat, also organic fertilizers. The quantities of biogas and the methane that can be produced from a substrate depend mainly on its content of carbohydrates, fats and proteins, its biodegradability and its carbon to nitrogen ratio. It is a commonly known fact that methanogenic bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes, ratios higher than 30:1 were found to be unsuitable for optimal digestion, and ratios lower than 10:1 were found to be inhibitory, because of low pH, poor buffering capacity and high concentrations of ammonia in the substrate. The results of the determination of the quality of substrates from the studied forage legume species and their biochemical methane production potential are presented in *table 4*. The nitrogen content in the studied substrates ranged from 24.5 to 33.0 g/kg, the estimated content of carbon – from 497.9 to 516.8 g/kg, the C/N ratio varied from 15.0 to 21. It is well known that fats are a good source of energy. Carbohydrates supply most of the energy for maintaining vitality. The two carbohydrate fractions commonly used in evaluating the carbohydrate content of substrates are crude cellulose and nitrogen-free extract. The capability of biomass methanization is tightly associated with nutrient digestibility and plant species. When crude cellulose content increases, digestibility usually decreases. Nitrogen-free extract contains the most digestible portion of the carbohydrates. Digestible organic matter or fermentable organic matter represents the proportion of organic matter which can be biologically degraded under anaerobic conditions and, thus, can be potentially utilized in biogas facilities. The digestible organic matter concentration in the tested forage legume substrates ranged from 580.9 to 640.8 g/kg, the biochemical methane production potential varied from 247 to 289 l/kg DM or 269 to 322 l/kg OM. The best results were achieved in chickpea substrates with high level of digestible organic matter and low content of crude fiber.

## CONCLUSIONS

The local chickpea cultivar *'Ichel'*, which has high content of crude protein and crude fats, can be used as an alternative forage source for farm animals or as co-substrate in biogas generators for renewable energy production.


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# RESEARCH ON THE INFLUENCE OF GROWTH REGULATORS ON THE WATER REGIME OF SOYBEAN PLANTS IN THE CONTEXT OF CLIMATE CHANGE IN CENTRAL MOLDAVIA

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**Abstract**

Soybean is a globally valued and sought-after crop due to its high protein and oil content in the beans, as well as its ecological adaptability. Water is an indispensable component for plant life, and its absence is the most important abiotic factor negatively influencing the quality and quantity of agricultural yields. Analyzing the climatic conditions in Central Moldavia, there has been an observed increase in temperatures in recent years, coupled with a reduction in precipitation. This paper presents the results obtained from a bifactorial experiment aimed at determining the influence of growth regulators on the water regime of soybean plants in response to climate change, achieved through the application of different growth regulator treatments. The experiment was conducted in the experimental field of A.R.D.S. Secuieni in the year 2023. According to the determinations made, the rate of dehydration varied both according to the soybean variety and the applied treatment. In the first hour of dehydration, the percentage values of total water content ranged from 79.92 % (untreated Onix variety) to 92.79 % (Iris variety treated with Toprex), and after 24 hours, the values of total water content ranged from 25.69 % (Ziana variety treated with Moddus Evo) to 33.54 % (Iris variety treated with Toprex).

**Key words:** water, growth, drought, soybean

Soybean is a herbaceous crop belonging to the botanical family Fabaceae, subfamily *Fabioideae*, genus *Glycine L*. The most important and widely cultivated species of this plant is *Glycine max. L*., also known as *Glycine hispida* (Moench.) Maxim (Mogârzan A., 2012).

The word "soia" (soybean) comes from the Chinese word "Shiang-yu," pronounced "shoyu" in Japanese. It's important to note that it originally referred not to the whole plant, but to soy sauce. The word evolved to "so-ya" in Japan and was subsequently adopted by the countries where the crop was introduced, eventually encompassing the entire plant (Dencescu S. *et al*, 1982).

In the early 1900s, the properties of soybean were discovered outside of Asia. Today, soybean is a globally valued and sought-after crop due to its ecological adaptability (Celeac V., Budac A., 2013).

Soybean is also referred to as the plant of the future, potentially capable of meeting the global protein demand. As such, soybean is cultivated for its seeds which are rich in protein (33.0 – 45%) and lipids (18 – 24.5%) (Roman G.V. *et al*, 2011).

Water is an indispensable component for the life of plants and plays a crucial physiological role. At the cellular level, it provides the optimal environment for vital biochemical reactions. In plants, water acts as a thermoregulator through its circulation within the plant organism and the process of transpiration. Additionally, water is involved in cell elongation, ensuring the normal volume of tissues and plant organs during the growth process (Jităreanu C.D., Toma L.D., 2007).

Reducing the water content in plants leads to the inhibition of the growth process, a decrease in the speed of assimilate translocation, and a reduction in the intensity of photosynthesis. Furthermore, by negatively influencing enzymatic activity, biochemical processes within the plant are affected (Burzo I., 2015).

Crop yield and quality are directly influenced by temperatures and precipitation. Initial studies on climate change effects on crops have focused on the impact of elevated carbon dioxide levels ($CO_2$), global average temperatures, precipitation, and nutrition on agricultural yield. Crops react differently to climate change as they

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are subjected to multiple stress factors that affect their growth, development, and productivity (John R.P., Mikhail A.S., 2005).

## MATERIAL AND METHOD

The results presented in this study originate from the experimental field of A.R.D.S. Secuieni, Neamț (Agricultural Research and Development Station Secuieni, Neamț), from a bifactorial experiment aimed at determining the influence of growth regulators on the water regime of soybean plants. The experiment was bifactorial in nature, with Factor A represented by the Soybean Variety, and Factor B represented by the growth regulator. Both Factor A and Factor B had four levels, represented by the soybean varieties Eugen, Onix, Iris, and Ziana for Factor A, and the untreated control along with the growth regulators Moddus Evo, Ormet, and Toprex for Factor B.

In this study, four Romanian soybean varieties (Eugen, Onix, Iris TD, Ziana TD) creation of by A.R.D.S. Turda were utilized. All four varieties are early-maturing, belonging to the maturity group OO. Productivity and quality-wise, these varieties have a yield potential of over 4500 kg/ha, protein content exceeding 40%, and oil content surpassing 20%. Additionally, these varieties exhibit excellent resistance to lodging, shattering, and powdery mildew.

For the purposes of the proposed research, namely, determining the influence of growth regulators on the water regime of soybean plants, the four soybean varieties underwent treatments using 3 commercial products with specific growth regulator actions.

Moddus Evo is a product with a plant growth regulator action, containing 250 g/l Trinexapac-ethyl. When applied to growing crops, it helps produce shorter, stronger plants with improved root systems. It is predominantly absorbed through leaves and stems and is translocated to meristematic areas, where it inhibits internode elongation. (https://www.verdon.ro).

Ormet is a growth regulator containing 480 g/l Ethephon. This product is rapidly absorbed by plants and translocated to meristematic areas where it optimizes internode elongation, thus limiting the risk of lodging. It also results in stem thickening, increased leaf surface, and ease of harvest. (https://www.adama.com/romania/ro/plant protection products/growth regulators).

Toprex is a product that combines the protection of a fungicide with the benefits of a growth regulator. It contains two active substances, 125 g/l paclobutrazol (growth regulator) and 20 g/l difenoconazole (fungicide). The active substances are rapidly absorbed by plants and distributed acropetally in the xylem. The benefits of the product include optimizing plant height growth, healthy root system development for better plant stability, and crop uniformity. (https://www.adama.com/romania/ro/plant-protection products/fungicides/toprex).

The field layout for this experiment employed a subdivided plot design, with three replications. The soil type used was typical Cambic Phaeozem (Chernozem), characterized by a very good supply of phosphorus and potassium, a well-supplied active humus content and a low supply of nitrogen *(table 1)*.

Table 1

**Characterization of the soil type on which the experiments were located**

| Characterization of the soil type | A.R.D.S. Secuieni | |
|---|---|---|
| | phaeozem (chernozem) cambic | |
| | Value | Interpretation |
| $pH_2O$ | 7.29 | neutral |
| Texture | 40 | muddy |
| $CaCO_3$ (%) | 0.91 | slightly chalky |
| Humus Content | 2.3 % | well |
| Total Nitrogen Content (mg/kg) | 9.4 | poorly supplied with nitrogen |
| Phosphorus Content (mg/kg) | 189 | very well supplied with mobile phosphorus |
| Potassium Content (mg/kg) | 304 | very well supplied with mobile potassium |

During the establishment of the experiment, all technological steps were followed. The difference was made by the treatments applied during soybean flowering (BBCH 51 - 55). Determinations regarding the water content of the plants were carried out seven days after the treatment application, by collecting five plants (leaf samples) from each variant in the field.

To highlight the influence of the applied treatments on the water content of the plants, the rate of leaf dehydration was monitored through hourly weighing for 4 hours and after 24 hours. Additionally, the dry matter content of the leaves was determined by chopping the plant material and drying it in an oven for 4 hours at a temperature of

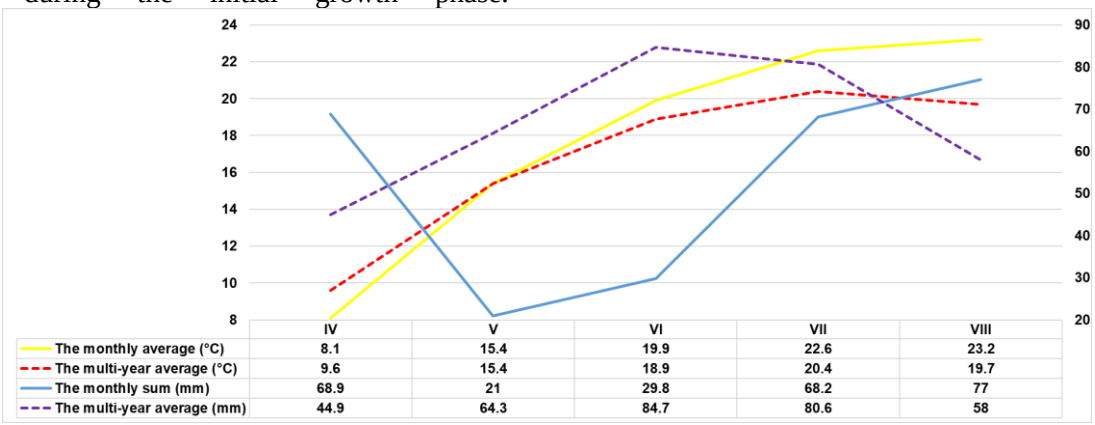
105 °C. Weighing of the leaves was done using an analytical balance.

## RESULTS AND DISCUSSIONS

The agricultural year 2022-2023 was characterized as highly atypical for field crops, especially for soybeans, which experienced a vegetation period marked by high temperatures and severe drought conditions *(figure 1).*

The soybean crop exhibited uniform germination, thanks to the precipitation in April and the first decade of May. The crop developed rapidly during the initial growth phase. Unfortunately, the lack of precipitation in May and June slowed down the crop's growth, which also suffered due to the high temperatures during this period *(figure 1).*

Throughout the vegetation period of the soybean crop, the recorded precipitation was unevenly distributed, and their total sum was below the multi-year average. Analyzing the pluviometric characteristics of the vegetation period (as shown in *figure 1*), it is evident that it was exceptionally dry, with significant negative effects on the growth and development of the soybean plants.




| | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|
| The monthly average (°C) | 8.1 | 15.4 | 19.9 | 22.6 | 23.2 |
| The multi-year average (°C) | 9.6 | 15.4 | 18.9 | 20.4 | 19.7 |
| The monthly sum (mm) | 68.9 | 21 | 29.8 | 68.2 | 77 |
| The multi-year average (mm) | 44.9 | 64.3 | 84.7 | 80.6 | 58 |

Figure 1 **Climate conditions recorded at A.R.D.S. Secuieni, 2023.**

At the onset of flowering, the relative air humidity ranged from 74 % to 83 %, which positively favored soybean fructification. During this period, the average temperatures varied between 19.4 °C and 24.9 °C. The maximum temperatures ranged from 23.4 °C to 33.8°C, while the minimum temperatures ranged from 14.0°C to 18.6°C. These temperatures negatively affected fructification, leading to flower abortion. In addition to the high temperatures recorded at the beginning of flowering, there was also a soil drought, with May and June being very dry. All of these factors had negative influences on the soybean plants. Consequently, the treatments were applied when the soybean plants were under significant stress (*figure 2).*

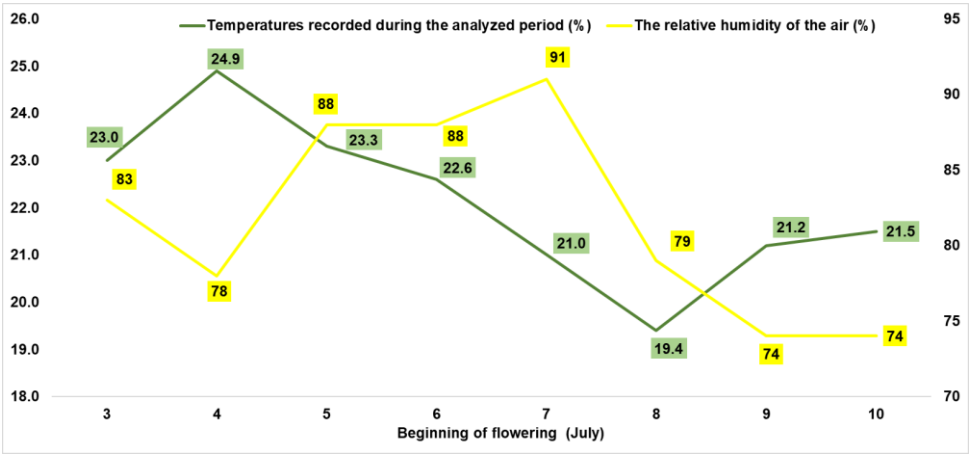



Figure 2 **Climate conditions recorded at the onset of soybean blooming (July)**

Leaf mass directly influences the amount of captured light energy by soybean, thus directly affecting photosynthesis, transpiration, and the plant's final yield. It is important to determine the effects of drought-induced stress and find solutions to mitigate it.

Regarding the results obtained during soybean blooming, it is observed from figures 3-6

that the dehydration rate in soybean varied significantly depending on the cultivated variety and the applied treatment.

For instance, in the case of the Eugen variety, in the untreated variant, a water content of 31.90 % was determined after 24 hours. In the first four hours, the dehydration rate per hour ranged between 6.16 % (4 hours) and 9.92 % (3 hours). After 24 hours, in the variants treated with growth regulators, the water content ranged from 27.59 % (Toprex) to 29.96 % (Ormet). The dehydration rate per hour varied between 6,3 % (4 hours) and 11,52 % (3 hours) in the variant treated with Moddus Evo, between 7.82 % (2 hours) and 11.26 % (3 hours) in the variant treated with Ormet, and between 4.83 % (4 hours) and 9.88 % (3 hours) in the variant treated with Toprex *(figure 3).*

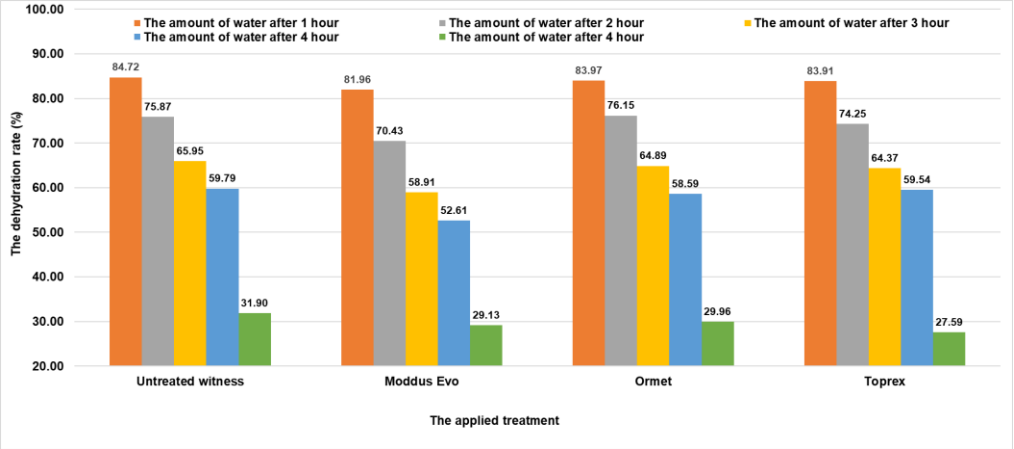



Figure 3 **Influence of treatment on the dehydration rate in the Eugen variety**

For the Onix variety, in the untreated variant, a water content of 26.64 % was determined after 24 hours, with the dehydration rate per hour ranging from 10.42 % (4 hours) to 13.13 % (3 hours) in the first four hours. In the variants where growth regulators were applied, the water content varied between 26.81 % (Ormet) and 27.81 % (Toprex) after 24 hours. The dehydration rate per hour ranged from 6.24 % (4 hours) to 13.36 % (2 hours) in the Moddus Evo treated variant, between 6.63 % (4 hours) and 12.62 % (3 hours) in the Ormet treated variant, and between 6.38 % (4 hours) and 15.05 % (2 hours) in the Toprex treated variant *(figure 4)*

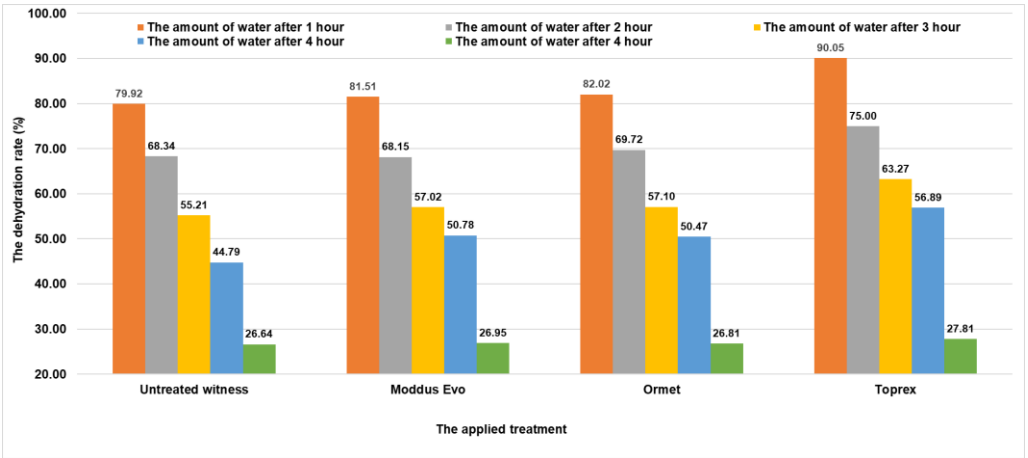



Figure 4 **Influence of treatment on the dehydration rate in the Onix variety**

For the Ziana variety, in the untreated variant, the water content was 27.07 % after 24 hours, with the dehydration rate per hour varying between 5.86 % (4 hours) and 14.55 % (2 hours) in the first four hours. In the variants where growth regulators were applied, the water content ranged between 25.69 % (Moddus Evo) and 28.41 % (Ormet) after 24 hours. The dehydration rate per hour varied between 6.42 % (4 hours) and 16.77 % (3 hours) in the Moddus Evo treated variant, between 5.51 % (4 hours) and 14.78 % (2 hours) in the Ormet treated variant, and between 5.71 % (4 hours) and 15.47 % (2 hours) in the Toprex treated variant *(figure 5).*

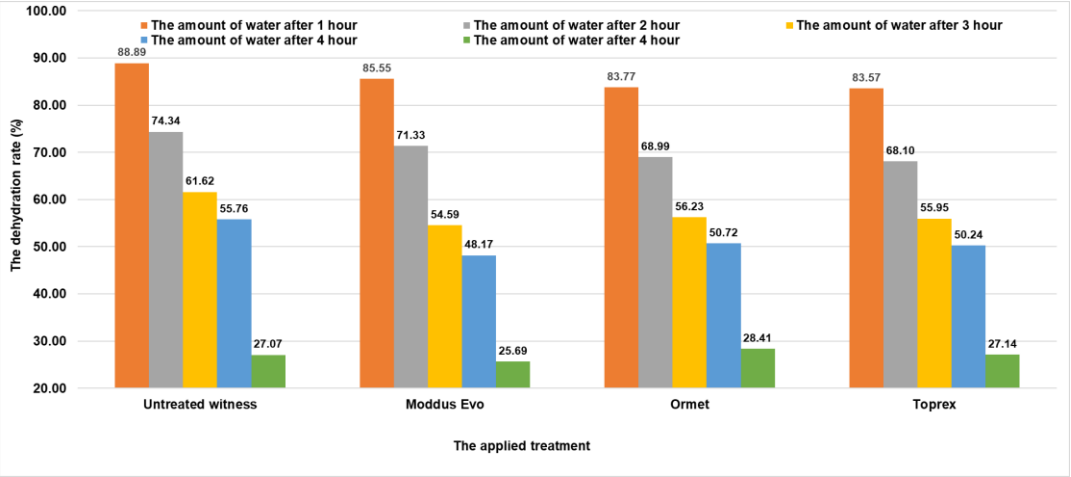



Figure 5 **Influence of treatment on the dehydration rate in the Ziana variety**

At the Iris variety, in the untreated version, a water content of 30.96 % was recorded after 24 hours. In the first four hours, the dehydration rate per hour ranged from 5.33 % (4 hours) to 10,4 % (2 hours). After 24 hours, in the versions treated with growth regulators, the water content ranged from 30.38 % (Ormet) to 33.54 % (Toprex). The dehydration rate per hour varied between 5.31 % (4 hours) and 11.33 % (2 hours) for the version treated with Moddus Evo, between 5.77 % (4 hours) and 13.08 % (2 hours) for the version treated with Ormet, and between 4.39 % (4 hours) and 11.29 % (2 hours) for the version treated with Toprex *(figure 6)*.

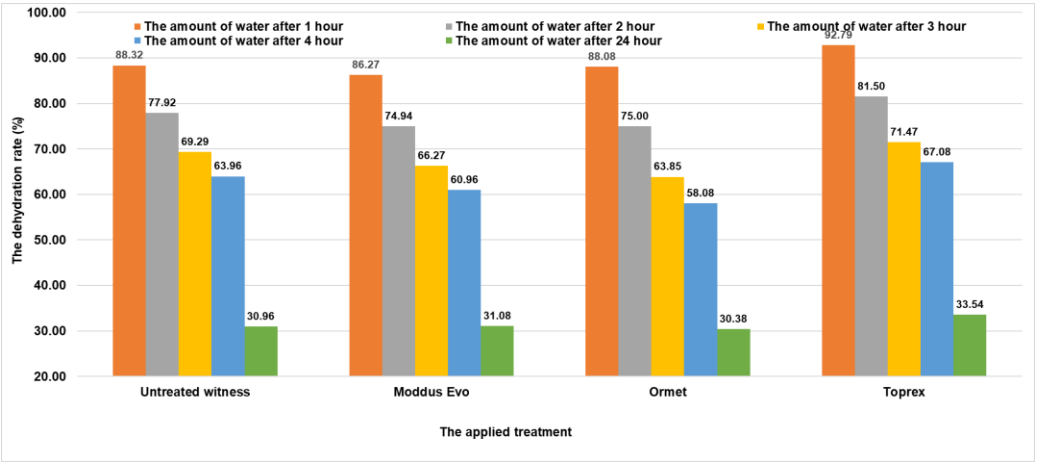



Figure 6 **Influence of treatment on the dehydration rate in the Iris variety**

The dry matter content also varied, both depending on the variety and the applied treatment. For the Eugen variety, it ranged from 18.70 % (Ormet) to 19.20 % (Toprex). It is worth noting that in the untreated variant seeded with the Eugen variety, the dry matter content was high, at 19.14% *(figure 6)*.

In the variant seeded with the Onix variety, the dry matter content varied between 17.93% (Toprex) and 19.25 % (untreated control), while in the variant seeded with the Ziana variety, the values of dry matter content ranged from 18.35 % (untreated control) to 19.81 % (Ormet). In the variant seeded with the Iris variety, the variation in dry matter content was between 18.35 % (Moddus Evo) and 22.63 % (Toprex) *(figure 7)*.

Analyzing the results obtained for the interaction between the studied factors (variety x growth regulator), it can be observed that the slowest dehydration rate was recorded in the variant sown with the Iris variety, to which the commercial product Toprex was applied. Its value after 24 hours was 66.46 %, with a water content of 33.54 N% *(figure 6)*.

Additionally, this variant also had the highest dry matter content, which was 22.63 % *(figure 7)*. Considering that this variant stands out for its high dry matter content, it provides us with the premise that a larger proportion of the total plant mass is represented by solid substances, including proteinaceous substances.

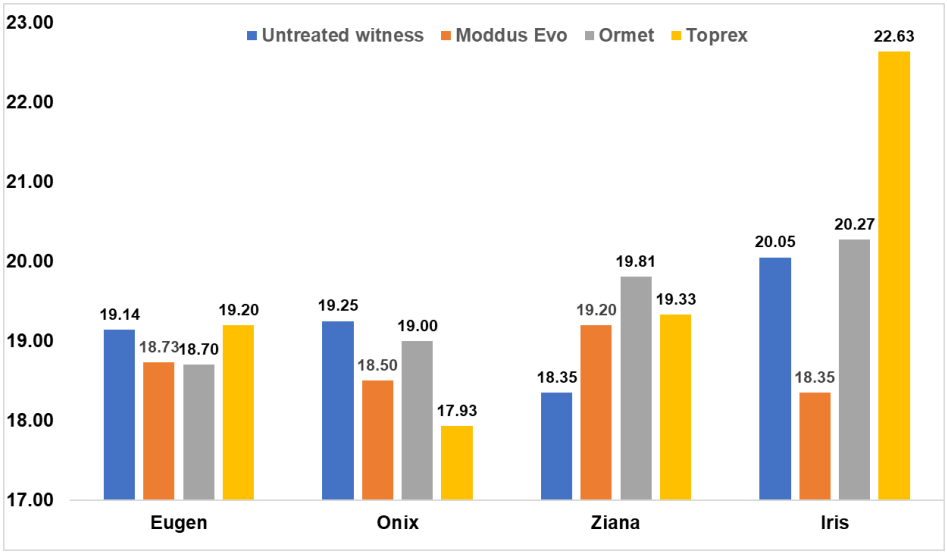



Figure 7 **The Influence of treatment on soybean dry matter content**

## CONCLUSIONS

Soybean is a globally esteemed and sought-after crop due to its ecological adaptability and its seeds, which are rich in proteins and lipids.

Water is an indispensable component for plant life and plays an essential physiological role. However, the water content of plants is directly influenced by climatic conditions.

The water content of the plants varied both depending on the variety and the applied treatment.

The lowest dehydration rate was recorded in the Iris variety. The water content determined after the first hour ranged from 86.27 % (Moddus Evo) to 92.79 % (Toprex), and after 24 hours, it fluctuated between 30.38 % (Ormet) and 33.54 % (Toprex).

Additionally, the highest dry matter content was recorded in the Iris variety, with values ranging from 18.35 % (Moddus Evo) to 22.36 % (Toprex).

The slowest dehydration rate was observed in the variant sown with the Iris variety, to which the commercial product Toprex was applied.

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# CONTRIBUTION OF AGRITOURISM TO THE SUSTAINABLE DEVELOPMENT OF RURAL COMMUNITIES

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**Abstract**

Agritourism encompasses a wide range of experiences and activities related to agriculture and rural life, offering real opportunities for rural development. The primary aim of this paper was to highlight the links that exist between agritourism and sustainability, based on a systematic review of the scientific literature. In this regard, all three dimensions of sustainability (economic, ecological and social) were considered. Numerous studies and researches have focused on the analysis of the effects generated by tourist activities on rural environment, their findings being essential for the management of tourist destinations and rural sustainability strategies. According to the specialized literature, the development of agritourism can have both negative and positive consequences. The contribution of agritourism to the sustainable development of rural communities may include several directions (job creation, additional source of income, environmental and cultural conservation, preservation of agricultural heritage, infrastructure improvement etc.). Achieving positive effects requires efficient planning, community involvement and responsible practices in agritourism management. Avoiding environmental degradation, overdevelopment and negative impact on local traditions and culture is essential for the rural sustainability.

**Key words**: agritourism, rural development, rural sustainability, impact of agritourism

Among the topics covered by the scientific literature and scholars, very frequent are those related to the effects of agritourism on the rural area and local communities, as s well as regarding the potential of tourist activities to contribute to the sustainable development of the rural environment. Despite the different visions and conclusions of the researchers, they agree that agritourism could be the right means to create new opportunities to diversify the rural economy and to achieve the sustainability of rural areas (Barbieri C., 2013; Morau R.A. *et al*, 2022), especially in the case of the poorest and unattractive for investments (Moraru R.A., 2019; Park D.B. *et al*, 2012) or of the isolated areas, where the cultural and environmental heritage exerts a strong attraction for visitors (Shen F. *et al*, 2009; Ciolac R. *et al*, 2019). The concept of "rural development" can be described as "an overall improvement in the economic and social well-being of rural residents and the institutional and physical environment in which they live" (Hodge I.D., 1986). To be sustainable, the development must be based on local resources, especially natural ones. These resources are under the control of farmers and the local population, these being the main actors who can influence the future of rural space through their development options (Van der Ploeg J.D *et al*, 2000).

## MATERIAL AND METHOD

The main objective of this paper is to highlight on what grounds agritourism can be considered as a tool for sustainable development. This article also aims to highlight the positive effects of farmers' involvement in tourist activities and to show if they are in accordance with the requirements imposed by the concept of sustainability. To achieve these objectives, the specialized literature review method was applied and the international experience in this field was analyzed. The approach started from the hypothesis that, by developing agritourism in the right way, it has the potential to promote the sustainability of villages.

## RESULTS AND DISCUSSIONS

In the last decades, the concepts and objectives of sustainability and sustainable development have been among the main concerns of researchers and the whole society (Harrington B.L.M., 2016). As a result of globalization, intensive urbanization and the continuous increase in the costs of agricultural inputs as well as the

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pressures on the natural environment, the rural area faces numerous problems and challenges, undergoing important changes at an economic, social and ecological level (McGehee N.G., 2007; Yang, L., 2012) (*figure 1*).

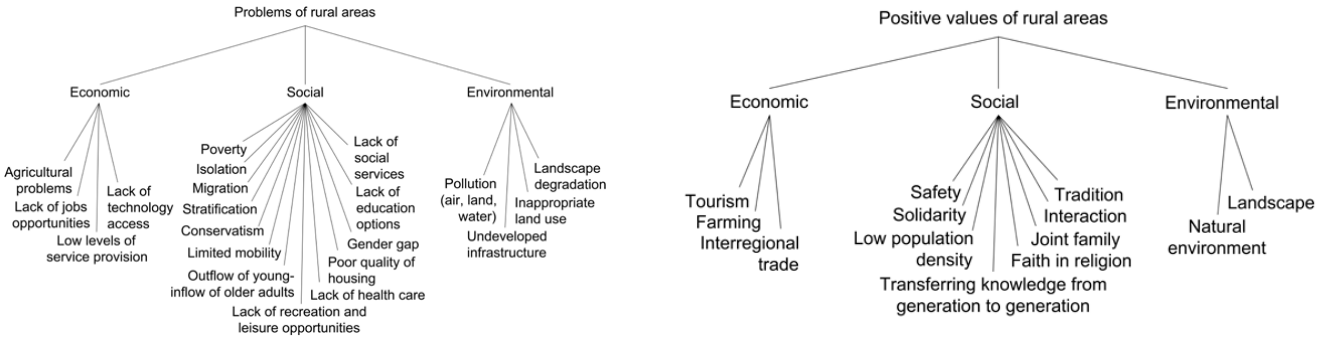


Figure 1 **Problems and values of rural areas** (Lekić O. *et al*, 2018)

For a sustainable future in rural development, three basic conditions must be fulfilled: improving the quality of life of the rural population; protecting and preserving natural and cultural resources in rural areas; ensuring sustainable agricultural production (Sonnino, R., 2004). Sustainable agriculture consists of ecological agricultural practices that allow obtaining plant and animal productions without affecting natural and human systems, avoiding the negative impact on habitats, water, soil and local resources from upstream and downstream of agricultural activities (Kothari H. and Perwej A., 2021).

Rural sustainability is central to global sustainability, as rural areas are very important to the whole society due to the multiple functions and services they provide (Harrington B.L.M., 2016). The preservation of the rural environment requires knowing and highlighting its values and the factors that influence them (*figure 1*), in order to find the most appropriate solutions to neutralize possible negative effects (Lekić O. *et al*, 2018). That is why multi-functional development of rural areas is necessary, which means, in addition to sustainable agricultural production, the development of other functions of agriculture (Sobczyk W., 2014) (*table 1*).

Table 1

**Functions of traditional and sustainable agriculture**

| Traditional agriculture | Sustainable agriculture |
|---|---|
| • food production<br>• production of raw materials for the farming industry and food processing industry | • production of safe food,<br>• identity of rural areas,<br>• generation of renewable materials for the production of bioplastics<br>• production of energy from biomass (biogas, bio-alcohols, biodiesel) and renewable energy: solar, wind, water fall and geothermal |

The scientific community shows a growing interest in the relationship between the diversification of farms through agritourism and the implementation of sustainable rural development (Kothari H. and Perwej A., 2021), all three classic pilars of sustainable development, which are best known as the Triple Bottom Line - TBL (Sonnino, R., 2004), being covered: ecological, economic and socio-cultural (*figure 2*).

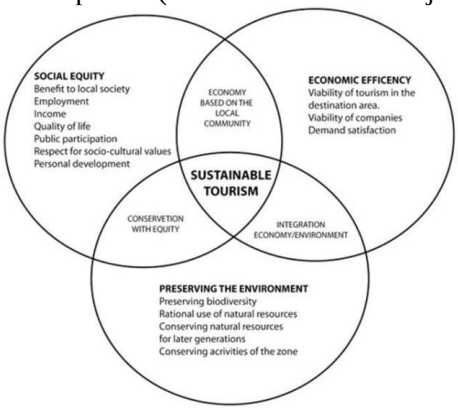


Figure 2 **Sustainable Agritourism development model (Kothari H. and Perwej A., 2021)**

Ensuring the sustainability of the rural area requires the achievement of a sustainable development of agritourism, which implies the minimization of negative effects on the environment, the reuse of resources and the recycling of materials, the increase of the quality of services and the number of visitors, the preservation of the local culture and activities (Tseng M.L. *et al*, 2019), as well as applying the Best Management Practices for an agritourism business: providing an authentic farm experience, an educational experience and excellent customer service; providing adequate public facilities; maintaining a accessible and safe environment; creating good community relations (Sachaleli N., 2022). The U.S. Natural Resources Conservation Service (NRCS) takes into account six essential elements of agritourism sustainability: authenticity, relationships, fun, learning, values and involvement (Sustainable Agriculture Research & Education Program, 2021).

Agritourism activities are very diverse, but regardless of their nature, they must obey the principles of sustainable tourism: the touristic activity should be managed by the local community, by its own means; to create new jobs for local people, thus improving their quality of life; international standards in tourism must be respected, with the establishment of acceptability limits for different areas and monitoring the impact of tourist activities; various educational and training programs should be implemented, in order to improve the management of the protection of cultural and natural resources (Jamieson W. and Noble A., 2000).

The evaluation of the three dimensions of agritourism can be done based on some indicators of sustainability, as summarized in *table 2*.

Table 2

**Sustainability indicators for agritourism** (Rodrigues Ferreira D.I. *et al*, 2023)

| Dimension | Description | |
|---|---|---|
| Economic | • Farm household income<br>• Farm gross sales<br>• Diversification effect on farm profits<br>• Employment of family members (in numbers)<br>• Number of farm employees<br>• Number of full-time employees | • Diversification effect on employees<br>• Revitalization of local economies<br>• Enhancing the quality of life of local people<br>• Enhancing the tourism appeal of rural areas |
| Environmental | • Environmentally friendly farming<br>• Stewardship practices<br>• Preserving natural resources and ecosystems | • Providing scenic beauty and landscapes<br>• Educating visitors about agriculture or nature |
| Socio-cultural | • Attachment to agriculture<br>• Off-farm employment<br>• Historic and cultural preservation<br>• Preserving rural heritage and traditions | • Sharing cultural heritage with visitors<br>• Providing recreational activities for visitors<br>• Attracting and retaining a population |

***Socio-cultural sustainability.***

The socio-cultural pillar of sustainable rural development refers to people and their living conditions (Hull Z., 2008), the impact of agritourism from this point of view being dependent on the individuality of the tourist destination and the interaction between tourists and residents (Bello F.G. *et al*, 2017; Muresan I.C *et al*, 2021). The positive social and cultural effects determined by the development of agritourism, may include:

- stopping rural depopulation and improvement of public infrastructure and community services (Lupi *et al*, 2017).
- maintaining traditions, folklore and popular customs, conservation and revitalization of local culture and heritage (Contini C. *et al*, 2009).
- providing jobs for the farmer's family members (Santeramo F.G. and Morelli M., 2014), especially for young people (Ashley C. *et al*, 2007).
- opportunities for the emancipation of women and their employment in non-agricultural activities (Barbieri C., 2013; Kizos T. and Iosifides T., 2007).
- restores pride and identity for rural residents and increase social cohesion in rural communities (Sachaleli N., 2022)
- educating tourists about rural life, agriculture and food production (Sonnino, R. *et al*, 2014).

***Economic sustainability***

Not only for tourism entrepreneurs, but also for the host community, agritourism provides numerous economic benefits:

- additional income for farmers (Veeck G. *et al*, 2006; Sonnino, R. *et al*., 2014).
- stimulating local businesses (Sharpley R. and Sharpley J., 1997; Veeck G. et al, 2006;

Ollenburg C. and Buckley R., 2007; Tew C. and Barbieri C., 2012).

- new marketing and distribution channels for agricultural products. Agritourism increases the demand not only for the agri-food products obtained at the local level but also for other related rural goods and services. (Tew C. and Barbieri C., 2012).
- stimulating public investments, developing and strengthening local infrastructure (Sharpley R. and Sharpley J., 1997).
- attracting capital from outside the rural area (Sonnino, R. *et al.*, 2014).
- opportunities for business diversification in the rural area (Veeck G. *et al*, 2006; Ammirato S. and Felicetti A.M., 2014). By connecting the agri-food economy with that of tourism, agritourism generates opportunities for farmers and rural areas to prosper through diversification, value addition, and efficient use of spare or underutilized assets and capacities. (Sachaleli N., 2022).

***Ecological sustainability***.

Among the favorable benefits generated by agritourism for the host community, those related to the environment are of particular importance for rural sustainability:

- landscape preservation (Lupi C. *et al*, 2017; Ammirato S. *et al*, 2020) and ecological improvements in degraded rural areas (Choo H. and Jamal T., 2009; Calza F. *et al*, 2018).
- protection of the environment (Lupi C. *et al*, 2017), of ecosystems (Barry J. and Hellerstein D., 2004; Choo H. and Jamal T., 2009), of biodiversity (Mastronardi L. *et al*, 2015; Ammirato S. *et al*, 2020; Kothari H. and Perwej A., 2021)
- contribution regarding soil protection and prevention of hydrogeological disasters (Ollenburg C. and Buckley R., 2007).
- conservation of natural resources (Choo H. and Jamal T., 2009; Ammirato S. *et al*, 2020) and the optimal and responsible use of natural resources and raw materials (Shen F. *et al*, 2009; Ammirato S. *et al*, 2020).
- waste reduction (Ammirato S. *et al*, 2020; Brandth B. and Haugen M.S., 2011).
- encouraging farmers to switch to ecological agriculture, thereby increasing the production of organic and healthier food items ((Kothari H. and Perwej A., 2021), and to adopt environmentally friendly farming practices (Shah C. *et al*., 2020).
- the implementation in farms of some programs for recycling materials, water conservation or educating tourists on issues related to the conservation and reduction of resource consumption (Clarke J., 2007; Sharpley R. and Sharpley J., 1997).
- awareness of local population regarding the need to protect the natural environment (Barry J. and Hellerstein D., 2004).
- increasing tourist interaction with farms and rural life may promote understanding and appreciation for agricultural production and landscapes, helping to maintain and improve natural resources (Sachaleli N., 2022).
- stimulating the use of renewable energy and sources of energy with zero impact on the environment (Tew C. and Barbieri C., 2012; Santeramo F.G. and Morelli M., 2014).
- opportunities for revalorizing or reusing agricultural products that could not be sold in traditional ways (Brandth B. and Haugen M.S., 2011).

On the other hand, specialized literature has drawn attention also to the negative impacts that agritourism and rural tourism can generate in rural areas, mainly on the environment, if there is no proper planning and responsible monitoring of tourist activities: excessive use of natural resources, inappropriate land use, soil and footpaths erosion, trampling of vegetation, littering problems, increase in waste production, conflicts between residents and tourists, overcrowding in the rural area, damage to local traditions and customs, disruption of wildlife, air and noise pollution etc. (Grant M. *et al*, 1997; Almeida F. *et al*, 2015; Tiraieyari N. and Hamzah A., 2012; Pramanik P.D. and Ingkadijaya R., 2018; Martin J.M. *et al*, 2018).

Numerous academic papers underlines that, while women are more concerned about the impact of agritourism on the environment and more receptive to the socio-cultural benefits (Lepp A., 2007; Mason P. and Cheyne J., 2000; Vargas-Sánchez A. *et al*, 2011), young people are more interested in the economic impact (Greene S., 2005) and men more enthusiastically support the development of agritourism and are more willing to get involved in this activity (Almeida G.F., 2015).

Most of the time, the inhabitants of the rural environment do not consider "agritourism" as a solution to their problems, and the majority of farmers being followers of traditional agricultural practices (Kothari H. and Perwej A., 2021). But, a key factor for the development of agritourism is represented by the perception and attitude of the rural community regarding its impact, which, in turn, influences the support for its development and sustainability (Martínez González J.A. *et al*, 2017; Vargas-Sánchez A. *et al*, 2011). For the overall development of the rural environment, local authorities and governance policy play a vital

role (Khongsatjaviwat D. and Routray J., 2015; Živojinovi´c, I. *et al*, 2019).

## CONCLUSIONS

The study of specialized literature highlights that the scientific community has a very positive view on agritourism. It is seen as a synergy linking two important sectors for rural areas: tourism and agriculture. Sustainable agritourism is a part of the overall sustainability of rural area and has the potential to be a way of revitalizing local communities, generating benefits for both farmers and the rest of the rural population. In this regard, the existence of a sustainable development strategy and the support of the authorities and the host community are necessary.

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# ASPECTS REGARDING GLOBAL FOOD SECURITY

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**Abstract**

At a time when global food security is of utmost importance, the Global Food Security Index (GFSI) shows that the global food environment is deteriorating. After hitting its peak in 2019, the GFSI has since declined amid skyrocketing food prices and hunger on an unprecedented scale. In GFSI 2022, affordability drags the index down. The index's affordability score has fallen by 4%, from 71.9 to 69, between 2019 and 2022 as shocks like the covid-19 pandemic and the war on Ukraine have led to rising costs for food. In addition, weakening trade freedom and an inability to fund safety nets have made it harder for people to afford food around the world. Meanwhile, social and political barriers to access have dampened the availability of food. In the past three years, the GFSI has shown rising risks from armed conflicts and political instability, indicators which have seen scores fall by 4% and 6% respectively. This has been accompanied by a growing dependency on chronic food aid, the score for which has dropped by 8% since 2019. Eight of the top ten performers in 2022 come from high-income Europe, led by Finland (with a score of 83.7), Ireland (scoring 81.7) and Norway (scoring 80.5). These nations score strongly on all four pillars of the GFSI. Japan (scoring 79.5) and Canada (scoring 79.1) round out the remainder of the top ten.

**Key words**: food security, Global Food Security Index (GFSI), risks, resilience

At a time when global food security is of utmost importance, the Global Food Security Index (GFSI) shows that the global food environment is deteriorating. After hitting its peak in 2019, the GFSI has since declined amid skyrocketing food prices and hunger on an unprecedented scale. Based on 11 years of data, the index highlights that the food system has been weakening over the years, with shocks in 2020-22, including the covid-19 pandemic and high commodity prices, showcasing this fragility. These shocks exacerbate the systemic issues that are threatening food security and weakening the resilience of the food system. The downward trend in food security is a reversal from the GFSI's early days, which saw eight years of strong growth before a slowdown began. This subsequent stalled progress reflects structural issues and significant risks in the global food system, which include, but are not limited to, volatility in agricultural production, scarcity of natural resources, increasing economic inequality, and trade and supply-chain volatility. The economic and socio-political shocks of the past few years have only exacerbated an already-weakening food environment. As these shocks become more frequent and severe, global food security will be increasingly threatened.

In GFSI 2022, affordability drags the index down. The index's affordability score has fallen by 4%, from 71.9 to 69, between 2019 and 2022 as shocks like the covid-19 pandemic and the war on Ukraine have led to rising costs for food. In addition, weakening trade freedom and an inability to fund safety nets have made it harder for people to afford food around the world. Meanwhile, social and political barriers to access have dampened the availability of food. In the past three years, the GFSI has shown rising risks from armed conflicts and political instability, indicators which have seen scores fall by 4% and 6% respectively. This has been accompanied by a growing dependency on chronic food aid, the score for which has dropped by 8% since 2019. However, new metrics incorporated in this year's GFSI model, including new metrics to gauge the inputs that farmers use on their farms and in the "first mile" (the segment that links farmers to the nearest market), show that agricultural inputs have seen some of the biggest increases in GFSI scores in the past few years (albeit, from a very low base, as these are some of the lowest-scoring indicators in the index). For example, scores measuring commitments to empowering female farmers and food security strategies have increased by 19% and 13% respectively. In addition, despite a 10% fall in public expenditure on research and development since the index's inception in 2012, there has been a strong reorientation towards innovation, with big

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improvements in access to agricultural technology, education and resources, and in commitments to using innovative technology. The growth in the use of these inputs is crucial in improving agricultural productivity and enhancing food security (these measures have proven critical in staunching further declines in the GFSI in 2022).

## MATERIAL AND METHOD

This research is based on, online versions of several journals, brochures and book volumes to analyze the perspectives of different authors on the notion of food security. Quantitative research is carried out by the method of observation and by the procedure of analysis of statistical data (secondary data), which covers the national level and the international or global level. All data used were taken from the official websites of organizations, ministries and governments.

## RESULTS AND DISCUSSIONS

Also key in halting the index's slide in 2022 are big jumps in political commitments to agricultural adaptation and sustainability, especially related to financing. On average, scores for political commitments to adaptation increased by 10% from 2019 to 2022. In 2022 89 countries have a current climate strategy in place with specific measures for agriculture or food security, compared to just 74 countries in 2019. Improvements in political commitments to adaptation also include score increases in environmental economic accounting, risk management coordination and climate finance flows as central banks around the world push for green finance. Eight of the top ten performers in 2022 come from high-income Europe, led by Finland (with a score of 83.7), Ireland (scoring 81.7) and Norway (scoring 80.5). These nations score strongly on all four pillars of the GFSI. Japan (scoring 79.5) and Canada (scoring 79.1) round out the remainder of the top ten *(Fig. 1).* Consistent with previous years of the index, six of the bottom ten scoring nations in 2022 come from Sub-Saharan Africa. The Middle East and North Africa, along with Latin America, are home to the three worst performing nations. Syria sits at the bottom of the list (with a score of 36.3), followed by Haiti (scoring 38.5) and Yemen (scoring 40.1). The gap between the best performing country and the worst performer is stark—Syria scores less than half the score of Finland. The difference between the top performer and the country at the bottom of the ranking has continued widening since 2019, reflecting the inequity in the global food system.

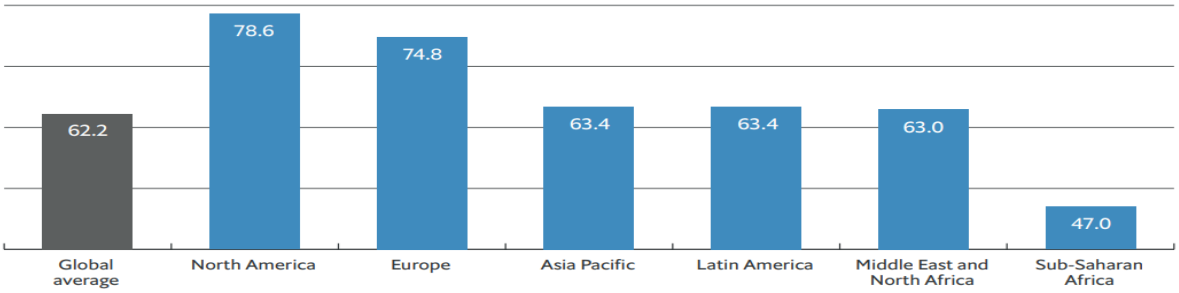


Figure 1. **Overall GFSI 2022 scores, by region**

***Source:*** Global Food Security Index 2022

The early years of the GFSI (2012-15) saw the biggest improvements, with the average overall food security environment score jumping by 6%. However, the GFSI saw slower growth between 2015 and 2019 and then has weakened from 2019 to 2022, plateauing over the past three years as the world faces its highest-ever food prices and hunger on an unprecedented scale *(https://www.wfp.org/publications/global-report-food-crises-2022).* The GFSI score topped 62.6 out of a possible 100 in 2019 but currently stands at 62.2. In 2022 the index was dragged down by falls in two of its strongest pillars—affordability, and food quality and safety—and saw continued weakness in its other two pillars—availability, and sustainability and adaptation. In this report, the theme of resilience will be examined as it plays into each of the four pillars of the GFSI: economic resilience (affordability), production and agricultural resilience (availability), nutritional resilience (quality and safety), and environmental resilience (sustainability and adaptation). This report will examine this data to see what works best, especially when it comes to helping stakeholders to navigate an increasingly volatile world.

Affordability is a key component of food security. Whenever safe and nutritious food is not available at a price affordable to all, it jeopardises people's welfare. Affordability, the top-scoring pillar of the GFSI, dropped by 4% in 2019-22, from 71.9 to 69.0, dragged down by sharp rises in

food costs, declining trade freedom and decreased funding for food safety nets. Meanwhile, big falls in nutritional standards, particularly in national nutrition plans and monitoring, triggered a drop in scores, from 67.1 to 65.9, for the quality and safety pillar. Countries from all regions have dropped the ball on nutritional plans in 2022. Around onethird of countries (35 out of 113) have no national nutrition plan or strategy in 2022, nearly double the number that lacked one in 2019. In addition, 25 of 113 countries are not regularly monitoring the nutritional status of their population (compared with 15 in 2019). Without regular monitoring, policymakers cannot identify nutritional deficiencies and deploy resources where needed. Concurrently, the index's remaining two pillars—availability, and sustainability and adaptation—remain weak.

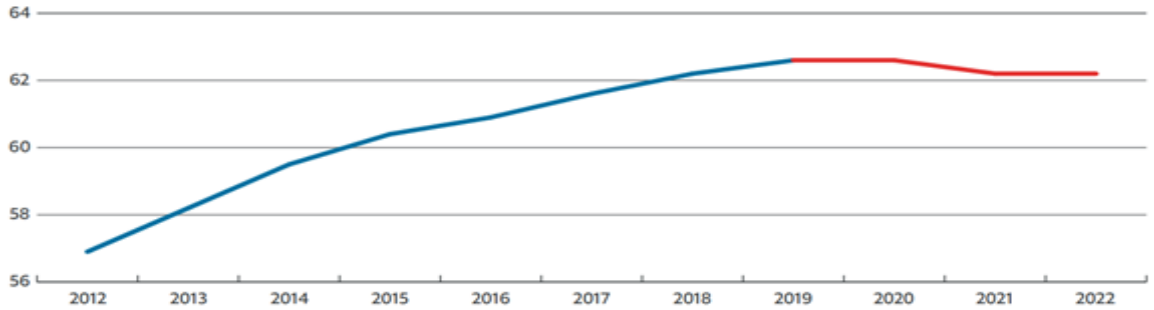


Figure 2. **GFSI average overall score, global 2012-22**

***Source:*** Global Food Security Index 2022

To boost availability, farmers need inputs like finance, but also community support, extension services and strong infrastructure, both on the farm and in supply chains. In 2022 the score for the availability pillar is only 57.8, while sustainability and adaptation trail behind at 54.1. Farmers need political and social support to access markets and infrastructure, but the 2022 index shows that armed conflicts and political instability are being accompanied by a growing dependency on chronic food aid. Moreover, political upheaval and worsening climate change threaten to pull these pillars down further This weakening of the index's overall food security score comes as the world is experiencing an unprecedented level of global shocks. These shocks are placing great pressure on food security with the UN World Food Programme (WFP) seeing the highest number of people in crisis (or worse) since it started releasing its food crisis reports six years ago. Already, 811m people face hunger, and in 2020 one in three global citizens did not have access to adequate food. Experts say that shocks such as pandemics, conflict and extreme weather events due to climate change are going to become the new norm in a global food system of 600m food producers and 8bn consumers living in a degrading environment. Even before the impacts of these unpredictable, recent shocks were being felt, longer-term stresses were adversely affecting the global food system, both directly and indirectly. The most advanced countries were not immune to these structural risks in the global food system, which include volatility in agricultural production, scarcity of natural resources, and trade and supply-chain volatility. Looking ahead, most respondents to a recent World Economic Forum survey on global risks ranked "climate action failure" as both the top long-term threat to the world and the risk that had the potential for the most severe impacts over the next decade, with a disorderly climate transition exacerbating inequalities. To counter these stresses and shocks, and to ensure food security in the future, stakeholders will need to adopt a systemic approach and build resilience in the supply of food and in the environment upon which food is grown and distributed. Looking at the effects of covid-19 on the food supply system, the longer-term issues highlighted by the pandemic—such as the limitations of cost-efficient and streamlined supply chains and lack of agility in redistributing supplies between parts of the food sector—will have to be addressed to build resilience to future shocks. To be resilient, a food system needs to deliver desired outcomes, even when exposed to these stresses and shocks. Research shows that a resilient food system is robust (resists disruptions), is able to recover quickly after any disruption (bounces back) and re-orients (bounces forward) towards more sustainable food system outcomes. All of these responses involve reorganising and adapting to the way that the food system operates. However, given the complexity and connectedness of the food system, multiple stakeholders need to work together to overcome the different food system stressors and shocks, and to define resilience collectively. Conflict is one of the main drivers of food insecurity, as evidenced in the GFSI, which shows that armed conflict is strongly linked to lower food security scores. Conflict negatively

affects almost every aspect of the food system, from production, harvesting, processing and transport to input supply, financing, marketing and consumption. The GFSI shows that armed conflict most negatively impacts supply-chain infrastructure, which is key to moving food from farm to fork. Hunger and food insecurity were already concentrated in conflict zones even before the Ukraine invasion. The GFSI shows that 17 out of 113 nations were already at high or very high risk of conflict (Fig.3). Indeed a 2022 WFP report said that the war in Ukraine is "supercharging a threedimensional crisis—food, energy and finance—with devastating impacts on the world's most vulnerable people, countries and economies." Conflict is also closely connected to climate change. Of the 25 nations most vulnerable to climate change, 14 are mired in conflict. The ability of these countries to adapt to climate change is weakened when more urgent short-term issues such as safety and daily access to food are at stake and authorities and institutions are preoccupied with security. The natural environment can also be a casualty of conflict if it is attacked or damaged by warfare, leading to water, soil or land contamination, or air pollution. Those living in conflict areas are more vulnerable to food insecurity. The GFSI shows a link between armed conflict and water pollution, with conflict impacting the quality and availability of this key resource for agriculture.

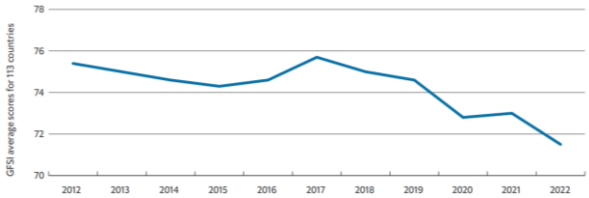


Figure 3. **Risk of armed conflict, 2012-2022**
*Source: Global Food Security Index 2022*

Food price shocks are both an effect and a determinant of conflict. Robust demand, spurred by a recovery from covid-19 contractions, was pushing up food prices even before Russia's invasion of Ukraine, but the war has pushed prices even higher with the added pressure of supply constraints. The 2022 GFSI data show that armed conflict has had a negative effect on affordability. The costs of energy, fertiliser and commodity prices have surged since the Ukraine conflict started, triggering price increases of up to 30% for staple foods. Some areas in the US are reporting 300% increases in fertiliser costs. Higher prices for agricultural inputs such as fertiliser and fuel are being felt on the global markets through higher transport costs, logistical hurdles and disruption of supply chains, with the GFSI showing armed conflict has had a particularly harmful effect on supply chain infrastructure. Systemic issues in the food system, including excessive commodity speculation, have also contributed to record prices. The 2022 GFSI data shows that the affordability of food has declined by 4% relative to 2019. GFSI scores measuring average food costs are poor—performance has plummeted by 11.4%, indicating soaring food prices between 2019 and 2022 *(Fig.4).*

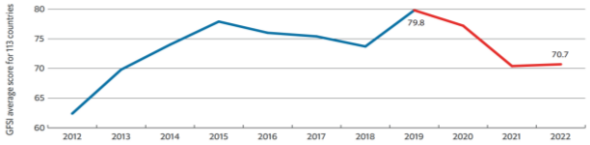


Figure 4 **Change in global average food costs, 2012-2022**

The world is now facing the third global food price crisis in 15 years and policymakers are keen to avoid a repeat of 2008, when food prices also reached record highs. But they face a daunting task. "Climate change, widespread poverty and conflicts are now combining to create 'endemic and widespread' risks to global food security," the International Panel of Experts on Sustainable Food Systems has noted, "which means higher food prices may be the new normal unless action is taken to curb the threats,".

## CONCLUSIONS

The scores in the 2022 GFSI reflect a fragile global food system that is under immense pressure and facing some of its worst outcomes ever. Food prices and hunger are hitting record highs, while affordability is plummeting as shocks like the covid-19 pandemic, armed conflict and climate change compound systemic stresses. These stresses and shocks pose risks that could get worse as threats to food security become the new normal.

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# STUDIES ON THE MORPHOLOGICAL CHANGES OF BEEF DURING FREEZING PROCESSES

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**Abstract**

The freezing process can cause several morphological changes in meat, with the most significant ones being the formation of ice crystals, damage to muscle fibers, and protein denaturation. During the freezing process, water molecules transform into ice crystals, which can lead to the breakdown of meat fibers. The aim of this study was to analyze the changes that occur in beef after freezing and to observe the differences between refrigerated and thawed samples using two different storage methods: vacuum-packed (wet chilling/ freezing) and unpacked (dry chilling/ freezing). Beef samples were subjected to slow freezing, both dry and wet freezing, with a temperature drop of 0.45 - 0.48ºC/hour. The most notable changes after thawing were observed in physical parameters, pH, and texture indicators. The pH of the samples decreased during thawing, with the dry freezing method showing a more pronounced decrease (from a pH value of 6.43 in the chilled sample to a pH of 5.69 after thawing). Shear force (N) and the energy required for shearing (mJ), which reflect the textural qualities of the meat, demonstrated an increase in tenderness as the values of these parameters significantly decreased after thawing. The average values shifted from 87.08 N/1351.02 mJ (wet-chilled sample) and 89.29 N/1389.80 mJ (dry-chilled sample) to the lowest values observed in the wet thawed sample, with averages of 49.14 N/778.14 mJ. The impact of the studied factors on the chemical components was minimal. Humidity decreased from initial values of 75.66% (wet refrigeration) and 75.64% (dry refrigeration) to 75.46% (wet thawing) and 74.94% (dry thawing), with a more noticeable decrease in the case of dry thawing.

**Key words**: beef quality, freezing / thawing, texture

The consumption of meat is largely influenced by factors such as its availability, price, and cultural traditions. Meat production is a highly intricate process, contingent not only upon consumer demand, often driven by economic factors like price and income, but also subject to a multitude of socio-economic influences, including government policies, price support mechanisms, and various interrelated factors (FAO, 2021).

In European legislation, specifically Regulation (EU) No 1169/2011 of the European Parliament and of the Council dated 25 October 2011, the term 'meat' pertains to the edible portions obtained from the carcasses of domestic animals, encompassing livestock such as cattle, pigs, sheep, goats, as well as poultry and game.

Meat ranks among the most widely consumed food items worldwide and holds significance as a vital component of a well-rounded and nutritious diet, thanks to its inherent nutritional properties. Nevertheless, despite its popularity, the meat industry is not immune to controversy. In recent years, concerns have arisen regarding the environmental impact of meat production and the welfare of animals raised for meat production (Pereira P.M. & Vicente A.F., 2013; Hartmann C., Siegrist M., 2020).

Meat and meat products constitute a significant source of essential nutrients, including protein, fats, vitamins, and minerals, which are essential for dietary nutrition (González N. *et al*, 2020). The overall quality of meat is influenced by various attributes such as taste, texture, juiciness, appearance, and odour. In the fast-paced routines of daily life, meat is often purchased in bulk for future use, necessitating a thawing process that can result in changes in nutritional quality. In many instances, the primary concern with frozen food lies in the quality of the thawing process, which often receives less attention (Akhtar S. *et al*, 2013; Balan P. *et al*, 2019).

Freezing is among the most prevalent and effective preservation methods for meat products, extending their shelf life considerably (Setyabrata

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D., Kim Y.H.B., 2019; Wang Z. *et al*, 2021). The advantage of freezing meat, is, as opposed to distributing it chilled, its prolonged storage duration (Warner R.D. *et al*, 2022). However, the quality of frozen meat and subsequently thawed meat is generally regarded as inferior to that of fresh, never-frozen meat (Leygonie C. *et al*, 2012; Hou Q. *et al*, 2020).

The quality of frozen meat is influenced by the specific processes employed in freezing, storage, and subsequent thawing. While freezing is considered a relatively mild preservation method, it does induce alterations in food, and the extent of these changes is closely linked to the speed at which freezing occurs. The rate at which freezing occurs can have a significant impact on meat quality due to the structural alterations that take place during the freezing process, primarily driven by the formation of ice crystals, known as nucleation. Nucleation is influenced by temperature, with rapid freezing rates resulting in the generation of both extra- and intra-cellular ice nuclei. Moreover, the size and shape of these ice nuclei are contingent upon the rate of freezing. For instance, conventional freezing at -20°C leads to the development of irregular and relatively large ice crystals, which in turn can increase structural damage to the meat (Leygonie C. *et al*, 2012; Muela E. *et al*, 2010; Oliveira M.R. *et al*, 2015; Wang Y. *et al*, 2020).

While freezing is a crucial method for preserving the nutritional value and sensory qualities of meat during various stages such as processing, storage, and transportation, before using the frozen meat in subsequent procedures it needs to undergo a thawing process. Thawing is the process of transforming the ice in a food product into its liquid phase, essentially representing the reversal of the freezing process. Thawing meat is often associated with protein degradation, fat oxidation, diminished color quality, and decreased water retention due to the melting of ice crystals. As a result, the adoption of suitable thawing techniques becomes imperative to enhance the quality of thawed meat (Min S.-G. *et al*, 2015; Gan S. *et al*, 2022; Köprüalan Aydın Ö. *et al*, 2023).

There are numerous thawing methods described in the literature, including thawing under refrigeration conditions, at room temperature, in cold water, in tap water, in hot water, using microwaves, heat convection, infrared, radio frequency, ohmic, pressure or even thawing while cooking (Eastridge J.S., Bowker B.C., 2011). Despite those mentioned methods, to limit the microbial growth, the increase of drip loss and other quality changes that may occur in the thawed meat, it is recommended that thawing should be performed at temperatures up to 4 °C or under controlled conditions (Eastridge J.S., Bowker B.C., 2011; Oliveira M. R. *et al*, 2015; Köprüalan Aydın Ö. *et al*, 2023).

In the case of beef consumption, the overall gustatory enjoyment derives from its harmonious blend of tenderness, juiciness, and flavour. Among these attributes, tenderness holds particular prominence for a substantial segment of consumers and is concurrently recognized as one of the most variable quality characteristics. It is well-established that the process of freezing beef has an impact on its tenderness. Notably, beef that has undergone a tenderization period of 7 days followed by freezing and subsequent thawing exhibited comparable shear force values to chilled beef that had undergone a 21-day tenderization process (Lagerstedt Å. *et al*, 2008).

Hence, this study aims to determine the morphological alterations that occur in beef during freezing and thawing processes, with the objective of discerning differences in both texture and the physico-chemical attributes of the analyzed meat. Moreover, the investigation examines the impact of the freezing process on meat quality, with particular emphasis on texture—a pivotal quality parameter known to exert a significant influence on product juiciness, tenderness, and chewiness.

## MATERIAL AND METHOD

The biological material employed in this study consisted of beef (M. *longissimus dorsi*) sourced from the local market. The total quantity of beef was divided into four portions and subjected to distinct treatments as part of the experimental design:

1. Wet refrigerated
2. Dry refrigerated
3. Wet freezing for a duration of 24 hours, followed by thawing through wet refrigeration
4. Dry freezing for a duration of 24 hours, followed by thawing via dry refrigeration

The preparation procedures for the refrigerated samples involved the wet storage method, which included vacuum-sealing and placement in refrigerated cabinets maintained at temperatures between 2-4°C. For the dry refrigeration procedure, the meat was placed outside of the vacuum-sealed bag and stored in the refrigeration cabinets.

In the freezing process, a probe thermometer was inserted into the meat structure to monitor temperature changes during freezing. Subsequently, the meat was vacuum-sealed (only in the case of wet freezing) and stored in freezer crates. The thawing stage followed either the wet or dry method, with the meat left to thaw at chilling

temperatures either inside or outside a vacuum-sealed bag.

The determination of the proximate chemical composition was conducted using the FoodCheck automatic analyzer, which operates on the principle of infrared spectrophotometry. This device analyzes meat samples based on their infrared absorption properties as identified in the sample spectrum.

The pH determination for the meat samples was executed using a digital pH meter equipped with automated measurements for both acidity and temperature values. Before assessing the acidity of the samples under investigation, the pH meter underwent calibration using two buffer solutions with known pH values (solution 1 - pH = 4.01; solution 2 - pH = 7.01). After calibration, the electrode was inserted into the prepared meat sample. Preceding and following each measurement, the electrode was cleaned with distilled water.

Texture determination tests were conducted using a mechanical tester Mark 10 (USA), and a Mark 10 series 7 dynamometer featuring a range spanning from 0 to 1000 N and a resolution of 0.2 N. The test probe employed was of the V-blade type known as WERNER BRATZLER.

Meat samples were collected using a cylindrical stainless steel probe measuring 6 cm in length, 2 cm in width and 25 mm in diameter, ensuring uniform sample size. The maximum cutting force required to shear the cylindrical meat samples was measured, and subsequently, the mechanical work or energy needed to cut each sample was computed. The cutting process involved a knife displacement speed of 200 mm/min, and the resulting data were graphically represented as a force-versus-displacement curve. Data acquisition was performed using the MESUREGauge+ program, while further data analysis was conducted using Excel and GraphPadPrism9 software.

Each analysis entailed a minimum of five determinations for each type of refrigeration and freeze-thaw treatment applied to the meat samples. An average value was calculated based on the results obtained. The statistical analysis was carried out using the XLSTAT statistical and data analysis program (Addinsoft 2023, New York, USA), performing the Tukey test (HSD) / Analysis of the differences between the categories with a confidence interval of 95%.

## RESULTS AND DISCUSSIONS

To monitor temperature variations during the freezing process for each meat type, a thermometer probe was inserted into each meat sample. These measurements were taken at a depth of 1 cm from the surface of the meat, and the rate of freezing front advancement was estimated in degrees Celsius per centimetre per hour (ºC/cm/h). After 48 hours of continuous monitoring, the average rates of freezing and thawing were calculated. This process resulted in the construction of freezing temperature curves (*figure 1*) and thawing temperature curves (*figure 2*), aiding in the characterization of the freezing process as either slow or fast.

For wet freezing, the product's temperature decreased from an initial 4.2ºC to -18.2ºC over a 24-hour period, translating to an average rate of 0.48 ºC/h, indicative of a slow freezing process. Conversely, in dry freezing, the product's temperature decreased from 4.1ºC to -18.6ºC over 24 hours, with an average rate of 0.45ºC/h, signifying a slow freezing process.

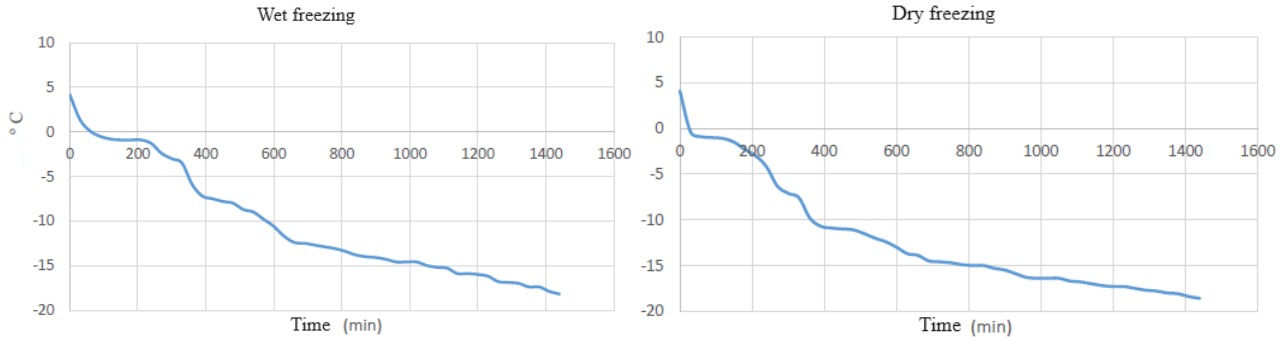


Figure 1 **Temperature graph for freezing beef**

In the wet thawing phase, the product's temperature exhibited an increase over a 24-hour period, rising from -18.1 ºC to 2.3 ºC, with an average rate of 0.42 ºC/h. Conversely, during the dry thawing process, the sample's temperature rose from -18.4 ºC to 2.4 ºC over the same 24-hour duration, at an average rate of 0.43 ºC/h (*figure 2).*

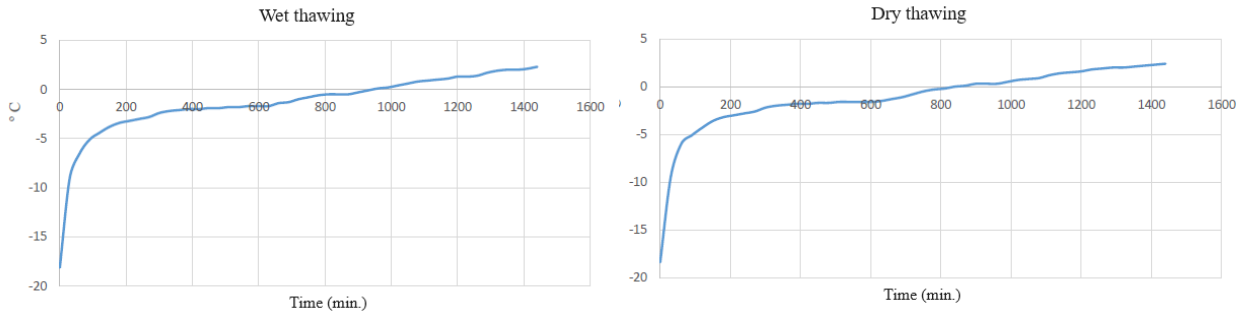


Figure 2 **Temperature graph for thawing beef**

The physicochemical properties examined for the studied meat included pH values, shear force, and cutting energy (*table 1*), as well as water content, dry matter, protein, collagen, and fat content (*table 2*). The obtained values were compared among themselves based on the applied treatments.

The results obtained for the physical parameters of the four samples are presented in *table 1*. The pH values of the samples ranged from 5.69 ± 0.05 (thawed dry sample) to 6.43 ± 0.13 (refrigerated dry sample). Higher pH values were observed for the fresh meat samples compared to the thawed ones, with similar findings reported by Leygonie C. *et al* (2012b). Min S.-G. *et al* (2015) also reported decreases in pH values for beef following conventional thawing. This decrease in pH after thawing can be attributed to the production of exudate, which leads to denaturation of buffering proteins and the release of hydrogen ions, subsequently resulting in a decrease in pH. Statistical analysis revealed significant differences in pH among the four samples, as determined by the Tukey test. Based on the Type III sum of squares, the variable that does not bring significant information to explain the variability of the dependent variable pH is the storage method, whereas variable storage type is the most influential.

The highest shear force was observed in the dry chilled sample, measuring 89.20 ± 1.79 N, whereas the lowest shear force was noted in the wet thawed sample, amounting to 49.14 ± 1.71 N. Statistically, a significant decrease in shear force was observed following the thawing process, with the storage type variable exerting the most substantial influence. The lower shear forces observed in the thawed samples can be attributed to the thawing process, which induces the breakdown of muscle fibers as a result of protein denaturation, thereby diminishing the cutting force.

The storage method (wet/dry) exhibited statistically significant differences solely in the thawed samples, where the shear force was notably higher in the dry thawed sample by a margin of 10.84 N. Elevated shear forces in the dry refrigerated / thawed samples can be explained by the enhancement of beef tenderloin cutting resistance due to the reinforcing effect of connective tissue on the meat's structural integrity, along with moisture loss resulting from exposure to air currents.

These findings align with previous studies by other researchers who have reported an increase in meat tenderness as a consequence of freezing and thawing (Lagerstedt Å. *et al*, 2008; Leygonie C. *et al*, 2012a).

Table 1

**Analysis of the differences between the categories for physical parameters.**

| Parameter | Refrigerated | | Thawed | | Significance levels of p-value | | |
|---|---|---|---|---|---|---|---|
| | W. | D. | W. | D. | A | B | A x B |
| **pH** | 6.19 $^{c}$ ± 0.06 | 6.43 $^{d}$ ± 0.13 | 5.88 $^{b}$ ± 0.05 | 5.69 $^{a}$ ± 0.05 | *** | ns | *** |
| **Shear force (N)** | 87.08 $^{c}$ ± 2.16 | 89.20 $^{c}$ ± 1.79 | 49.14 $^{a}$ ± 1.71 | 59.98 $^{b}$ ± 1.42 | *** | *** | *** |
| **Total energy (mJ)** | 1351.02 $^{c}$ ± 4.11 | 1389.80 $^{d}$ ± 8.15 | 778.14 $^{a}$ ± 2.40 | 909.48 $^{b}$ ± 3.06 | *** | *** | *** |

a, b, c, d - The same superscript letter within the same row means there is no significant difference between the same parameter analysed (p>0.05). W. - wet refrigeration / thawing; D. - dry refrigeration / thawing; A - storage type (R/T); B - storage method (W/D)

The results obtained for the energy required for cutting are inherently linked to the shear force, as a greater force needed to cut the meat sample corresponds to higher energy consumption. In this context, the energy required for cutting in the case of thawed samples was notably lower (wet thawed sample: 778.14 mJ; dry thawed sample: 909.48 mJ) compared to that observed for the chilled samples. Consequently, thawing demanded considerably less energy for meat cutting, with

reductions of up to 44%, indicative of increased tenderness in the samples.

Moisture loss in meat is an inherent post-mortem occurrence owing to pH fluctuations. However, the extent of these losses varies depending on various influencing factors. In the present study, the moisture content of the samples fell within the range of 74.94 ± 0.18 (dry thawed samples) to 75.66 ± 0.09 (wet refrigerated samples). Significant differences were only noted in the case of the dry thawed sample, which exhibited the lowest moisture content, potentially attributed to its higher lipid content (2.96%). Given that the samples underwent a slow freezing process, water losses were minimal.

In terms of dry matter content, a higher concentration was observed in the dry thawed sample (25.12%), which also exhibited the highest fat level. The dry matter content, inversely related to moisture content, displayed higher values in the thawed samples compared to the chilled ones, regardless of the storage method.

Regarding the protein content in the analyzed samples, the average values ranged from a minimum of 21.54% (wet thawed sample) to a maximum of 21.86% (dry refrigerated sample). Lower values were noted in the frozen and thawed samples, indicating some degree of protein denaturation likely induced by slow freezing, resulting in the formation of ice crystals.

Collagen content ranged from 19.88% (wet thawed sample) to 20.24% (dry thawed sample). Collagen is a structural protein found in connective tissues of meat, and it plays a crucial role in determining the meat's texture and tenderness. Collagen does not undergo major alterations when meat is frozen and subsequently thawed.

Table 2

**Analysis of the differences between the categories for chemical components**

| Parameter | Refrigerated | | Thawed | |
|---|---|---|---|---|
| | W. | D. | W. | D. |
| **Moisture** | 75.66 $^{b}$ ± 0.09 | 75.64 $^{b}$ ± 0.09 | 75.46 $^{b}$ ± 0.15 | 74.94 $^{a}$ ± 0.18 |
| **D.M.** | 24.32 $^{ab}$ ± 0.17 | 24.09 $^{a}$ ± 0.13 | 24.54 $^{b}$ ± 0.15 | 25.12 $^{c}$ ± 0.11 |
| **Protein** | 21.84 $^{b}$ ± 0.05 | 21.86 $^{b}$ ± 0.05 | 21.54 $^{a}$ ± 0.05 | 21.78 $^{b}$ ± 0.04 |
| **Collagen** | 20.22 $^{b}$ ± 0.08 | 20.18 $^{b}$ ± 0.08 | 19.88 $^{a}$ ± 0.04 | 20.24 $^{b}$ ± 0.11 |
| **Fat** | 2.26 $^{a}$ ± 0.11 | 2.22 $^{a}$ ± 0.13 | 2.46 $^{ab}$ ± 0.15 | 2.96 $^{b}$ ± 0.11 |

a, b, c, d - The same superscript letter within the same row means there is no significant difference between the same parameter analysed (p>0.05). D.M. – dry matter; W. - wet refrigeration / thawing; D. - dry refrigeration / thawing; A - storage type (R/T); B - storage method (W/D)

## CONCLUSIONS

Freezing is an extensive preservation method widely employed in the meat industry, known for its capability to extend the shelf life of meat by several months. However, it is crucial to acknowledge that the freezing process can induce significant morphological alterations in the meat's structure, which can significantly influence its physico-chemical and textural attributes. In this study, following the process of slow freezing applied to beef tenderloin samples, noteworthy changes were observed, primarily in terms of texture. Post-thawing, a notable reduction in shear force was evident, particularly in the case of the wet thawed sample when compared to the chilled sample.

Concerning the chemical characteristics of the samples, despite the slow freezing process, there were minimal differences in moisture content between the refrigerated and thawed samples, attributed to the controlled thawing conditions maintained at 2-4°C. The freezing process indeed impacts the morphological attributes of the meat, notably its texture, rendering it softer and more tender upon thawing. However, depending on the thawing method employed, it is possible to preserve elasticity and juiciness while minimizing moisture loss.

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# STUDIES ON THE ORGANOLEPTIC CHARACTERISTICS OF SOME ROSÉ WINES FROM STRUNGA WINERY

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**Abstract**

Climatic conditions, genotype and appropriate technology are very important for successful wine cultivation and obtaining quality products. To describe a wine-growing climatic zone, climatic parameters were used, which show the binary and ternary interaction between climatic conditions: light, temperature, humidity, precipitation and solar radiation. Through current research, it was aimed to highlight the influence of climatic conditions on the organoleptic characteristics of rosé wines from the Fetească neagră and Busuioacă de Bohotin grapes obtained during the years 2020-2021. This study includes the description of the Strunga viticultural center, the technological flow of obtaining the chosen wines, results of physical-chemical analyzes and sensory characteristics of wines varieties respectively V1 and V2, V3 and V4 .This two wine variants from 2020 have a lower acidity but at the same time a higher alcohol concentration compared to those from the 2021 harvest. The results of the research in terms of sensory evaluation revealed that both variants of the year 2021 were the most appreciated for most of the characteristics.

**Key words**: Fetească neagra, Busuioacă de Bohotin, climatic conditions, quality, *terroir*

Wine is a significant example of human ingenuity, even if it is not necessary for our existence. When we talk about the many repercussions of climate change, wine may not be the first thing you think of, but vineyards around the world are already experiencing disruption. Solar radiation, light intensity, temperature, soil and air humidity, wind movement, and quality are the main factors used to assess the climate of a pogdoria. Overall, while the significance of rosé wine to humanity may not be earth-shattering, it is undoubtedly an important part of human culture and history and one that impacts our social, economic, and natural well-being.

The basic environment of the vineyards has remained fairly consistent over the millennia, being dramatically altered only by extreme changes in the world's climate. Such variations in wine-growing regions as well as changes in vine phenology have been caused by such changes occurring as temperature increases or decreases. (Malheiro A.C. *et al,* 2010; Santos J.A. *et al,* 2012).

One of the most commonly used but least understood winemaking words is terroir. When this idea was applied to wines, it led to the acceptance that a wine region is a collection of *terroirs*, some of which are better than others, and that each *terroir* gives the wine a distinctive quality that cannot be found elsewhere (James J., 2014).

High-quality wines are produced from grapes grown in a variety of soil types, as no one soil is considered optimal from a soil perspective. However, each type of soil gives a variety of its distinct flavor and mouthfeel (Cotea V.D., 1985)

In addition to translating the composition of the grapes into the best possible wine by using appropriate winemaking procedures, winemakers can also affect the so-called *terroir* favorably or less favorably (Seguin G., 1986). The vast majority of wine writers agree that human influence on terroir in one form or another is a factor. Each wine-growing region in Romania differs from one another in that it has a unique *terroir*, to which the varieties studied, respectively Fetească neagră and Busuioacă de Bohotin, have adapted and which result in wines of exceptional quality. The combination of all these factors creates a unique *terroir*, specific to a particular vineyard or wine-producing region.

Strunga Winery is a relatively new winery, which started its activity only in 2017, realizing in the first years a small production of 10 thousand bottles, currently they have produced half a million

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bottles of wine. Due to the favorable climatic circumstances of the area (rainfall, climate, exposure, slope, duration of sunshine), it is possible to grow varieties with a high yield, and ultimately to produce wines of superior quality.

The main purpose of this study is to analyze some climatic categories that can affect the organoleptic and physico-chemical properties of the wines obtained from the Feteasca neagră and Busuioacă de Bohotin grape varieties during the years 2020-2021.

## MATERIAL AND METHOD

The hills characteristic of the wine production we are analyzing, where the grapes are planted, have a popular name for several hundred years. The Pârjolita Hill has an area of approximately 100 hectares, of which the Strunga Winery owns 41.66 ha and the Calda hill 45 ha.

Cold winters and hot summers with irregular and more frequent winds from the north and west, winter and south and south-west in spring, with significant rains in early summer are characteristics of the region where the Strunga Winery is located. The range of average annual temperature values is 8.3$^{o}$C -9$^{o}$C. The typical annual amount of atmospheric precipitation is 500-550 mm, with irregular frequency.

The technological versions used for the production of rosé wines in the Strunga Winery follow the stages of the general technological flow. The location of the weather station Strunga (47.17°N 26.98°E, 171m), is chosen by standards that guarantee the regional representativeness of the measured elements, is the place where meteorological measurements are made.

*Determination of temperatures*- thermometers are mounted at a height of 2 m from the ground on the meteorological platform in the shelter.

*Determination of precipitation* - The rain gauge is buried at least 60 cm below the ground surface. When the water level is halfway between two divisions, the data obtained by measuring the amounts of precipitation are entered in a register that corresponds to the respective time calculated in the interval 7:00-19:00.

*Determining the duration of sunshine*- Automatic equipment with a global radiation sensor was used to calculate the effective glow duration. They are mounted on the station's metal pole.

The analyses were completed by the methodology of the *International Organization of Vine and Wine 164/2015* which is based on the techniques described in state and international standards, as well as those found in the specialized literature. The determination of the alcoholic concentration and the total acidity were carried out according to STAS 6182/6-70 respective STAS 6128/1-79

*Determination of the color of wines by the CIE Lab 76 method*- Analytik Jena Specord 200 UV-VIS spectrophotometer was used to identify the color. To measure absorbances, cuvettes with an optical path were used that were suitable for each wine sample by 0.2 cm to reduce absorbance errors. According to STAS 6182/35-75, it involves expressing the color of rosé wines by shade and intensity. Based on the absorption spectra observed for each sample, the chromatic parameters (L, +a, +b, -a, -b) of Feteasca neagră and Busuioacă de Bohotin rosé wines were calculated using CIE Lab 76 techniques.

*Sensory analysis*

The proposed evaluation method, Closed Tasting, was approached for the first time by the International Union of Oenologists (UIO) for the organoleptic evaluation of the technological variants obtained as a result of the winemaking process in 2020 and 2021.

## RESULTS AND DISCUSSIONS

The information presented in the following tables was provided by the private meteorological station within the Strunga Winery for two consecutive years.

Table 1

**The results obtained regarding the analyzed climatic factors**

| Month/Year | Precipitation (mm) | | Annual temperatures ($^{o}$C) | | Duration of sunshine (hour) | |
|---|---|---|---|---|---|---|
| | **2020** | **2021** | **2020** | **2021** | **2020** | **2021** |
| **January** | 4.6 | 26.9 | 2.4 | 0.3 | 103.4 | 48.1 |
| **February** | 44.7 | 28 | 4.7 | -0.3 | 91.7 | 89.4 |
| **March** | 17.6 | 42.6 | 7.3 | 4.1 | 139.2 | 115.6 |
| **April** | 2.8 | 42.6 | 12.1 | 8.5 | 244.3 | 129.6 |
| **May** | 95 | 81.2 | 14.1 | 15.2 | 156 | 167.1 |
| **June** | 136.8 | 107.2 | 21 | 20.2 | 180.2 | 173.6 |
| **July** | 48.2 | 86 | 22.2 | 23.9 | 213.2 | 231.7 |
| **August** | 11.4 | 79.2 | 23.5 | 21.5 | 257.3 | 208.9 |
| **September** | 69.8 | 27 | 19.9 | 15.8 | 207.2 | 149.1 |
| **October** | 68.6 | 0.4 | 13.9 | 10.2 | 81.9 | 142.4 |
| **November** | 7.8 | 6.8 | 5.2 | 7.3 | 62.8 | 85.6 |
| **December** | 50 | 90.8 | 1.7 | 0 | 21.5 | 21.8 |
| **Annual average** | **46.5916** | **51.5583** | **12.3** | **10.6** | **1758.7** | **1562.9** |

*Table 1* shows us that the year 2020 recorded an average of only 46.59 mm, the value obtained from the average of each individual month. Compared to 2020, 2021 turned out to be much wetter, accumulating an average of 51.55mm.

On the other hand, due to the more abundant precipitation in 2021, the average annual temperature was 10.6ºC, thus resulting in a lower value than in 2020. The duration of sunshine (insolation) registers significant values in the station**:** 1758.7 h in 2020 and 1562.6 h in 2021. Since the climatic circumstances vary from year to year, the grapes used in the winemaking process have different physico-chemical properties, which also implies a change in the finished product obtained.

The wines obtained during the two consecutive years 2020-2021, obtained from the Busuioacă of Bohotin (V1 and V2) and Fetească neagră (V3 and V4) grape varieties, vinified rosé, constituted the research material that was subjected to the following determinations.

Table 2

**The main physico-chemical characteristics of the analyzed wines**

| Composition features | Experimental variants | | | |
|---|---|---|---|---|
| | V1 | V2 | V3 | V4 |
| Alcohol concentration (% vol.) | 12.1 | 12 | 13.3 | 13 |
| Total acidity (g/L tartaric acid) | 4.3 | 4.5 | 3.5 | 3.8 |
| Malic acid (g/L) | 1.7 | 4.2 | 3.1 | 4.3 |

The alcohol content of the examined variants did not change appreciably during two years. Due to the climatic conditions of 2020, varinates V1 and V3 have accumulated a higher concentration of 12.1% and 13.3%, respectively. Similar results were obtained by the V2 and V4 variants from 2021, which showed modest variations compared to the previous year by 12% in the case of V2 and 13% for V4. 2020 was a dry year with high temperatures that affected the studied wines and resulted in a lower acidity of 4.3 for sample V1 and 3.5 for sample V3 due to dryness.

In contrast, the year 2021 turned out to be rainy and cooler as a result the wines have more acidity, 4.5 in the case of the V2 sample and 3.8 in the case of the V4. The difference in malic acid content is caused by the fact that 2021 was a rainy year which resulted in higher acid accumulation.

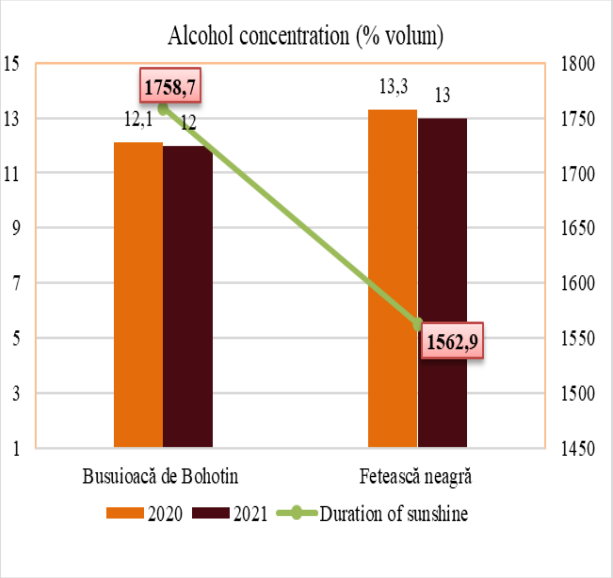


Figure 1 **Correlation alcohol concentrationduration of sunshine**

In the first graph, it can be observed that the duration of sunshine in 2020 totaled 1758.7 hours. This indicates that 2020 was a dry, arid year with little rainfall which caused the grapes to ripen faster and accumulate more sugars. As a result, the wine had an alcohol content of 12.1% in V1 and 13.3% in the case of sample V3. Due to other factors at play, the year 2021 is significantly shorter, totaling only 1562.9 hours.

As a consequence, the grapes accumulated less sugars, causing a lower alcohol concentration. In the case of analysed samples for the 2021 studied year, the sample highlights 12% for the V2 sample and 13% in the case of the V4 sample.

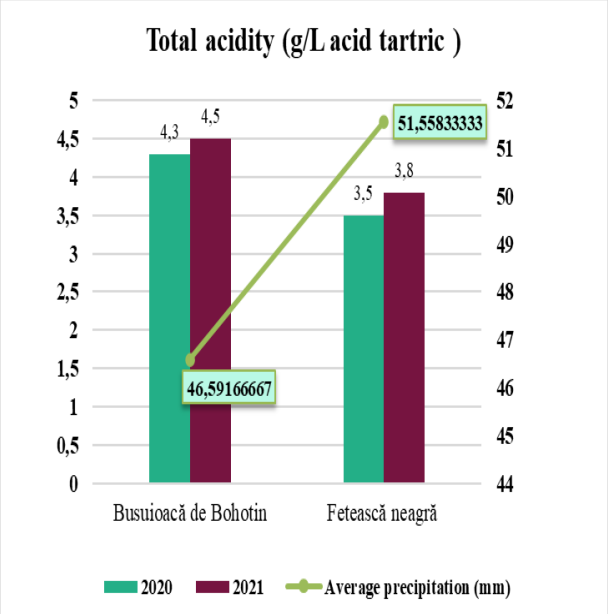


Figure 2 **Correlation between total acidity and precipitation**

In the case of the wines obtained from Busucioacă of Bohotin, a difference of 0.2 g/L tartaric acid can be observed, more in the case of

sample V2. In the case of the wines obtained from Feteasca neagră, the same difference can be observed this time of 0.3 g/L in the case of the V4 sample, both obtained in 2021.

Malic acid is a natural acid founded in grapes in a certain amount making it unstable in all circumstances, but especially in rosé wines.

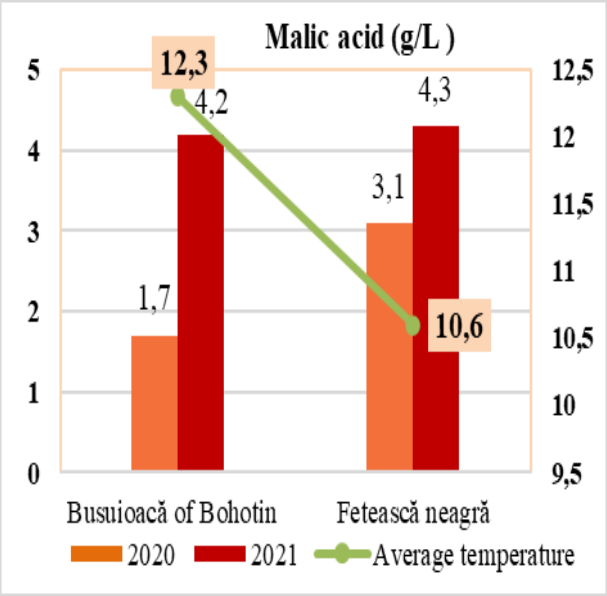


Figure 3 **Correlation between malic acid and average annual temperatures 2020-2021**

Due to the high temperatures of 2020, the malic acid in the samples highlighted less values as can be observed in *figure 3*, because it was metabolized leading to a decrease in it. As a result, it is visible in sample V1 with 1.7 g/L.

The fact that 2021 stood out to be a wet year, with low temperatures, the average being 10.6ºC, encouraged the accumulation of malic acid in the grapes and later to be found in the analyzed wines. As a result, a higher amount of malic acid was found in samples V2 (4.2g/L) and V4 (4.3g/L).

The determination by the software DIGITAL COLOR ATLAS 3.3 of the chromatic properties of the wines made it possible to emphasize the color differences through a visual separation.

According to the data in *table 3*, the wine from the Fetească neagra variety from 2020 (V3) is the most intensely colored, unlike the one from 2021 with a slightly lower shade. In the case of the wine from the Busuiocă de Bohotin variety, the one obtained also in 2020 (V1) stood out as more intense. This is the result both of the grape variety of which it is part, as the amount of anthocyanins is in greater quantity in the 2020 samples, but also of the technique that the oenologist chooses to use to produce the desired wine in perfect balance.

In terms of clarity (L*), all samples present high clarity, but sample V2's values of 98.07 stood out as the highest. However, the wines tend to have a distinct peach-salmon hue. With high values between 5.01 and 4.75 in samples V3-V4, the indication stood out as a strong predictor of green color intensity. The specific values of the red-green coordinates had lower values in the Busuioacă de Bohotin samples with 2.1 in the V1 sample and 1.74 in the V2 sample, which is also due to the fact that the variety it belongs to is lighter.

In the case of sample V3, it can be clearly observed that the b parameter belonging to the yellow-blue coordinate had higher values. In contrast, the b-values of samples V1 and V2 range from 5.31 to 3.12, while those of samples V3 and V4 range from 8.84 to 7.50.

It can deduce that the parameter a, has higher values in 2021, and the color, which is more intense in the samples of the year 2020, are both due to the more extreme heat conditions that emphasize these factors.

Table 3

**CIE Lab 76 color parameters and chromatic color stimulation for the analyzed rosé wines**

| Experimental variants | Clarity L* | Chromaticity | | Saturation/Chroma C | Tone H | The brightness | Tempt | Computer simulation of wine color |
|---|---|---|---|---|---|---|---|---|
| | | a | b | | | | | |
| **V1** | 97.26 | 2.17 | 5.31 | 5.73 | 67.73 | 0.31 | 1.73 | |
| **V2** | 98.07 | 1.87 | 3.12 | 3.64 | 58.98 | 0.19 | 1.57 | |
| **V3** | 95.09 | 5.01 | 8.84 | 10.16 | 60.43 | 0.57 | 1.48 | |
| **V4** | 95.48 | 4.75 | 7.50 | 8.88 | 57.67 | 0.50 | 1.42 | |

Based on the criteria listed above, the chromaticity of the tested samples corresponding to the two grape varieties considered was determined. From the low chromaticity and excellent clarity of the wines, it can be observed that the high temperatures and low rainfall had a semnificative impact on the color.

Saturation shows how much white is incorporated into the color. The saturation in the analyzed samples had a maximum value in the V3 sample (10.16) and a minimum value in the V2 sample (3.64), which indicates that no wine blending was used in the technological process.

The tonality of the analyzed varieties from the two years is comparable, which indicates that there is not much variation between the sample values.

The sample from the Fetească neagră variety from 2020, V3, had a score of 60.43, instead in 2021 it had 57.67.

The samples from the Busuiocă de Bohotin variety in 2020 had a tonality of 67.73, while in 2021 it was slightly lower at 58.98. This indicates that temperature fluctuations, solar radiation and the acquisition technology have imprinted on this aspect.

According to the tasters, all versions are similar to each other, the difference is the intervention of climatic conditions as well as the unique technology, and the decision of the oenologist. *Figure 4* presents the diagrams made following the mathematical calculation of the wine bonus points received.

Olfactory profile of the obtained wines was slightly influenced by the climatic conditions, both wines vinified in the 2020 harvest year present the characteristics of rich wines, such as ripe fruit, dried fruit, forest fruit and peony that transport you to a state of spirit of a pleasant summer night.

On the other hand, the wines produced in the 2021 harvest year had an aromatic profile nuanced by the aromas of citrus fruits, exotic fruits, wildflowers and rose, a fact due to the accentuation of the colder climatic conditions. The character of honey, basil, mineral and vegetal green were imprinted in all four varinates in a balanced way.

Regarding the taste profile of the 2021 samples, it was observed that due to the low temperatures and precipitation throughout the maturation period, the acidity of the wines is increased and a decrease in the intensity of the sweet, bitter and phenolic characteristics is observed. On the other hand, as can be seen in *figure 4*, the persistence and structure of the wines examined in 2021 stand out as increasing, compared to the unctuous character which is less

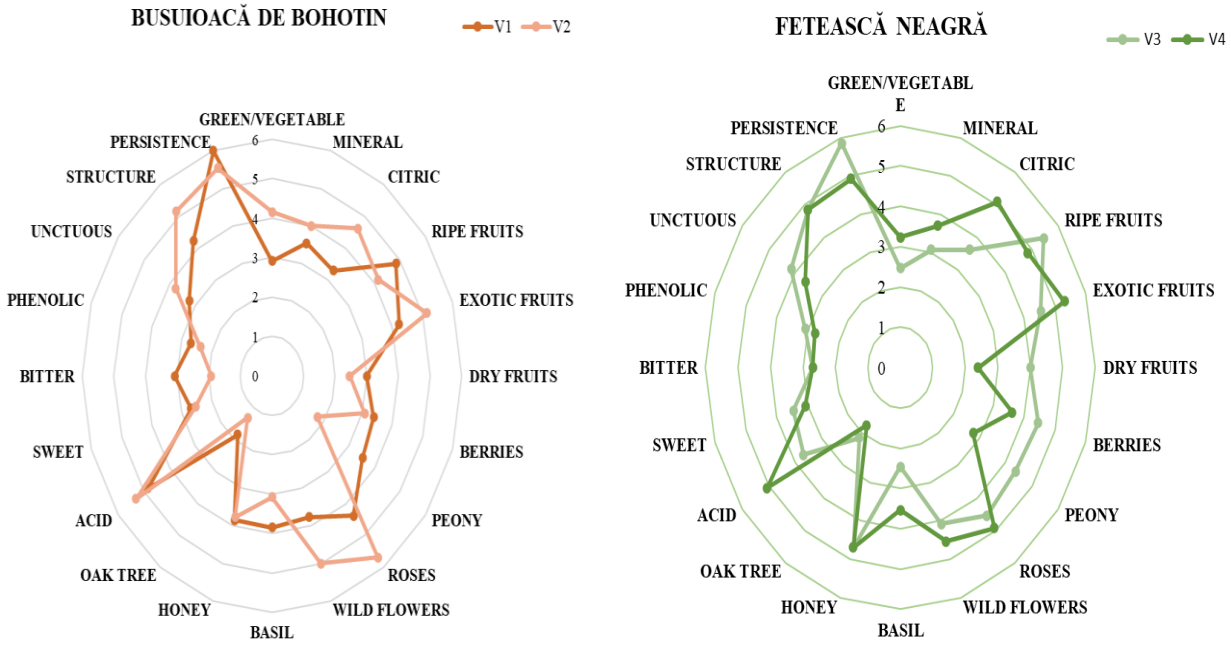


Figure 4 **Graphical representation of the tasting data for Fetească neagră and Busuioacă de Bohotin wine**

On the other hand, the taste profile of the wines made in 2020 reveals a decrease in acidity as a result of the warmer weather, which leads to the loss of freshness of the wines, which is not desirable. In both cases, the sweet character is perceived more strongly, weakening the other qualities. The V1 variant is reported to have a more consistent structure and high persistence after tasting, leaving a more pronounced aroma, although the unctuous character of both samples is noted to be in balance.

## CONCLUSIONS

After conducting this study, the following were found: the year 2020 was warmer with an average temperature of 12.3°C, which caused a faster ripening of the grapes implicitly and a greater accumulation of sugars, leading to a higher alcohol concentration in V1 and V3 wines. From a sensory point of view, both wines show the characteristics of rich wines, such as ripe fruit, dried fruit, wild berries and peony that transport you to a pleasant summer night's mood. The

olfactory profile reveals a decrease in acidity as a result of the warmer weather, which leads to a loss of freshness in the wines, which is not desirable.

However, the year 2021 stood out as being the opposite of 2020, being marked by more significant amounts of precipitation with a total of 559.1 mm, which also dictated a decrease in average annual temperatures to 10.6 °C. This found an alcohol concentration slightly lower in both wines, 12 and 13, but with more acidity, 4.5 g/L tartaric acid in the case of sample V2 and 3.8 g/L in the case of V4. The aromatic profile of the wines is nuanced by the aromas of citrus fruits, exotic fruits, wildflowers and rose, a fact due to the accentuation of the colder climatic conditions.

In the end, it can conclude that no wine is the same from year to year, without a doubt. Nature is truly a lucky charm of Strunga Winery.

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# COMPARATIVE STUDY OF THE EFFECT OF DRY AND WET AGING ON BEEF MEAT COLOUR PARAMETERS DURING MATURATION

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**Abstract**

Meat colour remains one of the most important quality parameters influencing consumers and attracting the attention of meat scientists around the world. The objective of this scientific article was to follow the colorimetric differences produced by two types of maturation - wet and dry, on meat from intensively reared cattle. Colorimetric tests were performed on samples of the beef round for a 20-day maturation period, the first sample being analyzed less than 24 hours after slaughter and the others on days 4, 8, 12, 16, and 20 of maturation. Colorimetric measurements were performed both on the meat surface and in sections for both types of maturation. The colour of beef outside showed non-significant differences ($p > 0,05$) between the two types of maturation studied for all three colorimetric parameters studied (L*, a*, and b*), with highly significant differences ($p < 0.001$) only between the type of maturation and advancement of maturation (TM*Days) for the same colour parameters. As regards the colour of the beef round in the section, the differences identified were highly significant ($p < 0.001$) for the parameters L* and b*, but significant ($p < 0.05$) for the parameter a*. As for the colour on the outside of the meat, highly significant differences ($p < 0.001$) were identified between the type of maturation and the advancement of the maturation period in the section of the beef round.

**Keywords**: beef meat colour, dry-aging, wet-aging, pH

The beef industry is a vital factor for the agricultural economy, with global production reaching approximately 73.9 million tons in 2022, a value that is 1.4% higher compared to 2021 (FAO, 2022).

The color of meat is essential for consumer acceptability. Dark or overly pale colors are associated with a decrease in consumer preference and a more pronounced rejection compared to a bright red hue (Hughes J. *et al*, 2017; Jeremiah L.E. *et al*, 1972; Viljoen H.F. *et al*, 2002). Among the quality attributes of beef, maintaining a red color is of major importance in terms of its attractiveness, being interpreted as an indicator of freshness and safety. All changes that occur inside or on the surface of the muscle are reflected in the resulting color (Gašperlin L. *et al*, 2001). Beef cuts that exhibit changes in color are often sold at reduced prices or are ground to produce lower-value products, such as ground beef, and if the color change is very pronounced, the product will be discarded. All of these practices result in economic losses. A very important factor that affects meat color is meat aging, according to numerous studies (Smith G.C. *et al*, 2000).

In general, aging can be dry aging (where beef carcasses or primary/subprime cuts are stored at a refrigerated temperature without packaging materials) or wet aging (primarily, meat cuts are vacuum-sealed). Dry aging is typically intended for higher-quality meat and occurs in well-controlled environmental conditions in terms of temperature, relative humidity, and air ventilation (Smith R.D. *et al*, 2008).

Wet aging is an aging process introduced in the 1970s, where vacuum packaging is used to protect the meat from spoilage and dehydration when it is stored for aging in a refrigerated environment for a period of 3 to 83 days. This type of aging offers several economic advantages, including significant reductions in weight and trim losses. It also requires less storage space and is suitable for an automated and efficient production process. In addition to these benefits, wet aging extends the shelf life of meat by controlling microbiological factors, without compromising palatability characteristics (Kim Y.H.B. *et al*, 2018).

The main mechanism of meat aging is attributed to the proteolysis of essential myofibrillar and cytoskeletal proteins (Koohmaraie

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M., 1996; Ouali A., 1990; Valin C., 1988). Boakye K. and Mittal G.S. (1996) argue that the duration of aging influences all CIE color parameters (the values of L*, a*, b* parameters and color attributes: hue, saturation, and intensity) of beef.

The surface color of meat is largely determined by the concentration and chemical state of myoglobin, as well as the depth of myoglobin layers (Faustman C. *et al*, 2010), and also by changes in post-mortem muscle structure. In the case of an early drop in muscle pH after slaughter, there is evidence of structural changes and the formation of drip channels (Heffron J.J.A., Hegarty P.V.J., 1974; Bertram H.C. *et al*, 2004), which could result in differences in oxygen absorption, reflection, and penetration into the muscle. As the muscle enters rigor mortis, there is a 14-16% reduction in muscle fiber diameter, leading to an increase in extracellular space (Heffron J.J.A., Hegarty P.V.J., 1974). Additionally, myofibrils can contract, creating opportunities for water formation and loss (Diesbourg L. *et al*, 1988; Bertram H.C. *et al*, 2004). The magnitude of these changes is a pH-dependent process and is a key factor in the alteration of post-mortem muscle color. A slight decrease in pH results in a higher pH (pH > 5.7) and a darker color. In contrast, a complete drop in pH to 5.4-5.5 leads to a bright red-purple color (Murray A.C., 1989).

Rapid post-mortem metabolism (rapid pH decline) can lead to the denaturation of proteins, resulting in changes in light diffusion and the loss of sarcoplasmic proteins, such as myoglobin in the muscle (Swatland H.J., 2008), and hence the appearance of a paler color of beef. For these reasons, it has been hypothesized that meat color is influenced by the drop in pH and temperature after slaughter, with these parameters being determined by factors of animal origin and processing conditions (Hughes J.M. *et al*, 2014).

## MATERIAL AND METHOD

In the present study, beef sirloin from intensively raised animals was used to analyze color parameters and pH. A total of 6 sirloins were used, each of which was divided into two pieces, resulting in a total of 12 pieces (samples). Six of these were dry-aged, and six were wet-aged. The meat was acquired on the day of animal slaughter and aged for 20 days, with analyses performed on the day of slaughter and on days 4, 8, 12, 16, and 20 of aging, totaling 6 analysis periods. The first sample was dry-aged, while the second was wet-aged in vacuum-sealed bags. Both samples were stored during aging in refrigerated aging rooms with controlled microclimate parameters. For dry aging, the microclimate parameters were set in accordance with the recommendations established by Kim Y.H.B. *et al* (2016) following a consumer evaluation, which were as follows: a refrigeration temperature of 3°C, relative humidity of 49%, and air current velocity of 0.2 m/s. The refrigeration temperature for wet-aged beef sirloin was 0-2°C, as per Jaspal M.H. *et al* (2021).

For conducting instrumental meat color analyses, a portable Konica Minolta CR-410 colorimeter with a measurement diameter of 50 mm was used, previously calibrated on a white standard plate. To measure the color of the samples, Illuminant D65 with a 10° observation angle was employed. This angle is considered the most representative for reproducing colors as perceived by the human eye, in accordance with the method provided by Kim Y.H.B. *et al* (2016).

The data were analyzed in the CIELAB color space, which expresses the quantitative relationship between colors on three axes: L*, a*, and b*. L* indicates values from 0 (black) to 100 (white). The a* component indicates the presence of red colors (for positive values) and green colors (for negative values), while the b* parameter indicates the presence of yellow colors (for positive values) and blue colors (for negative values). Colorimeter measurements were conducted on both the meat's surface (exterior) and in its cross-section, with 10 measurements for each aging and section analyzed.

To determine the pH value, a digital pH meter specially designed for meat and meat products, Hanna Instruments HI 99163, was used. pH measurements were performed five times for each type of aging on the following days of aging: 0, 4, 8, 12, 16, and 20.

The aging of the meat subjected to the analysis took place within the Meat Microproduction Workshop at the U.S.V. Iași, and the color and pH analyses were carried out in the Meat Technology and Quality Control Laboratory of the same university.

The data obtained from pH and colorimeter evaluations were processed using the ANOVA (Analysis of Variance) statistical test within the XLSTAT software for Microsoft Excel.

## RESULTS AND DISCUSSIONS

*Table 1* presents the results regarding the pH of the meat samples analyzed for the two types of aging studied: dry aging and wet aging. The aim was to investigate the influence of the type of aging, the influence of the aging progression (days of aging), and the interaction between these two factors on the pH value.

Following the analysis of the results in *table 1*, we can observe that the type of aging, aging progression, and the interaction between the type of aging and aging progression exhibit highly significant differences ($p < 0.001$) in the mean pH values. These results contradict those obtained by

Kim Y.H.B. *et al* (2016), who found nonsignificant differences ($p > 0.05$) regarding the influence of the type of aging on pH values.

Table 1

**The average pH values during the aging period and the influences produced by the type of aging, its evolution, and the interaction between these two characteristics on the pH values**

| Aging time | Type of aging | |
|---|---|---|
| | Dry | Wet |
| 0 | 5.630±0.006$^{a}$ | 5.514±0.009$^{a}$ |
| 4 | 5.726±0.009$^{b}$ | 5.546±0.004$^{a}$ |
| 8 | 5.796±0.007$^{b}$ | 5.758±0.010$^{bc}$ |
| 12 | 5.660±0.005$^{ab}$ | 5.710±0.011$^{b}$ |
| 16 | 5.976±0.012$^{c}$ | 5.716±0.007$^{b}$ |
| 20 | 6.004±0.016$^{d}$ | 5.922±0.015$^{c}$ |
| p-value | | |
| Type of aging | **< 0.0001 (***)** | |
| Days of aging | **< 0.0001 (***)** | |
| Type of Aging*Days of aging interaction | **< 0.0001 (***)** | |

a, b, c, d - Superscripts on different means within the same column differ significantly, $p \leq 0.05$

The lowest average pH value was recorded on day 0 for both dry aging and wet aging (5.630±0.006 and 5.514±0.009, respectively). Subsequently, in the case of dry aging, this value gradually increased until the 12th day of aging when a slight decrease in pH was observed, reaching a mean pH value close to the initial pH (5.660±0.005), as can be seen in *figure 1*. After this decline, there was a gradual increase in pH values until the end of aging (day 20), resulting in a final pH with an average value of 6.004±0.016 (*table 1*).

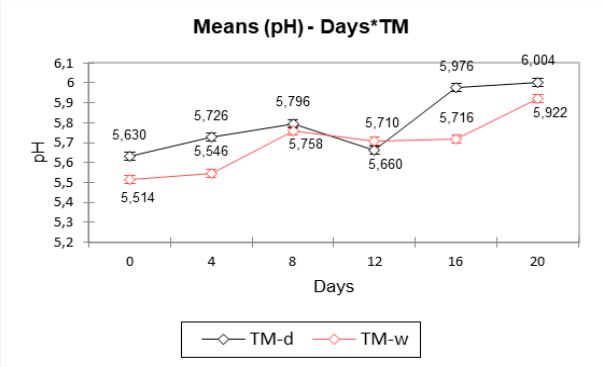


Figure 1 **The mean values of pH during wet and dry aging**

*TM – type of maturation/aging; d – dry aging; w – wet aging*

Similar to dry aging, wet aging also exhibited a decrease in pH values on the 12th day (5.710±0.011). However, this decrease was not as significant as that observed in dry aging, but it was only slightly lower by 0.048 compared to the previous day's pH measurements (day 8). Additionally, the subsequent increase in pH values in wet aging was less pronounced after the drop on day 12 compared to dry aging. At the end of the aging process, a lower final pH was observed in wet aging (5.922±0.015) (*table 1, figure 1*) than that obtained in dry aging (6.004±0.016) (*table 1, figure 1*).

The instrumental results of the color parameters performed on the surface of the beef sirloin are presented in *table 2*. The results are differentiated according to the type of aging applied.

The results of the statistical analysis of the studied color parameters (L*, a*, and b*) on the exterior of beef sirloin indicate a nonsignificant influence ($p > 0.05$) of the type of aging on all of them. Highly significant differences ($p < 0.001$) were identified in all the mentioned parameters for the interaction between type*days of aging. Concerning only days of aging, the CIE b parameter exhibited distinct significant differences ($p < 0.01$), while the other two analyzed colorimetric parameters (CIE L* and CIE a*) showed highly significant differences ($p < 0.001$), as can be seen in *table 2*.

The differences between the types of aging on the same days when colorimeter measurements were taken (day 0, 4, 8, 12, 16, and 20) were determined using the Tukey (HSD) test. The results obtained are presented in *table 2* and highlight highly significant differences ($p < 0.001$) for the L* parameter between the two types of aging throughout the entire aging period (from day 0 to day 20). Regarding the CIE b* parameter, significant differences ($p < 0.05$) were recorded only on days 4 and 20 (*table 2*), while for the other analyzed days, the differences were nonsignificant ($p > 0.05$). The CIE a* parameter only exhibited nonsignificant differences ($p > 0.05$) concerning the influence of the type of aging on the same aging day (*table 2*).

The highest value for the entire aging period of the CIE L* parameter was achieved by dry aging on the 8th day (45.442±0.627) of color measurements. Similarly, for the CIE a* and CIE b* parameters, the highest values were also

recorded during dry aging, specifically 21.866±1.973 (on the 4th day of aging) and 9.462±1.888 (on the first day of aging), as indicated in the data presented in *table 2*.

Table 2

**The effects of aging type, evolution of aging, and the interaction between aging type and evolution of aging on color parameters (L*, a*, and b*) on the exterior of the meat**

| Type of aging | Aging time | Parameters | | |
|---|---|---|---|---|
| | | L* | a* | b* |
| Dry | 0 | 40.944±0.741$^{d}$ | 21.798±1.493$^{c}$ | 9.462±1.888$^{c}$ |
| | 4 | 42.300±0.745$^{d}$ | 21.866±1.973$^{c}$ | 9.162±1.148$^{c}$ |
| | 8 | 45.442±0.627$^{e}$ | 19.200±0.621$^{bc}$ | 7.616±1.023$^{bc}$ |
| | 12 | 33.800±0.697$^{b}$ | 11.058±0.521$^{a}$ | 6.974±1.233$^{abc}$ |
| | 16 | 30.234±0.915$^{a}$ | 9.770±1.265$^{a}$ | 3.802±0.718$^{ab}$ |
| | 20 | 29.862±0.309$^{a}$ | 9.554±2.767$^{a}$ | 2.702±0.810$^{a}$ |
| Wet | 0 | 36.780±0.395$^{c}$ | 19.378±1.874$^{bc}$ | 7.104±0.805$^{abc}$ |
| | 4 | 36.954±0.454$^{c}$ | 16.686±1.226$^{abc}$ | 4.604±0.240$^{ab}$ |
| | 8 | 37.332±0.197$^{c}$ | 15.440±1.249$^{abc}$ | 5.644±0.351$^{abc}$ |
| | 12 | 38.072±0.456$^{c}$ | 14.692±1.917$^{abc}$ | 7.608±0.417$^{bc}$ |
| | 16 | 37.698±0.459$^{c}$ | 13.674±1.084$^{ab}$ | 6.246±0.424$^{abc}$ |
| | 20 | 38.050±0.302$^{c}$ | 16.780±1.007$^{abc}$ | 7.452±0.332$^{bc}$ |
| p-value | | | | |
| Type of aging | | 0.245 | 0.527 | 0.738 |
| Days of aging | | **<0.0001 (***)** | **<0.0001 (***)** | **0.004 (**)** |
| Type of aging*Days of aging interaction | | **<0.0001 (***)** | **0.001 (***)** | **<0.0001 (***)** |
| Tukey Honest Signifficant Difference (HSD) for different type of aging (wet aging versus dry aging) in the same day | | | | |
| Day 0 | | **0.000(***)** | 0.993 | 0.791 |
| Day 4 | | **<0.0001 (***)** | 0.440 | **0.037 (*)** |
| Day 8 | | **<0.0001 (***)** | 0.847 | 0.923 |
| Day 12 | | **0.000(***)** | 0.873 | 1.000 |
| Day 16 | | **<0.0001 (***)** | 0.814 | 0.752 |
| Day 20 | | **<0.0001 (***)** | 0.067 | **0.025 (*)** |

a, b, c, d - Superscripts on different means within the same column differ significantly, $p \leq 0.05$

At the end of aging, it can be observed that dry-aged beef sirloin has lower average values on the exterior for the colorimetric parameter L* compared to the average values obtained for the same colorimetric parameter in the case of wet aging. Analyzing these data, it can be inferred that dry-aged beef sirloin has a darker color on the meat's surface due to the formation of a crust, dehydration in the superficial layer, and, consequently, the concentration of color compounds. This observation is also supported by the comparative analysis of the data in *table 2* and *table 3* regarding the CIE L* parameter, which are relatively similar for wet aging, with no significant differences. The lack of significant differences is attributed to good color compound diffusion within the beef sirloin and the absence of dehydration. The data obtained by us for the exterior of dry-aged beef sirloin are in line with the findings of Kim Y.H.B. *et al* (2016).

*Table 3* presents the results of the color parameters from the cross-section of beef. By analyzing the described table above, we can observe that the type of aging had a highly significant influence ($p < 0.001$) only on the CIE L* and CIE b* parameters, while for the CIE a* parameter, its influence was significant ($p < 0.05$). As for the characteristics of days and the type of aging*days of aging interaction, highly significant differences ($p < 0.001$) were identified for all the studied colorimetric parameters (*table 3*).

After applying the Tukey (HSD) statistical test, the most significant differences between the types of aging on the same aging day were observed within the CIE b* color parameter *(table 3*). Highly significant differences ($p < 0.001$) were recorded on the 8th day of aging, while significant differences ($p < 0.05$) were observed on days 4 and 12. The colorimetric parameter a* was affected by the type of aging according to the Tukey test only on the 16th day of meat aging, where highly significant differences ($p < 0.001$) were observed, as indicated in *table 3.*

The average values of the L* parameter (*table 3*) in the meat's cross-section exhibit a relatively high degree of similarity and some linearity (minimal fluctuations in the average values between the days of analysis). The same characterization can also be attributed to the a* parameter in the meat's cross-section (*table 3*), especially in the case of dry aging, as wet aging shows more significant differences in the average values towards the end of the aging period. The b* parameter displays the largest fluctuations in average values during the meat's aging in its cross-section (*table 3*).

Table 3

**The effects of aging type, evolution of aging, and the interaction between aging type and duration on meat color parameters (L*, a*, and b*) in the section of the beef sirloin**

| Type of aging | Aging time | Parameters | | |
|---|---|---|---|---|
| | | L* | a* | b* |
| Dry | 0 | 37.436±0.578$^{bc}$ | 19.468±0.701$^{d}$ | 4.618±0.801$^{ab}$ |
| | 4 | 38.906±0.609$^{de}$ | 19.106±0.415$^{bcd}$ | 6.604±0.715$^{cd}$ |
| | 8 | 37.882±0.336$^{cd}$ | 19.060±0.808$^{bcd}$ | 7.414±0.603$^{d}$ |
| | 12 | 38.038±0.256$^{cde}$ | 18.038±0.647$^{bc}$ | 7.022±0.608$^{d}$ |
| | 16 | 37.910±0.526$^{cd}$ | 18.288±0.563$^{bcd}$ | 5.298±0.482$^{bc}$ |
| | 20 | 36.660±0.218$^{ab}$ | 18.440±0.192$^{bcd}$ | 4.536±0.328$^{ab}$ |
| Wet | 0 | 36.050±0.336$^{a}$ | 19.330±0.279$^{cd}$ | 4.108±0.296$^{ab}$ |
| | 4 | 36.890±0.428$^{abc}$ | 19.378±0.152$^{cd}$ | 4.164±0.194$^{ab}$ |
| | 8 | 36.104±0.160$^{a}$ | 18.918±0.172$^{bcd}$ | 4.134±0.171$^{ab}$ |
| | 12 | 35.958±0.372$^{a}$ | 18.820±0.330$^{bcd}$ | 4.598±0.202$^{ab}$ |
| | 16 | 39.090±0.370$^{e}$ | 14.470±0.711$^{a}$ | 6.668±0.482$^{d}$ |
| | 20 | 36.884±0.451$^{abc}$ | 17.912±0.189$^{b}$ | 3.700±0.242$^{a}$ |
| p-value | | | | |
| Type of aging | | **0.000 (***)** | **0.040 (*)** | **<0.0001 (***)** |
| Days of aging | | **0.000 (***)** | **<0.0001 (***)** | **0.000 (***)** |
| Type of aging*Days of aging interaction | | **0.000 (***)** | **0.000 (***)** | **0.000 (***)** |
| Tukey Honest Signifficant Difference (HSD) for different type of aging (wet aging versus dry aging) in the same day | | | | |
| Day 0 | | 0ș428 | 1.000 | 1.000 |
| Day 4 | | **0.044 (*)** | 1.000 | **0.030 (*)** |
| Day 8 | | 0.119 | 1.000 | **0.001 (***)** |
| Day 12 | | **0.033 (*)** | 0.992 | **0.032 (*)** |
| Day 16 | | 0.665 | **<0.0001 (***)** | 0.667 |
| Day 20 | | 1.000 | 1.000 | 0.982 |

a, b, c, d, e - Superscripts on different meanswithin the same column differ significantly, $p \leq 0.05$

Within dry aging in the beef's cross-section (*table 3*), there is a noticeable trend of decreasing average values for all the color parameters analyzed. This could be attributed to the lack of atmospheric oxygen and, consequently, the formation of oxymyoglobin. The same can be observed in wet aging in the meat's cross-section for the colorimetric parameters a* and b* (*table 3*). These results are contrary to those obtained by Abril M. *et al* (2001). They did not analyze color parameters based on the type of aging but rather based on the final pH of the meat. Although the final pH for both types of aging used in this study fell within the pH values < 6.1 that they examined, the instrumental results for the color parameters were contradictory.

## CONCLUSIONS

This scientific study aimed to investigate the influence of two types of aging (wet aging and dry aging) on the CIE L*, a*, and b* color parameters and on the pH value.

As a result of the conducted analyses, it was found that the type of aging, the stage of aging, and the interaction between the type of aging and the stage of aging significantly influence the pH value.

The CIELAB system parameters on the meat's exterior are insignificantly affected ($p>0.05$) by the type of aging, but the interaction between type and days of aging significantly affects ($p < 0.001$) all the analyzed colorimetric parameters.

In the case of the meat's cross-section, the three analyzed color parameters (CIE L*, CIE a*, and CIE b*) were significantly influenced ($p<0.001$) by the stage of aging (days of aging) and by the interaction between type and days of aging. The type of aging significantly influenced ($p<0.001$) the L* and b* parameters, but for the a* parameter, the influence was only significant ($p<0.05$).

The most significant colorimetric differences at the end of aging compared to the beginning are caused by dry aging on the meat's exterior. In the case of this type of aging, dehydration occurs on the meat's surface, leading to the formation of a brownish crust (which darkens progressively during aging). This darker color is the result of the concentration of pigment substances (mainly myoglobin) on the surface, resulting in darker meat. The most noticeable changes on the meat's exterior began to appear from day 12 of aging, intensifying until the end of the process.

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# QUANTITATIVE CHARACTERISATION OF CHICKEN BREAST OPTIMISED WITH DIFFERENT AMOUNTS OF BRINE

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**Abstract**

Chicken meat is considered an affordable source of high-quality protein and a complete provider of essential nutrients required for proper bodily functions. It has gained popularity not only in developed countries but especially in nations with growing economies. While the meat processing industry emphasizes the technological aspects of meat, consumers are keen on its sensory and nutritional attributes. This study aimed to characterize and compare three batches of chicken breast pastrami injected with three different brine percentages (5%, 8%, and 12%) in terms of physicochemical attributes (pH, colour, moisture, protein, lipid, and salt content) as well as sensory characteristics. The study results revealed significant differences between the pastrami batches concerning moisture and protein content ($p<0.05$). The brine percentage had a notable impact on the colour and pH properties of the meat products. In terms of cross-sectional colour, the data analysis indicated that meat samples injected with the higher LBI3 brine percentage exhibited increased lightness (73.13) and a more pronounced yellow hue (9.40) compared to the other two samples injected with 5% brine (70.97, and 8.90, respectively) and 8% brine (71.25, and 8.73, respectively). In terms of sensory evaluation, the samples were assessed for attributes such as colour, texture, juiciness, flavour, and overall acceptability. The batch of chicken breast pastrami injected with 8% brine (LBI2) received favourable scores for overall quality and colour, whereas the LBI3 batch distinguished itself with texture and juiciness, which were highly appreciated by the evaluators.

**Key words**: meat technology, chicken breast, brine injection

The increasing global population is driving a growing demand for animal protein on a global scale as efforts are made to meet the expanding protein needs of people. In this context, poultry meat consumption is steadily on the rise (Weimer L.S. *et al*, 2022). Modern consumers, in particular, exhibit a strong preference for the animal protein found in poultry meat, which has led to an increased focus on the quality of poultry meat products (Grasso A.C. *et al*, 2021).

In recent years, there has been a shift in consumer behaviour from the consumption of whole chickens to portioned chicken, with a particular emphasis on chicken breast fillets and processed products. These changes have been motivated by the need for convenience in meal preparation in today's fast-paced, industrialized era, as well as the desire to cater to consumer preferences for specific chicken parts (Nusairat B. *et al*, 2022). Different production methods have varying impacts on carcass and meat quality. The price of poultry meat is primarily influenced by the commercial characteristics of carcasses. While the meat processing industry emphasizes the technological aspects of meat, consumers are increasingly interested in the sensory and nutritional qualities of meat. The visual appearance of meat is also gaining importance. Health considerations remain a significant concern for all parties involved, from producers to consumers (Baéza E. *et al*, 2022).

For consumers seeking poultry meat with exceptional quality and sensory characteristics, the product range has expanded to include meat produced under the national food quality assurance program. Regarding the superior quality attributes of 'premium' meat, external sensory features, such as the absence of external hematomas and a colour ranging from light pink to pink, are taken into account, as well as physical attributes indicating meat safety and durability (Grzybowska-Brzezińska M. *et al*, 2023).

The objective of this study is to determine the physicochemical and sensory quality of three batches of poultry meat preparations with a compact structure, manufactured using the chicken breast pastrami production method, with the specification of injecting brine at three different percentages: 5%, 8%, and 12%.

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## MATERIAL AND METHOD

The research material comprised three batches of chicken breast pastrami, with each sample batch containing six samples. The samples from each batch were utilized for assessing physicochemical characteristics such as pH, color, chemical composition, salt content, and for the analysis of sensory properties. The chicken breast underwent trimming, tendon removal, and was adjusted to a uniform weight. Subsequently, brine was prepared, consisting of water, ice flakes, and salt, within a double-walled electric kettle filled with glycerol.

The injection treatment involved brine injection at 5%, 8%, and 12%. An INWESPOL AN-10 machine equipped with ten needles was used for the injection process.

The pH analysis was conducted using a portable meat pH meter, Hanna model HI99163, by inserting the penetration electrode at three different points on each piece of pastrami. The instrument used for these measurements was pre-calibrated for pH values of 4 and 7. Between calibrations and readings, the instrument's electrode was cleaned with distilled water to prevent any influence on the results obtained.

Colour determinations of the chicken breast pastrami samples were performed using a colourimeter programmed with the CIE Lab* system (Konica Minolta CR-410T), which was calibrated before assessing the colour of each sample batch.

For the measurement of proximate chemical composition, which includes water, protein, fat, and salt content, approximately 100 g of each sample was finely shredded and placed in a Petri dish. The proportions of the mentioned parameters were determined via near-infrared transmission spectroscopy using a FoodCheck analyzer.

For the sensory analysis, the samples were sliced into uniform pieces and stored under refrigerated conditions until the actual analysis. Descriptive sensory testing was conducted with the voluntary participation of a group of 5 evaluators, each possessing moderate experience in sensory profiling. These evaluators were pre-trained on attributes that could effectively describe the samples being assessed. They individually analyzed sensory attributes, including colour, odour, aroma, texture, juiciness, and overall impression for each sample. Scores were assigned on a scale ranging from 0 (no perception) to 100 (highly perceptible).

The results obtained for the physicochemical parameters, along with the findings from the sensory analysis, were subjected to ANOVA statistical testing, and mean values were compared using Tukey's test at a significance level of 5% ($p < 0.05$).

## RESULTS AND DISCUSSIONS

The average pH values observed in the chicken breast pastrami samples, post-heat treatment, fell within the range of 5.15 to 5.79 (*table 1*). Notably, the percentage of injected brine significantly influenced the pH value, with sample LBI3, injected with 12% brine, demonstrating a pronounced increase in acidity. pH serves as a quantifiable indicator of a food product's acid-base equilibrium. The observed slight elevation in pH value can be attributed to the heightened salt content incorporated into the product. It is well-established that salts exert an influence on the acidity-alkalinity balance in meat.

For comparison, Kim Y. *et al* (2015) reported pH values of 6.31±0.05 for injected and heat-treated chicken breast, which were notably higher than those observed in the present study. Results consistent with the findings of the present study were reported by Ha H. *et al* (2019), who documented pH values of 6.15±0.01 for a control sample of chicken breast that underwent a 30% brine injection.

In terms of colour attributes, both surface and cross-sectional analyses indicated that the sample injected with the highest brine percentage (12%) exhibited markedly higher lightness (L*) values (46.640 ± 0.959 and 73.128 ± 1.390, respectively), with a statistically significant distinction from the remaining results. The escalation in meat lightness with the increasing percentage of injected brine can be attributed to the dilution effect that occurs following brine injection, which introduces water and salts. Furthermore, the resultant meat colour is perceptibly influenced by the volume of salt incorporated. In parallel with the changes in lightness, the mean values for parameter b* (yellow-blue) exhibited a discernible increase solely in the case of the 12% brine-injected sample, while the 5% and 8% injection batches displayed negligible distinctions.

In terms of the colour parameter a*, there was a notable decrease in the intensity of the red hue, observed with the progressive rise in injection percentage. This phenomenon was apparent on both the product surface and in the cross-section, where values transitioned from 2.980±0.730 and 2.110±0.711, respectively, to a level of 1.780±0.506 and 1.670±0.311, respectively (*table 1*). However, statistically significant differences ($p<0.05$) were only discerned for the LBI3 sample.

Similar outcomes were observed in a study conducted by Kim D. H. *et al* (2021), where an increase in lightness (L*) and b* values, coupled with lower a* parameter values, was noted following the injection of beef with a 30% brine, as compared to an uninjected control sample. Kim Y. *et al* (2015) reported values for lightness (L*) similar to those observed in our study, at 70.59±2.66, but exhibited

higher values for a* (4.83±0.72) and b* (23.56±0.93) for injection-marinated chicken breast.

Chotyakul N. and Tanakamolpradit T. (2023) documented a comparable trend in colour parameters L* and b*, with values increasing as the injection percentage elevated from 10% (L*=73.79±0.64, b*=14.95±0.39) to 20% brine (L*=77.86±1.59, b*=15.21 0.29).

Contrastingly, Luckose F. *et al* (2017) reported a divergent phenomenon in the context of chicken jerky production. In their study, the application of salting and drying treatments led to results opposite to those obtained in our research, where injection salting and smoking heat treatments were employed. These authors noted that a reduction in salt content, in conjunction with high drying temperatures, resulted in increased lightness (L*) and decreased a* values. This effect was attributed to Maillard reactions and the reduction of myoglobin and metmyoglobin during the drying process.

Table 1

**pH and colour of injected chicken breast pastrami**

| | | Section colour | | | Surface colour | | |
|---|---|---|---|---|---|---|---|
| | ***pH*** | ***L**** | ***a**** | ***b**** | ***L**** | ***a**** | ***b**** |
| **LBI1** | 5.752±0.290$^{a}$ | 70.974±2.822$^{a}$ | 2.110±0.711$^{b}$ | 8.902±0.961$^{a}$ | 43.140±1.335$^{a}$ | 2.980±0.730$^{b}$ | 14.720±1.198$^{ab}$ |
| **LBI2** | 5.798±0.210$^{a}$ | 71.250±1.922$^{a}$ | 2.080±0.228$^{b}$ | 8.730±0.272$^{a}$ | 43.216±0.868$^{a}$ | 2.740±0.472$^{b}$ | 13.760±0.470$^{a}$ |
| **LBI3** | 6.156±0.097$^{b}$ | 73.128±1.390$^{b}$ | 1.670±0.311$^{a}$ | 9.400±0254$^{a}$ | 46.640±0.959$^{b}$ | 1.780±0.506$^{a}$ | 15.380±0.589$^{b}$ |

a, b, c, - The same superscript letter within the same column means there is no significant difference between any two means (p>0.05).
L * = Lightness, a * = redness, b * = yellowness; LBI1 – Brine injection 5%; LBI2 – Brine injection 8%; LBI3 – Brine injection 12%.

The proximate analysis results of the chicken breast patrami according to the injected concentration are presented in *table 2*. The moisture content of the three samples fell within the range of 74.32% to 76.82%. Notably, the moisture content of the starch samples exhibited an increasing tendency in direct proportion to the increasing percentage of brine injected, with the highest moisture level observed in the sample subjected to 12% brine injection (LBI3).

The lipid content of the chicken breast samples remained largely unaffected by the injection parameters (p>0.05). The lipid content ranged from 1.48% (LBI2) to 1.66% (LBI1). These findings align with those reported by Ha H. *et al* (2019) in their study, where chicken breast injected resulted in a lipid content of 1.59±0.25% for the control sample within their research.

In the case of chicken pastrami, the protein content displayed a diminishing trend with increasing injection percentages, with statistically significant differences (p<0.05) observed only in the instance of sample LBI1, which exhibited the highest protein level at 21.75%. This variance in protein content can be attributed to the capacity of brine to facilitate the solubilization of certain meat proteins. This effect stems from the denaturation process undergone by proteins in the presence of salts and water within the brine. Moreover, during the brine injection procedure, some proteins may be leached or washed out from the meat, potentially leading to a reduction in overall protein content.

The results derived from the chemical analysis in this study yielded values lower than those presented by Kim Y. *et al* (2015), who conducted a comparative study of three chicken breast maturation methods (tumbling, tenderization, and injection). Kim Y. *et al* (2015) reported moisture levels of 73.77% and a protein content of 22.86% for injected samples. In contrast, the samples in the current study exhibited higher levels of fat and salt compared to the findings of Kim Y. *et al* (2015). The salt content exhibited a subtle increase that was directly proportional to the increased percentage of brine injection. However, statistically, the differences observed between the three sample batches were not deemed significant (p>0.05).

Table 2

**Proximate analysis of brine injected chicken breast patrami**

| | Moisture | Fat | Proteins | Salt |
|---|---|---|---|---|
| **LBI1** | 74.320±0.334$^{a}$ | 1.660±0.167$^{a}$ | 21.750±0.418$^{a}$ | 1.608±0.391$^{a}$ |
| **LBI2** | 75.420±0.909$^{a}$ | 1.480±0.178$^{a}$ | 20.540±0.219$^{b}$ | 1.708±0.285$^{a}$ |
| **LBI3** | 76.820±0.936$^{b}$ | 1.620±0.083$^{a}$ | 19.260±0.180$^{b}$ | 1.802±0.122$^{a}$ |

a, b, c, - The same superscript letter within the same column means there is no significant difference between any two means (p>0.05).
LBI1 – Brine injection 5%; LBI2 – Brine injection 8%; LBI3 – Brine injection 12%.

The analysis of the descriptive profile of chicken breast pastrami yielded pertinent outcomes for characterizing the sensory quality of the investigated products. *Figure 1* illustrates the mean results achieved by aggregating the scores provided by five evaluators during the sensory evaluation session.

A comprehensive assessment of the average results for the sensory attributes assessed reveals a relatively harmonious sensory profile among the three product batches studied. Generally, the

distinctions observed between the LBI1, LBI2, and LBI3 batches were less pronounced for attributes associated with aroma (6.58 points), colour (8.98 points), and overall acceptability (8.87 points), and slightly more significant for texture (14 points) and juiciness (14.5 points). In the context of these scores, it was discerned that the most substantial disparities resulting from the injected brine volume were discernible in the realm of textural attributes, with juiciness being particularly affected.

In regards to the overall acceptability of the products within each analyzed batch, it is notable that the LBI2 batch obtained the highest scores, tallying 80.12 points, followed by the LBI1 batch with 75.58 points, while the L3 batch achieved a score of 80.12 points.

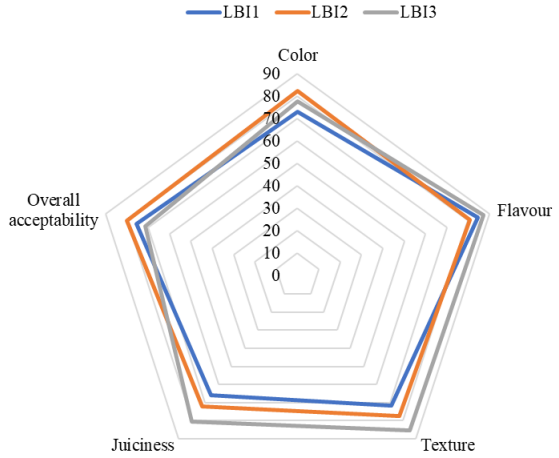


Figure 1 **Sensory traits of chicken breast pastrami**

## CONCLUSIONS

The study has revealed that brine injection and heat treatment can exert a considerable influence on the physicochemical and colour characteristics of chicken breast pastrami. These changes are influenced by the percentage of brine used and may vary depending on the specific preparation methods.

The pH values of the samples demonstrate variance in response to the brine percentage injected, with a conspicuous trend towards heightened acidity (a reduction in pH values), particularly evident in the sample injected with 12% brine. This escalation in acidity can be ascribed to the amplified quantity of salt introduced into the product through the brine mixture. The colouration of the samples is significantly influenced by the brine injection percentage, with an increasing percentage of injection correlating with increased lightness (L*) and yellow-blue coordinate (b*), alongside a decrease in the a* colour parameter.

In terms of chemical composition, the brine injection process exerts a marked influence on the moisture and protein content of chicken pastrami, while demonstrating no statistically significant impact on fat and salt content.

Chicken breast pastrami, subjected to varying brine injection percentages, displayed noticeable disparities in terms of texture and juiciness. However, a relatively evenhanded sensory profile was observed concerning attributes such as flavour, colour, and overall acceptability. Among the batches, LBI2 garnered favourable evaluations regarding the overall impression. These findings underscore the noteworthy impact that selecting an appropriate brine percentage can have on the sensory quality of the product.

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# CHARACTERIZATION OF A SAUSAGE ASSORTMENT MADE FROM BEEF AND PORK LIVER: A COMPARATIVE STUDY

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**Abstract**

The study aimed to determine the influence of the amount of liver introduced into two varieties of liver sausages, pork and beef, on the quality properties of these products. The sausages were made from raw materials from two species (pork and beef) with additions of 25% liver and 50% liver, respectively, resulting in four experimental batches of products: BL25 (sausages with 25% beef liver), BL50 (sausages with 50% beef liver), PL25 (sausages with 25% pork liver), PL50 (sausages with 50% pork liver). Product quality was assessed in terms of chemical composition, instrumental color, pH, and sensory quality. The results showed higher protein contents for batches made with 50% liver, both for pork (19.18%) and beef (19.34%), compared to batches where only 25% liver was added. The same trend was observed for the moisture content; samples made with 50% liver showed higher moisture content compared to those with 25% liver. Increasing the percentage of liver added in the technological process caused a decrease in the lightness (L*) both in the external appearance and cross-sectional aspects of the beef-based batches. In contrast, the increse in liver content led to a rise in the average values for the a* (red-green) coordinate. These two parameters are directly influenced by the percentage of liver in the sausages, through the presence of myoglobin and hemoglobin, which are proteins that contain heme iron.

**Key words**: meat processing, beef/pork liver sausages, quality indicators

Over the last 60 years, there has been a significant shift in meat consumption trends. Bonnet C. *et al* (2020) reported that in 1961, plant products, mainly wheat products, were the primary source of available protein, providing 26 g per capita per day, which accounted for 52% of available protein in the European Union (EU). In contrast, meat contributed only 17 g per capita per day.

Currently, according to González N. *et al* (2020), animal products now account for up to 58% of protein availability, with meat products being the leading source of protein, supplying 28 g per person per day and covering 30% of total calorie consumption.

The growth of the global middle-class population and changes in food supply have led to substantial shifts in dietary patterns, particularly in the consumption of meat and meat products. An increase in meat consumption has been observed among the population, with Basu S. (2015) noting a 204% increase between 1960 and 2010. Other authors have reported an even higher increase in meat consumption for the more recent period from 1992 to 2016, with a 500% rise (Katare B. *et al*, 2020). Future projections also indicate a potential increase in consumption of up to 388 million tonnes by 2030 and 460–570 million tonnes by 2050 (Libera J. *et al*, 2021).

The definition of meat, as per Council Regulation (EC) No. 700/2007 dated June 11, 2007, also encompasses offal, which is a by-product of animal slaughter and is recognized as a high-nutritional-value product. Certain nutrients, particularly minerals such as iron, zinc, magnesium, and calcium, are found in higher levels in the calf and beef liver compared to muscle tissue (Florek M. *et al*, 2012; Biel W. & Kowalczyk A., 2019).

By-products in general and liver in particular, are processed and incorporated into various food products, offering competitive nutritional value. The manufacturing of liver sausage is a well-known practice in many countries, resulting in a wide range of product varieties distinguished by raw materials, optional additions, and seasoning blends (Biel W. & Kowalczyk A., 2019; Florowski T. *et al*, 2021).

Meat products containing liver are renowned for their smooth, rich, and spreadable texture, as they are primarily emulsified products with high-fat content, occasionally reaching percentages as high as 40%. While this aspect enhances textural

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and flavour properties, it may also be viewed negatively by consumers due to the association of high-fat and saturated fatty acid products with the risk of certain cardiovascular diseases (Barbut S. *et al*, 2021; Florowski T. *et al*, 2021).

The challenge for processors in the meat industry is to create high-quality products that are both nutritionally and sensorially appealing, in order to meet market and consumer demands, while also considering the optimal utilization of raw and auxiliary materials to promote sustainability.

The present study aimed to produce and characterize liver sausages, diversified by the species of origin of the liver (pork / beef) and the percentage of added liver (25% / 50%). This assortment is traditionally known as 'caltaboși' and is made in Romania using pork offal and meat, with or without the addition of rice. These products can be consumed fresh or smoked. For the four batches produced, the objective was to characterize them based on pH value, instrumental color, chemical composition, and sensorial quality, while also identifying the extent of influence of these two factors on the finished products.

## MATERIAL AND METHOD

The research took place at the Meat Processing Section and the Meat and Meat Products Technology Laboratory of the University of Life Sciences in Iasi.

***Experimental design***

For the fabrication of the four experimental batches of sausages, the necessary raw materials were procured from the local food market. Table 1 provides an overview of the raw and additional materials essential for producing each of the four assortments.

The experimental protocol consisted in the manufacture of four categories of sausages: two batches with beef liver and two batches with pork liver (25% liver, 5% fat, 15% offal and 50% liver, 10% fat, 10% offal). The other ingredients were added in the same proportions in all samples: pork trimmings, rice (10%), salt (2%), onion powder (1.5%), black pepper (0.5%), nutmeg (0.15%).

Table 1

**Formulations to prepare the sausages**

| Batch code | Ingredients (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Beef liver** | **Pork liver** | **Pork trimings** | **Offal** | **Pork backfat** | **Rice** | **Total** |
| **BL25** | 25 | - | 45 | 15 | 5 | 10 | 100 |
| **BL50** | 50 | - | 20 | 10 | 10 | 10 | 100 |
| **PL25** | - | 25 | 45 | 15 | 5 | 10 | 100 |
| **PL50** | - | 50 | 20 | 10 | 10 | 10 | 100 |

***Sausage production***

The raw meat was passed through a grinder (GRINDER WP - 105) through a 0.8 cm diameter sieve. The rice used in the technological process of obtaining the sausages is washed beforehand, then it is pre-cooked for 20-30 minutes, after which it is cooled so that it can be mixed with the raw materials and spices. To produce the final composition, the meat, liver, fat and pre-cooked rice are transferred into the mixer (CUTTER TITANE V 45L) and mix to homogenize for 10-15 minutes, depending on the quantity and the binding state of the raw material. After mixing, the composition is transferred to the filling machine (REX RVF 220), which is equipped with vacuum and continuous operation. The edible pork membranes used were prepared at least 30 minutes prior to the filling stage by immersing in water at a temperature of approx. 50°C in order to remove excess salt and obtain membrane elasticity.

After filling, the next step is to tie the sausages and then place them on the racks. The sausages are twisted one by one to form individual pieces of 10 cm each, then tied at both ends in the shape of the letter 'U'.

The shaped sausages are then stamped to remove any air voids formed during the filling process, placed on the smoker trolley's grates and placed in the cell for heat treatment (Table 2).

Table 2

**Heat treatment scheme of the sausage batches**

| Heat treatment stage | Time | Temperature inside the cell | Temperature in the thermal centre | Humidity |
|---|---|---|---|---|
| | minutes | °C | °C | % |
| Air drying | 20 | 62 | 45 | 30 |
| Boiling | - | 72 | 69 | 99 |
| Hot air drying | 10 | 86 | 69 | 30 |
| Cooking | 30 | 86 | 69 | 30 |

The products were stored under refrigerated conditions (2-4°C) until the proposed analyses were performed.

***Proximate analyses***

The chemical characterization involved the determination of the proximate chemical

composition, including the assessment of moisture, fat content, and protein levels. These measurements were conducted using a spectrophotometer, specifically the Food-Check analyzer, which utilizes an infrared light source.

The physical characterization of the samples encompassed instrumental color assessment, and evaluation of pH values. Color analysis of the samples was performed using the portable Konica Minolta Chroma Meter CR-410, within the three-dimensional CIE color system. The measurements included L*, a*, and b* color parameters, employing the D65 illuminant at an observation angle of 10 degrees. Prior to measurements, calibration of the instrument was executed on a white calibration plate to establish standard values. pH measurements were conducted with the aid of a HANNA HI99163 digital pH meter. This device is equipped with an amplified pH electrode that incorporates an integrated temperature sensor.

***Sensory evaluation***

For the sensory evaluation of the liver sausages, a group of 30 potential consumers from the student body at the University of Life Sciences were selected. The panel members, ageing from 20 to 25 years, were selected according to Lawless H. T. *et al* (2010).

The sensory evaluation involved conducting a hedonic acceptance test on a 9-point scale. In this test, participants were instructed to assess the samples based on attributes such as appearance, color, flavour, taste, firmness, and overall acceptance (with 1 being extremely undesirable and 9, extremely desirable). The samples were presented for the evaluators in approximately 2 cm thick pieces, each identified by a unique three-digit number for codification.

***Statistical analysis***

To analyze the rezults, the values were compared for proximate composition, color parameters, pH and the acceptability test using an analysis of variance (ANOVA) followed by Tukey's test at a 5% significance level ($p < 0.05$). The statistical analyses were conducted using the XLStat software (version by Addinsoft, 2023).

## RESULTS AND DISCUSSIONS

In terms of pH levels, the examination of liver sausages revealed average pH values ranging from 6.38±0.04 (PL50) to 6.89±0.07 (BL50). These values exceeded those documented by Belleggia L. *et al* (2022), who reported a mean pH of 5.25 ± 0.28 for fermented liver sausages from various producers. This discrepancy can be attributed to the inherent characteristics of the sausages. It is important to emphasize that the safety of fermented sausages is intricately linked to their pH levels. An acidic pH inhibits the proliferation of spoilage and potentially harmful microorganisms naturally present in the raw ingredients. In the case of fermented sausages, the pH gradually decreases as fermentation commences, typically reaching as low as 4.4 (Belleggia L. *et al*, 2022). Conversely, the sausages analyzed in this study underwent heat treatment, which prevented a decline in pH. Furthermore, the observed pH values exceeded those reported by Florowski T. *et al* (2021) for liver sausages enriched with walnut paste, where the control sample exhibited a pH value of 6.13 ± 0.03. Similar pH values were documented by Di Cagno *et al* (2008), describing liver sausages with pH values ranging from 5.99 to 6.62. These variations underscore the significance of pH levels in ensuring the safety and characteristics of fermented sausages, with differences arising due to product type and processing methods.

Regarding colour measurements, the study followed both the surface colour and section colour of the products. When analyzing the surface colour it was found that increasing the liver content caused a significant descrease in lightness (L*), only in case of the beef liver sausages. In terms of redness, there was a significant increase in a* value for both beef liver and pork liver sausage samples due to the increase in the percentage of liver in the recipe. Furthermore, the values of the colour parameter a* were higher for the sausage samples that included pork liver. However, statistically, the species of origin of the liver significantly influenced the intensity of the red colour only in the case of the 25% liver formulations. The colour parameter b* (yellow-blue) showed a decreasing trend on the surface of the products, more pronounced for the beef liver formulations. Nevertheless, statistically, only the type of liver introduced had a significant influence on this parameter ($p<0.0001$).

The colour per section of the four batches of sausages was significantly affected by both factors under consideration (type and percentage of liver). An increase in the colour parameters a* and b* was observed with the increase in the percentage of the liver, which may be attributed to the more intense colour of the liver due to a higher amount of pigments, such as myoglobin and hemoglobin. The brightness parameter L* per section exhibited a significant increase in value for the pork liver formulation, potentially due to the higher percentage of added fat in this particular sausage sample. The amount of fat in sausages significantly impacts the final colour of the product (Serdarogl M. & Ozsumer M. S., 2003; Estèvez M. *et al*, 2005). When comparing the results obtained in the present study to those described by Florowski T. *et al* (2021), we observed lower values for lightness, higher values for the a* parameter (green-red), and

values that are approximately similar for the b* parameter (yellow-blue). Moreover, the values for lightness and yellowness differed from those documented by Estèvez M. *et al* (2005), who reported values of approximately 66 for lightness and 13 for yellowness.

Table 3

**Effects of different liver type and various percentages of added liver on pH and colour (CIE L*, a*, and b* values) of sausages**

| Parameters / Samples | | BL25 | BL50 | PL25 | PL50 | Significance of influence factors (p value) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | F1 | F2 | F3 |
| | *pH* | 6.64±0.25$^{b}$ | 6.89±0.07$^{c}$ | 6.39±0.008$^{a}$ | 6.38±0.04$^{a}$ | **<0,0001** | 0.059 | **0.041** |
| **Surface colour** | *L** | 39.51±1.04$^{c}$ | 33.99±1.31$^{b}$ | 31.51±0.94$^{a}$ | 30.99±0.51$^{a}$ | **<0,0001** | **<0,0001** | **<0,0001** |
| | *a** | 10.33±0.22$^{a}$ | 14.46±0.41$^{c}$ | 13.05±0.49$^{b}$ | 15.20±0.45$^{c}$ | **<0,0001** | **<0,0001** | **<0,0001** |
| | *b** | 10.50±0.43$^{b}$ | 9.63±0.56$^{b}$ | 7.36±0.63$^{a}$ | 7.34±0.47$^{a}$ | **<0,0001** | 0.078 | 0.093 |
| **Section colour** | *L** | 51.22±1.78$^{c}$ | 49.67±0.54$^{bc}$ | 42.28±1.51$^{a}$ | 47.54±0.42$^{b}$ | **<0,0001** | **0.004** | **<0,0001** |
| | *a** | 10.80±1.03$^{a}$ | 13.53±0.05$^{b}$ | 13.30±0.47$^{b}$ | 15.44±0.12$^{c}$ | **<0,0001** | **<0,0001** | 0.271 |
| | *b** | 10.01±0.50$^{b}$ | 11.71±0.03$^{c}$ | 9.12±0.62$^{a}$ | 10.13±0.03$^{b}$ | **<0,0001** | **<0,0001** | 0.068 |

BL25 - sausages with 25% beef liver; BL50 - sausages with 50% beef liver, PL25 - sausages with 25% pork liver, PL50 - sausages with 50% pork liver, F1 - liver type; F2 - % of liver; F3 – liver type and % interaction;

The proximate compositions of the sausage formulations are presented in *Table 4.* It was observed that formulations containing 50% liver content, both from pork and beef batches, exhibited significantly higher moisture content and notably lower fat content. The elevated moisture content in these finished products can be attributed to the liver's higher water content in comparison to muscle tissue, as supported by previous findings (Biel W. & Kowalczyk A., 2019), which reported a moisture percentage of 70.00% in beef liver as opposed to 66.00% in *Musculus semitendinosus*.

Similarly, fat content displayed higher average values for sausages with 25% liver, a result of the elevated lipid content in the pork trimmings used in the formulation, along with the fat content naturally present in the liver (12.14%, Biel W. & Kowalczyk A., 2019), indicating an inverse relationship with moisture content.

Regarding the protein content of liver sausage formulations, it was observed that higher mean values were present in formulations with a greater amount of liver. However, statistically significant differences were only noted for beef liver formulations *(table 4).*

These variations arise from the distinct chemical compositions of the raw materials employed in the formulation. The liver exhibits a higher protein content in comparison to muscle tissue, with beef liver containing approximately 20.30% protein (Biel W. & Kowalczyk A., 2019), pork liver containing about 22.05% protein (Seong P. N. *et al*, 2014), and pork meat ranging from 18.13% to 19.19% protein (Zomeño C. *et al*, 2023).

The results obtained in this study are consistent with other research findings, albeit influenced by the specific composition of the raw materials and the processing methodology. Consequently, the protein content data obtained in our study surpass the 11.3% protein content reported by Yessimbekov Z. *et al* (2021) for liver patties, yet they are lower than the figures documented by Florowski T. *et al* (2021), who reported protein content of 24.0% in the control sample of liver sausage formulations that incorporated peanut paste.

Table 4

**Proximate composition of liver sausages formulations**

| Samples | BL25 | BL50 | PL25 | PL50 | Significance of influence factors (p value) | | |
|---|---|---|---|---|---|---|---|
| | | | | | F1 | F2 | F3 |
| *Moisture* | 60.90±0.22$^{a}$ | 66.4±0.20$^{d}$ | 62.34±0.33$^{b}$ | 65.76±0.15$^{c}$ | <0,0001 | <0,0001 | <0,0001 |
| *Fats* | 20.32±0.25$^{d}$ | 13.4±0.18$^{a}$ | 18.48±0.45$^{c}$ | 14.20±0.18$^{b}$ | 0.001 | <0,0001 | <0,0001 |
| *Proteins* | 17.68±0.04$^{a}$ | 19.34±0.13$^{b}$ | 18.14±0.11$^{b}$ | 19.18±0.08$^{b}$ | <0,0001 | <0,0001 | <0,0001 |
| *Collagen* | 15.82±0.08$^{a}$ | 17.54±0.34$^{b}$ | 16.3±0.12$^{b}$ | 17.4±0.07$^{b}$ | <0,0001 | <0,0001 | <0,0001 |

Means with different superscripts within the same row are significantly different ($p < 0.05$). Data are means ± standard error.
BL25 - sausages with 25% beef liver; BL50 - sausages with 50% beef liver, PL25 - sausages with 25% pork liver, PL50 - sausages with 50% pork liver, F1 - liver type; F2 - % of liver; F3 – liver type and % interaction.

The sensory attributes, including appearance, colour, flavour, hardness, and overall acceptability, as perceived by the group of evaluators, are depicted in Figure 1 for sausages formulated with two different percentages of pork and beef liver. The values for appearance were higher for the beef liver formulations. Furthermore, the most noticeable differences in the perception of appearance were observed between formulations with different liver types, with formulations containing 25% liver being generally more favoured.

The evaluators' assessment of colour was strongly associated with brightness intensity, where samples formulated with beef liver exhibited the highest L* lightness values both on the surface and in cross-section, and were thus more highly appreciated. Sample PL25 received the lowest score in terms of colour, possibly due to the effect of lower lightness intensity, which was correlated with the heightened intensity of the red hue. This variance altered the evaluators' perception and made it distinct from the other samples.

In terms of flavour, the primary distinctions noted by the evaluators were between the two levels of liver content introduced, namely 25% and 50%. Consequently, the flavour of the samples with 25% liver was more positively assessed, receiving higher average scores.

Firmness, as defined, represents the force required to penetrate a product, serving as an indicator of the degree of hardness of its constituent parts and their level of cohesion. In the current study, the samples containing 25% liver (BL25 and PL25) were noted by the evaluators to exhibit greater firmness when compared to those formulated with 50% liver. This evaluation by the evaluators could be attributed to the fact that the liver, being an organ, lacks the elasticity provided by muscle fibres and is characterized by its smooth muscle texture. Moreover, firmness may be closely linked to the chemical composition, with higher firmness often associated with lower moisture content.

Lastly, the overall acceptance of the sensory quality of the four sausage samples was predominantly influenced by the perceived flavour and external appearance, with the highest rating being awarded to PL25.

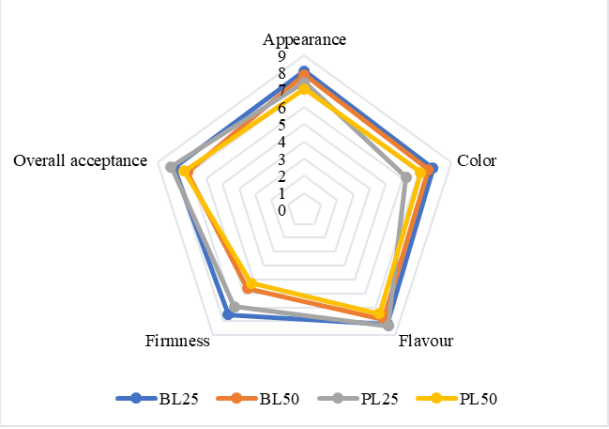


Figure 1 **Sensory traits of liver sausages; BL25 - 25% beef liver; BL50 - 50% beef liver, PL25 - 25% pork liver, PL50 - 50% pork liver**

## CONCLUSIONS

The type and percentage of liver exert a notable influence on the colour of liver sausages. Specifically, increasing the liver percentage led to significant alterations in product colour. These alterations included a decrease in lightness (L*) for beef liver sausages and a noteworthy increase in the red hue (a*) for both beef and pork liver sausages. However, the impact of liver type was more pronounced in the case of the 25% liver formulations. The colouration per section of the sausages was also influenced by the type and liver percentage, resulting in significant increases in a* and b* as the liver percentage increased.

Based on the provided information, it was evident that the chemical composition of the produced sausage varieties exhibited significant variations in response to the percentage of liver incorporated into the recipe. Formulations containing 50% liver, both beef and pork, displayed higher moisture content and lower fat content when compared to formulations containing 25% liver. These distinctions can be attributed to the liver's elevated water content relative to muscle tissue and the fat content of the meat trimmings employed. Furthermore, protein content increased with a higher liver percentage, particularly in the case of beef liver formulations.

The sensory perception of the evaluators was influenced by the two factors, with the percentage of liver in the manufacturing technology most obviously influencing the perception and preference of the evaluators. The overall acceptability was higher for formulations with 25% liver.

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# ASSESSMENT OF UTILIZING ANNATTO SEEDS POWDER AS A NATURAL FOOD INGREDIENT FOR CHEDDAR CHEESE

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**Abstract**

Food products with desirable sensory qualities, safety, and nutrition are in high demand in industrialized countries. Annatto (*Bixa orellana L.*) is a small tree cultivated in tropical and subtropical America and is appreciated for the pigmented seeds that come from which the yellow-orange achiote or annatto natural food color is made. This study investigated the effect of the annatto seeds powder on the phytochemical and physicochemical properties as well as the textural and color of the value-added cheddar cheese. Two types of cheddar cheese were produced containing different concentrations of annatto seeds powder, 0.25 and 0.5% (ASP0.25, ASP0.5), and a control (ASP0). Based on the results, annatto seed powder appeared to show good antioxidant activity (79.432 ±1.015%). The addition of annatto seed powder positively influenced the textural and color characteristics of the value-added cheese. The obtained samples highlighted the satisfactory content in phytochemicals. The sensory analysis showed that the addition of annatto seed powder had no detrimental effects on the cheese's general acceptance, the improved color being appreciated. Therefore, annatto seed powder might be a good source of natural antioxidants for the production of dairy products being a natural alternative to synthetic food coloring ingredients.

**Key words**: Annatto seeds, antioxidant activity, pigments, food ingredients, cheddar cheese

One of the most significant aspects of food product that affects consumer preference, taste perception, and ultimately purchasing decision is color (Sukkwai S. *et al*, 2018).

The pericarp of the seeds of the tropical tree *Bixa orellana L*. (Bixacea), which is native to the woods of Central and South America, is used to produce the red-orange-yellow natural colorant known as annatto (achiote). It is widely grown throughout the world's tropical regions, particularly in Mexico, Colombia, Ecuador, and the Peruvian Andes. The name "annatto" refers to the crude pigment extract derived from achiote that contains bixin, norbixin, and other carotenoids in varying amounts. Achote is now one of the most intriguing plant sources of vegetable colorants due to the possibility of acquiring both water-soluble (norbixin) and oil-soluble (bixin) colorants depending on the kind of extraction, the solvent, and the temperature utilized (Smith J., 2006).

The seeds contain 4.5–5.5% pigment, which about 80% of the total carotenoids in the color made from achiote are represented by bixin. Norbixin, bixin dimethyl ester, and by-products of lycopene breakdown are additional carotenoids that are also present but at smaller levels. The carotenoid 9′-cis-bixin is the main coloring agent in the oily soluble annatto extract, while the carotenoid 9′-cis-norbixin is the main coloring agent in the alkaline aqueous annatto extract (Viuda M. *et al*, 2012). According to research by Chiste R.C. *et al* (2011), achiote includes significant levels of tocotrienols, tocopherols (vitamin E), terpenes, and flavonoids in both the seeds and the leaves.

Due to the emergence of degenerative diseases, the use of some artificial food colors, (carmoisine, Ponceau 4R), has been prohibited in the USA and Europe. Instead, the use of natural colorants, such as the dye that comes from the surface of the seeds of *B. orellana L.* (E 160b, annatto extract), has been suggested. Because it doesn't change flavor and is mostly non-toxic, annatto extract has a significant economic impact on the entire world and is one of the natural colorants used most frequently in the food, cosmetic, and pharmaceutical industries (Lourido P.H., Martınez S.G., 2010).

Some bioactive substances extracted from annatto seeds have demonstrated antioxidant and antibacterial properties of special significance for food product manufacturing. In fact, because of their coloring ability, these extracts have been used in food matrices like dairy, meat, and baked goods.

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Many different food products, including butter, margarine, cheese, beverages (soft drinks and juices), ice creams, poultry, breakfast cereals, several desserts and confectionary, use annatto seeds as a natural color (Zhang *et al*., 2018). Researchers reported that 0.065 mg/kg body weight/day of bixin is an appropriate consumption for people and that feeding experimental animal diets high in bixin and norbixin did not have any harmful or carcinogenic effects (Lauro G., Francis J., 2000).

The aim of the present study was to obtain value-added cheddar cheese. The cheese's physicochemical, phytochemical and color qualities, as well as their textural and sensory analyses, were examined in order to show the added value of the products.

## MATERIAL AND METHOD

Material and method. The cow's milk (200 L) was donated by the University of Life Sciences' Rediu Iași Research Station. Annatto seeds (moisture content of 10.11±0.87 %) were ground using a blender (Blender Nutribullet Original). Hexane, acetone, ethanol, methanol, sodium carbonate, 2,2-diphenyl1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, gallic acid solution, sodium hydroxide were purchased from Sigma Aldrich (Schnelldorf, Germany).

Extraction of bioactives from annatto seeds powder (ASP). The ultrasound-assisted extraction method described by Quintero Quiroz J. *et al* (2019) was utilized to extract the bioactive components from ASP with a few minor modifications. 1.0 g of ASP were mixed with 10 mL of a solvent mixture of 3:1 n-hexane:acetone or 70% ethanol (for the extraction of total polyphenols) before being treated with an ultrasound bath (Elmasonic S 180 H, Elma, Germany) for 30 minutes at 25°C and a frequency of 37 kHz. The resulting extract was then recovered, and it was centrifuged for 10 minutes at 6000 rpm and 10°C. After that, the ASP supernatant was examined for total carotenoids, total flavonoids, and total polyphenols.

The determination of carotenoids, phenolic compounds and antioxidant activity of annatto seeds powder (ASP) extract.

Total carotenoid content. Spectrophotometric analysis was used to measure and determine the total carotenoids concentration as described by Mihalcea *et al*. (2018). A UV–VIS Spectrophotometer, Analytik Jena - Specord 210 Plus, Germany was used to measure the absorbance at 450 nm for total carotenoids content of the extract. The results were reported as mg/100 g of dry weight (d.w.).

Total flavonoid content. The total flavonoid content values of ASP extract were determined using the aluminum chloride spectrophotometric technique (Dewanto V. *et al,* 2002). Briefly, 0.075 mL of 5% sodium nitrite ($NaNO_2$) and 0.25 mL of the extract were combined with 2 mL of distilled water. 0.15 mL of aluminum chloride (AlCl3) was added to the mixture after 5 minutes. 0.5 mL of sodium hydroxide (NaOH) 1 M was added to the mixture after 6 minutes, and the mixture was then measured at 510 nm. A calibration curve for catechin as a standard was employed and the results were expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g d.w.).

Total polyphenolic content. The Folin-Ciocalteu method was used to spectrophotometrically measure the ASP extract total polyphenolic contents (Cheok *et al*. 2013). The Folin-Ciocalteau reagent, 15.8 mL of distilled water, and 200 µL of the extract were carefully combined. 3 mL of NaCO3 20% was added to the mixture after 10 minutes. After 60 minutes of dark storage at room temperature, the resultant combination was measured at 765 nm. The results were expressed as milligrams of Gallic acid equivalents per gram of dry weight (mg GAE/g d.w.) and a standard curve for Gallic acid was used.

Antioxidant activity (DPPH). The DPPH technique was used to measure the antioxidant activity, and the results were expressed as µmol of Trolox equivalents per gram of dry weight (µmol TE/g d.w.) (Castro-Vargas H.I. *et al*, 2010). A calibration curve utilizing Trolox as standard was applied. The samples were prepared by mixing 0.10 mL of each extract with 3.90 mL of 0.1 M DPPH solution. The solutions were then left at room temperature in the dark for 30 minutes before the absorbances (Af) were measured. Instead of the extract (A0), the blank absorbance was measured at 515 nm using a 3.9 mL DPPH solution 0.1 M (in methanol) and 0.10 mL methanol. Also, the inhibition percentage was calculated. % Inhibition = (A0- Af)/A0 x 100.

Raw milk collecting, sampling and analysis.

200 L of milk were taken out of the farm's storage tank in sterile containers. It was refrigerated at 4°C for 24 hours. Milk was then completely homogenized and added to the analytical laboratory investigations.

The physicochemical parameters of milk samples (moisture content, solid non-fat content, total solid, fat content, protein content, and pH) were determined in according with methods of AOAC.

Cheddar cheese manufacturing. After being standardized to have a protein to fat ratio of 0.70:1, pasteurized at 65°C for 60 minutes, cooled to 30°C, and inoculated with lactic bacteria (*Str. Lactis, Str. Cremoris*). The raw cow's milk was then added calcium chloride solution and allowed to stand for 30 minutes. After two minutes, milk was mixed with liquid rennet (Chymax Plus).

After cutting the coagulum, the curd/whey mixture was left to heal for 10 minutes before being continually churned. In a 40-minute period, the

curd was heated from 38 to 42°C. The acidity of whey should not exceed 19-20ºT. The cedarization stage is carried out when the acidity of the whey approaches 60-70ºT with a pH 4.6-6.5. The curd was separated into three batches, each of which received ASPs and 0.15 kg dry salting. Cheeses were prepressed at 0.13 kPa for 30 minutes after 20 minutes of mellowing, then overnight at 2.5 kPa. At an interval of about 2 hours during the pressing, the cheeses were twisting. The temperature of the room where the pressing takes place must be between 25-27°C. The cheese is to be subjected to drying at temperatures of 12-14°C, for 7-10 days, humidity 85%. Ripening takes place at temperatures between 2-6°C in spaces free of foreign odors and the relative humidity of the air must reach 80-90% for 90-110 days. At intervals of 2 days each piece of cheese is turned. The cheese pieces are stored at a temperature between 2-4°C until being analyzed.

Sensory, physical and chemical analyses were performed with regard to the part of the qualitative analyses carried out to establish the qualitative parameters of the samples obtained with the addition (cheeses with annatto seeds powder (ASP) - ASP0.25– cheese with 0.25% ASP, ASP0.50– cheese with 0.5% ASP) and the control sample (ASP0).

Texture analysis. Using a texturometer with a digital dynamometer of 25N, the Mark 10 ESM 300 texturometer (Mark-10 Inc., USA) was used to analyze the texture of cheddar cheeses samples by the Texture Profile Analysis (TPA) Method. The data were recorded and processed utilizing the TexturePro CT V1.5 software. For each sample, four tests were performed.

Color analysis. The color of samples was determined using the Konica Minolta Chroma Meter CR-410 (Konica Minolta, Osaka, Japan) with a CIE Lab scale against a white standard. Color coordinates. were reported as lightness (L*, from 0 = black, to 100 = white), redness (a*, from red = +a, to green = -a) and yellowness (b*, from yellow = +b, to blue = -b).

Sensorial analysis. The seven attributes scale, which was based on unit numbers, was used to evaluate the value-added cheeses samples. Color, external appearance, scent, flavor, consistency, aftertaste, and overall acceptability are the evaluated attributes. A panel of 10 different panelists conducted a sensory assessment at 20 °C, in white light, and 45–47% relative air humidity.

Statistical Analysis. Using Minitab 17 software, the outcomes of each analysis were statistically examined in duplicate. To evaluate the variations between the samples, a one-way ANOVA and Tukey test was applied. The mean (n = 3) ±SD was used to express all experimental results.

## RESULTS AND DISCUSSIONS

The phytochemical content and antioxidant activity of the ASP extract were determined and the results are displayed in *Table 1*

Table 1

**Phytochemical content of the ASP extract**

| ***Parameters*** | ***Sample ASP*** |
|---|---|
| Total carotenoids (mg/100g d.w.) | 14.205±0.622 |
| Total flavonoids (mg CE/g d.w.) | 2.285±0.028 |
| Total polyphenols (mg GAE/g d.w.) | 3.960±0.055 |
| DPPH (µmol TE/g d.w.) | 12.158±0.102 |
| Inhibition (DPPH) % | 79.432 ±1.015 |

*Table 1* shows that ASP extract had a high carotenoid content of 14.205±0.622 mg/100g d.w. and antioxidant activity of 12.158±0.102 µmol TE/g d.w. Our results comply with other studies, that reported a total polyphenol content of 3.81 mg GAE/g d.w. (Quintero Quiroz *et al*. 2019) after the ultrasound assisted extraction of ASP bioactives with ethanol 96% for 20 minutes treatment time. The extracts from the seeds of annatto appear to be remarkable due to their coloring capacity and antioxidant activity.

Chemical composition of raw cow's milk.

In order to establish the quality parameters for the raw material milk, the main quality indices were determined. Results of chemical composition of cow's milk samples are in *table 2*.

Table 2

**Chemical composition of raw cow's milk samples**

| ***Parameters*** | ***Mean*** |
|---|---|
| Water (%) | 87.17±0.07 |
| Total Solids (%) | 12.83±0.08 |
| Fat (%) | 3.98±0.02 |
| Protein (%) | 3.25±0.04 |
| Solid-non fat (%) | 8.86±0.06 |
| pH | 6.59±0.01 |

Regarding the water content, it had an average value of 87.12±0.094% and that of total solids was 12.89±0.094%. Milk quality indices meet the conditions of freshness, safety, and general milk quality.

The phytochemical profile and antioxidant activity of the obtained samples are shown in *table 3*. The obtained food products were analyzed in terms of the global phytochemical profile.

Table 3

**Phytochemical characterization and storage stability of added-value cheese samples**

| ***Parameters*** | ***Storage time(days)*** | ***Type of cheddar cheeses*** | |
|---|---|---|---|
| | | **ASP0.25** | **ASP0.50** |
| Total carotenoids (mg/100g d.w.) | 0 | 8.22±0.18[a] | 10.19±0.20[b] |
| | 110 | 8.62±1.11[a] | 12.01±1.28[b] |
| Total flavonoids (mg CE/g d.w.) | 0 | 3.28±0.20[a] | 4.83±0.24[a] |
| | 110 | 3.45±1.13[a] | 6.63±1.14[b] |
| Total polyphenols (mg GAE/g d.w.) | 0 | 5.03±0.15[a] | 6.07±0.17[a] |
| | 110 | 5.85±1.78[a] | 9.75±1.88[b] |
| DPPH (µmol TE/g d.w.) | 0 | 5.75±0.14[a] | 7.22±0.19[b] |
| | 110 | 5.92±1.79[a] | 9.88±1.98[b] |

Different letters within rows indicate significant differences between samples (ANOVA test, $P < 0.05$).

As expected, the amount of powder added correlates with the differences in bioactives and antioxidant activity between the two samples. The phytochemicals content and antioxidant activity was higher in cheese with 0.50% addition. The obtained samples highlighted the satisfactory content in phytochemicals. During the storage period, the phytochemicals increased until the end of the ripening for the ASP0.50 sample while for the ASP0.25 sample the values were constant. The phytochemicals content of all cheeses increased during the ripening phase, which is consistent with the findings of Rashidinejad A. *et al* (2013), who investigated the influence of catechin addition on the phenolic content and antioxidant capabilities of low-fat cheese. Reduced analytical precision and the presence of milk-derived compounds may be responsible for the increased phenolics values.

Chemical composition of value-added cheddar cheese. The chemical composition of the prepared Cheddar cheeses is described in *table 4*.

Table 4

**Chemical composition added-value cheese samples**

| ***Parameters*** | ***Type of cheddar cheeses*** | |
|---|---|---|
| | **ASP0.25** | **ASP0.50** |
| Water (%) | 36.22±0.18[a] | 36.19±0.18[a] |
| Dry mattter (%) | 63.78±0.18[a] | 63.81±0.19[a] |
| Fat (%) | 40.03±0.03[a] | 39.07±0.03[a] |
| Fat in dry matter (%) | 62.77±0.16[a] | 61.23±0.15[a] |
| Protein (%) | 23.86±0.04[a] | 25.81±0.04[b] |
| Ash (%) | 4.24±0.08[a] | 6.11±0.09[b] |
| Salt(%) | 2.08±0.06[a] | 2.13±0.03[a] |

Different letters within rows indicate significant differences between samples (ANOVA test, $P < 0.05$).

Table 5

**Texture of value-added cheese samples**

| ***Component*** | ***Type of cheddar cheeses*** | |
|---|---|---|
| | **ASP0.25** | **ASP0.50** |
| Cohesiveness | 0.275 ± 0.01[a] | 0.655 ± 0.01[b] |
| Springiness, mm | 0.50± 0.01[a] | 1.06 ± 0.01[b] |
| Hardness, N | 65.80 ± 0.02[a] | 79.70 ± 0.04[b] |
| Gumminess, N | 0.43 ± 0.01[a] | 0.54 ± 0.02[b] |
| Chewiness, mJ | 4.97± 0.03[a] | 5.76± 0.04[b] |

Different letters within rows indicate significant differences between samples (ANOVA test, $P < 0.05$).

The analysis of the obtained media indicates differences between the sample with de addition of powder obtained from annatto seeds for protein and ash parameters. The other parameters that were not influenced by the addition of ASP used by us was fat, dry matter, fat in dry matter and salt.

With the addition of more annatto powder, the ash content showed a significant increase, increasing from 4.24% in the case of the sample

with an addition of 0.25% to 6.11% in the case of the sample with an addition of 0.50%.

Value-added cheddar cheeses texture analysis. In order to estimate the effect of ASP over the texture of cheddar cheeses, the hardness, cohesiveness, springiness, gumminess and chewiness were tested using the Texture Profile Analysis method. The results of these parameters are presented in *table 5*. Cheese texture is an extremely important attribute that results from a combination of physical properties, perceived with the help of the tactile senses. Hardness, described as the maximum force needed to compress the samples in the first cycle, was between 65.80 ± 0.02N for ASP0.25 and 79.70 ± 0.04N for ASP0.50. The ASP0.50 sample displayed the greatest values for cohesiveness and springiness, which are measured as the difference in deformation between the two compression cycles and measured as the ratio between the samples' resistances during the second and first compression, respectively. The energy needed to break down the food during mastication is known as chewiness, and it increased from 4.97± 0.03 mJ for ASP0.25 and 5.76± 0.04 mJ for ASP0.50.

Color evaluation of value-added cheddar cheese samples. A food's color is a crucial physical characteristic that influences the consumer's preference. The samples were analyzed in terms of CIELAB colorimetric parameters. The terms L* (brightness), a* (trend towards red or green), and b* (trend towards yellow or blue) were used to express the results. Color data of the value added cheeses where shown in *table 6*.

Table 6

Color data of ASP0.25 and ASP0.5 cheeses

| ***Parameters*** | ***Storage time(days)*** | ***Type of cheddar cheeses*** | |
|---|---|---|---|
| | | **ASP0.25** | **ASP0.50** |
| L* | 0 | 71.43±0.05[a] | 68.59±0.16[b] |
| | 110 | 70.62±0.11[a] | 67.21±0.28[b] |
| a* | 0 | 11.86±0.02[a] | 14.16±0.02[a] |
| | 110 | 12.45±0.13[a] | 15.63±0.14[b] |
| b* | 0 | 47.80±0.01[a] | 49.73±0.03[b] |
| | 110 | 48.92±0.79[a] | 50.88±0.98[b] |

For each type of cheese, letters indicate a comparison across color parameters; means denoted by distinct letters in each row indicate significantly different results (ANOVA test, P 0.05).

As expected regarding the colorimetric parameters, a* and b* values suggested a red-orange to orange-yellow color, with a pleasant taste due to the presence of ASP. Both samples show high levels of lightness and yellowness, which are characteristics of carotenoid compounds, according to the L* and b* values. The significant increase of b* value with the powder concentration suggests a tendency to yellow, offered by the biologically active compounds (carotenoids) from the annatto seeds used as a functional ingredient ($p < 0.05$). All cheese samples containing spirulina showed a decrease in L* value during storage, which might be explained by the rise in acidity and proteolysis that took place during this time (Chudy *et al*. 2020) The a* and b* values, a measure of redness and yellowness, of annatto fortified samples were significantly higher at the end of storage.

Sensory evaluation of value-added cheddar cheese. To evaluate the sensory aspects of the product, we used the quantitative descriptive analysis method, which is frequently used in studies of various products, including cheese (Stone H. *et al*, 2021). *Table 7* outlines the mean intensity ratings of descriptive attributes of evaluated cheese samples.

Table 7.

**Sensory attributes values given by the panelists to ASP0.25 and ASP0.5 cheeses**

| ***Sensory attributes*** | ***Type of cheddar cheeses*** | |
|---|---|---|
| | **ASP0.25** | **ASP0.50** |
| Color | 5.71[a] | 6.13[b] |
| External appearance | 5.77[a] | 4.12[b] |
| Scent | 4.20[a] | 4.60[a] |
| Flavor | 5.22[a] | 6.00[b] |
| Consistency | 5.30[a] | 6.12[b] |
| Aftertaste | 3.93[a] | 4.22[a] |
| Overall quality | 5.81[a] | 6.92[b] |

Different letters within rows indicate significant differences between samples (ANOVA test, $P < 0.05$).

The addition of ASP in cheese samples increased the yellow color, so the testers noted that the cheeses varied in color, with ASP0.5 being the most yellow. The annatto seeds powder-infused

cheddar cheeses were praised for having an agreeable flavor, scent, and color. The samples' crumbly, layered, dense consistency was also appreciated. The panel of tasters gave high marks to all of the suggested samples, ASP0.50 being the most preferred cheddar cheese. Sensory analysis showed that cheese with ASP could potentially be well accepted among consumers, having quite acceptable quality attributes.

## CONCLUSIONS

Research has shown that adding annatto powder to cheese, regardless of quantity, does not significantly affect the nutritional composition of the cheese, without adding significant amounts of fat or protein.

Due to the ability of the seeds to give an intense orange-yellow shade, coloring cheese with annatto is a common practice in the food industry, without affecting the taste or aroma, it is a neutral additive that does not change the organoleptic characteristics of the product and does not interfere with the natural flavor of the product.

The addition of this powder, provides an attractive visual appearance by giving the color vibrant and attractive, which can influence consumer perception and increase the product's appeal in the market. The annatto seeds powder may be explored as valuable ingredients for the development of added-value food dairy products.

These results showed that value-added cheeses obtained with annatto seeds powder could be an alternative to synthetic food coloring, whereas providing a pleasant color, with potentially beneficial effects on human health.

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# STUDY REGARDING ESTIMATION OF HERITABILITY FOR MILK PRODUCTION TRAITS IN HOLSTEIN CATTLE

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**Abstract**

The goal of this study is to estimate the heritability of milk, fat, and protein yields in Holstein cows from a dairy farm in Iași, Romania. The heritability of these traits during the first lactation is examined using 16 bulls and their 542 progeny and was estimated the heritability of milk, fat, and protein yields during the first lactation. Data was statistically processed with ANOVA. The findings reveal a lower heritability for milk yield (0.15 ± 0.06), a moderate heritability for fat yield (0.30 ± 0.12), and a strong heritability for protein yield (0.64 ± 0.23). These results showed that protein yield exhibits the most significant genetic influence, while milk yield demonstrates the least. The findings regarding milk yield and fat percentage should be interpreted with caution due to their proximity to the limits of the specialized data range. However, it is important to note that the observed heritability for protein percentage may be of particular difference, potentially influenced by human error or the transmission of data in an improper manner.

**Key words**: dairy cattle, improvement, breeding plan

A breeding program is a systematic approach used in genetics and animal husbandry to improve desired traits through controlled mating strategies. Heritability, a key parameter in breeding programs, quantifies the extent to which genetic differences contribute to the observed variation in a trait. It helps breeders identify traits that are more responsive to selection and guides the selection intensity and breeding methods employed. Higher heritability values indicate traits that can be improved more effectively through selective breeding, while lower heritability may require alternative strategies. By considering heritability, breeders can optimize their breeding programs to achieve genetic progress and targeted objectives in an objective and efficient manner. (Ivancia M., 2020).

The study of genetic parameters in animal breeding assumes a important role in comprehending the phenotypic and genotypic structure of populations. (Creangă Șt. *et al*, 2008). It provides valuable insights into heritability, repeatability, and correlations between traits, which are necessary in developing effective breeding programs. This article specifically focuses on estimating genetic parameters, particularly heritability, for milk, fat, and protein yields in Holstein cattle from a dairy farm in Romania.

The initial step in any improvement process involves a comprehensive description of the phenotypic and genotypic structure of the population under analysis. Phenotypic assessment of quantitative traits necessitates the use of statistical methods, while genotypic evaluation relies on determining genetic parameters for production traits. Among these parameters, heritability assumes significant importance as it elucidates the proportion of total variation attributed to the average effect of genes (Meuwissen T. *et al*, 2022).

Numerous researchers have estimated heritability values for milk, fat, and protein yields during the first lactation in Holstein cows. Studies conducted by Jamrozik (1997) and Strabel (2006) reported heritability estimates ranging from 0.31 to 0.36 for milk yield, 0.28 to 0.36 for fat yield, and 0.12 to 0.28 for protein yield. These estimates reflect the proportion of phenotypic variation in these traits that can be attributed to genetic factors.

This study aims to bring a contribution in animal breeding by estimating the genetic parameters, particularly heritability, associated with milk, fat, and protein yields during the first lactation in Holstein cows from Iași, Romania. These estimates will provide valuable insights into the level of genetic control and the potential for selection and improvement within these specific conditions (Djaalab I. *et al.*, 2021). The findings

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will contribute to the development of more targeted breeding strategies focused on enhancing milk production and composition in Holstein cattle from the studied dairy farm. Consequently, this research contributes in the current understanding of animal breeding by investigating the genetic parameters, specifically heritability in Holstein cows from Iași, Romania. The obtained estimates offer comprehensive insights into the underlying genetic control mechanisms that influence these important production traits. The study's results will have practical implications for dairy farmers and breeders, informing the development of more effective breeding programs and enabling the selection of superior animals with improved productivity. This investigation contributes significant findings to the scientific literature on genetic parameters and advances targeted breeding strategies aimed at enhancing the overall production potential of Holstein cattle in the specific conditions under examination.

## MATERIALS AND METHODS

16 bulls of the same breed were the biological material studied and they were selected based on the criterion of having at least two daughters, totalizing a number of 542 daughters. The study ensures a diverse representation of genetic variations within the Holstein population under investigation, by including bulls with multiple daughters. The data was obtained from Holstein Ro Association. The selected bulls' daughters were obtained in 5 farms (*table 1)*.

Table 1

**Daughters repartition by father**

| Crt. | Bull code | Number of daughters |
|---|---|---|
| **1** | 54031 | 16 |
| **2** | 52987 | 17 |
| **3** | 53302 | 17 |
| **4** | 53227 | 21 |
| **5** | 53743 | 21 |
| **6** | 53400 | 24 |
| **7** | 53742 | 24 |
| **8** | 52943 | 25 |
| **9** | 52505 | 27 |
| **10** | 53022 | 28 |
| **11** | 53642 | 42 |
| **12** | 53188 | 44 |
| **13** | 53349 | 45 |
| **14** | 52811 | 56 |
| **15** | 52533 | 60 |
| **16** | 52938 | 75 |
| **TOTAL** | | **542** |

To assess the production traits, monthly milk samples were gathered from the farm and sent to the laboratory of the Holstein Ro Organization. The analyse of production traits, including milk, fat, and protein yields, was carried out using the Combifoss equipment that provides accurate measurements and enables the quantification of these essential production traits.

The 305-day milk, fat, and protein yields were calculated using the Test Interval Method, as recommended in the ICAR Guidelines *(ICAR Recording Guidelines*, 2022). This method considers variations in test intervals, which can commonly occur in practical dairy farming (Onaciu *et al.*, 2015). The Test Interval Method ensures precise estimation of the 305-day yields for each trait, by incorporating the appropriate coefficients from the ICAR Guidelines. This approach is preferred over other methodologies due to its ability to account for variations in test intervals, thereby providing reliable and consistent results for the estimation of milk, fat, and protein yields (Maciuc V., 2003).

To ensure data quality, cows with fewer than seven tests were excluded from the analysis. This exclusion criterion helps maintain a sufficient number of observations for robust statistical analyses and reduces the potential impact of unreliable or incomplete data on the estimation of heritability.

The heritability of the milk, fat, and protein yields was estimated based on standardized lactation records from the first lactation of cows. These standardized records enable the comparison and evaluation of production traits across different cows and lactation periods (Kim B.W. *et al*, 2009). The data were processed using ANOVA, including the genetic component that contributes to the heritability estimates.

The formula employed for estimating heritability of a trait, including milk yield, fat yield, or protein yield, is given as:

$$\mathbf{h^2 = (\sigma^2_a / \sigma^2_t)}$$, (Ivancia M., 2020).

- $h^2$ represents the heritability of the trait,
- $\sigma^2_a$ corresponds to the additive genetic variance specific to the trait, and
- $\sigma^2_t$ denotes the total phenotypic variance including the trait's overall variation within the population.

To calculate heritability for a particular trait, such as milk yield, fat yield, or protein yield, the formula was adapted accordingly. The heritability of the interest traits was determined using:

$$\mathbf{h^2X = (\sigma^2_aX / \sigma^2_tX),}$$ where

- X means MY, FY and PY (Ivancia M., 2020).

The additive genetic variance ($\sigma^2_a$) captures the extent of genetic influence on the trait's variation, while the total phenotypic variance ($\sigma^2_t$) accounts for the overall variation observed in the trait across the population.

Variance was calculated using the formula:

**Variance = (Σ(X - μ)²) / n**, where:

- Σ denotes summation,
- X represents individual observations,
- μ represents the mean of the observations, and
- n represents the total number of observations (Ivancia M., 2020)

In addition to estimating heritability, the standard deviation for each trait was calculated. The standard deviation provides an understanding of the variability within the traits and assesses the precision of the estimated heritability values (Jamrozik J., Schaeffer L.R., 1997).

The standard error (SE) is calculated as:

**SE = sqrt (MSW / n)**, where

- MSW is the mean square error from the ANOVA, and
- n denotes the number of observations.

Justification of the chosen methodology lies in its established reliability, statistical rigor, and wide applicability in the field of animal breeding. These methods have been extensively used and validated in previous studies, ensuring accurate estimation of heritability for milk, fat, and protein yields in the Holstein breed from the Moldovian region.

## RESULTS AND DISCUSSIONS

The data analysis estimates heritability for milk yield, fat yield, and protein yield. The heritability values obtained in this study were 0.15 ± 0.06 for milk yield, 0.30 ± 0.12 for fat yield, and 0.64 ± 0.23 for protein yield.

The heritability estimate for milk yield (*table 2*) indicates a lower heritability compared to the findings reported by other authors.

This suggests that the genetic contribution to milk yield variation in the studied population is relatively smaller. This observation aligns with previous studies (Creangă Șt. *et al.*, 2008; Toghiani S., 2012; Roșca N. *et al.*, 2018) that have also reported lower heritability values for milk yield in Holstein cattle populations.

The heritability estimate for fat yield (*table* 2) indicates a moderate level of heritability. This finding suggests that genetic factors play a more substantial role in the variation of fat yield compared to milk yield. The moderate heritability of fat yield is consistent with the results reported by other researchers (Jamrozik J., Schaeffer L.R., 1997; Strabel T., Jamrozik J., 2006; Stoop W. M. *et al*, 2008), indicating a consistent genetic influence on fat production in Holstein populations.

**Table 2**

**Heritability Estimates for Milk Yield from Various Studies and Own Results**

| Source | $h^2$ | | |
|---|---|---|---|
| | MY | FY | PY |
| **Own results** | **0.15** | **0.30** | **0.64** |
| **Jamrozik, 1997** | 0.32 | 0.28 | 0.28 |
| **Strabel, 2006** | 0.18 | 0.12 | 0.13 |
| **Kim, 2009** | 0.15 | 0.13 | 0.12 |
| **Nixon, 2009** | 0.14 | 0.26 | 0.20 |
| **Toghiani, 2012** | 0.26 | 0.15 | 0.24 |

The heritability estimate for protein yield (*table* 2) indicates a strong heritable character, with a value above 0.4. This finding highlights the substantial genetic contribution to the variation observed in protein yield among the Holstein cows in the studied population. The high heritability of protein yield aligns with previous research (Kim B.W. *et al.*, 2009; Wongpom B. *et al*, 2017) that has reported significant genetic effects on protein production traits in Holstein populations.

The substantial disparity observed between the heritability estimates of fat yield and protein yield in this study may be attributed to variations arising from the employed methodology when compared to existing data from the specialized literature. The ANOVA did not incorporate the complete equation of the mathematical model, including the consideration of fixed or random factors, environmental effects, and animal pedigree. This restricted information in the model equation could potentially lead to biased or even negative heritability estimates. Future studies may consider more comprehensive statistical methodologies that account for additional factors and assumptions to obtain more accurate heritability estimates for production traits.

The heritability estimates derived from this study provide significant insights into the genetic influence on milk yield, fat yield, and protein yield in Holstein cattle. This suggests that solely relying on genetic selection may not yield substantial improvements in milk production. In contrast, moderate heritability for fat yield suggests the influence of both genetic and environmental factors. Conversely, the high heritability of protein yield indicates a strong genetic influence, making genetic selection a potential key strategy for enhancing protein yield in milk.

Further research could focus on exploring environmental factors that impact milk, fat, and protein yield, including feed quality, milking practices, and living conditions. Understanding these factors could facilitate the development of a holistic breeding strategy that considers both genetic and environmental optimization. Investigating the genetic correlation between these

traits could also be beneficial in uncovering common genetic factors influencing them and informing genetic selection strategies.

Gene-mapping studies or Genome-Wide Association Studies (GWAS) could be conducted to identify specific genes or genomic regions with significant influence on these traits, enabling the development of genomic selection strategies for more efficient breeding programs. However, it is important to acknowledge that the heritability estimates in this study were limited by the methodology employed, which did not fully incorporate all relevant factors such as fixed or random effects, environmental influences, and animal pedigree. Future research should consider employing more comprehensive statistical methodologies, including mixed model approaches or machine learning algorithms, to obtain more accurate heritability estimates.

These findings contribute significantly to our understanding of the heritability of milk, fat, and protein yield in Holstein cows. However, they also underscore the need for further investigation in several areas to develop more effective and sustainable breeding strategies.

## CONCLUSIONS

The study revealed that milk yield exhibited a lower heritability compared to the other production traits, indicating a relatively smaller genetic influence. Conversely, fat yield showed a moderate level of heritability, while protein yield demonstrated a strong heritable character. These findings have important implications for breeding programs, as they highlight the potential for genetic improvement in specific production traits. Further research and selection strategies should focus on enhancing the genetic potential for protein yield, while considering the limitations of ANOVA in accurately estimating heritability.

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# PHYSIOLOGICAL ASPECTS REGARDING THE SWEET CHERRY WATER REGIME IN THE CLIMATIC CONDITIONS OF 2023

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**Abstract**

The lack of water in the fruit trees ecosystem can cause atmospheric and pedological drought, under the action of which the plants suffer from cell dehydration through various biochemical and physiological changes. The purpose of this study is to evaluate the water regime by assessing the state of hydration of some sweet cherry cultivars ('Van', 'Andreiaș'and 'Margonia') cultivated at Research Station for Fruit Growing (RSFG) Iași (N-E Romania) in the climatic conditions of the year 2023. The physiological indices analyzed were the determination of the water potential and the evaluation of the rate of dehydration at leaf level in three different phenological stages according to the BBCH scales: 65 (full flowering); 78 (fruits approximately 80% of final size) and 89 (fruit ripening) in two different areas of the crown: internal and external. Regarding the rate of dehydration, the results recorded statistical differences between cultivars but also at the level of crown areas within the same phenophase. The results oscillated between the minimum value of 45.62 at the 'Andreiaș' in the flowering phenophase in the internal area of the crown and the maximum of 72.28 in the outside of the crown at 'Margonia'. The water content of the leaves recorded maximum average values in the flowering phenophase of 69.90%. The climatic conditions in the growing season (March-August) were characterized by an average temperature of 17°C, with an increase of 1.9°C compared to the multiannual average and a rainfall deficit of 88 mm. The physiological response of fruit trees to drought conditions caused by high temperatures associated with a lack of precipitation was to increase the content of reserve substances and total dry matter.

**Key words**: *Prunus avium* L., water content, leaves, drought stress

Being widely distributed throughout the world, species of the *Prunus* genus show a variety of physiological strategies when faced with the impact of current climate change that includes water scarcity, making it possible to identify and select genotypes with high tolerance to water limitation (Jimenez *et al*, 2013; Opazo *et al*, 2020; Gonçalves *et al*, 2021).

Compared with other tree fruits or grapes, there has been relatively little research on the need to optimize water potential of sweet cherry.

Although, as irrigation water supply has become increasingly uncertain, increased interest has developed in the need to cover water shortages to improve yield, fruit quality, and control the development of phenological stages (Quero-Garcia *et al*, 2007).

Sweet cherry (*Prunus avium* L.) has become one of the most valued and economically prolific fruit crops around the world (Gonçalves *et al*, 2021) which is mainly grown in arid and semi-arid areas, with about 550 mm of annual precipitation, but in the last decade, these areas have experienced a dramatic reduction in the water regime, which has led to some adaptation changes for sweet cherry trees (Toro *et al*,2023).

Regarding the phenological stage, an adequate amount of water has a great importance in the phases of flowering and fruit development, as well as in the post-harvest period (Predieri *et al*, 2003).

Atmospheric and pedological drought causes the disruption of some physiological and biochemical processes that can have important repercussions on the ultrastructure and metabolic processes in cells (Jităreanu *et al*, 2009). The negative effects of water stress are highlighted at the foliar level primarily by decreasing stomatal opening, transpiration and photosynthesis, respectively decreasing $CO_2$ supply (Burzo *et al*, 1999). For the sweet cherry, water regime influences numerous parameters such as vegetative growth, fruit quality and production yield, water and gas exchange status (Predieri *et al*,2003; Vosnjack *et al*,2021).

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The aim of this study was to evaluate the water regime at the foliar level of some sweet cherry cultivars during the phenological stages in the climatic conditions of the year 2023 in the North-East area of Romania.

## MATERIAL AND METHOD

**Experimental site**

The study was carried out during the year 2023 in the experimental field of the Research Station for Fruit Growing (RSFG) Iași, North-Eastern Romania (47°20´N; 27°60´E at 165 m altitude).

**Plant material and growth conditions**

The biological material used was represented by three cultivars of sweet cherry ('Van', 'Andreiaș' and 'Margonia') grown on rootstocks of medium vigor of *Prunus mahaleb* L., with planting distances of 5×4 m from, trained as a free palm tree, without an irrigation system.

The meteorological conditions in the experimental field in the last three years are characterized by an average annual temperature of 11.3°C, and total precipitation of 475 mm with a deviation of 43 mm from the multiannual values.

The soil is of meso-relief type with a gently sloping plateau and predominantly cambic chernozem soil with good natural drainage (Iurea *et al,* 2022), 3.7-4.3% humus content; pH 6.6-8.1; and 0.150-0.220% N.

**Experimental determinations**

Analysis of physiological parameters was performed at three different phenological stages, according to the BBCH scale (Meier, 2001) at 65 (full flowering), 78 (fruits has 80% of final size) and 89 (fruit ripening), in different areas of the crown: internal and external.

The rate of foliar dehydration was monitored by repeated weighing at intervals of 1, 2, 3, 4 and 24 hours, determining the percentage of water lost in the first hour and up to 24 hours (Jităreanu and Marta, 2020). Foliar dehydration was calculated using the formula:

DR (%) = $(x_{1\text{-}n}/x_0)\times 100$, where DR - foliar dehydration (%); $x_0$ - the first weighing (g);

$x_{1\text{-}n}$ - leaf weight after 1 to 24h periods of time (g).

Analysis of total water content (RW) of sweet cherry leaves was performed by laboratory oven drying method (Zhang *et al,*2012) at 104°C for 2–72 h at 80°C. Leaf water content was calculated with the equation:

RW (%) = (Fw-Dw)/ Fw × 100, where Fw-fresh leaves weight (g); Dw - weighed dry matter leaves resulted (g).

Meteorological conditions in the experimental field during the research period were monitored by an Agroexpert system.

**Statistical Analysis**

The data were statistically processed by the Duncan's multiple range test performed for mean separation at $p \leq 0.05$.

## RESULTS AND DISCUSSIONS

The analysis of climatic conditions data from the vegetation period (March-August) showed that in the experimental field at RSFG Iasi, the average monthly temperatures were higher than the multiannual average, with an deviation of +1.88 varying between -0.6°C (April) and +4.95°C (August), associated with a precipitation deficit of -87.7 mm. The relative air humidity during the analyzed period had an average value of 55.5% (*table 1*).

The absence of rain and the high day and night temperatures led to the pedological, atmospheric and physiological drought, thus shortening the phenophases (Jităreanu *et al*, 2009).

The amount of precipitation recorded during the time period that was analyzed was characterized by a deficit in March, April, May and August and an excess of +51.2 mm in July, during the period of fruit ripening, which can leads to the phenomenon of fruit cracking (Engin *et al*, 2009).

In sweet cherry, the absence of rain and high day and night temperatures produced variations in flowering, productivity and fruit quality from year to year. Changes in the rainfall distribution pattern led to the manifestation of water stress with direct effects on the species phenological development specific (Jităreanu *et al*, 2009; Bhattacharjee *et al*, 2022).

Table 1

**Climate conditions during the experimental period (RSFG Iași-Romania, 2023)**

| Period | Air temperature (℃) | | | Precipitation (mm) | | | Relative humidity (%) |
|---|---|---|---|---|---|---|---|
| | Average | Multiannual | Deviation | Sum | Multiannual | Deviation | Average |
| March | 6.60 | 3.10 | +3.50 | 5.80 | 28.90 | -23.10 | 58.00 |
| April | 9.70 | 10.30 | -0.60 | 2.00 | 28.90 | -26.90 | 66.00 |
| May | 16.20 | 16.10 | +0.10 | 5.00 | 27.40 | -22.40 | 54.00 |
| June | 20.70 | 19.40 | +1.30 | 10.00 | 28.10 | -18.10 | 53.00 |
| July | 23.30 | 21.30 | +2.00 | 91.50 | 40.30 | +51.20 | 51.00 |
| August | 25.45 | 20.50 | +4.95 | 9.20 | 57.60 | -48.40 | 51.00 |
| **Average/sum** | **16.99** | **15.12** | **+ 1.88** | **123.50** | **211.20** | **- 87.70** | **55.50** |

The rate of dehydration in the flowering phenophase oscillated both between cultivars and between the two areas of the crown analyzed.

The lowest rate of dehydration was recorded in the 'Van' cultivar (33.14% in the internal area of the crown and 37.70% external) (*figure 1*). Analyzing the obtained data, it is highlighted that in the external area of the crown, the rate of dehydration was more pronounced at all cultivars.

Statistically significant differences were recorded between the analyzed experimental variants.

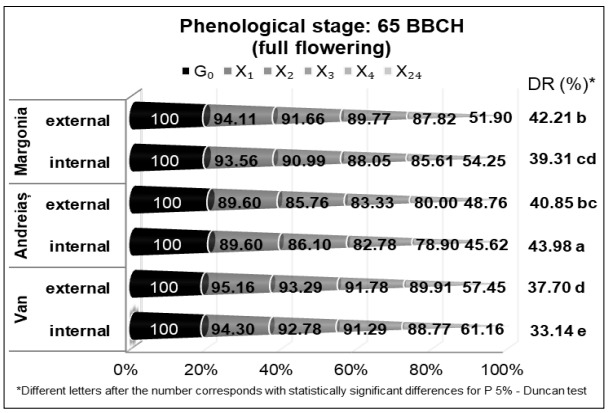


Figure 1 **The rate of dehydration in the phenophase of full flowering (RSFG Iași-Romania, 2023)**

In the phenophase of fruit growth, the obtained results of the dehydration rate were on average lower compared to the phenophase of flowering, highlighting a slowing down of the dehydration process, except for the 'Van' cultivar.

During this phenological stage, the lowest rate of dehydration was obtained on average in the 'Andreiaș' cultivar outside the crown (32.83%) and 'Margonia' in the internal area, with 32.61% (*figure 2*).

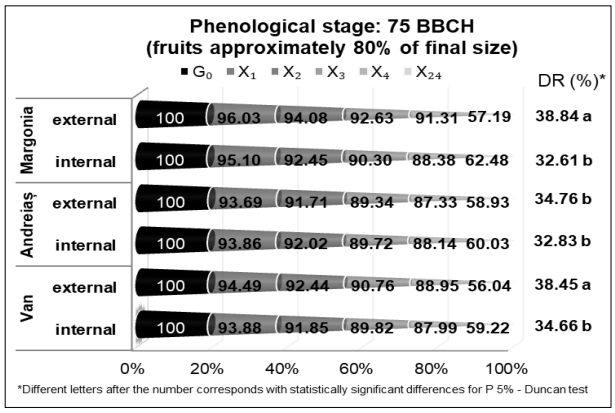


Figure 2 **The rate of dehydration in the phenophase of fruit growth (RSFG Iași-Romania, 2023)**

With regard to the phenophase of fruit ripening, each cultivar evaluated has its own specificity.

The 'Margonia' cultivar maintained its rate of dehydration compared to the phenophase of fruit growth, while a significant decrease was recorded in the 'Van' cultivar (from 34.66 to 23.03% and from 38.45 to 24.02%). The 'Andreiaș' cultivar recorded a decrease in the rate of dehydration inside the crown (to 24.46%), with a significant difference compared to the outside of the crown (32.91%).

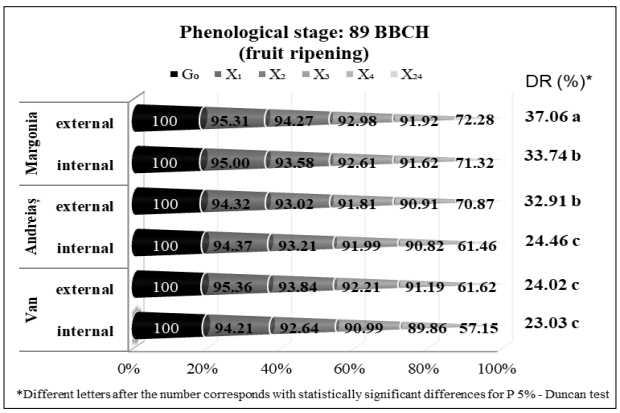


Figure 3 **The rate of dehydration in the phenophase of fruit ripening (RSFG Iași-Romania, 2023)**

Comparing the three phenophases studied, in the flowering phenophase the maximum values of the dehydration rate were obtained between 33.14 and 43.98%, in the fruit growth phenophase, constant values between 38.84 and 32.61% and the lowest dehydration rate was recorded in the phenophase of fruit ripening with values between 23.03 and 37.06 %.

The variability of the evaporation process during the fruit ripening period could also be explained by the excess of precipitation recorded during that period (+ 51.2 mm), after a period of severe drought associated with higher temperatures than the multiannual average.

The assessment of water content and total dry matter was done dynamically during the growing season (*figure 4*). Thus, in the flowering phenophase, average values of the water content of 69.35-70.62% were obtained, with statistically insignificant differences between the analyzed experimental variants. In the phenophase of fruit growth, the water content remained constant at values between 67.83 and 69.81%, although in terms of precipitation, a deficit was recorded during that period.

In the phenophase of fruit ripening, the water content at leaf level decreased significantly, reaching the minimum value from 61.56% at the 'Andreiaș' cultivar in the external part of the crown.

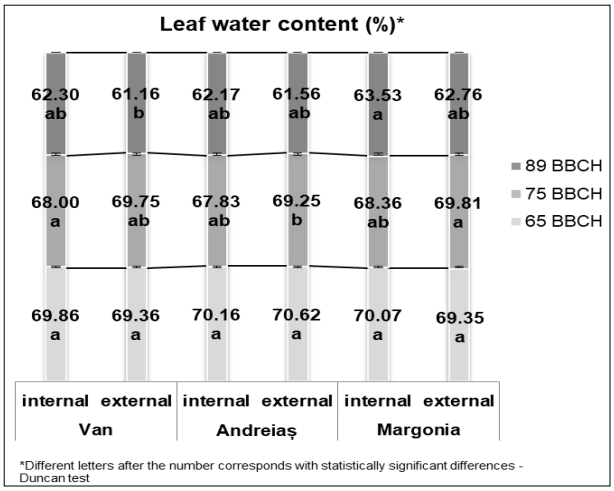


Figure 4 **The leaf water content during the phenophases (RSFG Iași-Romania, 2023)**

Correlated with the climatic conditions, it was highlighted that the transport of assimilates to the fruits was not negatively influenced by the high temperatures and the sudden excess of precipitation during this phenophase, the final production not being affected. In all analyzed phenophases, no very significant statistical differences were recorded between cultivars.

In this case, the levels of water use efficiency differed more from one cultivar to another, but also from the crown area, depending also on the phenophase, due to the variability of climatic factors in these periods.

## CONCLUSIONS

The analysis of the evolution of climatic conditions during the vegetation period (March-August) of 2023 showed that the studied sweet cherry cultivars can be characterized by a high capacity to absorb and maintain water in conditions of low water availability, although there were conditions of thermal and water stress.

The water content of the leaves of the shoots generally has relatively close values in all the cultivars studied, confirming the traits of the species.

The rate of dehydration varied even within the same cultivar in different areas of the crown. The evaporation process was lower inside the crown.

The most intense rate of dehydration and water content at the foliar level was recorded in the 'Andreiaș' cultivar, in the flowering phenophase.

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# THE EFFECT OF USING LECITHIN ON THE TEXTURAL AND RHEOLOGICAL PROPERTIES OF ARTISANAL CHOCOLATE

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**Abstract**

The main objective of current research is to evaluate the influence of lecithin as an emulsifer on the textural properties of artisanal chocolate. With its origins as a cacoa drink in antiquity, chocolate has evolved throughout history, culminating in a wide variety of chocolate types, including both industrially produced and artisanal varieties. In this context, the role of lecithin in altering the density, viscosity, and texture of chocolate was analyzed. Two chocolate samples were prepared, one containing lecithin as an emulsifier and a control sample. These samples were subjected to analysis to evaluate density, viscosity with the EVO Expert R digital viscometer, SPAIN and texture using a Mark 10 texturometer, USA. Following studies carried out, the results showed that the chocolate sample with lecithin exhibited a softer texture, making it easier to compress and manipulate in fracture section. Additionally, it also showed lower viscosity compared to the control smaple.

**Key words**: artisanal chocolate, density, viscosity, texture, lecithin

Chocolate is a suspension composed mainly of solid particles of cocoa, sugar, milk and cocoa butter, which at temperatures above 36°C behave as a Newtonian fluid (Afoskwa E., 2010).

Each individual ingredient has an important role in the chocolate manufacturing technology, therefore a series of factors that appear during the manufacturing process, as well as after its completion, must be followed.

Currently, chocolate is one of the most appreciated confectionery products in the world, being an important source of polyphenols. According to data from the National Institute of Statistics, there is an increase in chocolate consumption in Romania, so that in 2016 the average monthly consumption per person was 157g of chocolate, while in 2022 a person consumed an average of 206g of chocolate (roaliment.ro).

Lecithin is extracted from soya beans. It is a natural emulsifier that has been used in the food industry, including chocolate production, since the 1930s, as it helps modify the flow properties of chocolate mass. Due to their special molecular structure, these surface-active ingredients reduce the surface tension between the dispersed and continuous phases (Minife B.W., 1980).

Emulsifiers help mix two or more liquids that don't naturally mix. Soy lecithin is added to chocolate to prevent the ingredients from separating so that the final product has a uniform consistency and can be molded and shaped into an ideal shape, as well as to achieve a smooth texture (Elevina S., 2015).

Also, soy lecithin is added to chocolate to obtain the maximum benefit of reducing viscosity and yield value in a ratio of 0.5-0.7%, this ratio is made according to the fat content, the content of moisture and particle size distribution. At higher levels than these, depending on the other ingredients used, lecithin can have the opposite effect *on the yield value* (Ferenc A.M., 2017).

The amount of lecithin needed to optimise rheology increases as the fineness (particle size) of the chocolate decreases. Thus, the ability of lecithin to influence rheological properties decreases as the fat content of the chocolate increases. Lecithin can bind particularly strongly to sugar, and this makes it very effective in chocolate making. (Beckett S.T., 2000).

The main objective of the research is to evaluate the impact of the use of lecithin addition on textural and rheological parameters by making two samples of chocolate, a control sample and a sample with lecithin as emulsifier.

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## MATERIAL AND METHOD

The research was carried out at the "*Ion Ionescu de la Brad"* University of Life Sciences in Iași, in the laboratory "Unitary Operations for the Food Industry", and the two chocolate samples were made in the pastry processing workshop, located in building Technology of food products processing.

A technological flow was established for the production of the two samples of artisanal chocolate that includes each step, the only difference between the two products being the use of lecithin addition for the experimental sample.

The technological flow starts with the boiling of water with added sugar to form a thick and sticky syrup by dissolving sugar in water at a temperature of 110-115ºC. To avoid the formation of sugar crystals during this boiling process, mixing of the two ingredients should be avoided until the composition starts to boil. Then the butter is melted into the resulting syrup.

The homogenization operation consists in bringing all the ingredients into contact. Thus, over the syrup made of water and sugar, the butter is added, after which the solid substances (cocoa, milk powder) are added, and the temperature that must be recorded during the process is 40-50ºC. It is important not to exceed this temperature range to avoid the deterioration of the flavor and the loss of the chocolate's nutritional properties.

The molding operation is done in the form of bars in cylindrical plastic matrices with a diameter of 10 mm and a height of 12 mm. The operation of cooling the cast mass consists in the solidification of the mass and the formation of the structure. The specific cooling temperature is between 2-3ºC, since at a temperature higher than 10ºC "fatty bleaching" occurs. During the demoulding operation, the temperature of the room must be between 15-18ºC, the relative humidity of the air must be between 65-70ºC, and the temperature of the chocolate must be between 12-15ºC in the moment of removal from the form.

After making the two samples, the following determinations were made to see the influence of lecithin: density (g/cm³), viscosity (cP) with the digital viscometer EVO Expert R, Spain, texture with the texturometer Mark 10, USA.

To determine the density, the chocolate was melted and transferred into a cylinder, thus the mass of the sample was determined by weighing on a balance. The density formula ($\rho$=m/v) was applied.

The viscosity of the melted chocolate samples was determined in the case of the control sample, at temperatures between 30-65ºC, and as for the lecithin sample, the temperatures were between 30-55ºC. The samples were prepared and tested with the Digital EVO viscometer, with the probe corresponding to the type of food, namely cylindrical pin type R7.

Regarding the texture determination, 4 types of probes were used, which were attached to the MARK 10 texturometer *(table 1).* The chocolate samples were prepared according to the probe type, thus the samples were bar type, 2.5 cm cube type L, l, h.

Table 1

**Mark 10 Texturometer Probes**

| Warner Bratzler knife | Cone type probe | Cylinder type probe | Bend type probe |
|---|---|---|---|
|  |  |  |  |

## RESULTS AND DISCUSSIONS

Regarding the determination of the density of the two chocolate samples, a difference can be observed between the two results in *table 2*, so that for the control sample a lower result was obtained compared to the result obtained by the lecithin sample, the result closer to the value the reference being that of the sample with lecithin, an aspect determined by the presence of lecithin as an emulsifier that slightly increases the density of chocolate.

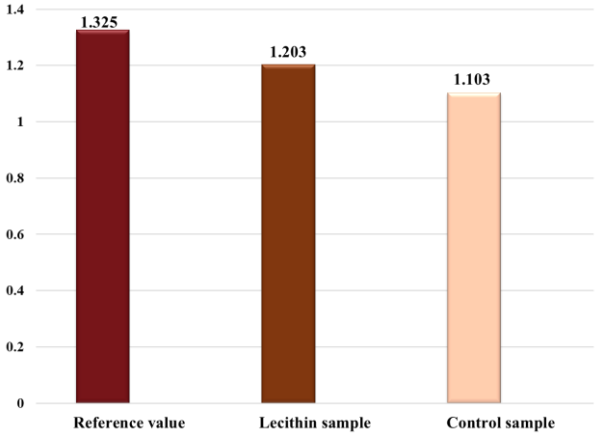


Figure 1 **Results on density, g/cm³**

*Figures 1* and *2* show the viscosity curves as a function of temperature, thus comparing the two figures it can be seen that the addition of lecithin caused a decrease in viscosity, the viscosity curve being in a more pronounced decrease in the lectin sample, suggesting that the incorporation of 5 g of lecithin into the recipe of the lecithin sample resulted in a decrease in viscosity. Lecithin is considered an emulsifier that reduces the cohesive forces between cocoa solids and fat particles.

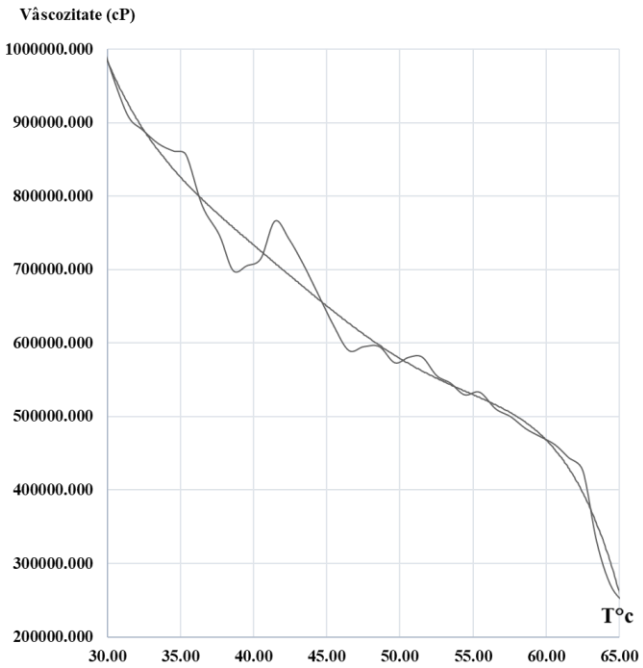


Figure 2 **Viscosity variation with temperature for the lecithin sample**

In order to highlight the influence of lecithin on the texture of chocolate, texture parameters obtained by four methods were analysed: compressive stress with a cylinder-type probe, shear stress with a knife-type probe, compressive stress with a cone-type probe, bending breaking stress. Of the four methods, the most representative were the methods of compressive stress with a cylinder-type probe and bending breaking stress.

By the method of compressive stressing with a cylinder probe the following texture parameters were obtained: cohesivity [-], elasticity [-], resilience [-], gumminess [N], chewability [N]. The results obtained for each parameter show that lecithin had a beneficial effect on them (*figure 3*).

Analysing the cohesivity, the lowest value was obtained in the lecithin sample (0.112) and the highest value was obtained in the control sample (0.397), which means that the lecithin sample showed a softer texture than the control sample, so it is also more crumbly, the value being closer to 0 in this case.

As far as elasticity is concerned, it is observed that the values are very close, so it can be said that both samples fall into the category of plastic products, the values being close to 0.

For resilience, values below 1 were obtained for both samples, suggesting that upon compression the samples could not recover to their original shape.

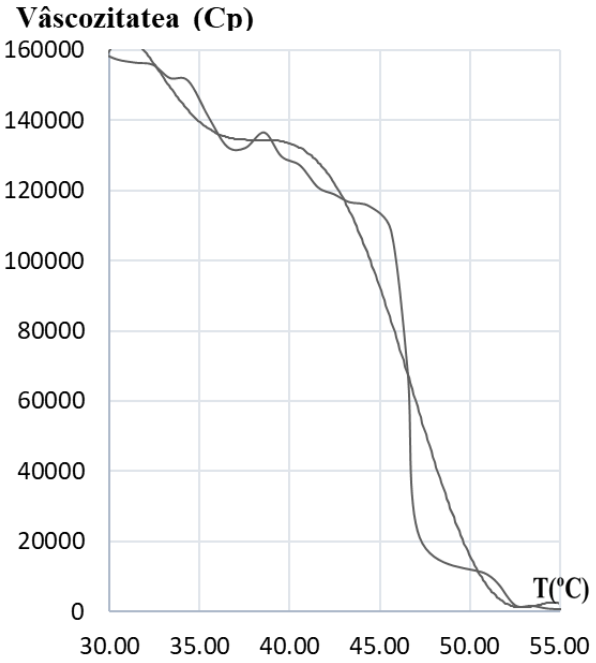


Figure 3 **Variation of viscosity with temperature for the control sample**

The gumminess evaluation showed a higher compressive force for the control sample and a lower force for the lecithin sample.

The results obtained for chewability were defined as 1.379 N for the lecithin sample and 3.257 N for the control sample, it can be stated that the lecithin sample has a lower chewability compared to the control sample.

In order to analyse which type of chocolate breaks more easily, the bending stress method was used. According to the values in *table 3*, it can be seen that lower values were obtained for the sample with lecithin and higher values in terms of the maximum force required for bending in the control sample, a fact due to the presence of lecithin which makes the chocolate softer and more easy to handle when broken into sections.

Table 3

**Bending breaking request**

| **Maximum force required [N]** | **Control sample** | **Lecithin sample** |
|---|---|---|
| | 81.4 | 55.2 |

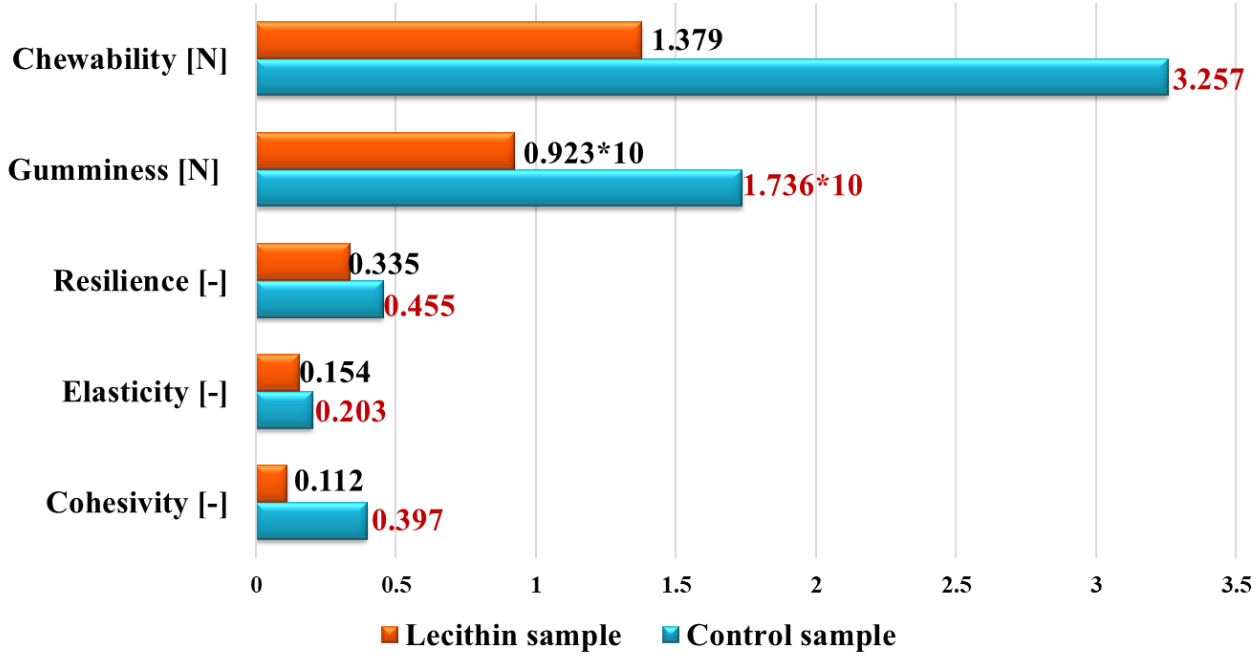


Figure 4 **Compression demand with a cylinder type probe**

## CONCLUSIONS

Lecithin has a an important role both in the manufacturing process and on physical parameters such as viscosity, density and texture.

In order to obtain a product with a specific viscosity it is necessary to add lecithin to the composition of the chocolate, since according to the curves obtained the lecithin sample obtained an ideal curve in terms of viscosity.

Analysing the data and comparing them shows that lecithin played an important role in terms of texture, so lecithin chocolate had a smoother and more uniform texture. In terms of the textural parameter of chewability lecithin chocolate is easier to chew, being softer and having a finer texture.

Also in terms of the cutting process of the two samples, the lecithin sample recorded lower values, indicating that the addition of lecithin to the chocolate facilitates the cutting process both analytically (using instruments) and by mastication in the first phase.

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# THE VM PROJECT: THE PARALLEL TEXTS METHOD PROMOTING DEEP LEARNING ENGAGEMENT OF MEDICAL STUDENTS

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**Abstract**

The article draws on the VM project, Digital Transformation of Histology and Histopathology by Virtual Microscopy (VM) for an Innovative Medical School Curriculum, funded by the European Union under the Erasmus+ framework. The project has as its focus VM, which has come to be regarded as a modern tool boasting increased quality and utility in microscopy education in European medical schools. We aim therefore at digitally transforming microscopy teaching and learning in order to achieve a cost-effective implementation and successful use of virtual microscopy technology towards the improvement of the histology and histopathology curriculum. The paper looks into the project's objectives and results, among which standard E.U. curricula for histology and histopathology, a virtual slide library, a training guide on advanced VM teaching in microscopy, and an open online course on VM. As an add-on, we also introduce the parallel text reading approach and its beneficial effects on the quality of foreign language learning by medical students. In view of the transferability potential of its deliverables and its relevant methodology, we are confident that the VM project contributes to the overall modernization of the educational system.

**Keywords**: virtual microscopy, multilingual VM library, online course, language learning, parallel texts

The COVID-19 pandemic shifted education to online delivery. To achieve this goal, several medical universities from Greece, Spain, Romania, Bulgaria, Greece and Poland introduced programmes based on Virtual Microscopy (VM), which has already come to be regarded as a modern tool boasting increased quality and utility in microscopy education in European medical schools. Innovative platforms, stored teaching libraries, study sets, and individual cases were used to improve didactic teaching, monitor the acquisition of new skills (e.g., stain interpretation), and assess competencies. The use of VM enhances student learning and their overall performance in a dynamic learning environment. State-of-the-art technology integration in the classroom encourages student participation, improves learning through strong linkages to the real world, and promotes the development of trustworthy multilingual learning resources. This in turn contributed to the internalization of higher education and brought about the accomplishment of international academic standards and quality in research, teaching, and services. The multilingual learning materials produced by such programmes also met young people's needs related to mobility, another constant of our world. Mobility provides young people with opportunities in terms of their personal and professional development depending, however, on their language competencies in the new context.

Arguably, VM programmes and their multilingual materials are beneficial for students in terms of their medical and language development being in line with societal requirements. Nevertheless, in spite of their advantages, there are few VM standard programmes in Europe.

The European VM project aims to bridge this gap and establish a dynamic virtual microscopy educational system in response to the need for improving the histology and histopathology curriculum in our medical schools and increasing the digital transformation of the educational systems.

## MATERIAL AND METHOD

The project aims to adapt the medical school curriculum of Histology and Histopathology at EU level, digitize the teaching of microscopy in medical universities, decrease the differences between education systems in Eastern EU countries and enhance undergraduate and postgraduate students' vocational skills in new medicine domains. The project addresses health care specialists, medical university staff from to the Histology and Histopathology departments, medicine students and postgraduate students in

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Histology and Histopathology training, specialists in Histology and Histopathology, medical universities and medical educational centers.

The project's results draw on partners' knowledge and competencies as well as on Covid experience: standard European curricula on histology and histopathology, a virtual slide library in histology and histopathology, a training guide for advanced VM teaching in microscopy and an open online course on VM aiming to increase students' competencies in microscopy/morphological sciences. The library, guide and training online course for advanced VM teaching in microscopy are available in six languages – English, Romanian, French, Bulgarian, Polish and Spanish. Thus, students can benefit from the project's multilingual online materials not only for their medical professional development but also for their foreign language learning. As an add-on of the project, in view of the transferability potential of its deliverables we introduce the parallel text reading approach and its beneficial effects on the quality of foreign language learning by medical students.

The term parallel text is generally understood to refer to versions of a text in two or more languages (Floros, G., 2004; Simard, M., 2019). The parallel texts reading method enables learners to understand the text with less difficulty since there are other versions of the text in other languages (including their mother tongue). If the students lack the knowledge of a lexical item, they can try to figure out its meaning from the context or resort to the version in the language they master.

According to Krashen, learners can take in a great deal of grammar and vocabulary when exposed to large amounts of linguistic material just above their current ability (comprehensible input: at least 70% of learners' known vocabulary) in a low-anxiety environment; thus, they feel comfortable and maximize their learning (Krashen, S., 1982; Krashen, S., 2013 ). Butzkamm and Caldwell (2009) identify two levels of comprehension: functional (understanding meaning) and formal (noticing specific linguistic instances and how things are expressed). Noticing is an important part of language acquisition because it demonstrates awareness and focus during the language-learning process (Schmidt, R., 2010; Nhung, N. T. P., 2020). Only when reaching the two levels of comprehension, can learners turn input into intake, which enables them to be independent readers (Butzkamm, W. and Caldwell, J., 2009). Butzkamm and Caldwell claim that the mediation of the first language or mother tongue (L1) plays a critical role in developing a comprehensible input. The parallel texts method can help learners become successful independent readers by exposing them to vast amounts of reading material.

The effectiveness of the strategy also depends on the learners' profession or language experience. The parallel texts reading method can take advantage of medical students' native language vocabulary base and subject background knowledge (medical background knowledge), which highly influence their acquisition of medical vocabulary in a foreign language (Drumhiller, M.F., and Schwanenflugel, P. J., 2013). Since the vocabulary is often similar to the target language, if the students already know the field-related vocabulary in their mother tongue and they also have topic knowledge, that will facilitate their understanding of the unknown words. As conceptual knowledge is not stored in different parts of the brain for the two languages, it is easily retrievable in either language, which facilitates and accelerates second-language vocabulary learning. Secondly, they may know one or two languages, which can help them with vocabulary acquisition in terms of comparison and commonality, as they can bring the language learning skills acquired through their rich language experience to the acquisition of another language.

## RESULTS AND DISCUSSIONS

Having the opportunity to teach foreign languages to medical Romanian and international students taking up English and French courses and also interested in other languages, our teachers use the multilingual texts produced by the project; the discussions with the teachers highlight that most of the activities using this method focus on students' reading and work on the texts accompanied by vocabulary/grammar exercises they encounter in the texts.

Our experience and research reveal that activities based on parallel texts are either student-driven, in which case students are autonomous or teacher-driven, when students are given concrete tasks to perform under the teacher's guidance (Abdallah, A., 2021; Becker, D. P., 2012). While the former case encourages the student to work independently having a relatively rich experience with the language, the latter relies on the teacher's feedback or digital language learning exercises with automatic answers.

**Student-driven activities** The student-driven activities are usually carried out as a home assignment as students want to deepen their medical and language knowledge. Such activities are first modeled in class and then done at home. Students also get some tips on how to proceed and are encouraged to find their own strategies while reading the parallel texts. We recommend the following activities:

- Students read the target language/ foreign language (L2) text first and try to understand as much as they can without resorting to the L1 version. This helps them improve their comprehension and identify the words and phrases that they need to look up; these are the words which impede the understanding of the text.
- Students look the identified words up in an L2-L1 dictionary if they don't understand a whole sentence. This will help them enrich their vocabulary and improve their understanding of the text.
- Students compare the L2 text to the L1 text to identify and notice the grammatical forms/ structures that are used in context. This will help them improve their grammar and comprehension.

- Students write down new words and phrases. This will help retain the new vocabulary.
- When reading students do not read both texts. Students can have a look at the text in L1 as a backup to reinforce or confirm their understanding.

Another strategy invites students to read the text first in the language they are familiar with, then in L2. They are advised to start with a small text, depending on how much they can digest and their mind can process. They can start gradually from a sentence to a paragraph at a time. They are encouraged to put down new words or phrases they have encountered and revise them from time to time.

Once they feel comfortable with the target language, they can reverse the process: read only in the target language and stop when they come across a word or phrase that hinders their understanding. Then they have to check out its meaning in the text written in the L1 language.

Some teachers advise students to read the passage in the foreign language out loud. Reading out loud is known to enhance fluency and pronunciation.

**Teacher-driven activities** The teacher-driven activities are carried out in class. The teacher introduces the topic of the parallel texts and invites students to discuss it, which activates their prior knowledge on the topic. The teacher can also use the KWL chart stimulating students to think about what they know (K), what they want to know (W), and what they have learned about a topic at the end of the learning session (L). This strategy activates students' background knowledge, develops a purpose for learning, summarizes and revises the learning input. The discussion is in the target language (L2), focusing on the topic, the title or pictures and eliciting students' knowledge and key vocabulary words. After the discussion with the whole group, students study the texts in L2 but they can use the L1 version whenever necessary. Students are given a task (e.g. comprehension questions on the text) to do either in pairs or in groups and are instructed to read the text in their pairs or groups and answer the questions.

During the following class, the text is read and discussed as an activity involving the whole class. Each group presents their answers to the questions and participates in the discussion. Students discuss the challenges they came across while reading and the differences in sentence structures of both texts. When needed, for instance in case of exceptionally difficult passages and concepts, it is always advisable for teachers and students to use the original L1 text. Teachers can provide students with explanations and exercises on problematic structures. When these sections are identified and solved, the passages and entire text may be read again in L2 to consolidate the vocabulary and structures.

In addition, the parallel reading method is supported by reading, writing and speaking activities when students practice and use the recently acquired language input. For example, the teacher introduces new vocabulary and asks students to try to guess the meaning from the context (L2 text) and then check (L1 text).

Other times, the text can be used to recycle vocabulary:

- The teacher gaps the L2 text and asks the students to fill in the missing words (multiple choice quizzes) and then check their solution with the parallel texts (either L1 or L2 or both).
- The students get the L2 text cut up and jumbled up in separate sentences or paragraphs and have to rearrange it based on the L1 (or another language they are familiar with) text.
- The students can practice vocabulary by matching lists of L1 words with L2 words in the text, matching lists of L2 words with synonyms in the text; doing true or false exercises, questions and answers; summaries etc.
- The students get a modified version of the L1 text in L2, where the L2 contains errors 'planted' by the teacher. The learners have to spot and fix the mistakes.

**Benefits of using the parallel texts reading method**

Our experience and research highlight that the method impacts foreign language learning.

The method stimulates learners' appetite for reading. The parallel text reading method encourages less confident learners to read by providing them with a text in the language they know to use whenever they have problems understanding the text in L2. The existence of parallel texts reduces the anxiety levels of such learners and provides a solid affective scaffold. On the other hand, the method should be used sensibly when students feel stuck or want to check to see whether their assumptions are correct.

The method improves comprehension: Parallel texts help learners to understand the content (especially at the beginner and intermediate levels) and also develop learners' knowledge of cognate words.

The method expands vocabulary. Reading parallel texts also exposes students to new words and phrases in authentic contexts, facilitating the recycling or expansion of vocabulary.

The method improves grammar. By comparing the texts in different languages, students can see how sentence structures and grammatical forms are used in context, which can help them improve their grammar skills.

The method provides reading practice. Reading parallel texts is a great way to practice reading skills in the language, which is an essential part of language learning. Parallel texts make it possible for learners to access higher-level texts by providing valuable support by means of multilingual translations.

The method draws learners' attention to similarities and differences between languages. Through parallel texts, teachers can draw learners' attention to linguistic as well as cultural differences that can be referenced in the texts. Teachers can draw learners' attention to meaning, structure, and vocabulary-related aspects.

The method provides scaffolding using L1 text as reference material. While reading a text in L2, students

find it difficult to comprehend it as they encounter unfamiliar words. In such a situation, they lose interest and stop reading the text. But if they have a translation side by side, they can fall back on the text in L1, find the meaning of the unfamiliar words and proceed to read. Such scaffolding can be withdrawn as they become self-reliant.

The method encourages self-reading and stimulates students to become independent readers. Parallel texts can facilitate self-reading without depending on the teacher. Learners can find meanings in the text and read them in context.

The method is less time-consuming and more effective. While reading parallel texts students do not have to resort to the dictionary all the time.

The method enhances learners' self-esteem. It confirms learners' decisions and encourages them to make choices and change decisions based on the parallel texts.

**Disadvantages of using parallel texts**

The method focuses on reading, which is only one of the language skills; however, the development of the other skills must not be ignored if the learners want to use the language. The method cannot be used as standalone but alongside other methods engaging students in integrated skills activities.

Students may become too dependent on the L1 translation, making it hard for them to understand the target language texts without it. The method relies on the silent reading of texts and depends on the use of parallel texts for clarification or confirmation.

## CONCLUSIONS

The VM project, Digital Transformation of Histology and Histopathology by Virtual Microscopy (VM) for an Innovative Medical School Curriculum, funded by the European Union under the Erasmus+ programme, aims at digitally transforming the teaching and learning of microscopy in order to achieve a cost-effective implementation and successful use of virtual microscopy technology. The project's results, among which standard E.U. curricula for histology and histopathology, a virtual slide library, a training guide on advanced VM teaching in microscopy, and an open online course on VM will have a major contribution to the modernization of medical universities. The parallel text reading approach and its beneficial effects on the quality of foreign language learning by medical students draws attention to the transferability potential of the project's deliverables and its relevant methodology.

The multilingual character of the results increases the accessibility to VM learning material. The parallel text reading approach will enhance students' reading skills. Moreover, by supplementing it with other techniques teachers can help students improve their listening, writing and conversational skills.

## ACKNOWLEGMENTS

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# THE INFLUENCE OF SALINE STRESS ON THE WATER REGIME IN BITTER CUCUMBERS (*MOMORDICA CHARANTIA*)

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**Abstract**

Bitter cucumber (*Momordica charantia*) is a tropical and subtropical plant widely distributed in China, Malaysia, India and tropical Africa, with a long history of use in traditional medicine. Soil salinity is a major abiotic stress worldwide that affects plant morphology and physiology leading to reduced growth, reduced production yield or in some cases plant death. Due to the reduction of water accessibility, plants try for its optimal absorption, increasing the concentration of vacuolar juice as a method of combating the hypertonicity of the external environment. To determine the adaptation capacity to saline stress, which is increasingly common, two varieties (Brâncusi and Rodeo) and three experimental lines (Line 1, Line 3 and Line 4) of bitter cucumber were treated with saline solutions of different concentrations (100 mM NaCl and 200 mM NaCl), and following the treatments determinations of free water and bound water content were performed. Research has shown that with the onset of salt stress, the amount of free water in bitter cucumber leaves decreased and the amount of bound water increased in most of the studied plants, which highlights the triggering of a pronounced reaction to this type of abiotic stress. The statistical analysis shows significant differences between the control and the treated variants both in the case of free and bound water.

**Key words**: *Momordica charantia*, salinity, water regime

Bitter cucumber (*Momordica charantia*) commonly known as bitter melon or bitter gourd is a tropical and subtropical plant. It is widely distributed in China, Malaysia, India and tropical Africa with a long history of use as a hypoglycemic agent (Agarwal S., Shaheen R., 2007; Blum A., *et al*, 2012; Grover J., Yadav S., 2004).

Soil salinity is a major abiotic stress worldwide that affects plant morphology and physiology leading to reduced growth, reduced production yield or in some cases their death (Balal *et al*, 2011). The accumulation of salts in the soil is harmful to plants, because it increases the concentration of the soil solution and disrupts the root absorption process, having a toxic effect on them (Jităreanu D.C., 2007). Due to the reduction of water accessibility, plants try for its optimal absorption, increasing the concentration of vacuolar juice as a method of combating the hypertonicity of the external environment (Farooq M., *et al*, 2015).

Research has shown that plants with better resistance to abiotic factors are characterized by cuticular transpiration and a lower rate of leaf dehydration (Petcu E., *et al*, 2007).

Water is the mineral component of all living organisms, predominant over other substances. The water content is variable depending on the species, organ and tissue. Variations in the water content are also found in the different organs and tissues of the same plant (Ievinsh G., 2023; Maggio A., *et al*, 2002).

The regulation of water absorption in plant cells is achieved by processes of simple, facilitated diffusion, osmosis and active transport, which occur at the level of plasma membranes. Less than 1% of the total amount of absorbed water is used in photosynthesis and growth processes, the rest being eliminated through transpiration processes (Lobet G., *et al*, 2014; Brendel O., 2021).

## MATERIAL AND METHOD

Bitter cucumber leaves (*Momordica charantia*) belonging to two Romanian varieties: Brâncusi and Rodeo and three experimental lines: Line 1, Line 3 and Line 4 were used to carry out the work. The seedlings were obtained in the greenhouse of the Agricultural Research Institute and Environment (ICAM) belonging to the "Ion Ionescu de la Brad" University of Life Sciences Iași. They were planted in 12-liter pots of

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vegetation, the cultivation being carried out in the greenhouses of the vegetable growing discipline within the didactic resort "Vasile Adamachi Farm".

The plants were treated with saline solutions of different concentrations: 0 mM NaCl, 100 mM NaCl, and 200 mM NaCl. 3 treatments were carried out 10 days apart, in different phenophases according to the BBCH scale: 201 which corresponds to the appearance of the first lateral shoot, 501-502 which corresponds to the appearance of the first flowers and 701 which corresponds to the appearance of the first fruit.

Seven days after the application of each treatment, determinations of free water and bound water were made. To carry out these determinations, leaves were harvested from the middle of the main shoot of the plants and inserted with the petiole into test tubes with water, then they were fixed with parafilm.

The analyzes were carried out in the laboratory of the Plant Physiology discipline within the Faculty of Agriculture. For the analysis of water loss at the foliar level, the free water was determined by weighing at 24 hours the leaves kept at room temperature. Before weighing, the petiole was removed and the surface of the section was covered with petroleum jelly to occlude the conducting vessels. The first weighing represents the initial weight of the turgid leaf (G0), and the last weighing (G24) gives by difference the amount of water lost by the leaves in the respective time interval. The water lost after 24 hours, as a percentage of the initial weight of the plant material, is considered free water (Jităreanu C.D., Marta A.E., 2020).

To determine the bound water, the plant material was placed in ampoules of known weights and placed in the oven at a temperature of 105 C° for 4 hours. After weighing, the vials were placed back into the oven and weighed until their weight remained constant. The amount of water lost through oven drying is considered bound water. This was determined by the difference between the initial weight and the dry matter (Jităreanu C.D., *et al*, 2011). Dry matter was calculated according to the formula:

b*100/a
a= initial weight
b=final weight.

## RESULTS AND DISCUSSIONS

In the body of the plant, water is found in two states, liquid and gaseous. Liquid water is the main component of all cells, being present in maximum quantity in vacuoles and minimum in cell organelles. This is in the form of free water and bound water (Toma D.L., Jităreanu C.D., 2007).

**Free water**

Free water is considered to be the amount of water lost through sweating and dehydration. It is retained with weak forces in the plant body and circulates easily intra and intercellularly.

Since the reaction to excess salts manifests itself, in a first phase, as osmotic stress, in order to identify the varieties and lines of bitter cucumbers tolerant to salinity, it is necessary to assess the capacity to retain water at the leaf level, expressed by a speed of dehydration of the leaves less.

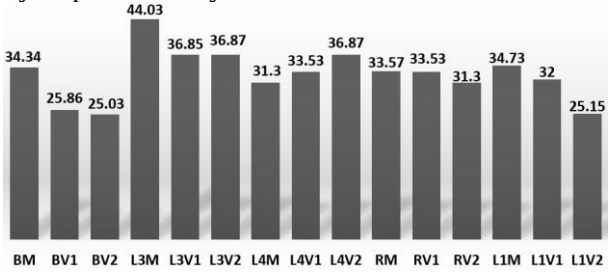


Figure 1 **Effect of salt stress on free water content after the first treatment**

After determining the amount of free water, it was possible to observe a tendency to decrease free water in plants treated with saline solutions, except for Line 4 where an opposite effect was highlighted, the lowest value of free water being observed in the control variant (31, 3%) and the highest value in the version treated with 200 mM NaCl (36.87%) (*figure 1*). This implies a poor adaptation of plants to salt stress. The most pronounced difference was observed in Line 3 where the control presented the highest value of free water (44.03%) in contrast to the variant treated with 100 mM NaCl (36.85%) and the variant treated with 200 mM NaCl (32 ,5%).

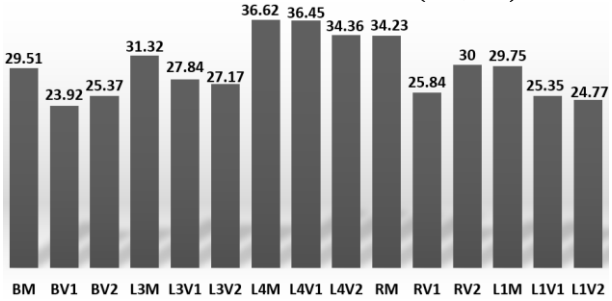


Figure 2 **Effect of salt stress on free water content after treatment two**

After the second treatment, the same phenomenon of decrease in free water content was observed in plates treated with saline solution. According to the graph (*figure 2*), pronounced differences between the control and the treated variants were observed in the Brâncusi variety, Line 3 and Line 1. In the case of Line 4, the highest value of free water was recorded in the control variant (36.32%) and the lowest amount in the variant treated with 200 mM saline solution,

which may imply a slight adaptation to the saline conditions to which the plants were subjected.

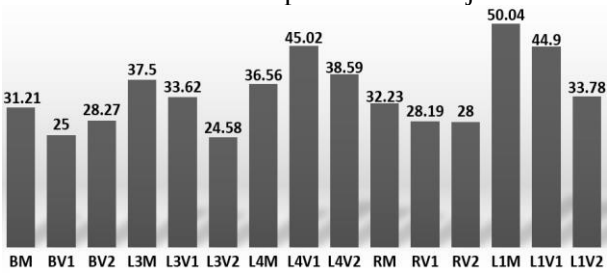


Figure 3 **Effect of salt stress on free water content after treatment 3**

After the application of the third treatment, the most pronounced differences between the control plants and those treated with saline solution were recorded in Line 1 where the control presented a free water value of 50.04% in contrast to the variant treated with 100 mM NaCl which recorded a value of 44.9%, and the variant treated with 200 mM with a value of 33.78%. In the case of Line 4, the highest value was recorded in the version treated with 100 mM saline solution (45.02%), and the lowest value in the control version (36.56%) (*figure 3*).

The T-test was used to compare the free water recorded in the control with the amounts of free water found in the 100 mM and 200 mM saline-treated variants. Following the application of the test, statistically significant differences were recorded both between the control (I) and the variant treated with 100 mM NaCl (II) and between the control and the variant treated with 200 mM NaCl (III). In the case of the two treatments, there are no significant differences, which may be due to the fact that they are close in terms of saline concentration (*tabel 1*).

Table 1

**Statistical differences between the control variant (I) and the variants treated with saline concentrations of 100 mM (II) and 200 mM (III), regarding the free water content of the leaves**

| *Variants compared* | *t-stat* | *P one-tail* |
|---|---|---|
| I-II | 2.848504699 | 0.00644394 |
| I-III | 3.636956011 | 0.00134681 |
| II-III | 1.565081015 | 0.06994084 |

t-Test Paired Two Sample for Means: P one-tail > α the null hypothesis is accepted; P one-tail ≤ α the null hypothesis is rejected;
Insignificant statistical differences (p≥0.05) between variants; significant statistical differences (p≤0.05) between variants; distinctly significant statistical differences (p≤0.01) between variants; very significant statistical differences (p≤0.001) between variants.

Bound water is retained in plants with high forces. This water is hardly lost by plants, because it consists of immobile molecules, lacking the property of diffusion. The bound water is removed by drying (Toma and Jităreanu, 2007).

In conditions of saline stress, when the vital activity of the plant decreases, there is an increase in the amount of bound water, thus ensuring survival in such conditions.

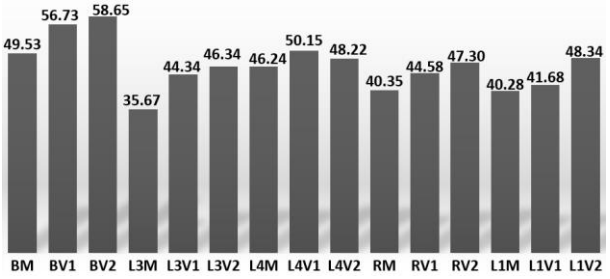


Figure 4 **The effect of salt stress on the bound water content after the application of the first treatment**

According to the determinations made after the first treatment, higher values of the bound water content were observed in the treated variants than in the control ones (*figure 4*). The most pronounced difference was observed in the Brâncusi variety where the bound water values increased directly proportional to the increase in NaCl concentration. The control showed the lowest value of bound water (49.53%) in contrast to the variant treated with 100 mM (56.73%) and the variant treated with 200 mM saline (68.65%).

Variants treated with 100 mM NaCl showed bound water values between 1.03-1.24 times higher than the control values. In the case of variants treated with 200 mM, the bound water values increased compared to the control by 1.04-1.29 times.

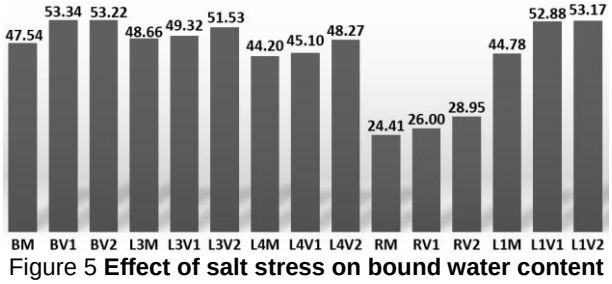


Figure 5 **Effect of salt stress on bound water content after application of treatment two**

After the second treatment, the same trend of increasing water values was noted, directly proportional to the increase in the concentration of the applied saline solution (*figure 5*). Pronounced differences were observed in the case of the Rodeo and Brancusi varieties, but also in the case of Line 1. In the variants treated with 100 mM saline

solution, the bound water increased by 1.01-1.18 times, and in the case of the variants treated with 200 mM saline solution bound water increased over control by 1.05-1.08-fold. Smaller differences between the two treatments compared to the previous treatment could be noted, which may be due to the small difference between the two saline concentrations.

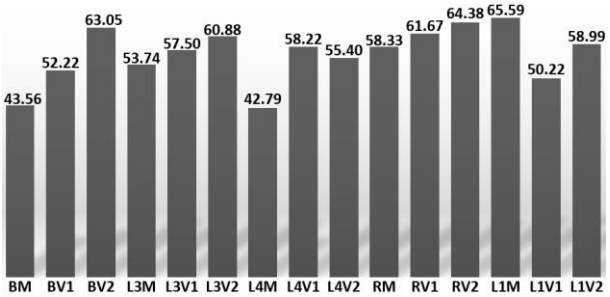


Figure 6 **Effect of salt stress on bound water content after application of treatment three**

Seven days after the application of the third treatment, a more pronounced difference in bound water was revealed between the control version and the variants treated with saline in the Brâncusi variety, where the control showed a value of 43.56% bound water and the version treated with 200 mM showed a value of 53.05% (*figure 6*). In the case of Line 1, the effect was opposite to the other varieties and lines, the control showing a higher bound water value (65.59%) than the variants treated with saline solutions. The lowest value was recorded for the variant treated with 100 mM (50.22%).

After the treatment, the most pronounced differences between the control and the treated variants were observed. The variety treated with 100 mM NaCl showed 3.76-15.43 times the amount of bound water and in the case of the variety treated with 200 mM saline, the amount of bound water was 6.5-19 times higher.

Following the application of the T test, statistically significant differences were observed both between the control and the two variants treated with saline solution and between the two concentrations of NaCl applied, which can highlight an adaptation of the bitter cucumber varieties and lines to saline stress (*tabel 2*).

Table 2

**Statistical differences between the control variant (I) and the variants treated with saline concentrations of 100 mM (II) and 200 mM (III), regarding the bound water content of the leaves**

| *Variants compared* | *t-stat* | *P one-tail* |
|---|---|---|
| I-II | 3.636956011 | 0.00134681 |
| I-III | -2.27403464 | 0.01961622 |
| II-III | -3.02802594 | 0.00451761 |

t-Test Paired Two Sample for Means: P one-tail > α the null hypothesis is accepted; P one-tail ≤ α the null hypothesis is rejected;
Insignificant statistical differences (p≥0.05) between variants; significant statistical differences (p≤0.05) between variants; distinctly significant statistical differences (p≤0.01) between variants; very significant statistical differences (p≤0.001) between variants.

## CONCLUSIONS

Following the applied treatments, smaller amounts of free water lost by the leaves of the plants subjected to saline stress were observed, in contrast to the control, a phenomenon specific to water and saline stress. After analyzing the content of bound water lost by the leaves treated with saline solutions and the control, a pronounced difference was observed between the control and the treatments, with the stressed plants showing higher amounts of bound water.

Following the statistical analysis of the content of free water and bound water, statistically significant differences were recorded between control plants and those treated with saline solutions.

Among the varieties and lines tested, the Brâncusi variety and Line 3 presented the highest bound water content, which demonstrates their good adaptation to saline stress conditions.

Line 4 recorded the lowest amount of water related to the variants treated with saline solutions, which shows a low capacity to adapt to saline stress.

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