



POLJOPRIVREDNI  
FAKULTET  
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Student Conference  
NOVI SAD

***49<sup>th</sup> Conference for Students of  
Agriculture and Veterinary  
Medicine with International  
Participation  
Proceedings book***



*University of Novi Sad, Faculty of Agriculture  
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POLJOPRIVREDNI  
FAKULTET  
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Student Conference  
NOVI SAD

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POLJOPRIVREDNI FAKULTET**

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**PROCEEDINGS  
OF THE 49<sup>th</sup> CONFERENCE FOR STUDENTS OF AGRICULTURE AND  
VETERINARY MEDICINE  
WITH INTERNATIONAL PARTICIPATION**

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Council of the University of Novi Sad, Faculty of Agriculture, at its fourth meeting on 18<sup>th</sup> of February 1981 made a decision that the Conference for students of agriculture and veterinary medicine with international participation, held each year at the Faculty of Agriculture will be held in the memory of academician ***Dr Petar Drezgić***, professor, and the prizes awarded at the Conference will bear his name.

## **PREFACE**

The first Conference for students of agriculture and veterinary medicine with international participation was held in Novi Sad 49 years ago. To the present date, many papers from national and international authors were presented (Hungary – Gödölö and Debrecen, Germany – Kassel, Slovakia – Nitra, Poland – Wroclaw and Warsaw, Romania – Timisoara, Cluj, Bucharest and Sibiu, Bulgaria – Plovdiv, Russia – Moscow and Kemerovo, Macedonia – Skopje and Bitola, Bosnia and Herzegovina – Sarajevo, Banja Luka, Croatia – Zagreb). Since 2009, papers presented at the Conference are published in a special publication named “PROCEEDINGS OF THE 49<sup>th</sup> CONFERENCE FOR STUDENTS OF AGRICULTURE AND VETERINARY MEDICINE WITH INTERNATIONAL PARTICIPATION” where all published papers have all elements of the original scientific paper. We encourage students and mentors who are interested in benefiting more from this Conference to publish their papers in the journal Contemporary Agriculture published by the University of Novi Sad, Faculty of Agriculture.

As the host and organizer of the Conference University of Novi Sad, Faculty of Agriculture has a special honor and a pleasure to wish a warm welcome to all participants, successful work and amusing socializing in Novi Sad.

**Vice-dean for science and international cooperation  
Dr Mila Grahovac, full professor**

**President of the organizing committee  
Dr Marta Loc, assistant professor**

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# EARTHWORMS-MEDIATED ENHANCEMENT OF SOIL PROPERTIES AND MAIZE YIELD

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*The study examined the effects of introducing *Lumbricus terrestris* into plowing and conservation tillage systems on soil properties and maize yield. Conservation tillage supported higher earthworm abundance, nutrient availability, and grain mass, while plowing favored early growth. Results confirm that reduced soil disturbance combined with biological activity improves soil fertility and sustainability.*

**Key words:** earthworms, tillage, maize, soil fertility, conservation agriculture

## INTRODUCTION

Earthworms play a pivotal role as “ecosystem engineers” due to their ability to modify soil’s physical, chemical, and biological properties through their burrowing and feeding activities (Arunachalama and Entoori, 2022). Their actions enhance aeration, porosity, water infiltration, and the formation of stable aggregates rich in organic matter, thereby improving the structure and fertility of soils (Lemtiri *et al.*, 2014). The abundance of earthworms has drastically decreased worldwide due to anthropogenic impacts such as soil tillage, pesticide use, and crop residue removal (Briones and Schmidt, 2017). Consequently, there is a growing emphasis on regenerative and conservation-based practices, which enhance biological activity and restore natural balance in arable soils (Lal, 2020; Reganold & Wachter, 2016). In Serbia, intensive agricultural systems characterized by frequent disturbance and low organic inputs have resulted in declining biological activity and soil structure degradation. Introducing native earthworm species into different tillage systems represents a potential solution for improving soil’s physical and biological properties and potentially enhancing crop yields (Šeremešić *et al.*, 2022; Sekulić *et al.*, 2022). Yet, studies examining the influence of earthworm activity under local agroecological conditions remain limited. While soils with higher earthworm populations are generally considered more fertile, quantifying the direct effects of earthworms on crop productivity remains challenging. Therefore, it is essential to conduct research that deepens our understanding of the interactions between different ecological groups of earthworms and cultivated plants.

The main objective of this study is to investigate the effects of introducing *Lumbricus terrestris* into different soil tillage systems (conventional plowing and conservation tillage) on the physical, chemical, and biological properties of soil, as well as on maize (*Zea mays L.*) yield and morphological characteristics. Therefore, this research aims to determine whether and to what extent earthworms can contribute to improving soil properties, which will subsequently influence the growth and development of plants.

## MATERIAL AND METHODS

The experiment was conducted during the 2021-2022 growing season at the “Rimski Šančevi” experimental field of the Institute of Field and Vegetable Crops, Novi Sad, within the long-term “Plodoredi” trial. The site is situated in a flat area with a moderately continental climate, an average annual temperature of 12.8°C, and average annual precipitation of approximately 640 mm. The soil is classified as loamy Haplic Chernozem with about 2.8% organic matter. Fertilization was applied according to agrochemical analyses and maize crop requirements. The trial was established within a three-year crop rotation plots (maize-soybean-wheat) as a two-factor experiment:

- 1) Factor A - Tillage system: mouldboard plowing (27-30 cm soil depth) vs. conservation tillage using a *Väderstad Tempo 6* planter after stubble mulching with a rotary tiller.
- 2) Factor B - Earthworm introduction: *Lumbricus terrestris* vs. control (without the enhancement).

Plots measuring 3 × 2.5 m, established in April, were enclosed with plastic barriers to prevent worm migration (Photo1). The design included three replications per treatment, totaling 12 plots: six under plowing (three with and three without earthworms) and six under conservation tillage (three with and three without earthworms). After sowing maize (*Zea mays L.*, hybrid NS4010) in May, *Lumbricus terrestris* were introduced following the small-plot monitoring protocol by Euteneuer and Butt (2025). All standard agronomic practices were applied according to the long-term experiment maintenance recommendations. Weeds were controlled according to the recommended procedure for maize. For pH<sub>CaCl<sub>2</sub></sub> measurement, the 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> extracts were performed in the ratio 1:10 w v<sup>-1</sup> (4 g of fresh soil (≤ 5 mm) 40 ml<sup>-1</sup> of extract) (Houba et al., 2000). The easily extractable glomalin (EEG) and was analyzed according to Wright and Upadhyaya (2004). In summary, 1.00 g of air-dried soil (<2 mm) was mixed with 8 mL of sodium citrate (20 mmol.L<sup>-1</sup> at pH 7.0 for EEG and 50 mmol.L<sup>-1</sup> at pH 8.0 for TG). The available phosphorus (P<sub>M3</sub>), potassium (K<sub>M3</sub>), calcium (Ca<sub>M3</sub>), and magnesium (Mg<sub>M3</sub>) content in the soil sample was determined using the Mehlich 3 extractant (Mehlich, 1984).



**Photo 1.** Experimental maize plots under different tillage systems:  
(a) Before maize emergence; (b) During maize growth.

Data were analyzed using analysis of variance (ANOVA) to evaluate the effects of tillage and earthworm treatments on maize yield. Differences between means were tested at the significance level  $p < 0.05$ . Statistical summaries and graphical outputs were produced using *Statistica 13.0* and *Microsoft Excel* software.

## RESULTS AND DISCUSSION

### Soil Chemical Properties

As shown in Table 1, the soil pH was slightly alkaline (7.6–7.7) across all treatments, indicating no major differences between tillage systems. Nitrate nitrogen (N-NO<sub>3</sub>) was higher under conservation tillage with earthworms, indicating more active mineralization and nutrient cycling. Ammonium nitrogen (N-NH<sub>4</sub>) was slightly higher in plowed soil, likely due to stronger aeration and faster nitrogen transformation. DOC values were similar among treatments, with slightly higher averages under conservation tillage. Overall, conservation tillage with earthworms enhanced nutrient availability compared to plowing.

**Table 1.** Average values of pH, available nitrogen (N-NO<sub>3</sub>, N-NH<sub>4</sub>), and dissolved organic carbon (DOC) under different tillage systems and treatments (measured in 0.01 mol/L CaCl<sub>2</sub> solution), ± standard deviation

Tillage system	Sample specification	pH (CaCl <sub>2</sub> )	N-NO <sub>3</sub> (mg/kg)	N-NH <sub>4</sub> (mg/kg)	DOC (mg/kg)
Conservation tillage	Earthworms	7.64 ± 0.03	20.3 ± 4.50	0.73 ± 0.23	17.3 ± 0.80
	Control	7.68 ± 0.04	9.8 ± 1.76	0.71 ± 0.07	18.7 ± 0.65

Plowing	Earthworms	7.67 ± 0.006	11.0 ± 2.01	1.31 ± 0.23	19.4 ± 4.52
	Control	7.70 ± 0.006	7.8 ± 1.82	1.42 ± 0.10	15.65 ± 0.05

Table 2 shows that easily extractable glomalin (EEG) levels were higher under conservation tillage, especially with earthworms, indicating greater biological activity and glomalin production (Meng *et al.*, 2022; Muchane *et al.*, 2019). Available phosphorus (P) remained below detection, but other macronutrients showed clear patterns. Available K, and Mg were also higher in conservation plots, reflecting improved nutrient retention and organic matter stabilization. In contrast, S was slightly higher under plowing, likely due to greater mineral exposure and oxidation. Overall, conservation tillage with earthworms enhanced glomalin accumulation and nutrient availability, supporting better soil quality and fertility.

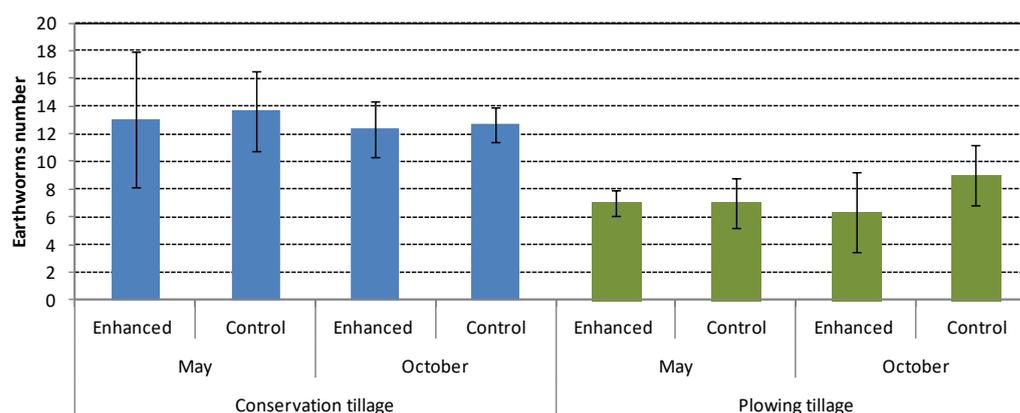
**Table 2.** Easily extractable glomalin (EEG) and available macronutrients (P, K, Mg, S) under different tillage systems and treatments (measured in 0.01 mol/L CaCl<sub>2</sub> solution), ± standard deviation

Tillage system	Sample specification	EEG (mg/kg)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	S (mg/kg)
Conservation tillage	Earthworms	460 ± 89.0	na	87.7 ± 26.6	172.5 ± 5.5	1.39 ± 1.2
	Control	391 ± 80.0	na	87.3 ± 54.7	151.0 ± 2.0	1.27 ± 0.05
Plowing	Earthworms	413 ± 45.5	na	76.9 ± 15.6	115.0 ± 1.0	1.81 ± 0.01
	Control	356 ± 68.5	na	68.0 ± 26.4	116.0 ± 0.0	1.73 ± 0.5

<sup>1)</sup> na - value under detection limit

### Earthworm Abundance

As shown in Figure 1, the number of earthworms was consistently higher under conservation tillage than under plowing, both in May and October. In conservation tillage, populations ranged between 13–14 individuals per 30 × 30 cm, while plowed plots averaged about 7 individuals per 30 × 30 cm. A slight decline in numbers from May to October was recorded across all treatments, reflecting normal seasonal dynamics rather than treatment effects. The introduction of *Lumbricus terrestris* caused a minor increase in abundance, more noticeable under plowing, where conditions were less favorable, but favored the anecic ecotype (deep-burrowers). These results confirm that reduced soil disturbance supports higher earthworm populations, as conservation tillage maintains structure, moisture, and organic residues essential for their survival and activity (Crittenden *et al.*, 2014; Briones and Schmidt, 2017).

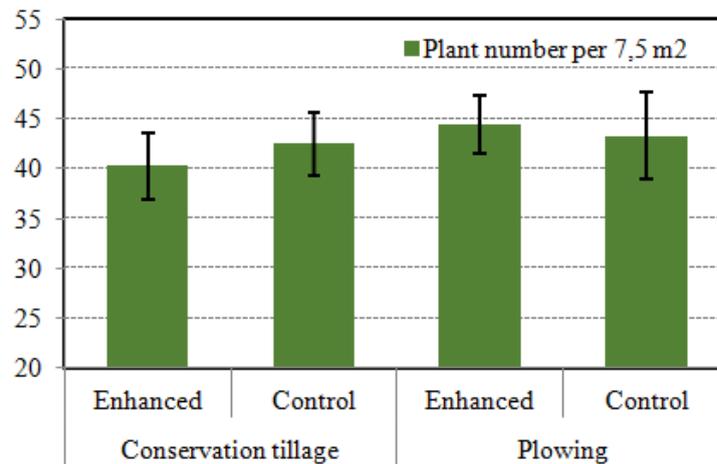


**Figure 1.** Earthworm abundance (individuals per 30 x 30cm) under different tillage systems (conservation tillage and plowing) and treatments (enhanced and control) during May and October

### Plant Number

Figure 2 presents the average number of maize plants per 7.5 m<sup>2</sup> under different tillage systems and treatments. The results show no statistically significant differences in plant number between conservation tillage and plowing, nor between enhanced and control plots. Plant populations ranged from 40 to 47 plants per 7.5 m<sup>2</sup>, with slightly higher values observed under plowing. This trend can be explained by the looser and more friable

seedbed created by plowing, which ensures better seed–soil contact and uniform germination (Blunk *et al.*, 2021). In contrast, conservation tillage often results in cooler and moister surface layers, which may delay germination and slightly reduce stand density in the short term (Obia *et al.*, 2020).

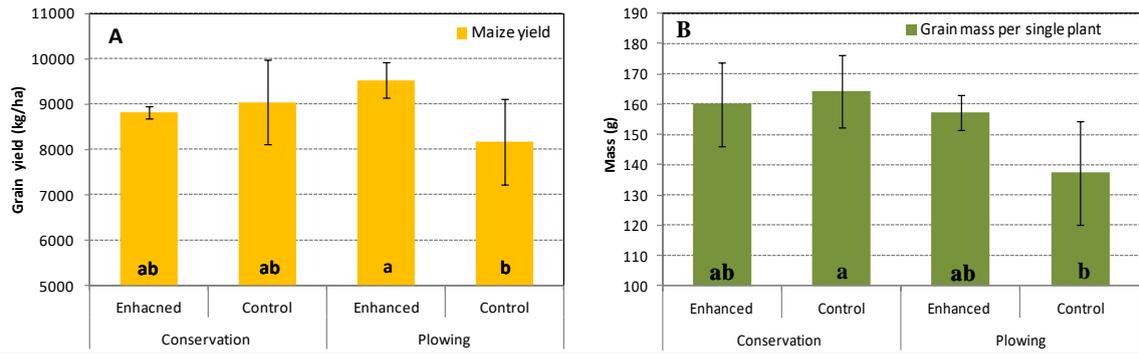


**Figure 2.** Number of maize plants per 7.5 m<sup>2</sup> under different tillage systems (conservation tillage and plowing) and treatments (enhanced and control)

The presence of earthworms did not influence germination success during the early growth stages, which may be explained by the fact that their positive effects on plant production are primarily expressed in later growth stages, through improved nutrient availability and soil structure rather than direct effects on seedling emergence (Van Groenigen *et al.*, 2014).

### Maize yields and grain mass per plant

Figures 3A and 3B show the maize yield under various tillage systems and treatments. Yields ranged from approximately 8.2 to 9.7 t/ha. The highest values were recorded in the plowing + earthworm (enhanced) treatment, followed by conservation tillage, while the lowest yields occurred in the plowing control plots. Although differences were statistically significant only within the plowing systems, this trend suggests that the combination of plowing and earthworm introduction could result in slightly higher yields, possibly due to improved aeration and better root development in loosened soil (Khan, 1996). In conservation tillage, yields were more uniform across treatments, indicating that soil structure and biological activity were already favorable for crop growth. The average grain mass per maize plant under different tillage systems and treatments showed a different trend compared with grain yield per hectare. The highest values were obtained under conservation tillage, ranging from 160 to 165 g per plant, while slightly lower values were recorded under plowing. The lowest grain mass was observed in the plowing control plots ( $\approx 140$  g per plant). The trend indicates that conservation tillage created more favorable conditions for grain filling per single plant, likely due to higher soil moisture retention, better biological activity, and a more stable structure that supports root function and nutrient uptake in the grain filling phase. In contrast, plowed soil, while better aerated, tends to lose moisture faster, which may limit nutrient assimilation during grain formation (Lal, 2020; Khan, 1996). Research in Central Europe shows that reducing tillage intensity through the use of conservation tillage improves the physical properties of the soil and increases the abundance of earthworms. These methods can also maintain or enhance crop yields compared to conventional plowing (Dekmati *et al.*, 2019).



**Figure 3.** Maize yield ( $t\ ha^{-1}$ ) under different tillage systems (A) and grain mass per maize plant (g) under different tillage systems (B)

Previous studies confirm that earthworm activity can enhance plant production by improving nutrient availability, soil aggregation, and water infiltration (Van Groenigen *et al.*, 2014; Bertrand *et al.*, 2015). However, these effects are often more pronounced over several seasons, as biological and structural changes in soil develop progressively.

## CONCLUSION

The results clearly demonstrate that conservation tillage provides a more favorable environment for soil biota and nutrient dynamics than conventional plowing. Earthworm abundance was consistently higher under conservation tillage, confirming that reduced disturbance, higher moisture retention, and surface residues support biological activity essential for soil regeneration. While plowing produced slightly higher plant counts, conservation tillage resulted in greater grain mass per plant and more balanced yields. Chemical analyses showed that conservation tillage with earthworms increased nitrate nitrogen, glomalin content, and available macronutrients (K, Mg), reflecting enhanced microbial activity and nutrient cycling. Overall, the combined findings highlight that conservation tillage integrated with biological components such as earthworms improves soil structure, fertility, and crop stability over time, whereas the short-term benefits of plowing are primarily physical. Long-term promotion of soil biological activity, especially through maintaining organic matter and supporting native earthworm populations, appears essential for sustainable soil productivity and resilient agroecosystems.

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## SELECTIVE UPTAKE OF MODEL VIRUSES BY WHEAT (*TRITICUM AESTIVUM* L.)

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*Many plant species important for human and animal consumption are capable of taking up and accumulating pathogenic bacteria, and to a lesser extent, viruses. While bacterial internalization is well-documented, the mechanisms and extent of viral uptake remain less well understood. In this study, the uptake of model viruses, i.e. bacteriophages Escherichia phage MS2 (Fiersviridae), Escherichia phage phiX174 (Microviridae), and Salmonella phage PRD1 (Tectiviridae) by three-week-old wheat (Triticum aestivum L.) was investigated. Wheat plants were grown in a nutrient solution under three experimental conditions: wheat with damaged roots grown in the presence of viruses; wheat with intact roots grown in the presence of viruses; and wheat grown in the absence of viruses (control). Detection of the viruses in extracts of wheat organs (leaves and stems) was performed 1, 3, and 7 days after inoculation of the nutrient solution with the viruses. Escherichia phage MS2 was detected in the stem of wheat with damaged roots one day after inoculation (187 PFU/g of plant tissue); in the leaf (320 PFU/g) and stem (240 PFU/g) of wheat with intact roots three days after inoculation; and in the stem (520 PFU/g) of wheat with intact roots seven days after inoculation. PhiX174 was not detected in wheat organs during the seven-day incubation period, likely due to its low stability in the nutrient solution, as its concentration decreased from the initial 10<sup>6</sup> PFU/mL to 10<sup>2</sup> PFU/mL after seven days. Despite its good stability in the nutrient solution, PRD1, which contains lipids, was not detected in wheat organs during the seven-day incubation. This may be due to the disruption of virion integrity in root tissues caused by a high osmotic pressure difference. Only Escherichia phage MS2 was internalized by wheat, highlighting that plant uptake of viruses is selective and influenced by viral stability and root condition.*

**Key words:** Wheat, MS2, phiX174, PRD1, nutrient solution

### INTRODUCTION

Many crop species important for human consumption are capable of taking up human pathogenic viruses and bacteria (Hirneisen et al., 2012). Plants can accumulate pathogenic bacteria and viruses in the root (Hirneisen et al., 2012), stem (Hirneisen et al., 2012), leaf (Hirneisen et al., 2012), and fruit (DiCaprio et al., 2015). The sources of pathogenic bacteria and viruses may include soil, nutrient solution, organic and mineral fertilizers, pesticides, or irrigation water (Hirneisen et al., 2012). The uptake of pathogenic microorganisms occurs through the root, and further transport to aboveground plant organs takes place via the xylem (Hirneisen et al., 2012).

Previous studies have demonstrated that certain plant species are able to take up viruses from their growth medium under both laboratory and field conditions. Plants such as bean (*Phaseolus vulgaris* L.) (Ward and Mahler, 1982), maize (*Zea mays* L.) (Ward and Mahler, 1982), green onion (*Allium fistulosum* × *Allium cepa*) (Chancellor et al., 2005; Hirneisen and Kniel 2012), romaine lettuce (*Lactuca sativa* O.) (Urbanucci et al., 2009; DiCaprio et al., 2012; Wei et al., 2011), spinach (*Spinacia oleracea* L.) (Hirneisen and Kniel, 2012), and strawberry (*Fragaria ananassa*) (DiCaprio et al., 2015) have been shown to absorb viruses under laboratory conditions, whereas tomato (*Solanum lycopersicum* L.) (Oron et al., 1995) can do so under field conditions as well (Table 1). Viruses can also be found in frozen berries and vegetables (Nasheri et al., 2019). The most significant human viruses that pose challenges to vegetable production particularly for crops consumed raw, are human norovirus and hepatitis A virus (Hirneisen and Kniel, 2012). One of the largest hepatitis A outbreaks occurred in 2003, when over 1,000 people became ill and four deaths were reported. The source of infection was green onion imported from Mexico (Hirneisen and Kniel, 2012). In addition to human and animal viruses,

bacteriophages are also used as model viruses in such studies (Ward and Mahler, 1982). Good model representatives for viruses of interest are the icosahedral bacteriophages MS2, phiX174, and PRD1. The MS2 bacteriophage (Riboviria, Orthornavirae, Lenaviricota, Leviviricetes, Norzivirales, Fiersviridae, Emesvirus, Emesvirus zinderi, Escherichia phage MS2) is an icosahedral virus with a single-stranded (ss) (+)RNA genome (Calanan et al., 2021). Its genome, consisting of 3,569 bases, encodes four proteins: the major capsid protein, the maturation protein, RNA polymerase, and the lysis protein (Fu and Li, 2015). The capsid has a triangulation number of 3 (T=3) and a diameter of 26 nm (Fu and Li, 2015).

The bacteriophage phiX174 (*Monodnaviria, Sangervirae, Phixviricota, Malgrandaviricetes, Petitvirales, Microviridae, Bullavirinae, Sinisheimervirus, Sinisheimervirus phix174, Escherichia phage phiX174*) is one of the most widely used model organisms in microbiology (Doore and Fane, 2016). It possesses an icosahedral capsid with a triangulation number of 1 (T=1) and a diameter of 27 nm (Doore and Fane, 2016). The viral genome is a single-stranded DNA (ssDNA) molecule of approximately 5.3 kilobases (Doore and Fane, 2016).

The PRD1 bacteriophage (*Varidnaviria, Bamfordvirae, Preplasmiviricota, Tectiliviricetes, Kalamavirales, Tectiviridae, Alphatectivirus, Alphatectivirus prd1, Salmonella phage PRD1*) is a virus with an icosahedral capsid symmetry enclosing a lipid vesicle beneath the capsid (Oksanen and Abrescia, 2019). The capsid has a triangulation number of 25 and a diameter of 66 nm, while the complex of protein spikes extends about 20 nm in length (Butcher et al. 2011). The viral genome is a linear double-stranded DNA (dsDNA) molecule with terminal proteins that play a key role in genome replication (Butcher et al., 2011). The genome length is approximately 15 kilobase pairs (Butcher et al., 2011).

The available data indicate that there is no information regarding the potential uptake and transport of viruses by wheat (*Triticum aestivum* L.), an extremely important cereal crop and a model species for other members of the *Poaceae* family that serve as feed for animals. The aim of this study was to assess the uptake and transport of virions of different structures and sizes into the aboveground organs of wheat (stem and leaf).

## MATERIAL AND METHODS

### Viruses

Escherichia phage MS2 (DSMZ 13767) used in this study was propagated in *Escherichia coli* (ATCC 15597). The bacterial host was grown on LBA (Luria–Bertani agar) medium (10 g/L tryptone (Torlak, Serbia); 5 g/L yeast extract (Biolife, Italy); 5 g/L sodium chloride (BetaHem, Serbia); and 15 g/L agar (BetaHem)) for 24 hours at 37 °C.

Escherichia phage phiX174 (DSMZ 4497) was propagated in *Escherichia coli* (DSMZ 13706). The bacterial culture was grown on LBA medium for 24 hours at 37 °C.

Salmonella phage PRD1 (DSMZ 19107) was propagated in *Salmonella enterica* serovar *Enteritidis* (ATCC 13076). The bacterial host was grown on LBA medium for 24 hours at 37 °C.

### Plant material

Wheat seeds (*Triticum aestivum* L.) purchased from a retail source were first allowed to germinate under sterile conditions, in the dark, at room temperature for seven days. The seedlings were then transferred to cultivation containers (Figure 1), with six plants per container. Wheat was grown in a nutrient solution (118.14 g/L Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O; 50.56 g/L KNO<sub>3</sub>; 27.2 g/L KH<sub>2</sub>PO<sub>4</sub>; 48.64 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O; 6.8 g/L Fe(CH<sub>3</sub>COO)<sub>2</sub>; 0.15 g/L ZnSO<sub>4</sub>·7H<sub>2</sub>O; 1.5 g/L H<sub>3</sub>BO<sub>3</sub>; 0.06 g/L CuSO<sub>4</sub>·5H<sub>2</sub>O; 7.5 g/L FeCl<sub>3</sub>·6H<sub>2</sub>O; 1.5 g/L MnCl<sub>2</sub>·4H<sub>2</sub>O; 0.02 g/L MoO<sub>3</sub>) with a total volume of 500 mL. The plants were grown in the containers for an additional two weeks at room temperature under natural light. The age of the wheat plants prior to virus inoculation into the nutrient solution was three weeks.

### Wheat exposure to viruses

A total of 72 wheat plants were used in the experiment, divided into three groups:

- a control group without virus exposure (2 containers with 6 plants each);
- a group grown in the presence of viruses (2 containers with 6 plants per virus); and
- a group with mechanically damaged roots (the roots were incised a few centimeters from the apical meristem) (2 containers with 6 plants per virus).

The viruses were added to the nutrient solutions of the second and third groups, each virus in a separate container. The concentration of viruses (MS2, phiX174, and PRD1) in the nutrient solution was 10<sup>6</sup> PFU/mL. Virus detection in plant organs was performed 1, 3, and 7 days after inoculation.

Detection of viruses in wheat plant organ extracts by plaque assay

Plant organs were carefully cut a few centimeters above the root. Leaves and stems were macerated separately. Maceration was performed manually in a mortar and pestle using PBS buffer (8 g/L NaCl; 1.44 g/L Na<sub>2</sub>HPO<sub>4</sub>; 0.2 g/L KCl; 0.24 g/L KH<sub>2</sub>PO<sub>4</sub>; pH = 7.4). Between 200 and 300 mg of plant tissue were sampled per extract, and this mass was macerated in approximately 1 mL of PBS buffer. The remaining plant debris and associated prokaryotic microorganisms were removed by centrifugation (Centrifuge 5424 R; Eppendorf, Germany) for 2 minutes at 16,000 × g. The supernatant was subsequently used for virus detection. The concentrations of MS2, phiX174, and PRD1 were expressed per gram of wheat leaf or stem tissue.

Virus detection in extracts of wheat organs grown in the presence of MS2, phiX174, or PRD1 was performed using three methods: Single Layer (SL), Double Layer (DL), and Double Layer–Spot (DLS).

In the SL method, 800 µL of plant organ extract and 100 µL of the corresponding bacterial suspension were added to 10 mL of molten λ-top agar (10 g/L tryptone; 5 g/L NaCl; 6.5 g/L agar).

In the DL method, 100 µL of extract and 100 µL of the corresponding host bacterial suspension were added to 4 mL of molten λ-top agar and poured over an LB agar plate.

In the DLS method, only 100 µL of the corresponding bacterial suspension was added to 4 mL of molten λ-top agar and poured over an LB agar plate. After the agar surface dried, five 20 µL drops of the corresponding extract were applied to the top layer. Incubation lasted 24 hours at 37 °C.

### **Virus stability in wheat extracts**

Viruses were added to extracts of wheat aboveground organs prepared as described above, and the stability of each virus was examined separately using plaque assays. For MS2, phiX174, and PRD1 changes in viral concentration were monitored. The initial concentration of each virus was 10<sup>6</sup> PFU/mL. Virus stability in the wheat extract was monitored over a period of 7 days. The extracts containing the viruses were incubated at room temperature.

### **Virus stability in nutrient solution**

The stability of each virus in nutrient solution was examined separately using plaque assays. Changes in viral concentration for MS2, phiX174, and PRD1 were monitored over time. The initial concentration of each virus was 10<sup>6</sup> PFU/mL. Virus stability in the nutrient solution was tracked over a period of 7 days, with incubation at room temperature.

### **Effect of wheat extract on viral plaque formation (Plaquing Efficiency)**

The effect of wheat extract on plaque formation was examined for MS2, phiX174, and PRD1. The test was performed separately for each virus. Viruses were inoculated into wheat extract and into SM buffer (5.8 g/L NaCl; 2 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O; 50 mL/L Tris-HCl) so that the final concentration in both inocula was identical, at 10<sup>2</sup> PFU/mL. One milliliter of the inoculum and 100 µL of the corresponding bacterial suspension were added to 10 mL of molten λ-top agar and poured into Petri dishes. Incubation was carried out for 24 hours at 37 °C. After incubation, the efficiency of plaque formation (EOP) was determined using the following formula:

$$\text{EOP} = \frac{\text{Number of plaques in SM buffer} \times 100\%}{\text{Number of PFU in wheat extract}}$$

### **Detection of viruses in wheat organ extracts by PCR**

Nucleic acids were isolated using the Viral RNA/DNA Purification Kit (cat. no. E3592; EurX Molecular Biology Products, Lithuania) according to the manufacturer's instructions. The RNA of the MS2 bacteriophage was first converted to cDNA using Reverse Transcriptase-PCR (RT-PCR) (Biometra TProfessional Thermocycler; Analytik Jena, Germany). The RT-PCR kit used was the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). RT-PCR was performed in a total volume of 20 µL, containing 2 µL of 10× RT buffer, 0.8 µL of 25× dNTP mix (100 mM), 2 µL of 10× random primers, 1 µL of reverse transcriptase, 4.2 µL of nuclease-free water, and 10 µL of RNA sample. The RT-PCR amplification process consisted of 40 cycles and the following steps: 10 min at 25 °C; 60 min at 37 °C; and 5 min at 85 °C.

The presence of viral DNA was subsequently assessed by real-time PCR (qPCR) using the CFX Connect Real-Time System (Bio-Rad, USA). The qPCR was performed using the PowerSYBR® Green PCR Master Mix (Thermo Fisher Scientific). Primers used in the qPCR (Invitrogen by Thermo Fisher Scientific) are listed in Table 1. qPCR was carried out in a total volume of 12 µL, comprising 6 µL Master Mix, 0.02 µL of each forward and reverse primer, 4.96 µL nuclease-free water, and 1 µL of DNA template. The amplification

program lasted 120 minutes and included the following steps: 2 min at 50 °C; 10 min at 95 °C; 39 cycles of 15 s at 95 °C and 1 min at 60 °C; followed by 5 min at 55 °C and a 5 min dissociation step at 95 °C.

**Table 1.** Forward and reverse primers of viruses MS2, phiX174 and PRD1, used for qPCR

Virus	Primers	Primer length
MS2	Forward-GCTCTGAGAGCGGCTCTATTG	21
	Reverse- CGTTATAGCGGACCGCGT	18
phiX174	Forward-ACTGCTGGCGGAAAATGAGA	20
	Reverse-TTGAACAGCATCGGACTCAG	20
PRD1	Forward- CCAGCAGGTTGAAACGCAAA	20
	Reverse- TGATAGCCCGCGTTACCTTC	20

## RESULTS AND DISCUSSION

Table 2 presents the presence and abundance of viruses, as well as the method of their detection, in specific wheat organs. The results indicate differences in virus detection depending on both the virus type and the plant organ analyzed.

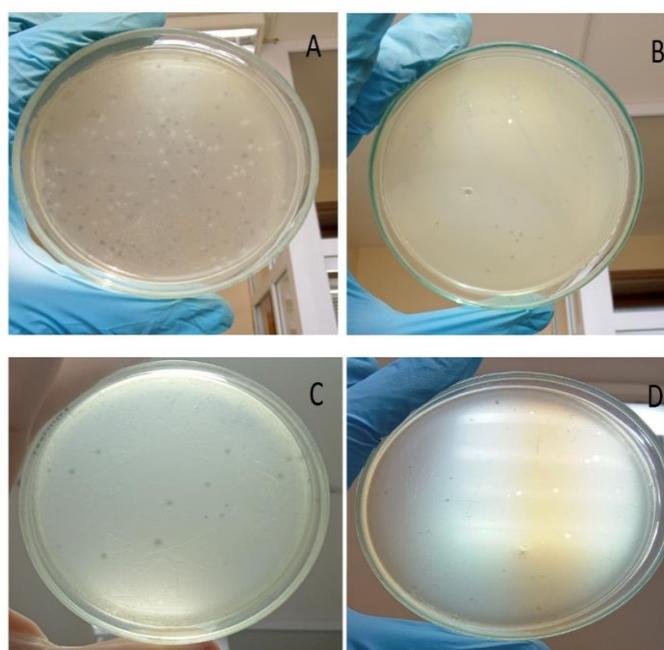
**Table 2.** Detection of viruses in wheat extracts using plaque assay and PCR methods.

Virus	Virus detection	Method of detection	Figure
MS2	Sc*, 1 DAI**, (187 PFU/g)	SL and PCR	Figure 1A
	L***, 3 DAI (320 PFU/g)	DL and PCR	Figure 1D
	S***, 3 DAI, (240 PFU/g)	DL	Figure 1B
	S, 7 DAI, (520 PFU/g)	DL and PCR	Figure 1C
phiX174			
PRD1			

\* Stem of wheat with cutted root

\*\* Days after inoculation

\*\*\* Stem and Leaf of uncutted root



**Figure 1.** Detection of MS2 in wheat organ extracts: (A) stem extract of plants with injured roots, 1 day after virus inoculation; (B) stem extract of plants with intact roots, 3 days after virus inoculation; (C) stem extract of plants with intact roots, 7 days after virus inoculation; (D) leaf extract of plants with intact roots, 3 days after virus inoculation.

In the present study, wheat plants were able to take up only MS2 from the nutrient solution in which they were grown. Previous studies have shown that the uptake of viruses from the substrate by plants depends on several factors, including the stability of the virus in the plant and the substrate, plant species and developmental stage, viral concentration, virus type, and environmental conditions during plant growth (Hirneisen et al., 2012).

The stability of viruses in the substrate is a crucial factor determining their uptake by plants. MS2 remained detectable in the nutrient solution; however, its titer decreased by 4 log units after 7 days of incubation in the plant extract (Table 3). Despite this reduction, the virus was successfully detected in wheat tissues using both plaque assay and PCR methods. In the study conducted by Ward and Mahler (1982), extracts of maize (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.), as well as the nutrient solution, showed no toxic effect on the Escherichia phage f2 (*Fiersviridae*; *Emesvirus*). For the f2 bacteriophage, the average efficiency of plaquing (EOP) was 89% in bean extract and 70% in maize extract, which is comparable to the 80% EOP obtained for MS2 in wheat extract in this study (Table 4). The similarity in EOP values between MS2 and f2 bacteriophages is expected, given the structural and genomic resemblance between these two viruses (van Duin and van den Wor 2005). The titer of Tulane virus (TV) (family *Caliciviridae*; *Recovirus A*) decreased from  $10^6$  PFU/mL to  $10^4$  PFU/mL in bell pepper (*Capsicum annuum*) puree after 7 days, indicating that TV is substantially more resistant to pepper extract than MS2 is to wheat extract (DiCaprio et al. 2015).

**Table 3.** Sustainability of virus in wheat extract and nutrient solution for period of 7 days.

Virus	Wheat extract	Nutrient solution
MS2	Quantity of virus decreased from $10^6$ to $10^2$ PFU/mL.	Quantity was unchanged.
phiX174	Quantity was unchanged.	Quantity of virus decreased from $10^6$ to $10^2$ PFU/mL.
PRD1	Quantity was unchanged.	Quantity of virus decreased from $10^6$ to $10^5$ PFU/mL.

**Table 4.** Test results: Effect of wheat extract on viral plaque formation (Plaquing Efficiency)

Virus	Plaquing Efficiency
MS2	80 %
phiX174	100 %
PRD1	72 %

The interior of a plant acts as a molecular sieve for viruses taken up through the roots, allowing only a certain portion of viral particles to pass to the next barrier (Ward and Mahler, 1982). This principle can also be applied to the results obtained for the detection of MS2 bacteriophage using the plaque assay. After three days, the virus accumulated in the stem (240 PFU/mL of nutrient solution) and leaves (320 PFU/mL of leaf extract) of wheat plants with intact roots. By day seven, the viral titer in the stem of these plants had more than doubled (520 PFU/mL). Interestingly, MS2 was no longer detected in the leaves of plants with intact roots on day seven, which deviates from the expected pattern. This result could be attributed to the limited stability of MS2 within the plant extract, that is, within the plant tissue itself.

The age of the plant is also an important factor influencing the uptake and accumulation of viruses. In our study, as well as in the work of Ward and Mahler (1982), wheat plants were three weeks old before the virus was introduced into the nutrient solution. Similarly, Wei et al. (2011) exposed 20-day-old lettuce plants to murine norovirus (MNV), which was later detected in the leaves. The minimum plant age before virus inoculation should be at least three weeks, and the plant should continue to grow with the virus until fruiting to enable potential detection of the virus in the fruit. The longer a plant grows in the presence of a virus, the more it is likely to accumulate in its aerial parts, assuming it can be taken up and transported (Ward and Mahler, 1982). In our study, the plants grew for seven days in the presence of viruses, which may not have been long enough for the uptake of bacteriophages phiX174 and PRD1. It is possible that the concentrations of phiX174 and PRD1 in the stems and leaves of wheat were too low for detection by plaque assay or qPCR.

The uptake of viruses from the substrate depends on their initial concentration in the medium (Ward and Mahler, 1982). In our experiment, the initial concentrations of MS2, phiX174, and PRD1 in the nutrient solution were  $10^6$  PFU/mL. The titer of phiX174 decreased by 4 log units over 7 days in the nutrient solution, which may explain why this virus was not detected in wheat even after 7 days of exposure. PhiX174 is the only virus in this study with a ssDNA genome, and salts in the nutrient solution could potentially have disrupted the capsid structure and/or DNA, contributing to its high sensitivity to the growth medium. Wei et al. (2011) observed that MNV achieved higher titers in lettuce when the initial concentration in the nutrient solution was  $5 \times 10^8$  RT-qPCR U/mL compared to inoculation over five consecutive days at  $5 \times 10^5$  RT-qPCR U/mL. Similarly, Ward and Mahler (1982) reported that when the initial concentration of f2 bacteriophage in the nutrient solution was  $10^{10}$  PFU/mL, a maximum of  $10^6$  PFU/g was detected in plant tissue.

Plants with injured roots require less time to take up viruses from the substrate compared to those with intact roots. In this study, wheat with damaged roots took up MS2 after just one day, a pattern similar to maize with injured roots, which acquired f2 bacteriophage within one day (Ward and Mahler, 1982).

It is important to note that phiX174 did not show a significant change in titer in plant extract over the 7-day period, and its efficiency of plaquing (EOP) was 100%. Based on these observations, it can be hypothesized that phiX174 could have been detected in wheat organs by both plaque assay and qPCR if it had been present in the tissues.

The virus type can influence plant uptake, and even different genotypes of the same virus species may show variable behavior (DiCaprio et al., 2012). This may explain why PRD1 was not taken up by wheat during the 7-day exposure, despite being relatively more stable in nutrient solution than phiX174. PRD1 is the only virus in this study that possesses a lipid vesicle beneath the capsid, which is necessary for penetration into the host cell (Oksanen and Abrescia, 2019). An osmotic gradient is required for vesicle transformation into a tube and for DNA transport into the bacterial cytoplasm (Santos-Perez et al., 2017). It is possible that the difference in osmotic pressure within plant tissue disrupts the structure of the viral vesicle and, consequently, its integrity. However, this does not fully explain why viral DNA was not detected in plant organs by qPCR.

The conditions under which plants are grown in the presence of a virus have a significant impact on viral uptake. In our study, all plants were grown in nutrient solution at room temperature. In other studies, plants grown in soil took up fewer viruses or did not acquire any at all compared to those grown in nutrient solution (Wei et al., 2011; Hirneisen and Kniel, 2012). Air humidity in the growth environment is also an important factor, as increased transpiration leads to more intensive water uptake from the substrate (Wei et al., 2011). In a preliminary experiment, wheat grown in a high-humidity room did not take up MS2, phiX174, or PRD1 (results not shown). When wheat was grown under lower humidity conditions, MS2 was successfully taken up, while all

other growth conditions remained unchanged. Similarly, Wei et al. (2011) reported that more lettuce samples tested positive for MNV at 70% relative humidity compared to 99% relative humidity.

The method used for virus detection in plant organs (plaque assay or qPCR) can influence the quality of virus detection. In our study, wheat organ extracts were only centrifuged at  $16,000 \times g$  for 2 minutes to remove large plant tissue debris and associated microorganisms present on or within the plant. The filtration step was omitted because filtration can further reduce viral titers in the sample (Gavrić and Knežević, 2022). The filtration step was also omitted in other studies using plaque assay for virus detection in plants (Oron et al., 1994; DiCaprio et al., 2012; DiCaprio et al., 2015). Oron et al. (1994) added a specific cocktail of fungicides and bactericides to prevent the growth of contaminating microorganisms, while DiCaprio et al. (2015) immersed plant samples in a 2000 ppm chlorine solution for 5 minutes to inactivate potential surface viruses. An additional sample purification step is necessary because associated microorganisms can interfere with plaque formation.

## CONCLUSION

Since no studies have addressed the uptake of bacteriophages by wheat, we aimed to investigate this phenomenon by assessing the uptake of model viruses: MS2, phiX174, and PRD1. Three-week-old wheat plants were able to take up MS2 from the nutrient solution, with the virus detected as early as one day after inoculation. In contrast, neither phiX174 nor PRD1 were detected in wheat extracts. The absence of phiX174 may be attributed to its limited stability in the nutrient solution. Although PRD1 proved relatively stable in the nutrient solution, its absence in plant organs may be due to disruption of viral integrity within the root tissue caused by high osmotic pressure.

These results demonstrate that wheat in early developmental stages is capable of virus uptake from the nutrient solution, and that plants with injured roots acquire viruses more readily. Future experiments should investigate the ability of wheat to take up viruses during the fruiting stage and the likelihood of virus presence in grain or straw. Additionally, experiments under field conditions, with wheat grown in soil, should be conducted, as this represents the primary method of wheat production.

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# BETALAIN CONTENT OF PEEL, LEAVES, PULP AND JUICE OF TWO BEETROOT (BETA VULGARIS VAR.CONDITIVA) CULTIVARS

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*The present study focused on the evaluation of betalain pigments in different parts of beetroot, as well as juice, using two different cultivars (Cylindra and Detroit). The total betalain content of aqueous extracts revealed significant differences between cultivars and plant organs, highlighting the superior potential of certain varieties for nutritional and industrial applications. These findings may suggest that betalain-rich cultivars, particularly Cylindra, may represent valuable sources for functional foods and natural colorants, supporting their broader use in nutrition and food industry applications.*

**Key words:** *Beta vulgaris var. conditiva, betalains, red beetroot, cultivars, spectrophotometry*

## INTRODUCTION

Red beetroot (*Beta vulgaris var. conditiva*), Amaranthaceae family, has long been valued both as a dietary component and for its health-promoting properties. It is particularly rich in bioactive compounds, including pigments, vitamins, minerals, and polyphenolic substances, which contribute to the prevention and management of various diseases. Among these compounds, betalains, the water-soluble pigments responsible for beetroot coloration, have attracted growing scientific attention due to their high antioxidant capacity and various therapeutic effects, such as anti-inflammatory, anti-diuretic, anti-hypertensive, hepatoprotective, anti-microbial and hypoglycemic effect (Bashir et al., 2024). Betalains are classified into two groups: betacyanins (red–violet) and betaxanthins (yellow–orange) (Sadowska-Bartosz & Bartosz, 2021). Their concentration, however, is not constant and can vary considerably across cultivars, being influenced by many factors. Temperature during the growing season is considered the primary determinant of pigment synthesis in beetroot tissues. Moreover, other environmental conditions — including soil fertility, moisture availability, irrigation practices, and the timing of harvest — can also contribute to variations in pigment content (Kujala et al., 2002). Rubóczki et al. (2015) found significant differences between cultivars in morphological and quality parameters.

The present study was designed to evaluate and compare the betalain content in two *Beta vulgaris var. conditiva* cultivars, “Cylindra” and “Detroit” by investigating aqueous extracts obtained from different plant parts (peel, leaves, pulp) and juice. Emphasizing the differences between these cultivars is important for identifying varieties with superior pigment concentrations, which may be more suitable for nutritional, therapeutic, and industrial applications.

## MATERIAL AND METHODS

### Plant material

The plant material comprised two beetroot varieties, *Beta vulgaris var. conditiva* Cylindra and *Beta vulgaris var. conditiva* Detroit, harvested from Hălmeag (Brașov county, Romania) in September 2024. After harvesting, the roots were thoroughly cleaned of remaining soil residues by washing, and the plants were left to air-dry at room temperature. For each cultivar, three plant parts (peel, pulp, and leaves) were separated, chopped with a blender, and transferred to containers labeled with the harvest date, plant part, and cultivar. Fresh juice was also obtained and treated as a separate sample type. The beetroot samples were stored at -20°C until analysis.

## Preparation of extracts

2 g of plant material was soaked in 40 ml of distilled water. Regarding the beetroot juice, 20 ml of distilled water were added to 1 g of juice. Extraction was performed under magnetic stirring (extraction time, 30 min; shaking rate, 3500 rpm; room temperature) using the apparatus RO-10 (IKA, Germany). The mixtures were further centrifuged at 8000 rpm for 10 min, using the refrigerated centrifuge (Universal 320-R, Hettich, Germany).

## Determination of betalain content

The betalain content was determined using the spectrophotometer Specord 200 Plus UV-Vis (Analytik Jena, Germany), at 538 nm and 476 nm, according to the method described by Pérez-Loredo et al. (2016).

The total betalain content, B ( $\mu\text{g/g}$ ), represented by the sum of betacyanins (Bc) and betaxanthins (Bx), was calculated according to the following equation.

$$B = \frac{A \times FD \times MW \times V}{\epsilon \times P \times L}$$

where:

A = absorbance at 538 nm for Bc and at 476 nm for Bx

FD = dilution factor

MW = molecular weight (Bc 550 g/mol and Bx 308 g/mol)

V = extract volume (mL)

$\epsilon$  = molar extinction coefficient for Bc = 60,000 L/mol\*cm, and Bx = 48,000 L/mol\*cm

P = sample weight (g)

L = cuvette path length (1 cm)

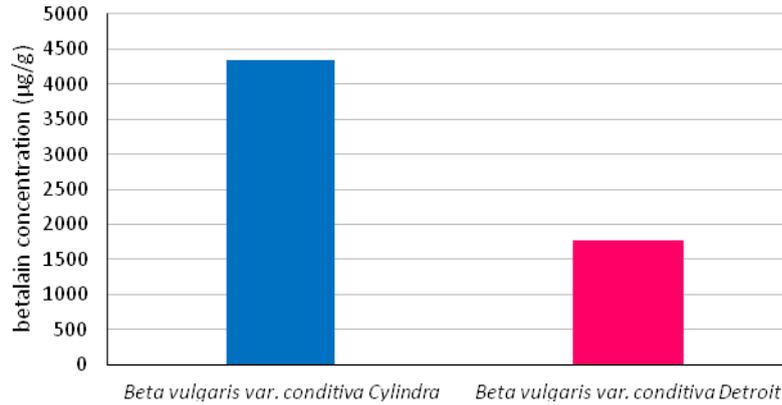
## RESULTS AND DISCUSSION

The moisture content of the investigated beetroot samples is presented in Table 1.

**Table 1.** Moisture content of beetroot extracts

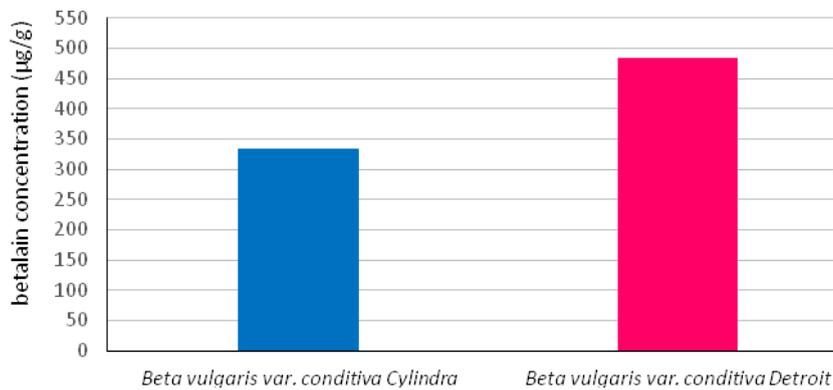
Sample no.	Samples description	Moisture (%)
1.	<i>Beta vulgaris</i> var. <i>conditiva</i> <i>Cylindra</i> peel	83.078
2.	<i>Beta vulgaris</i> var. <i>conditiva</i> <i>Cylindra</i> leaf	85.831
3.	<i>Beta vulgaris</i> var. <i>conditiva</i> <i>Cylindra</i> pulp	86.572
4.	<i>Beta vulgaris</i> var. <i>conditiva</i> <i>Cylindra</i> juice	—
5.	<i>Beta vulgaris</i> var. <i>conditiva</i> <i>Detroit</i> peel	87.219
6.	<i>Beta vulgaris</i> var. <i>conditiva</i> <i>Detroit</i> leaf	89.168
7.	<i>Beta vulgaris</i> var. <i>conditiva</i> <i>Detroit</i> pulp	89.433
8.	<i>Beta vulgaris</i> var. <i>conditiva</i> <i>Detroit</i> juice	—

The results on the betalain content in peel of the two beetroot cultivars are presented in Figure 1. The total betalain concentration was approximately 2.5 times higher (4345.641  $\mu\text{g/g}$ ) in the peel of the *Beta vulgaris* var. *conditiva* *Cylindra* cultivar, compared to that of the *Beta vulgaris* var. *conditiva* *Detroit* cultivar (1774.45  $\mu\text{g/g}$ ).



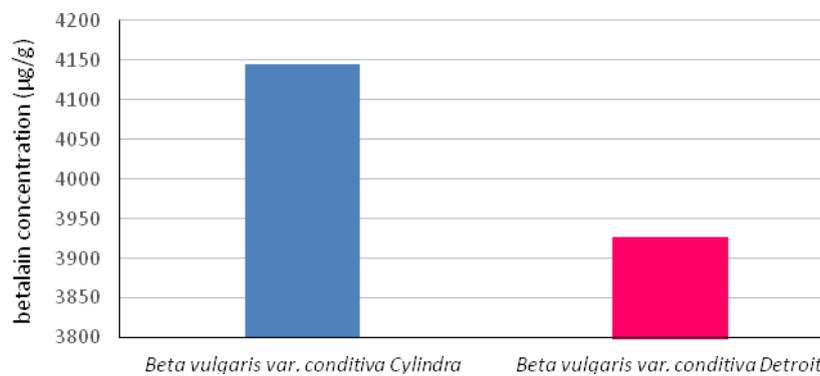
**Figure 1.** Total betalain content in red beetroot peel of selected cultivars

Figure 2 shows the concentration of betalains in the leaves. In the cultivar *Beta vulgaris var. conditiva Detroit*, the total betalain concentration in leaves was higher (484.721 µg/g) compared to that of the leaves of the *Beta vulgaris var. conditiva Cylindra* cultivar (333.31 µg/g). Thus, the leaves of *Detroit* contain approximately 1.45 times more betalains than those of *Cylindra*.



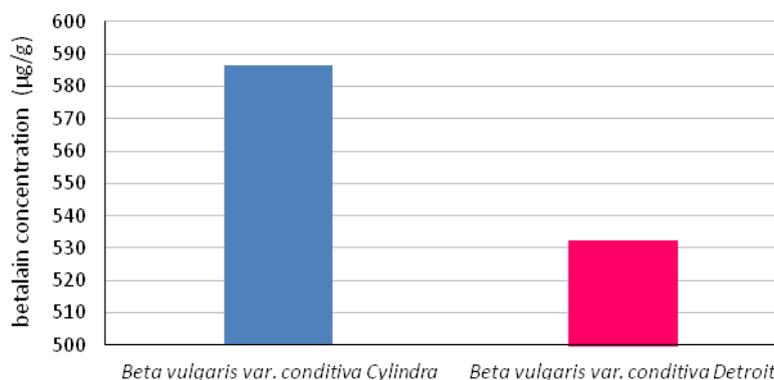
**Figure 2.** Total betalain content in red beetroot leaves of selected cultivars

Similar to the peel, *Beta vulgaris var. conditiva Cylindra* exhibits a higher betalain pigment concentration in the pulp (4144.729 µg/g) compared to *Beta vulgaris var. conditiva Detroit* (3926.518 µg/g), as shown in Figure 3.



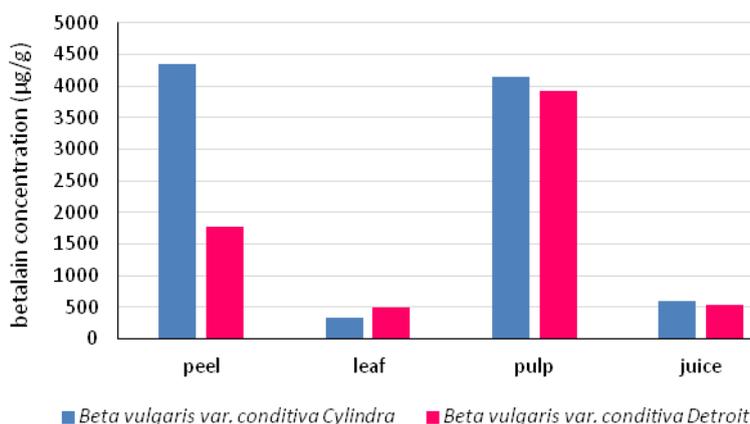
**Figure 3.** Total betalain content in red beetroot pulp of selected cultivars

As shown in Figure 4, the red beetroot juice obtained from *Beta vulgaris var. conditiva Cylindra*, contains higher amounts of betalains (586.685 µg/g) than those in juice obtained from *Beta vulgaris var. conditiva Detroit* (532.172 µg/g).



**Figure 4.** Total betalain content in red beetroot juice of selected cultivars

Figure 5 presents a comparative view on the total betalain content in different parts of beetroot, and juice, in the investigated cultivars.



**Figure 5.** Comparative presentation of the betalain content in different parts of the two investigated red beetroot cultivars.

The highest betalain content was found in peel of both cultivars. This finding is similar to other reported studies on by-products and root fractions, which indicated high betalain levels in the peel or peel powder (approximately 0.3–1.4 mg/g DW, influenced by solvent choice and extraction conditions) (Lazăr et al., 2021). The leaves showed the lowest levels of betalain pigments. This finding is consistent with recent profiling studies, which report total betaxanthin contents in leaves ranging from 3.3 to 20.4 mg/kg FW (including cv. Cylindra) (Spórna-Kucab et al., 2023). The values obtained for the pulp fall within the broad range reported for the beetroot: averages around ~120 mg/100 g FW for total betalains and ~20–210 mg/100 g FW for betanin alone, depending on cultivar (Milton-Laskibar et al., 2021; Thomsen et al., 2023). Regarding the fresh juice, typical reported values ranges ~0.8–1.3 g/L total betalains, ~893 mg/L betacyanins.

The differences on total betalain content in beetroot reflect the influence of variety, harvest stage, and applied processing, as described by other authors (Wruss et al., 2015; Trych et al., 2022). A wide genotype/environmental effects across red beetroots (~200–2100 mg/kg FW) and pigment profiles dominated by betacyanins (~75–95% of total) was previously reported (Sadowska-Bartosz & Bartosz, 2021).

## CONCLUSION

*Beta vulgaris* var. *conditiva* *Cylindra* exhibits the highest betalain concentration among the two investigated cultivars. The results indicate that, the peel, pulp, and juice of *Cylindra* contain significantly higher amounts of pigments among the two cultivars, whereas *Detroit*, has a higher betalain concentration only when the leaves are compared. The highest betalain concentration was recorded in the peel (4345.641 µg/g), followed by the pulp, with a slightly lower value (4144.729 µg/g). The concentration in the diluted juice was significantly lower (586.685 µg/g), while the lowest value was observed in the leaves (333.31 µg/g).

Regarding the *Beta vulgaris* var. *conditiva* *Detroit*, the betalain distribution showed a different pattern compared to the *Cylindra* cultivar. The highest concentration was recorded in the pulp (3926.518 µg/g), in contrast to the peel, which contained a considerably lower amount (1774.45 µg/g). The diluted juice contained 532.172 µg/g, while the leaves contained the lowest betalain content (484.721 µg/g), similar to the trend observed in the *Cylindra* cultivar.

Overall, the concentration of betalains in the investigated beetroot cultivars shows an organ-specific pattern (peel>pulp> leaves).

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# THE POTENTIAL OF *TRICHODERMA* SPP. FOR BIOLOGICAL CONTROL

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*This study evaluated the antimicrobial activity of four Trichoderma spp. isolates against Alternaria alternata using dual culture, diffusion, and volatile assays. All isolates inhibited pathogen growth, with Trichoderma sp. II showing the strongest and sp. IV the weakest effect, confirming the biocontrol potential of Trichoderma spp.*

**Key words:** *Trichoderma, Alternaria alternata, antifungal activity, biocontrol potential*

## INTRODUCTION

Fungi of the genus *Trichoderma* are widely distributed microorganisms that primarily inhabit soil and the rhizosphere of plants (Valeria et al., 2011). Because of their strong metabolic activity, they produce a variety of biologically active compounds including enzymes, volatile organic compounds, and antibiotics with antimicrobial properties. These metabolites inhibit the growth of numerous pathogenic fungi and bacteria through different mechanisms of action (Saba et al., 2012).

For these reasons, *Trichoderma* species have become an important focus of research as potential biological control agents. Their application offers an environmentally friendly alternative to synthetic pesticides and supports sustainable agricultural practices (Cordero et al., 2024). Moreover, several studies have shown that *Trichoderma* can stimulate plant growth by improving nutrient uptake and inducing plant defense responses (Saravanakumar and Wang, 2020).

The goal of this study was to investigate the antimicrobial activity of isolated *Trichoderma* strains against selected test microorganism *Alternaria alternata* using different experimental methods. The effects of both fungal mycelium and obtained extracts were analyzed.

## MATERIAL AND METHODS

Four isolates of *Trichoderma* spp. were obtained from substrates used in organic mushroom production and identified based on their morphological characteristics. The phytopathogenic fungus *Alternaria alternata* IHEM 3320 served as the test microorganism. All cultures were maintained in the BioSense Culture Collection of Fungi (BSCCF) at Institute BioSense, Novi Sad, Serbia.

Cultivation was performed on PDA, MEA, SL, and PDB media sterilized at 121 °C for 20 min.

The antimicrobial activity of *Trichoderma* isolates against *A. alternata* was evaluated using dual culture, diffusion, and volatile metabolite assays. Ethanolic extracts and culture supernatants were also evaluated for antifungal activity.

In the dual culture assay, 6×6 mm mycelial plugs of *Trichoderma* spp. and *Alternaria alternata* were placed on opposite sides of SL agar plates and incubated at 28 °C for 120 h. Growth inhibition was expressed as a percentage compared to the control.

Culture supernatants were obtained by incubating isolates in PDB medium at 28 °C and 150 rpm for 168 h, followed by centrifugation. Ethanolic extracts were prepared from 2.5 g of fungal biomass suspended in 80% ethanol, shaken for 24 h, centrifuged, and concentrated by rotary evaporation.

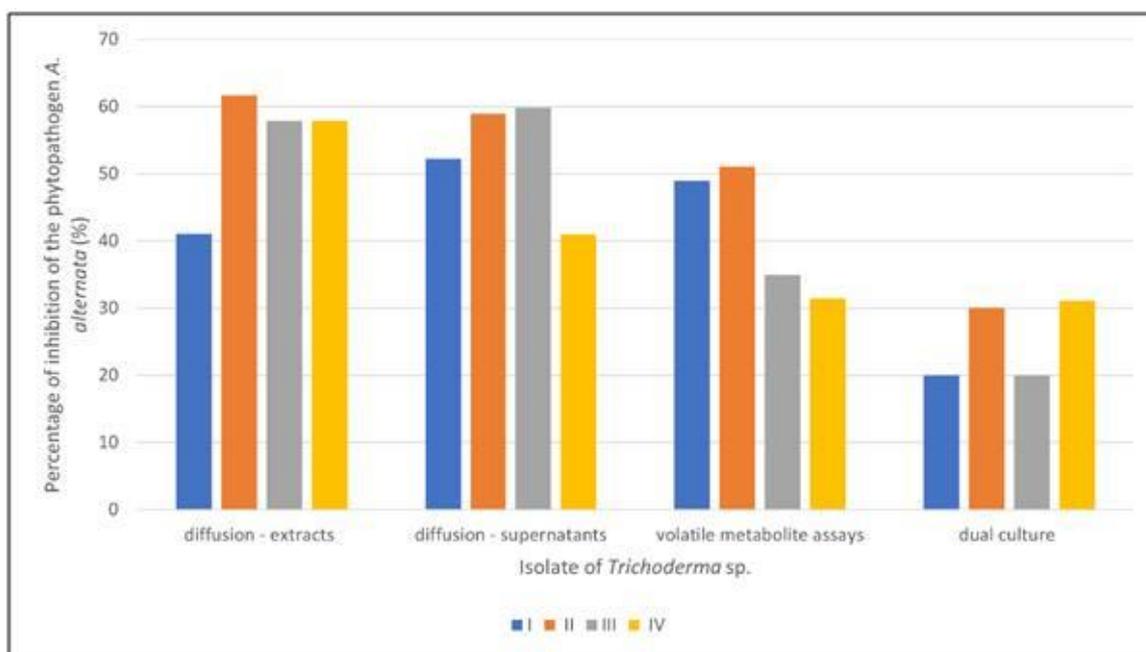
For diffusion assays, 200 µl of each extract or supernatant was incorporated into or spread onto PDA plates prior to pathogen inoculation. The volatile metabolite assay was performed by culturing *Trichoderma* and *A. alternata* on separate halves of sealed PDA plates and incubating at 28 °C for 96–120 h.

All experiments were conducted in triplicate. Data were expressed as mean ± SD and analyzed using one-way ANOVA followed by Tukey's test ( $p < 0.01$ ).

## RESULTS AND DISCUSSION

Based on the results obtained from all four applied methods (Figure 1), it can be concluded that all *Trichoderma* isolates exhibited antifungal potential against *Alternaria alternata*. However the degree of inhibition varied depending on the type of test. In all tests, the growth of the phytopathogen was reduced compared to the control, confirming the antagonistic interaction between *Trichoderma* spp. and *A. alternata*. Among the tested isolates, *Trichoderma* sp. II consistently showed the highest inhibitory activity, achieving the strongest suppression both through diffusible metabolites (extracts and supernatants) and volatile compounds. The highest inhibition rate was recorded in the extract assay (61.66%), followed by the supernatant (59%) and volatile assay (51.04%), indicating that soluble metabolites play a major role in pathogen inhibition. On the other side, *Trichoderma* sp. IV exhibited the weakest effect in most methods, suggesting possible differences in the metabolic profile and production of bioactive compounds among isolates.

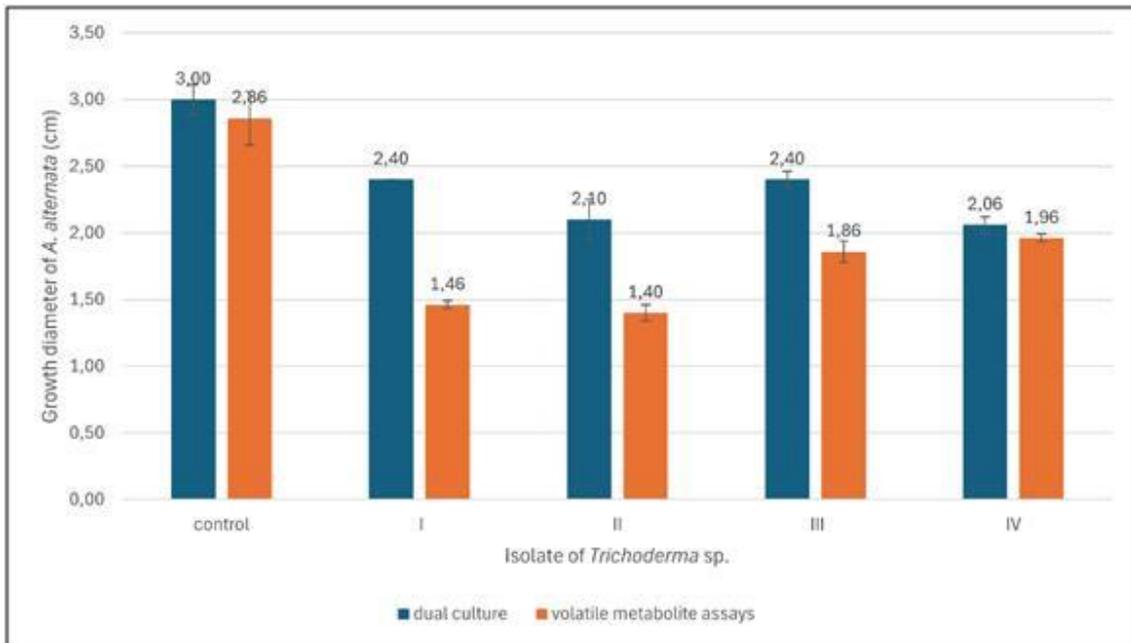
Overall, the results indicate that *Trichoderma* spp. possess multiple mechanisms of antagonism, including the production of diffusible and volatile metabolites that effectively limit the growth of *A. alternata*. The consistency of inhibitory effects across different assay types highlights the strong biocontrol potential of certain *Trichoderma* isolates, particularly *Trichoderma* sp. II, which could be further investigated for use as an effective biological control agent.



**Figure 1.** Percentage of inhibition of *A. alternata* by four *Trichoderma* sp. isolates obtained using different assay methods

Mean colony diameters and standard deviations of the phytopathogenic fungus in the presence of different *Trichoderma* spp. isolates and the control are presented in Figure 2, showing comparison between dual culture and VOC assays. In the dual culture assay, the smallest colony diameter of the test organism was observed with *Trichoderma* sp. IV (2.06 cm), corresponding to the highest inhibition percentage (Figure 1). In contrast, isolates which showed the lowest inhibition (*Trichoderma* sp. I and *Trichoderma* sp. II) allowed the largest growth of *A. alternata* (2.40 cm).

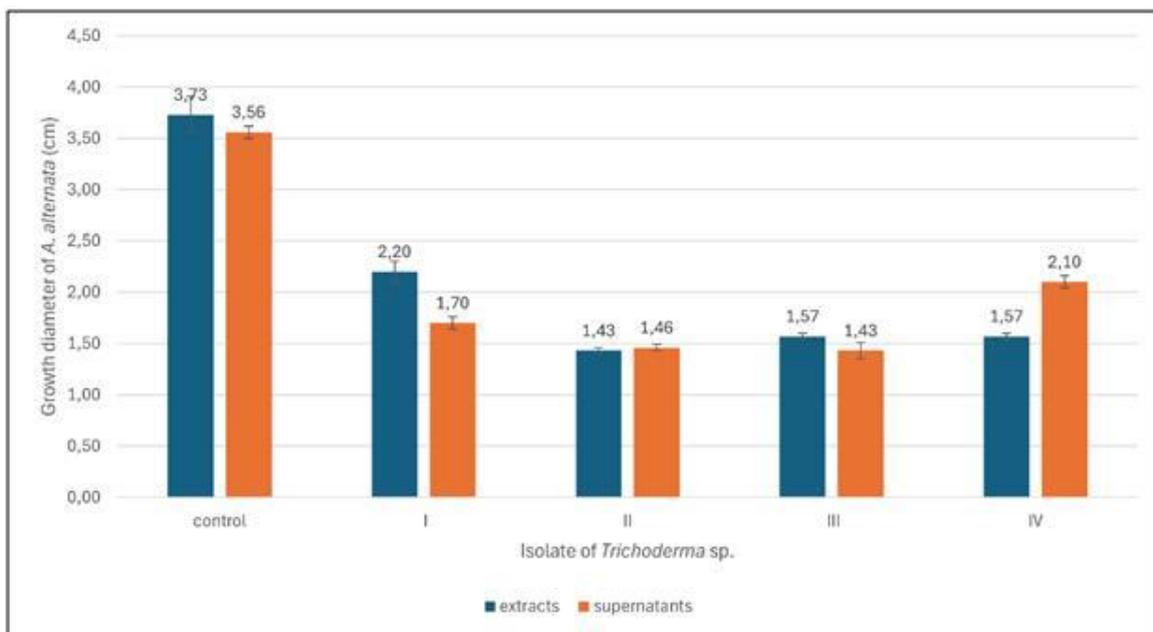
In the VOC assay, the pattern of inhibition differed slightly: the strongest growth reduction was observed with *Trichoderma* sp. II (1.40 cm), while the least inhibition occurred with *Trichoderma* sp. IV (1.96 cm). These results indicate that different isolates may exert varying inhibitory effects depending on whether direct contact or volatile-mediated interactions occur. Overall, all tested isolates slowed the growth of the phytopathogen to some extent, though the inhibition percentages were generally lower than those reported in other studies using similar methods.



**Figure 2.** Comparison of antifungal effects of *Trichoderma* sp. isolates on *A. alternata* growth in dual culture and VOC assays

Figure 3 presents the comparison of *A. alternata* colony diameters on plates with extracts and supernatants of *Trichoderma* sp. isolates. Growth inhibition was generally similar between treatments, except for *Trichoderma* sp. IV, where the supernatant showed a larger colony diameter and the lowest inhibition rate, suggesting weaker antifungal activity. On the other hand, *Trichoderma* sp. II and *Trichoderma* sp. III exhibited the strongest inhibition in both tests.

Previous studies reported comparable results: ethyl acetate extracts of *Trichoderma harzianum* contained several antifungal metabolites, with harzianopyridone being the most active (Ahluwalia et al., 2015). Yassin *et al.* also found that ethanol extracts of *Trichoderma harzianum* and *Trichoderma viride* inhibited several phytopathogens, with major active compounds including 6-pentyl- $\alpha$ -pyrone, palmitic acid, cyclooctanol, propylbenzene, 2-phenylethanol, and acetic acid (Yassin et. al., 2022).



**Figure 3.** Comparison of the antifungal activity of *Trichoderma* spp. supernatants and extracts against the pathogen *A. alternata* using the diffusion method

Based on the data presented in Table 1, the colony diameters of all tested *Trichoderma* sp. isolates showed statistically significant differences ( $p < 0.01$ ) compared to the control. However, the mutual comparison of the isolates revealed variations in statistical significance depending on the applied treatment. In the case of volatile compounds and diffusible metabolites, certain isolates, such as *Trichoderma* sp. IV, exhibited a statistically significant difference ( $p < 0.01$  or  $p < 0.05$ ) compared to the others, often corresponding to a lower inhibition percentage. On the other hand, in treatments involving extracts or supernatants, *Trichoderma* sp. I generally showed obvious inhibitory activity in relation to the remaining isolates. Overall, the results indicate that all *Trichoderma* sp. isolates effectively inhibited the growth of *A. alternata*, with notable differences in the intensity of inhibition depending on the isolate and the type of treatment applied.

**Table 1.** Mean values and standard deviations of the colony diameter of the test organism obtained using different applied methods

Isolate	Growth diameter of <i>A. alternata</i> (cm)			
	dual culture	VOC assay	diffusion extracts	diffusion supernatants
Control	3,00 ± 0,11 <sup>a</sup>	2,86 ± 0,20 <sup>a</sup>	3,73 ± 0,18 <sup>a</sup>	3,56 ± 0,06 <sup>a</sup>
<i>Trichoderma</i> sp. I	2,40 ± 0,00 <sup>b</sup>	1,46 ± 0,03 <sup>bc</sup>	2,20 ± 0,03 <sup>b</sup>	1,70 ± 0,06 <sup>b</sup>
<i>Trichoderma</i> sp. II	2,10 ± 0,15 <sup>b</sup>	1,40 ± 0,06 <sup>bc</sup>	1,43 ± 0,03 <sup>c</sup>	1,46 ± 0,03 <sup>b</sup>
<i>Trichoderma</i> sp. III	2,40 ± 0,06 <sup>b</sup>	1,86 ± 0,08 <sup>bc</sup>	1,57 ± 0,03 <sup>c</sup>	1,43 ± 0,08 <sup>b</sup>
<i>Trichoderma</i> sp. IV	2,06 ± 0,06 <sup>b</sup>	1,96 ± 0,03 <sup>c</sup>	1,57 ± 0,03 <sup>c</sup>	2,10 ± 0,06 <sup>c</sup>

a, b, c - different letters indicate statistically significant differences between isolates.

## CONCLUSION

The results of this study demonstrate that all tested *Trichoderma* isolates possess antimicrobial activity against the selected phytopathogen, but with differences in effectiveness. *Trichoderma* sp. II exhibited the strongest inhibition indicating the highest biocontrol potential. *Trichoderma* sp. IV showed the lowest inhibitory effect, while *Trichoderma* sp. I displayed moderate activity. Overall, all isolates could reduce pathogen growth in all applied methods. These results support the already known biocontrol potential of *Trichoderma* species and suggest their applicability in plant protection. Future research could focus on identifying the specific bioactive compounds responsible for this activity and testing additional phytopathogens.

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# MORPHOLOGICAL CHANGES IN RASPBERRY CANES INDUCED BY INFESTATION OF THE RASPBERRY GALL MIDGE (*LASIOPTERA RUBI*: DIPTERA, CECIDOMYIIDAE)

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*In a Fertodizamatos raspberry plantings in the Požega region, Lasioptera rubi infestation caused notable morphological changes in canes. Analysis of 105 collected infested canes from a 600 m<sup>2</sup> plantings revealed an average of 2.24 galls per canes, indicating a strong pest preference for this cultivar. High gall densities led to canes decay or breakage in the following growing season, resulting in plantings decline. These findings highlight the cultivar's high susceptibility and the significant impact of L. rubi on cultivar cane morphology.*

**Key words:** raspberry, gall, *Lasioptera rubi*, *Fertodi zamatos*

## INTRODUCTION

Pest organisms represent one of the most significant limiting factors in raspberry production, potentially reducing yields by up to 80% during years of severe outbreaks (Stamenković et al., 1996). To date, more than 80 pest species have been recorded on raspberry, including approximately 25 insect species and 5 mite species (Tanović et al., 2010; Tanasković and Milenković, 2012). Of economic importance in Serbia are the raspberry aphid (*Aphis idaei*, van der Goot, 1912), raspberry beetle (*Byturus tomentosus*, De Geer, 1774), raspberry blossom weevil (*Anthonomus rubi*, Herbst, 1975), as well as *Resseliella* and *Lasioptera* midges (Milenković and Tanasković, 2011).

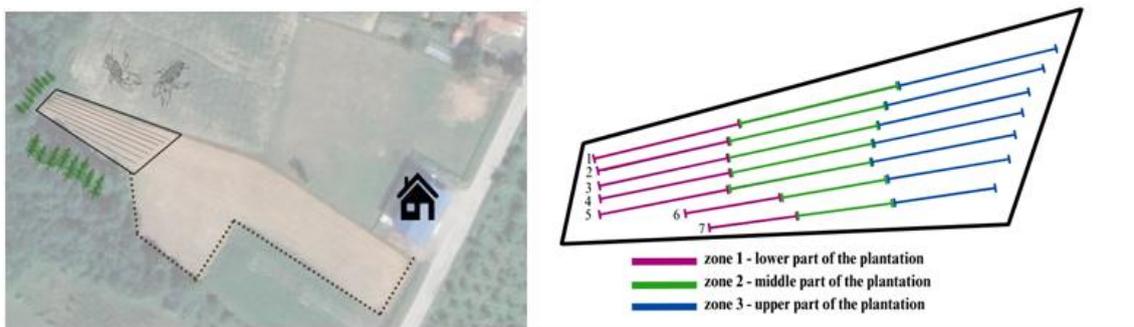
The species *Lasioptera rubi* Schrank (Diptera, Cecidomyiidae) is widely distributed and long time present in all raspberry-producing regions in Serbia (Simova Tošić, 1970; Tanasković and Milenković, 2012). According to the EPPO scheme for planting material production (1993), it is classified among pests requiring strict monitoring measures in raspberry plantings. This species induces gall formation on canes (primocanes and floricanes). Galls presence weakens the plant by disrupting the vascular system and causes cane decay and/or breakage.

The aim of this study was to determine the abundance and morphological characteristics of galls on canes of the *Fertodi zamatos* cultivar in a raspberry planting in the Požega region.

## MATERIAL AND METHODS

The study was conducted at the Trnava site (43°57'03"N, 19°54'14"E), Požega municipality, at an altitude of 521 m a.s.l. The plot is located in the immediate vicinity of the main road and a family home, bordered by forest on two sides, a maize field on the third side, and a fallow field where no crops were cultivated at the time of sampling (Fig. 1, left).

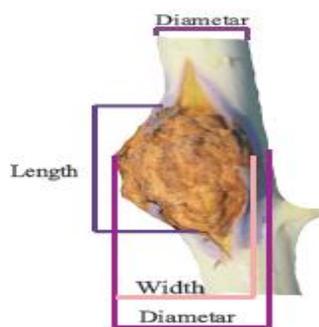
Sample collection of canes showing visible symptoms was carried out in September 2022 in a *Fertodi zamatos* raspberry plantings established in 2021. Samples were taken from all rows in the experimental field. Each row was divided into three zones (Fig. 1, right). Zone 1, marked in purple, represents the lower part of the plot adjacent to the forest. Zone 2, marked in green, corresponds to the middle part of the plantings, while Zone 3, marked in blue, represents the upper part of the plantings. Only canes with visible gall symptoms were collected. From each row zone (21 zones in total), five symptomatic canes were collected randomly. Sampling resulted in a total of 105 collected canes with galls.



**Figure 1.** Spatial layout of the experimental field and sampling row zoning

The Fertodi zamatos cultivar is of Hungarian origin and was selected in 1971. It is characterized by strong and vigorous canes, tolerance to high soil moisture, and registered resistance to certain pests (Vetek at al., 2008). The cultivar produces medium-large, light-red fruits. It's suitable for both - fresh consumption and processing. In the municipality of Požega, the number of plantings of this cultivar has increased rapidly over the past five years.

Collected samples were brought to the Entomology Laboratory at the Faculty of Agronomy in Čačak for analysis and measurements. The number of galls per canes was counted, and the diameters of the canes, as well as the length, width, and diameter of the galls, were measured. Measurements were performed using a sliding digital caliper ("Inox" calliper, precision  $\pm 0.05$  mm).



**Figure 2.** Positions for gall parameter determination

The obtained data were processed using Microsoft Excel 2007, where tables and graphs of mean values per row and zone were prepared.

## RESULTS AND DISSCUSION

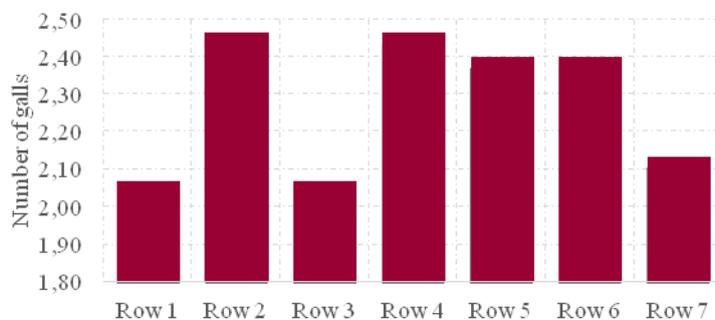
An examination of Fertodicanes in the field provided data on the number of galls per row (Table 1). The highest number of galls on a single cane (7) was recorded in the second row within the plot zone adjacent to the forest. Based on the assessments and recorded gall densities, it can be concluded that the highest number of galls per canes, and consequently the most intense infestation, occurred in the plot zone next to the forest, while the other two zones exhibited similar numbers of galls per canes (Table 1).

**Table 1.** Number of galls by rows and sampling zones

zone	Row						
	I	II	III	IV	V	VI	VII
1. cane forest	4	7	2	1	2	2	2
2. cane forest	2	3	2	2	2	6	5
3. cane forest	2	2	2	5	5	2	1
4. cane forest	2	4	2	2	4	4	1
5. cane forest	3	5	2	3	1	2	2
Average zone	2.60	4.20	2.00	2.60	2.80	3.20	2.20

1. cane middle	1	2	3	2	3	4	2
2. cane middle	2	2	1	2	5	2	4
3. cane middle	1	1	1	1	2	3	3
4. cane middle	2	1	2	4	1	1	1
5. cane middle	3	1	3	1	2	1	1
Average zone	1.80	1.40	2.00	2.00	2.60	2.20	2.20
1. cane upper	1	3	2	5	4	4	2
2. cane upper	3	1	5	3	1	2	4
3. cane upper	3	1	1	1	1	1	1
4. cane upper	1	3	1	3	2	1	1
5. cane upper	1	1	2	2	1	1	2
Average zone	1.80	1.80	2.20	2.80	1.80	1.80	2.00
Average row	2.07	2.47	2.07	2.47	2.40	2.40	2.13
Average plantings	2.24						

Analysis of cane infestation per row (Fig. 3) indicated that the highest number of galls per cane was recorded in the second and fourth rows (2.47 galls/cane), while the lowest numbers were observed in the first and third rows (2.07 galls/cane). In the analyzed canes of the Fertodi zamatos cultivar, the presence of the raspberry gall midge was confirmed, with an average of 2.24 galls per cane across the plantings.



**Figure 3.** Average number of gall per cane in the row

The smallest cane diameter measured in the Fertodi experimental field (Table 2) was 0.82 cm, recorded in the fourth row, zone 2. The largest cane diameter, measured in the seventh row, zone 1, was 1.18 cm. The mean cane diameter was approximately the same across all rows and zones, ranging from 0.96 cm in the second row to 1.01 cm in the seventh row.

**Table 2.** Cane and gall morphometry

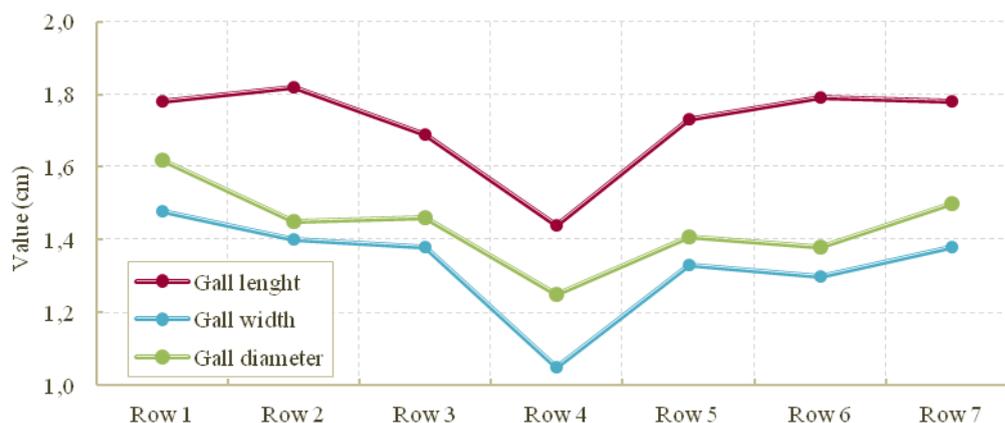
row	zone	Cane diameter	Galls		
			diameter	length	width
cm					
I	1	1.05	1.78	2.07	1.73
	2	1.05	1.57	1.56	1.33
	3	0.90	1.51	1.71	1.38
Average per row		1.00	1.62	1.78	1.48
II	1	1.14	1.45	1.74	1.29
	2	0.67	1.37	1.86	1.37
	3	1.08	1.54	1.86	1.54
Average per row		0.96	1.45	1.82	1.40
III	1	1.06	1.66	1.99	1.58
	2	0.98	1.35	1.55	1.26
	3	0.95	1.38	1.52	1.29
Average per row		1.00	1.46	1.69	1.38
IV	1	0.97	1.43	1.74	1.38
	2	0.82	0.86	0.93	0.50
	3	1.06	1.45	1.66	1.27
Average per row		0.95	1.25	1.44	1.05
V	1	1.02	1.36	1.73	1.28
	2	0.85	1.32	1.69	1.24

	3	0.92	1.55	1.77	1.48
Average per row		0.93	1.41	1.73	1.33
VI	1	0.96	1.35	1.78	1.23
	2	1.01	1.30	1.76	1.25
	3	1.02	1.48	1.83	1.43
Average per row		1.00	1.38	1.79	1.30
VII	1	1.18	1.64	1.85	1.37
	2	0.88	1.33	1.78	1.26
	3	0.98	1.54	1.71	1.50
Average per row		1.01	1.50	1.78	1.38

Unlike cane diameters, gall diameters varied considerably. The lowest mean gall diameter was recorded in the fourth row, zone 2 (0.86 cm), while the highest was observed in the first row, zone 1 (1.78 cm). The highest mean gall diameter by row was in the first row (1.62 cm), and the lowest in the fourth row (1.25 cm).

The cane and gall diameters, gall length and width were also measured. The minimum values for gall length and width were recorded in the fourth row, zone 2, whereas the maximum length was measured in the third row, zone 1, and the maximum width in the first row, zone 1. Gall length and width varied significantly. The smallest mean gall length was 0.93 cm, and the smallest mean gall width was 0.50 cm, both measured in the fourth row, zone 2 (Table 2). The largest galls were observed in the first row, zone 1, with mean length and width of 2.07 cm and 1.73 cm, respectively (Table 2).

Comparison of mean values by row (Fig. 4) showed that the greatest average gall length was recorded in the second row (1.82 cm), while the greatest average gall width was observed in the first row (1.48 cm).



**Figure 4.** Gall dimensions

The obtained results indicate that the average number of galls was higher than reported in the available literature data. Tanasković and Milenković (2010) reported a maximum of 1.75 galls per cane in the Latham cultivar. This suggests that the Fertodi zamatos cultivar demonstrate greater susceptibility to the raspberry gall midge compared to other cultivars. The mean gall diameter (1.44 cm) in our study was slightly lower than the values reported by Tanasković et al. (2008), which ranged from 1.75 to 2.7 cm in their work. These differences may be consequence to cultivar characteristics, as well as agro-ecological and climatic conditions. The mean gall width of 1.33 cm observed in our study was higher than in previously investigated cultivars, confirming the pronounced preference of the Fertodi zamatos cultivar for gall midge development. Available literature data does not report tolerance of this cultivar to this pest. However, according to Véték et al. (2008), during their studies, Fertodi zamatos presented tolerance to *Resseliella theobaldi* and *Agrilus aurichalceus*.

## CONCLUSION

The study conducted in a raspberry plantings at Trnava (Požega) demonstrated that the presence of *L. rubi* is significant and causes visible morphological changes on canes. The results indicate a substantial impact of *L. rubi* on the morphological characteristics of Fertodi zamatos canes. This cultivar becomes extremely popular in producers community, and introduced into pilot production in this raspberry-growing region from 2020 on different plot area. The average of 2.24 galls per cane highlights the pronounced susceptibility of this cultivar to *L. rubi*, but also emphasizes the need for further research to provide adequate recommendations to growers regarding the opportunities and risks of establishing plantings with this cultivar.

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# DRONE-ASSISTED BIOLOGICAL CONTROL OF EUROPEAN CORN BORER WITH *TRICHOGRAMMA BRASSICAE*

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*The European corn borer (ECB) is one of the most important pests in maize production and is mostly managed through the use of chemical insecticides. In this study, we evaluated the efficacy of an alternative biological control method using the egg parasitoid Trichogramma brassicae, applied via unmanned aerial vehicle (UAV). The effectiveness of T. brassicae was assessed based on the parasitism rate of ECB eggs. The results demonstrated a high level of egg parasitism, indicating that UAV-assisted releases of T. brassicae can serve as an efficient approach to maize pest management.*

**Key words:** maize, *Ostrinia nubilalis*, parasitism, UAV

## INTRODUCTION

Maize (*Zea mays L.*) is one of the most widely cultivated crops in Serbia (Statistical Office of the Republic of Serbia, 2024). Maize is often used for human and animal nutrition but also has significant industrial applications. However, maize production faces numerous challenges, among which insect pests represent a major problem. The European corn borer, *Ostrinia nubilalis* Hübner, 1796 (Lepidoptera: Crambidae) is a significant pest, with typically two generations per year in Serbia and, if environmental conditions are suitable, a partial third generation may occur (Ivezić et al., 2021). The second generation is considered the most damaging, as larval feeding causes substantial injury to stems and ears, disrupts nutrient transport within the plant, and reduces both yield and grain quality (Grčak et al., 2024; Pintilie et al., 2023).

Traditionally, the control of *O. nubilalis* has relied on chemical insecticides (Franeta et al., 2018), which carry several disadvantages, including negative impacts on human health and the environment (Ansari et al., 2013). Furthermore, insecticide application is often complicated by the growth stage of maize, as plants infested by the second generation can exceed two meters in height, requiring specialized high-clearance sprayers. As a sustainable alternative, the use of egg parasitoids from the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) has proven effective in controlling *O. nubilalis* (Razinger et al., 2016; Wright et al., 2002). Combining biological control with modern technologies, such as unmanned aerial vehicles (UAVs), can help to overcome the challenges posed by plant development stages and mitigate the negative effects of chemical treatments (Zhan et al., 2021). Precision and effectiveness of application can be enhanced using UAVs that enable rapid and uniform distribution of parasitoids across the field (Zhan et al., 2021).

The aim of this study was to evaluate the efficacy of *Trichogramma brassicae* Bezdenko, 1968 application via UAV in maize under local agroecological conditions, using locally produced maize hybrids, for the suppression of *O. nubilalis*.

## MATERIAL AND METHODS

The experiment was conducted at the Institute of Field and Vegetable Crops, located in Rimski Šančevi, Novi Sad. Two maize hybrids, NS640 and NS6000, were used in the study. Each hybrid was sown on an area of 1.1 ha. The treatment with a biological control agent was carried out on the area of 1 ha, while the control plot occupied 0.1 ha. Sowing of both hybrids was performed on 7<sup>th</sup> May 2024.

The application of the biological agent *T. brassicae* was carried out on 25<sup>th</sup> July of the same year. The product containing *T. brassicae* was supplied in capsules, each containing 2000 individuals. The capsules were imported from France, produced by Bioline Agrosciences. The applied amount was 125 capsules per hectare, corresponding to approximately 250,000 individuals of parasitic wasps per hectare. The application was

performed when the economic threshold level of 10% plants was infested with *O. nubilalis* eggs. The capsules were applied using a DJI Matrice 300 RTK drone equipped with a T-Drop dispenser for uniform release of capsules containing the parasitoids. The flight altitude of the drone was 20 m, and the flight speed was 10 m/s. Capsules were released every 10 m, with the distance between flight passes being 8 m.

The treatment efficacy was determined based on maize plant inspections conducted before and after the release of the parasitoids. Prior to release, the plants were inspected once on July 22. After release, both treated and control plots were examined. A total of 100 randomly chosen plants in the treated plot and 100 in the control plot were examined for each hybrid. During the inspections, the number of egg masses per 100 plants was recorded, and the percentage of parasitized eggs was calculated. A second inspection was performed five days later, on July 31<sup>st</sup> in the same area, recording again the number of egg masses and the percentage of parasitism.

To evaluate the treatment efficacy, the proportion of parasitized *O. nubilalis* eggs was compared among different groups. For each group, the numbers of parasitized and non-parasitized eggs were recorded. Comparisons were made using Pearson's Chi-square test (df = 1) in Statistica software (Version 14.0.0.15). The test was applied for the following comparisons:

Pre-treatment vs. Post-treatment in treated plots, to determine the effect of parasitoid application.

Post-treatment vs. Control after treatment, to assess the difference between treated and untreated plots.

For all tests, an  $\alpha$  value of 0.01 was used. The tables present Chi-square values and the corresponding p-values. Higher p-values (>0.01) indicate no significant differences, while  $p < 0.01$  denotes a statistically significant difference in the proportion of parasitized eggs between the groups.

## RESULTS AND DISCUSSION

The highest number of *O. nubilalis* egg masses per 100 maize plants was recorded in the hybrid NS640, where 44 egg masses were found (Table 1). Of these, 16 were parasitized and 28 were non-parasitized, resulting in a 36.36% parasitism rate prior to treatment. Five days after the release of *T. brassicae*, 22 egg masses were found on 100 randomly selected plants, 19 of which were parasitized, corresponding to a parasitism rate of 86%. In the control plot, only 7 egg masses were detected after treatment, and none were parasitized.

In the hybrid NS6000, 29 egg masses were recorded prior to treatment, with a 37.9% parasitism rate. Following treatment, 25 egg masses were found on 100 plants, of which 24 were parasitized, resulting in a parasitism rate of 96% by *Trichogramma* wasps. In contrast, 11 egg masses were recorded in the control plot, with only 2 parasitized, corresponding to a parasitism rate of 18.18%. The lower number of *O. nubilalis* egg masses observed in the control plots was probably due to the poor physiological condition of the plants in that section, resulting from the extreme weather conditions experienced during the trial year. The plants were dry and stunted, which may have influenced the oviposition preference of *O. nubilalis*.

**Table 1.** Percentage of parasitism, number of parasitized, nonparasitized and total number of eggs (pre\_T – before treatment; post\_T – after treatment; post\_C - control)

Hybrid	Group	Number of parasitized eggs	Number of nonparasitized eggs	Total number of eggs	Percentage of parasitism
NS640	pre_T	16	28	44	36.36%
	post_T	19	3	22	86%
	post_C	0	7	7	0%
NS6000	pre_T	11	18	29	37.90%
	post_T	24	1	25	96%
	post_C	2	9	11	18.18%

pre\_T – before treatment; post\_T – after treatment; post\_C – control

Pearson's Chi-square test indicated statistically significant differences among all groups in both hybrids (Table 2). Significant differences were detected in the proportion of parasitized egg masses between pre-treatment and post-treatment groups, as well as between post-treatment and control groups.

**Table 2.** Comparison of the proportion of *O. nubilalis* eggs parasitized by *T. brassicae* in pre-treatment, post-treatment and control maize plots

Maize hybrids	Group	X <sup>2</sup>	p-value
NS640	pre_T x post_T	14.72	<0.0001

	post_T x post_C	17.53	<0.0001
NS6000	pre_T x post_T	19.85	<0.0001
	post_T x post_C	23.06	<0.0001

Similar results following the application of *T. brassicae* at a rate of 375,000 individuals per hectare were reported by Razinger et al. (2016), who observed post-treatment parasitism rates of 80%, 71%, and 49%, in France, Italy and Slovenia. Wright et al. (2002) reported parasitism levels of up to 100% in maize fields where *T. ostriniae* Pang and Chen, 1974 was released. In another study, *T. ostriniae* released at a rate of 75,000 individuals per hectare achieved parasitism rates ranging from 0 to 75%, with a higher frequency of parasitized egg masses recorded in plots where the parasitoid was released (Hoffmann et al., 2006). Oztemiz (2009) reported a parasitism rate of approximately 82% in plots where *T. evanescens* Westwood, 1833 was released, whereas natural parasitism was similar to that observed in the present study, averaging around 30%. Additionally, Zhan et al. (2021) emphasized the high efficacy of using UAVs for releasing *T. ostriniae*, with the exceptional field coverage by parasitoid wasps of 99.33%, while the average parasitism rate in treated plots reached 87.42%.

## CONCLUSION

The results of this study indicate that the use of unmanned aerial vehicles (UAVs) can serve as an effective and precise method for applying biological control agents in crop protection. The application of *T. brassicae* for the suppression of the ECB demonstrated high efficacy, as evidenced by a significantly higher percentage of parasitized egg masses after treatment compared with the control plots. These results suggest that biological control using *T. brassicae* can be a viable option for controlling maize pests such as *O. nubilalis*. Overall, these findings confirm the potential of UAV technology to enhance sustainable maize protection practices.

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## EFFECTS OF GAMMA RAY IRRADIATION DOSES ON STERILITY IN SERBIAN POPULATION OF Aedes albopictus

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*The sterile insect technique is an environmentally friendly method of mosquito control based on the mass rearing, sterilization, and release of sterile males into the environment to compete with wild males. This study aims to determine the egg sterility of invasive Aedes albopictus mosquitoes (Serbian strain), achieved by applying different irradiation doses of Gamma rays in adult stage. Doses of 20, 40, 55 and 65 Gy of Gamma ray were applied to sterilize the adult males. All applied doses significantly decreased the egg hatch rate.*

**Key words:** mosquito, Aedes albopictus, SIT, gamma ray, hatch rate, sterility

### INTRODUCTION

The Asian tiger mosquito (*Aedes albopictus* Skuse 1894) is a widespread invasive mosquito species. After an *Ae. albopictus* population was established in Serbia (in 2017), the rapid spread of the species has been recorded every year. Populations are currently established in both urban and rural areas (Kavran et al., 2019). Being an urban, day-biting, exophilic species, its presence strongly limits humans from using outdoor spaces and affects many outdoor activities. In addition, *Ae. albopictus* is a competent vector of many different arboviruses, including chikungunya, dengue and Zika viruses (Weaver et al., 2018), which makes it a species of high medical and veterinary significance.

Chemical insecticides are mainly used to control adult mosquitoes. However, many problems are associated to its application, such as insecticide resistance, injury to nontarget insects, environmental contamination, and human toxicity (Du et al., 2019). Therefore, novel methods and techniques to control these species are needed.

The sterile insect technique (SIT) is an environmentally friendly insect pest control method, which has been successfully applied to prevent, suppress and eradicate several major insect pest species (Bourtzis and Vreysen, 2021). It is based on the mass rearing, sterilization, and release of sterile males into the environment to compete with wild males. After mating of released sterile males and wild (fertile) females, no offspring is produced, leading to population reduction (Dyck et al., 2021). Ionizing radiation is the most commonly used method to induce sterility in area-wide integrated pest management programmes with an SIT component (Balestrino et al., 2024). Gamma radiation from a <sup>60</sup>Co or <sup>137</sup>Cs source and X-ray radiation are commonly used because of their high energy and penetration (Helinski et al., 2009).

The response of mosquitoes to irradiation is affected by many critical factors, including the dose rate, dose amount, environment during irradiation, mosquito species, strain, life stage, and handling procedures (Bimbilé et al., 2022).

The dose of gamma radiation is the most critical factor associated with the male insect's sterility level. The appropriate radiation dose is required to produce good quality sterile male insects with a high level of sterility to maintain overall quality and competitiveness in the field (Robinson, 2021). Increasing radiation doses were found to correlate with the induced sterility in irradiated male mosquitoes, however, negative impacts have been identified on several parameters, including emergence rate, longevity, flight ability, and competitiveness (Ernawan et al., 2022).

This study aims to determine the sterility of *Ae. albopictus* Serbian strain achieved by using different Gamma ray radiation doses in adult stage.

## MATERIAL AND METHODS

### Mosquito culture

*Aedes albopictus*, used in the present study, was originally collected from the four locations in Vojvodina, Serbia (Rivica, Pavlovci, Šatrinici and Neradin) and transferred to the Insect Pest Control Laboratory (IPCL) of the joint Food and Agriculture Organization of the United Nations/International Atomic Energy Agency (FAO/IAEA) Centre of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria. The population was reproduced and maintained under rearing protocols developed at the IPCL (FAO/IAEA, 2020b). Two days after pupation, pupae were collected, and sex separation was performed using a mechanical separator, based on sexual dimorphism – different pupal size of sexes. Males were kept for treatment and females were placed in individual tubes for emergence to ensure virginity for later mating.

### Radiation sources and dosimetry

The irradiation device used in these experiments was a Gammacell 220 (Nordion Ltd, Kanata, Ontario, Canada). At the time of the experiment, the irradiator had a dose rate of 32.22 Gy/min. Irradiation times were calculated based on the decay table of the isotopic source. The dosimetry system used to verify the dose received by the samples was based on Gafchromic MD-VD film (Ashland Advanced Materials, Bridgewater NJ, USA). Three films of MD film were packed in small (2 × 2 cm) paper envelopes and placed directly below the mosquito samples. Films were read with an optical density reader (DoseReader 4, RadGen, H-1118 Budapest, Sasadi út 36, Hungary) after 24 h of development.

### Sample preparation and irradiation

Two to four days old males were chilled (below 10°C) and separated in three replicates of 15 males per treatment. Mosquitoes were exposed to four different irradiation doses (20, 40, 55, and 65 Gy). Males were irradiated in 2cm diameter PMMA tubes, with three replicates placed together into the PMMA cylinder in the middle of the Gammacell chamber.

### Assessment of induced sterility

Irradiated males have been transferred into the 15 × 15 × 15 cm Bugdorm cages (MegaView Science Co. Ltd., Taichung 40762, Taiwan) with 20 nulliparous females of the same age (15 males:20 females per cage). Cages were supplied with 10% sucrose solution with an *ad libitum* access. Mosquitoes were allowed to mate for two days before females being blood-fed with fresh porcine blood on two consecutive days and then provided with oviposition cups containing 100ml of tap-water and lined with filter paper on the inner side. Blood feeding was repeated on the fifth and sixth day following the mating, to increase the number of laid eggs (blood-feeding was done on 2<sup>nd</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 6<sup>th</sup> post-mating days). Egg papers were collected, and eggs were matured for seven days before hatching in a hatching solution consisting of tap water, nutrient broth and brewer's yeast (FAO/IAEA, 2020a). The total number of eggs (hatched and un-hatched) was counted for each treatment using a stereomicroscope, to calculate the hatch rate. The hatch rate was determined as the percentage of hatched eggs out of the total number of eggs checked.

### Statistical analysis

Data was analysed using the Statistica package TIBCO Software Inc. (2020). Statistical significance was assessed using a one-way ANOVA. The significant difference between tested groups was analysed by Fisher's *post-hoc* test with confidence interval  $p < 0.05$ .

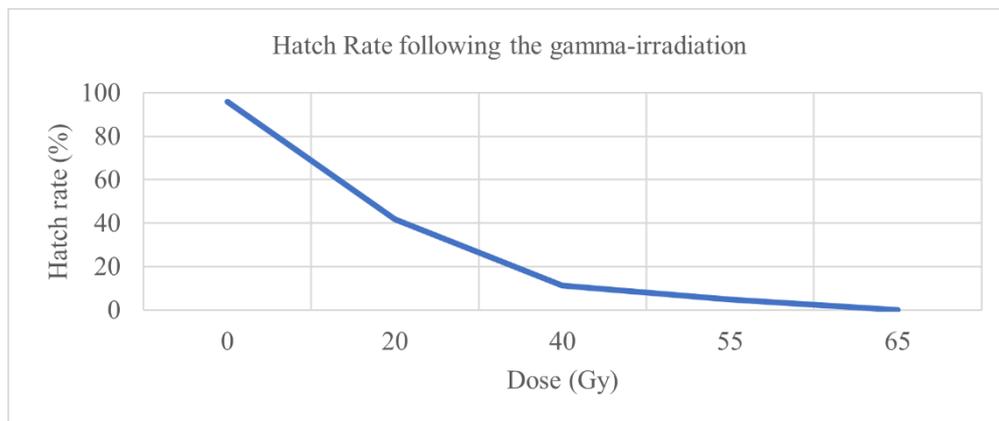
## RESULTS AND DISCUSSION

The dosimetry test confirmed that the actual irradiation dose received by the samples were  $20.30 \pm 1.32$ ;  $41.39 \pm 2.70$ ;  $59.35 \pm 3.87$  and  $73.98 \pm 4.82$  Gy at the dose setting of 20, 40, 55 and 65 Gy, respectively (calibration MD film lot# 10162302; 2025.09.19).

Total number of laid eggs was 202 in the control group and 358, 329, 692, 256 in treatment groups exposed to 20, 40, 55, 65Gy, respectively

The hatch rate is presented in Figure 1. The irradiation dose had significant effect on hatch rate (SS=18006.3; df=4; MS=4501.57; F=442.29;  $p < 0.001$ ). In the control group the average hatch rate was 95.99%, while there was a significant decrease in hatch rate from the dose of 20Gy (41.68%). When the dose of 40Gy

was used the hatch rate was 11.29%, while the hatch rate of 4.91% was achieved using the dose of 55Gy. Full sterility (no hatched eggs) was observed when mosquitoes were irradiated with the dose of 65Gy. Fisher's *post-hoc* test showed that all groups differed significantly, with the only exception being the 55 Gy and 65 Gy treatments, which did not significantly differ from each other ( $p > 0.05$ ).



**Figure 1.** Impact of irradiation dose on the egg hatch rate in *Aedes albopictus* following irradiation of adult males

Similarly, Bimbile et al. (2022) have determined that in the absence of irradiation, the egg hatch rate in *Ae. albopictus* was above 85%, while an approximate of 0% egg hatch rate was reached when adult mosquitoes were irradiated with gamma ray doses above 55 Gy. However, full sterility was observed with doses of 110 and 120 Gy.

Du et al. (2019) have determined that the hatch rate decreased significantly with increasing radiation dose in adult *Ae. albopictus* male irradiation. After irradiation at 40 Gy and 60 Gy, the average hatch rates were 1.4% and 0.7%, respectively.

In pupal gamma ray irradiation of *Ae. albopictus*, a consistent increase in the sterility was observed as the dose received by the male increased from 20 to 50 Gy. Residual fertility level of 1.0% was achieved by using 30-35 Gy, while the 100% sterility was achieved using 60Gy (Balestrino et al., 2010).

The presented data imply that increasing irradiation doses correlate with a decrease of hatch rates. The potential negative effects of irradiation on emergence rate, longevity, flight ability, and competitiveness should be considered. The series of trials showed the convenience of reducing the gamma ray radiation dose for pupal irradiation of *Ae. albopictus* from 60 Gy to 40 and 30 Gy in term of net capacity to introduce sterility into the females, indicating that the reduction in male sterility level is overcompensated by the increased competitiveness of partially sterile males (Bellini et al., 2013).

## CONCLUSION

In gamma ray irradiated adult *Ae. albopictus*, the hatch rate decreases with the increase of irradiation dose. Full sterility, with no hatch at all, can be achieved through use of 65Gy dose.

Additional tests need to be conducted to test the biological quality and competitiveness of the irradiated males.

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# INTEGRATION OF CIRCULAR ECONOMY CONCEPT AND ARTIFICIAL INTELLIGENCE IN THE DEVELOPMENT OF A FUNCTIONAL PLANT-BASED ALMOND YOGURT

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*This study developed a functional almond-based yogurt enriched with banana and lemon peel extracts. The incorporation of these by-products enhanced the yogurt's nutritional profile, increasing antioxidant activity, phenolic, and flavonoid content, while supporting the circular economy. AI-based sensory evaluation confirmed the product's high acceptability, demonstrating the potential of combining sustainable ingredients with advanced technologies.*

**Key words:** Almond yogurt, bioactive extracts, circular economy, AI sensory evaluation.

## INTRODUCTION

The global demand for plant-based dairy alternatives has risen significantly in recent years, driven by increased awareness of health, environmental, and ethical concerns. Plant-based yogurts, offering lactose-free, cholesterol-free, and vegan-friendly options, have emerged as popular alternatives to traditional dairy. However, these products still face several challenges, particularly related to sensory attributes, nutritional content, and consumer acceptance. Recent efforts have focused on enhancing the nutritional profile and sensory properties of plant-based yogurts by incorporating bioactive compounds from agro-industrial by-products, a practice that aligns with circular economy principles aimed at reducing food waste (Montemurro et al., 2021).

Among the various agro-industrial by-products, fruit peels, such as those from bananas and lemons, are rich in bioactive compounds like fibers, phenolics, flavonoids, vitamins, and essential oils. These components possess strong antioxidant, antimicrobial, and anti-inflammatory properties, making fruit peels ideal candidates for enriching functional foods (Yigit, 2020). While the use of fruit peels in food products has been explored in previous studies, the specific application of banana and lemon peel extracts in almond-based yogurts remains underexplored. Moreover, traditional extraction methods often involve the use of harmful solvents, which can degrade sensitive bioactive compounds, underscoring the need for greener, more efficient extraction technologies (Gorlov et al., 2019).

In this context, subcritical water extraction emerges as a promising green alternative. This method preserves the bioactive properties of plant materials without the need for toxic solvents, offering a more sustainable solution for extracting valuable compounds. Despite its advantages, the application of subcritical water extraction to banana and lemon peels for enhancing plant-based yogurts has not been widely studied, representing a gap in current research (Stojanova et al., 2024).

Furthermore, sensory and functional evaluations of enriched plant-based yogurts have traditionally relied on time-consuming, subjective methods. The advent of artificial intelligence (AI)-based digital sensor analysis presents a revolutionary solution for the rapid, objective, and precise assessment of sensory attributes, texture, aroma, and overall product quality. This approach improves the efficiency and consistency of sensory evaluations, making it an ideal tool for evaluating novel food products (Tangyu et al., 2019).

The aim of this study was to develop a novel functional almond-based yogurt enriched with bioactive extracts from banana and lemon peels, using subcritical water extraction. By repurposing agricultural waste materials in food production, this research reinforces the circular economy model by promoting resource efficiency and waste minimization. The research combined traditional nutritional and antioxidant assessments with AI-driven digital sensor analysis to comprehensively evaluate the product's functional and sensory characteristics. This integrated approach contributes to a sustainable food innovation pipeline, where circular economy principles are embedded from raw material sourcing to product evaluation. By integrating circular economy principles, green extraction methods, and AI technologies, the study contributed to the development of

sustainable, innovative, and high-quality plant-based functional foods, offering scalable solutions for future food systems.

## MATERIAL AND METHODS

Probiotic starter cultures of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium sp.* were prepared following the method of Stojanova et al. (2025). The cultures were propagated in appropriate broths, harvested by centrifugation, washed with diluted Ringer's solution, and resuspended to a final concentration of approximately 7 log CFU/mL for inoculation into almond milk. Almonds (200 g) were soaked in 200 mL water for 12 hours, then blended with incremental additions of water until the total volume reached 1 L. The resulting almond mixture was inoculated with the probiotic culture, and fermentation was carried out at 40 °C for 24 hours, targeting a final pH of about 4.5. After fermentation, the yogurt was cooled to 8 °C and homogenized to ensure uniform consistency. Subsequently, two extracts—banana peel and lemon peel—were added at a concentration of 4 g per sample, producing three variants of yogurt: functional almond-based yogurt (Sample 1), almond yogurt with banana peel extract (Sample 2), and almond yogurt with lemon peel extract (Sample 3) (Stojanova et al., 2025).

Subcritical water extraction was performed using a 450 mL high-pressure extractor (Parr Instrument Company, USA) at 120 °C, 30 bar for 25 minutes, as per Gavarić (2020). Banana peel and lemon peel powders (10 g each) were mixed with 100 mL distilled water, with nitrogen used to maintain pressure and prevent oxidation. A magnetic stirrer ensured uniform heat distribution. After extraction, the samples were cooled, depressurized, filtered, and stored at 4 °C.

The total phenolic content (TPC) of the extracts was determined using a microplate-adapted spectrophotometric method based on Stojanova et al. (2021), with gallic acid standards to create a calibration curve. TPC was expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g d.m.). Total flavonoid content (TFC) was determined via a modified aluminum chloride colorimetric method, with quercetin standards to construct the calibration curve. TFC was expressed as milligrams of quercetin equivalents per gram of dry matter (mg QE/g d.m.). Antioxidant activity was assessed using two assays: DPPH radical scavenging and the inhibition of lipid peroxidation in a linolenic acid system, as described by Stojanova et al. (2021).

For the yogurt samples, total carbohydrates and proteins were measured using the modified Fehling I and II titration methods and the modified Kjeldahl method, respectively (Stojanova, 2017). Fat content was determined by Soxhlet extraction, with the samples enclosed in cellulose bags and subjected to fat extraction with diethyl ether, as described by Stojanova (2017).

Sensory analysis involved 30 participants (15 women and 15 men, aged 25–60), who evaluated the three yogurt samples (approximately 30 g each) at 10 ± 2 °C, following the methodology of Gupta et al. (2021). Participants' facial expressions were recorded with PsychoPy software and analyzed using iMotions with the Affectiva facial recognition engine (Kulke et al., 2020). Participants also rated their liking on a nine-point hedonic scale. Statistical analysis was conducted using ANOVA and Tukey's test ( $p < 0.05$ ), as well as a T-test ( $p < 0.05$ ) with SPSS version 20.

## RESULTS AND DISCUSSION

As shown in Table 1, significant differences ( $p < 0.05$ ) were found between banana and lemon peel extracts in total phenolic content (TPC), total flavonoid content (TFC), extraction yield, and antioxidant activity (DPPH and conjugated diene assays). Lemon peel extract demonstrated superior bioactive potential, with higher TPC ( $34.72 \pm 0.06$  mg GAE/g d.m.) and TFC ( $15.84 \pm 0.10$  mg QE/g d.m.) compared to banana peel ( $28.45 \pm 0.15$  mg GAE/g d.m. and  $12.37 \pm 0.02$  mg QE/g d.m.), consistent with citrus by-products' known richness in flavanones and phenolic acids (González-Molina et al., 2010).

Lemon peel also showed a higher extraction yield ( $21.93 \pm 0.13\%$ ) than banana peel ( $18.26 \pm 0.07\%$ ), likely due to its more hydrophilic phenolic matrix suitable for subcritical water extraction. Correspondingly, antioxidant activity was higher in lemon peel, with both DPPH scavenging ( $81.47 \pm 0.22\%$ ), and lipid peroxidation inhibition reaching ( $73.06 \pm 0.16\%$ ), compared to  $72.84 \pm 0.18\%$ , and  $69.21 \pm 0.23\%$  in banana peel. These results confirm lemon peel extract's stronger antioxidant potential. The strong correlation between phenolic/flavonoid content and antioxidant capacity supports the use of these agro-industrial by-products as natural functional additives. From a circular economy perspective, valorizing such waste materials not only enhances product functionality but also contributes to sustainable food system development by reducing organic waste.

**Table 1.** Comparison of phytochemical and antioxidant properties of the tested extracts

Extract	n	Parameter				
		TPC (mg GAE/g d.m.)	TFC (mg QE/g d.m.)	Yield (%)	DPPH (%) (10 mg/mL)	Conjugated diene method (%) (10 mg/mL)
		$\bar{x} \pm SD$				
Banana peel	3	28.45 <sup>a</sup> ± 0.15	12.37 <sup>a</sup> ± 0.02	18.26 <sup>a</sup> ± 0.07	72.84 <sup>a</sup> ± 0.18	69.21 <sup>a</sup> ± 0.23
Lemon peel	3	34.72 <sup>b</sup> ± 0.06	15.84 <sup>b</sup> ± 0.10	21.93 <sup>b</sup> ± 0.13	81.47 <sup>b</sup> ± 0.22	73.06 <sup>b</sup> ± 0.16

<sup>a,b</sup> – values for the different extracts and the same parameter marked with different letters are statistically significantly different, T-test (p<0.05).

These results confirm the functional potential of lemon peel extract as a more effective antioxidant agent. Although not typically consumed, citrus peels are rich in bioactive compounds, especially polyphenols and flavonoids, with strong antioxidant and health-promoting properties (Shehata et al., 2021; Brezo-Borjan et al., 2023). The high phenolic and flavonoid content observed in the lemon peel extract in this study reflects this natural richness and highlights its suitability as a sustainable ingredient in functional food formulations.

Table 2 shows the chemical composition and bioactive content of the three almond-based yogurt variants, with significant differences (p < 0.05) in total carbohydrates, proteins, and phenolic and flavonoid contents. Total carbohydrates ranged from 4.73% in Sample 1 (control) to 5.12% in Sample 2 (banana peel), likely due to natural sugars and fiber from the fruit peels. Protein content varied slightly, with Sample 3 (lemon peel) showing the highest value (13.25%), possibly reflecting interactions between lemon peel phenolics and proteins. Fat content remained similar across all samples (9.62–9.68%), indicating that aqueous peel extracts did not affect lipid levels.

Notably, the inclusion of fruit peel extracts resulted in a dramatic increase in total phenolic content (TPC) and total flavonoid content (TFC). The control sample (Sample 1) recorded the lowest TPC (2.84 ± 0.11 mg GAE/g d.m.) and TFC (1.52 ± 0.09 mg QE/g d.m.), while the yogurts with banana (Sample 2) and lemon (Sample 3) peel extracts showed significantly enhanced values. Specifically, Sample 3 exhibited the highest TPC (27.76 ± 0.15 mg GAE/g d.m.) and TFC (14.42 ± 0.12 mg QE/g d.m.), consistent with the earlier observation that lemon peel extract had the highest antioxidant content among the tested extracts.

These results confirm the successful incorporation and functional enhancement of the almond-based yogurt through subcritical water-extracted fruit peel bioactives. The significant increase in TPC and TFC suggests that both banana and lemon peel extracts not only contribute to improved nutritional value but may also enhance antioxidant potential, supporting the development of functional plant-based dairy alternatives.

**Table 2.** Chemical composition of the almond yogurt

Yogurt type	n	Parameter				
		Total carbohydrates (%)	Total proteins (%)	Total fats (%)	TPC (mg GAE/g d.m.)	TFC (mg QE/g d.m.)
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Sample 1	3	4.73 <sup>a</sup> ± 0.12	13.12 <sup>a</sup> ± 0.08	9.65 <sup>a</sup> ± 0.10	2.84 <sup>a</sup> ± 0.11	1.52 <sup>a</sup> ± 0.09
Sample 2	3	5.12 <sup>b</sup> ± 0.09	13.19 <sup>a</sup> ± 0.07	9.68 <sup>a</sup> ± 0.13	24.18 <sup>b</sup> ± 0.13	11.63 <sup>b</sup> ± 0.10
Sample 3	3	5.05 <sup>c</sup> ± 0.11	13.25 <sup>b</sup> ± 0.06	9.62 <sup>a</sup> ± 0.11	27.76 <sup>c</sup> ± 0.15	14.42 <sup>c</sup> ± 0.12

<sup>a,b,c</sup> – values for the different samples and the same parameter marked with different letters are statistically significantly different, ANOVA, *post hoc* Tukey's test (p<0.05).

As shown in Table 3, the antioxidant activity of the almond yogurt samples, measured via DPPH radical scavenging and the conjugated diene method, exhibited statistically significant differences (p < 0.05) across the three formulations. The control sample (Sample 1) demonstrated the lowest antioxidant potential in both assays, reflecting its minimal content of phenolic and flavonoid compounds.

Samples enriched with fruit peel extracts showed markedly improved antioxidant profiles. Sample 2, containing banana peel extract, achieved 48.76 ± 0.19% (DPPH) and 41.35 ± 0.14% (conjugated diene), while Sample 3, containing lemon peel extract, reached the highest values: 56.82 ± 0.21% and 49.27 ± 0.17%, respectively. These findings strongly correlate with the increased total phenolic and flavonoid content observed in Table 2, confirming the contribution of the added extracts to the functional enhancement of the product. The results demonstrate the successful valorization of agro-industrial waste, specifically banana and lemon peels, as natural sources of antioxidants. Incorporating these by-products not only improves the nutritional and functional

quality of the almond-based yogurt but also supports the principles of the circular economy by reducing food waste and promoting sustainable ingredient sourcing. This strategy exemplifies how functional food development can align with environmentally responsible innovation, closing nutrient loops and extending the value chain of plant-based materials.

**Table 3.** Antioxidant activity of the almond yogurt

Yogurt type	n	<i>In vitro</i> antioxidant activity	
		Ability to capture DPPH radicals (%)	Conjugated diene method (%)
		$\bar{x} \pm SD$	$\bar{x} \pm SD$
Sample 1	3	21.34 <sup>a</sup> ± 0.16	19.61 <sup>a</sup> ± 0.11
Sample 2	3	48.76 <sup>b</sup> ± 0.19	41.35 <sup>b</sup> ± 0.14
Sample 3	3	56.82 <sup>c</sup> ± 0.21	49.27 <sup>c</sup> ± 0.17

<sup>a,b,c</sup> – values for the different samples and the same parameter marked with different letters are statistically significantly different, ANOVA, *post hoc* Tukey's test (p<0.05).

The significantly enhanced antioxidant activity observed in the almond yogurts enriched with fruit peel extracts reflects the beneficial effects of bioactive compounds introduced through fermentation. Plant-based beverages, derived from legumes, nuts, cereals, or pseudocereals, resemble milk but lack a standardized definition (Boukid et al., 2023). The almond-based yogurt developed here showed higher protein, fat, phenol, and flavonoid contents compared to traditional yogurt, demonstrating the nutritional advantages of precision fermentation with bioactive cultures such as *S. granulatus*. Fermentation has been shown to improve protein content and antioxidant properties in plant-based products, reinforcing the potential of these alternatives to rival conventional dairy products (Boukid et al., 2023; Brückner-Gühmann et al., 2019; Ogundipe et al., 2021).

Table 4 presents the results from the AI-driven facial expression analysis, used to assess the emotional and behavioral responses of participants consuming the three yogurt variants. Statistically significant differences (p < 0.05) were observed across all parameters, demonstrating the impact of formulation on consumer response. Sample 3, enriched with lemon peel extract, elicited the most favorable emotional reactions. It achieved the highest levels of *joy* (59.3 ± 2.63), *surprise* (42.7 ± 2.84), and *relaxed* (50.7 ± 2.33) expressions, as well as the strongest *smiley* emoji response (61.8 ± 2.50). Similarly, *lip press* (a common sign of aversion or uncertainty) was lowest for Sample 3 (24.1 ± 2.10), suggesting increased acceptability.

**Table 4.** Facial expressions, emotions, and emoji indicators in response to different yogurt samples

Type	Parameter	Sample 1 $\bar{x} \pm SD$	Sample 2 $\bar{x} \pm SD$	Sample 3 $\bar{x} \pm SD$
Facial Expression	Lip Press	35.2 <sup>a</sup> ± 2.73	28.5 <sup>b</sup> ± 2.34	24.1 <sup>c</sup> ± 2.10
	Lip Suck	18.7 <sup>a</sup> ± 1.89	26.3 <sup>b</sup> ± 1.97	30.4 <sup>c</sup> ± 1.81
Head Orientation	 Yaw	5.8 <sup>a</sup> ± 1.03	3.2 <sup>b</sup> ± 0.75	2.0 <sup>c</sup> ± 0.68
	Surprise	25.6 <sup>a</sup> ± 2.28	36.9 <sup>b</sup> ± 2.58	42.7 <sup>c</sup> ± 2.84
Emotion	Joy	33.4 <sup>a</sup> ± 2.74	52.8 <sup>b</sup> ± 2.91	59.3 <sup>c</sup> ± 2.63
	Relaxed	37.2 <sup>a</sup> ± 2.24	46.1 <sup>b</sup> ± 2.39	50.7 <sup>c</sup> ± 2.33
Emoji	 Smiley	36.5 <sup>a</sup> ± 2.41	54.6 <sup>b</sup> ± 2.64	61.8 <sup>c</sup> ± 2.50
	 Stuck Out Tongue	16.3 <sup>a</sup> ± 1.71	23.4 <sup>b</sup> ± 2.01	27.9 <sup>c</sup> ± 1.91
	 Smirk	28.2 <sup>a</sup> ± 2.07	39.7 <sup>b</sup> ± 2.29	45.6 <sup>c</sup> ± 2.37

<sup>a, b, c</sup> – values for the different samples and the same parameter marked with different letters are statistically significantly different, ANOVA, *post hoc* Tukey's test (p<0.05); (n = 30).

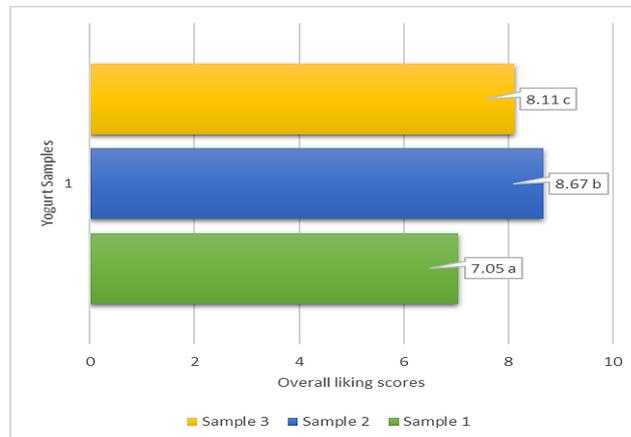
Sample 2, with banana peel extract, also showed positive emotional markers, though slightly lower than Sample 3. In contrast, Sample 1 consistently triggered less favorable expressions, including higher *lip press* and *yaw head movements*, which can indicate disengagement or hesitation.

These findings align closely with the biochemical and antioxidant data: the higher total phenolic content (TPC), flavonoid content (TFC), and antioxidant activity in Samples 2 and 3 likely contributed not only to enhanced health value but also to improved sensorial appeal, particularly in terms of taste and perceived freshness.

The integration of AI-based emotion recognition technology, such as Affectiva within the iMotions platform, enables objective, real-time, and non-invasive evaluation of consumer reactions. Unlike traditional

hedonic scales, this method captures subconscious emotional responses that often drive food preferences. As such, AI adds a powerful layer of precision and insight in product development, helping researchers and producers to fine-tune formulations according to emotional feedback, beyond self-reported liking.

Moreover, this method supports the broader goals of functional food innovation and circular economy, by validating the sensory acceptance of products developed from upcycled ingredients. It ensures that sustainable product development does not compromise consumer enjoyment – a critical factor for market success.



**Figure 1.** Hedonic sensory evaluation ratings for three almond-based yogurt samples

As shown in Figure 1, the overall liking scores from the nine-point hedonic scale confirmed the trends observed in the AI-based facial expression analysis. Sample 2 (banana peel extract) and Sample 3 (lemon peel extract) received significantly higher scores (8.67 and 8.11, respectively) compared to the control (Sample 1, 7.05), with all differences being statistically significant ( $p < 0.05$ ).

These results validate the AI-driven sensory findings, indicating a strong alignment between objective emotional responses and subjective consumer preference. The consistency between both methods strengthens the reliability of the conclusions regarding improved acceptability of upcycled, functional yogurt formulations. The reliability of Affectiva software for emotion recognition has been validated in studies showing accurate detection of facial expressions and faster emotional responses when viewing congruent facial cues (Kulke et al., 2020). Previous research on probiotic yogurts reported no significant differences in sensory quality or consumer preference compared to controls, despite minor variations in flavor or texture (Zhou et al., 2019; Mani-López et al., 2014). This supports the consistency and robustness of both AI-based and conventional sensory evaluations.

## CONCLUSION

This study developed a functional, plant-based almond yogurt enriched with bioactive extracts from banana and lemon peels. The incorporation of these by-products not only enhanced the yogurt's nutritional profile, increasing antioxidant activity, phenolic, and flavonoid content, but also contributed to the principles of the circular economy by valorizing food waste. Additionally, AI-based sensory evaluation provided objective insights into consumer preferences, reinforcing the product's acceptability. This innovative approach demonstrates the potential of combining sustainable food systems with cutting-edge technologies to create healthier, environmentally-friendly alternatives to traditional dairy products.

Future research should focus on optimizing extraction methods and fermentation processes to further increase bioactive compound yields and investigate the long-term health benefits of these plant-based alternatives.

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# OPPORTUNITIES AND CONSTRAINTS FOR FRUIT GROWING IN THE AGRO-ECOLOGICAL CONDITIONS OF TOPONICA, MUNICIPALITY OF KNIĆ

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*This paper analyzes the agroecological and agroeconomic conditions for establishing fruit orchards in the village of Toponica, Municipality of Knić (Šumadija District). The study included assessment of climate, soil properties, terrain characteristics, and market factors. The site is characterized by a temperate continental climate, Vertisol soils of heavy texture, and southwest to west exposure at elevations of 341–376 m. Climate variability and the absence of irrigation increase drought risk, while heavy soil structure limits root development in sensitive species. Soil analysis indicates moderate humus and nitrogen content but low phosphorus and potassium availability. The economic assessment shows that high-input crops such as raspberry, blueberry, peach, and apricot are less suitable under current conditions, while apple, pear, sweet cherry, walnut, hazelnut, and blackberry would require significant investment. Based on overall environmental and economic suitability, plum and sour cherry are identified as the most appropriate and sustainable fruit species for orchard establishment in this locality.*

**Key words:** orchard establishment; agroecological conditions; soil analysis; climate suitability; plum; sour cherry

## INTRODUCTION

Fruit production represents a significant agricultural sector in Serbia, particularly in the Šumadija region, where the village of Toponica is located. The area is characterized by diverse soil types and a temperate continental climate, which provide favorable conditions for cultivating various fruit species. However, the success of fruit growing depends on appropriate orchard establishment that considers agroecological factors such as climate, soil properties, and water availability.

Serbia is among the leading global producers of raspberries, contributing over 20% to the world market, while plum cultivation remains traditionally important (Ministry of Agriculture, 2020). Despite this potential, challenges such as climate variability, limited application of modern production technologies, and insufficient irrigation systems can constrain further development. Proper orchard planning including site selection, soil preparation, and selection of varieties adapted to local conditions is essential for achieving sustainable yields (Glišić and Cvetković, 2020). Additionally, climate change, expressed through irregular precipitation and increased temperatures, poses a growing threat to fruit production in Šumadija (Stričević et al., 2019).

The economic viability of orchards depends on balancing investment costs with expected yields, while also contributing to rural employment, landscape preservation, and biodiversity. Therefore, identifying optimal fruit species and agronomic practices is crucial for sustainable development.

This study analyzes the potential for fruit production in Toponica, to determine suitable fruit species and agro-technical measures that support sustainable and economically viable orchard establishment under local conditions.

## MATERIAL AND METHODS

The study was conducted in the village of Toponica, located in the municipality of Knić, Šumadija District, Central Serbia. The village lies at approximately 295 m above sea level, with a total territorial area of about 7 km<sup>2</sup>. The orchard establishment is planned within the hamlet of Matkovići, approximately 3 km from the village center, near the regional road leading toward Gornji Milanovac.

Two land parcels were selected for analysis.

Parcel 1 covers approximately 0.6 ha, situated at 341–358 m elevation with a southwest exposure and an average slope of 7.2%. The soil is classified mainly as Class IV, with a smaller portion belonging to Class III.

Parcel 2 occupies 0.1 ha, located at 368–376 m elevation, with a western exposure and an average slope of 9.5%.

The geomorphological characteristics of the site, including moderate elevation and favorable exposure, suggest its suitability for fruit cultivation. Agroecological conditions were evaluated using long-term climate data from the Kragujevac meteorological station (RHZS, 2024), soil samples taken from depths of 0–30 cm, and cartographic and GPS data for determining slope, elevation, and exposure. Climate, soil, and terrain data were compared with optimal requirements for orchard establishment based on relevant literature. Laboratory analyses were conducted at PSSS Kragujevac to determine pH, humus, nitrogen, phosphorus, potassium, and CaCO<sub>3</sub> content. Additionally, agroeconomic factors, such as investment costs, labor requirements, irrigation availability, frost protection, and expected yields, were considered to assess the feasibility of establishing cherry and raspberry orchards under local conditions.

## RESULTS AND DISCUSSION

### Climate Conditions

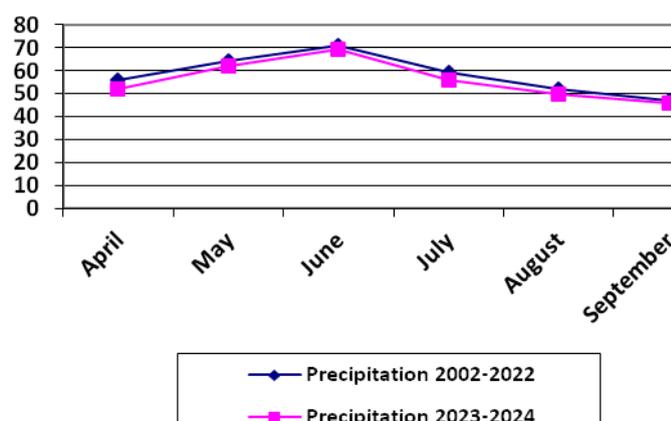
Climate conditions represent a key factor for successful fruit production, influencing growth, yield, and fruit quality. The study area is characterized by a temperate continental climate, and climate parameters were analyzed using long-term data from the Kragujevac meteorological station (18.5 km from the site).

The parcels exhibit southwest and west exposures, providing generally favorable light conditions. However, the presence of surrounding forested areas reduces light availability, which may limit the suitability of light-demanding fruit species such as apple, peach, apricot, raspberry, and blueberry. In contrast, plum, sour cherry, sweet cherry, walnut, hazelnut, and quince can tolerate moderate shading. Sufficient light availability is therefore an important factor in orchard design, requiring wider row spacing and regular canopy pruning to ensure adequate illumination.

Mean annual temperatures range from 11.8°C to 12.9°C. Minimum winter temperatures have reached -24°C (2012), while maximum summer temperatures have risen to 44°C (2007). These conditions are generally suitable for plum, sour cherry, sweet cherry, walnut, hazelnut, and quince, which tolerate temperatures down to -25°C to -30°C. Raspberry, blueberry, blackberry, peach, and apricot are more sensitive to freezing, particularly during spring bloom, and may require frost protection measures. High summer temperatures above 35°C can cause sunburn and reduce flower bud differentiation in multiple fruit species, which can be mitigated through drip irrigation and shading systems.

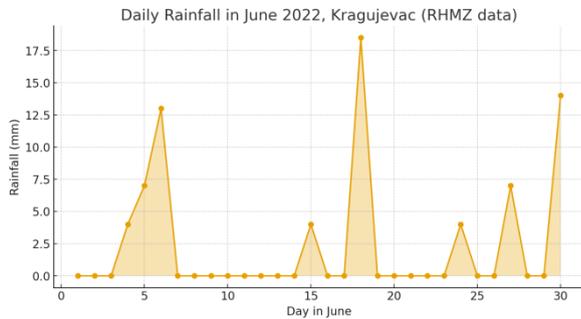
In recent decades, Serbia has experienced an increase in average temperatures and a higher frequency of extreme weather events, particularly summer droughts, which directly affect the stability of agricultural and fruit production. NAP (2015) reports that during the years 2000, 2003, 2007, 2011, and 2012, droughts caused significant losses in the agricultural sector.

Precipitation during the vegetation period (April–September) is essential for fruit growth. Average precipitation in this period was 349.12 mm for the 2002–2022 interval, compared to 334.65 mm in the 2023–2024 period, indicating a slight downward trend (Graph. 1). June was the rainiest month in both periods, while September was the driest. Species such as raspberry, blueberry, blackberry, apple, and pear require 400–600 mm of precipitation during the vegetation period and therefore depend on irrigation. Plum and sour cherry show higher drought tolerance and are more suitable for cultivation without intensive irrigation.

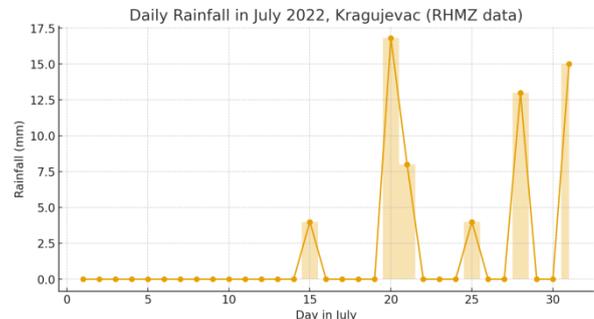


**Graph 1.** Monthly precipitation during the growing season for the period 2002-2022 and during the last two years (2023/2024)

Daily precipitation distributions for June, July, and August 2022 (Graphs. 2–4) show that rainfall was unevenly distributed. June exhibited favorable, evenly spaced rainfall events supporting fruit growth. July showed a mid-month concentration of rainfall following a dry period, increasing drought risk. August had a prolonged dry interval (1–26 August) followed by heavy rainfall at the end of the month, which is unfavorable for most fruit crops. These patterns highlight the importance of supplemental irrigation, particularly for berry crops and pome fruits.

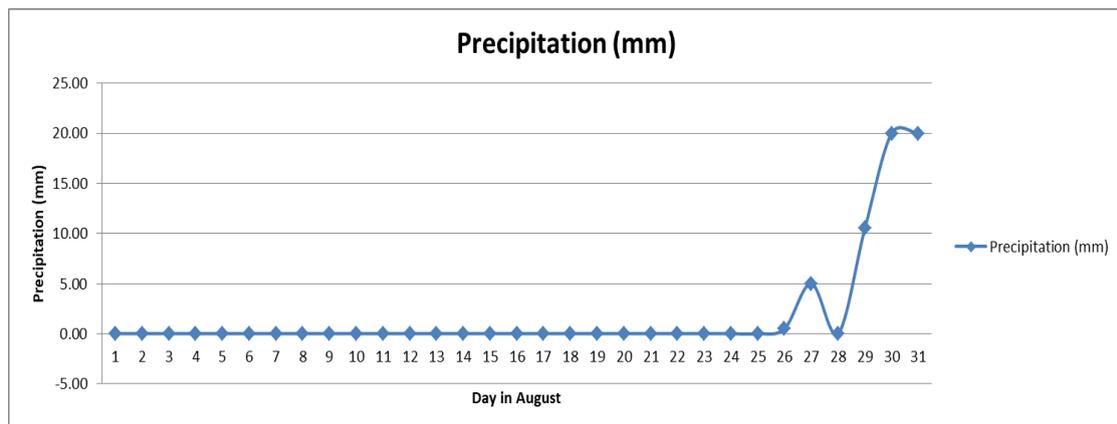


**Graph 2.** Precipitation distribution by day during June 2022



**Graph 3.** Precipitation distribution by day during July 2022

High air humidity and limited air circulation due to surrounding forest cover increase the risk of fungal diseases, especially in raspberry and blueberry production. Although prevailing northwestern winds provide some ventilation, canopy management remains crucial to reduce disease incidence.



**Graph 4.** Precipitation distribution by day during August 2022

Climate conditions in the Toponica area are suitable for fruit production, but the variability in precipitation distribution and the presence of shading require careful selection of species and management strategies. Species with moderate water and light requirements, such as plum and sour cherry, show the highest potential for extensive cultivation, while species more sensitive to drought and insufficient light will require irrigation and canopy management interventions Milošević and Milošević (2011). The use of hail nets, particularly colored ones, can influence the microclimatic conditions in the orchard by reducing fruit and leaf temperature, as well as affecting fruit physiology and coloration. Solomakhin and Blanke (2010) report that colored nets modify light and temperature within the canopy zone, influencing vegetative growth, fruit development, and fruit coloration.

### Soil Conditions

Soil characteristics in the study area are predominantly influenced by Vertisol (smonica), a humus-accumulative soil type with an A–C horizon profile, developed mainly over lacustrine and marl clays. The humus horizon is 50–100 cm deep, dark gray to black in color, and characterized by a heavy clay texture with low sand content. During dry periods, the soil exhibits pronounced shrinkage and cracking, which is typical for

Vertisol and may affect root development and soil aeration. The presence of small, scattered rock fragments (quartzite, argillite, andesite, marlstone) is minor and does not represent a limiting factor.

These physical properties make the soil suitable for plum, sour cherry, sweet cherry, walnut, hazelnut, and quince, which tolerate heavier soils when deep plowing and organic matter incorporation are applied. Species such as apple, pear, apricot, blackberry, and especially peach and raspberry require lighter, better-aerated soils (Dugalić and Gajić, 2012), while blueberry requires acidic, sandy substrates and therefore cannot be cultivated on Vertisol without substantial soil modification (Petrović et al., 2020).

Chemical analyses of soil samples taken from Parcels 1 and 2 are presented in Table 1. Both parcels show similar physicochemical characteristics, with pH values ranging from 5.8 to 6.1, which are generally suitable for most fruit species except blueberry, which requires a pH of 4–5. Humus content ranges from 3.58% to 3.82%, which is adequate for plum, sour cherry, sweet cherry, and hazelnut, but below the recommended values (4–5%) for apple, pear, apricot, peach, raspberry, blackberry, and blueberry. Total nitrogen values (0.18–0.19%) indicate medium availability but may limit growth in high-demand species.

**Table 1.** Chemical properties of the soil on the plots

	pH	Humus content (%)	N (%)	P <sub>2</sub> O <sub>5</sub> (mg/100g)	K <sub>2</sub> O (mg/100g)	CaCO <sub>3</sub> (%)
Parcel 1	5,80	3,58	0,18	1,18	16,07	0,00
Parcel 2	6,10	3,82	0,19	1,26	17,26	0,00

Available phosphorus levels (1.18–1.26 mg/100 g) are significantly below optimal values (5–10 mg/100 g), which may reduce yields, while potassium content (16.07–17.26 mg/100 g) is at the lower suitable boundary for plum, sour cherry, sweet cherry, apple, apricot, and peach, but insufficient for raspberry, blackberry, blueberry, and pear. Calcium carbonate was not detected, which is favorable for most fruit species and particularly beneficial for species sensitive to lime, including blueberry.

Overall, the soil conditions support the cultivation of plum, sour cherry, sweet cherry, walnut, hazelnut, and quince with moderate fertilization and organic matter application (Nekić, 2005). In contrast, apple, pear, apricot, peach, raspberry, blackberry, and especially blueberry require intensive soil improvement, including organic matter enrichment, phosphorus and potassium fertilization, and improved drainage. These measures are essential for achieving optimal growth and sustainable production outcomes in the study area (Mratinić, 2013).

### Orographic Conditions

Orographic factors, including elevation, slope, and exposure, directly influence microclimatic conditions and orchard design. The studied parcels are located at elevations of 341–376 m, which is within the optimal range for plum, sour cherry, apple, pear, quince, and walnut that commonly perform well up to 800–1000 m (Šoškić, 2016). Raspberry generally prefers slightly higher altitudes (400–800 m), but may be successfully cultivated at lower elevations with reliable irrigation systems (Petrović & Milošević, 2002). The hilly terrain also contributes to improved cold air drainage, thereby reducing the risk of late spring frost, which is particularly beneficial for sour cherry and pear (Šoškić, 2016).

Parcel 1 has a southwest exposure, while Parcel 2 is oriented toward the west. Southwest exposure provides favorable sunlight and heat accumulation, which is advantageous for plum, sour cherry, apple, and quince. In contrast, the west-facing exposure of Parcel 2 can increase afternoon heat stress, making it less suitable for raspberry, which performs better on cooler, northern slopes due to moderated soil temperatures (Keserović et al., 2016). When raspberry cultivation is considered on warmer exposures, supplemental irrigation and shading systems are recommended to prevent heat stress and moisture loss (Veličković, 2004).

Slope measurements indicate gradients of 7.2° on Parcel 1 and 9.5° on Parcel 2, which are acceptable for orchard establishment. While gentle slopes of 4–6° are generally optimal (Keserović et al., 2016), slopes up to 10° can support fruit cultivation when rows are aligned along contour lines to reduce erosion risk. On steeper sites, terracing or deep plowing along row direction may be required (Keserović et al., 2016). In the case of raspberry cultivation on sloped terrain, planting along contour lines is particularly important to prevent soil erosion (Petrović and Milošević, 2002). However, the observed slopes in this study do not require major land modification for most fruit species.

## Agroeconomic Conditions

Agroeconomic factors play a central role in determining the sustainability of fruit production in Toponica, particularly regarding establishment and maintenance costs, market accessibility, labor availability, and infrastructure. Local production traditions and existing demand influence the feasibility of different fruit species.

Market opportunities are most favorable for plum, sour cherry, and sweet cherry, which have stable local demand and established channels for processing into brandy, jam, dried fruit, and bakery ingredients. Plum yields of 15–20 t/ha with purchase prices of 0.3–0.5 €/kg can generate revenues of 4,500–10,000 €/ha, while sour cherry yields of 10–15 t/ha at 0.4–0.8 €/kg also provide viable returns. Apple and pear can generate higher revenues (10,000–20,000 €/ha), but require greater investments in irrigation and plant protection (Maksimović, 2021). Apricot and peach may yield similar revenues but are highly sensitive to frost and disease, increasing production risk.

Berry crops (raspberry, blueberry, and blackberry) have the highest earning potential, with revenues of 15,000–37,500 €/ha. However, they are also the most vulnerable to drought and disease and require reliable irrigation systems, intensive labor inputs, and access to cold storage. In the absence of local cooling and collection facilities, the risk associated with berry production increases significantly, limiting their economic feasibility. Walnut, hazelnut, and quince offer lower but steady yields and require minimal post-establishment labor, making them suitable for long-term, low-input production systems.

Labor is a critical cost factor. Manual harvesting of raspberry, blueberry, and blackberry may account for up to 40% of total production costs, whereas plum, sour cherry, sweet cherry, walnut, hazelnut, and quince require significantly less labor. Infrastructure limitations, including limited irrigation systems and challenging road access, further constrain intensive production. Although the electricity supply is sufficient for irrigation pumps, initial investments remain necessary.

State subsidies, while available, only partially support orchard establishment and generally do not cover irrigation systems or cold storage facilities, reducing the feasibility of high-input crops. Traditional species such as plum, sour cherry, and sweet cherry are more easily integrated into national support programs and local production systems.

## CONCLUSION

The analyzed parcels in Toponica are located at suitable elevations and slopes for fruit production, but the predominance of heavy Vertisol soil and the absence of irrigation significantly limit the range of viable fruit species. Due to irregular precipitation, drought risk, and sensitivity to frost, species such as raspberry, apricot, and peach are not recommended. Apple, pear, walnut, hazelnut, sweet cherry, and blackberry may be cultivated only with substantial investment in irrigation and soil improvement. Under current agroecological and economic conditions, plum and sour cherry represent the most suitable and sustainable options for orchard establishment on the studied site.

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## EXPLORING THE QUESTION OF BEHAVIORAL EUTHANASIA IN SERBIA

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*Behavioral euthanasia involves ending an animal’s life due to severe, unmanageable behavioral disorders conflicting with its welfare. While integral to animal welfare in developed countries, Serbia lacks legal regulation. A survey of 237 people showed low awareness and limited support. Respondents knew animal behavior basics but held conflicting views on aggression. Findings highlight Serbia’s need for education on animal behavior and its disorders.*

**Key words:** *behavior, euthanasia, animal welfare*

### INTRODUCTION

Behavioral euthanasia refers to the humane killing of an animal due to severe behavioral disorders that are unmanageable and incompatible with the animal’s welfare or public safety. Although recognized as part of animal welfare in many developed countries (Gibeault, 2021), Serbia currently lacks a legal and procedural framework for its application. (Animal Welfare Act, 2009)

The study aims to assess public awareness and attitudes toward behavioral euthanasia and general knowledge of animal behavior. Given the sensitivity and complexity of the topic, there is an emphasis on the need for public education and discussion in Serbia, where the issue remains largely unaddressed.

### MATERIAL AND METHODS

The primary aim of this study was to assess the level of public awareness and attitudes regarding behavioral euthanasia. Data were collected using a structured questionnaire consisting of 25 questions, with the final item allowing participants to provide additional comments or observations.

Except for the first and last items, all questions were closed-ended. The questionnaire included dichotomous, multiple-choice, Likert-scale, and importance-scale items. The first six questions gathered socio-demographic data such as age, gender, and educational level. Questions 7 through 16 (excluding question 11) focused on participants’ knowledge and opinions concerning euthanasia and behavioral euthanasia. Question 11 specifically assessed respondents’ understanding of animal welfare. The remaining questions examined general knowledge of animal behavior, including its principles and influencing factors.

The questionnaire was available online via the Google Forms platform and in both Serbian and Hungarian to ensure broader accessibility and inclusiveness of respondents.

The statistical analysis was conducted using Jamovi, an open-source software based on the R programming language.

### RESULTS AND DISCUSSION

A total of 237 participants completed the questionnaire. The largest number of respondents belonged to the 2003 birth cohort (n = 32; 13.19%), while the oldest participant was born in 1949 (76 years old) and the youngest in 2007 (18 years old).

Among all respondents, the majority were female (n = 151; 63.83%), followed by males (n = 85; 35.74%), while one participant (0.43%) preferred not to disclose their gender.

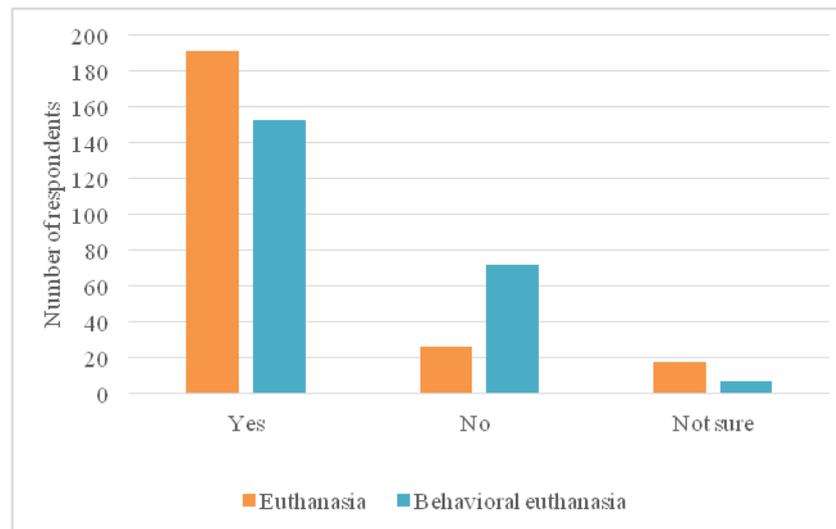
In terms of educational attainment, the majority of respondents (n = 132; 57.02%) had completed secondary education, followed by those with undergraduate or master’s university degrees, and a small number holding

doctoral degrees. Only four participants (1.69%) had completed only primary education. Furthermore, 22.5% of respondents had obtained or were pursuing medical or veterinary qualifications.

When asked to evaluate their fondness for animals on a five-point Likert scale (1 = does not like animals; 5 = likes animals very much), the majority (n = 169; 71.91%) identified as strong animal lovers, while only three respondents (1.27%) indicated that they did not like animals.

A large proportion of respondents (81.28%) were familiar with the general concept of euthanasia, while 11.06% had never heard of it, and 7.66% had heard the term but were uncertain of its meaning.

In contrast, awareness of behavioral euthanasia was markedly lower: 65.96% (n = 144) had never encountered the term, 3.02% (n = 7) had heard of it but were unsure of its meaning and 31.03% (n = 72) were familiar with the concept. Some participants noted that although they had not previously heard the term, they were able to infer its meaning from the words themselves.



**Figure 1.** Difference between knowledge about euthanasia and behavioral euthanasia

Among those familiar with the term, the primary source of information was the media (38%), followed by formal education, social contacts, and veterinarians; one participant (1%) cited ChatGPT as a source. Multiple answers were allowed, resulting in some overlap.

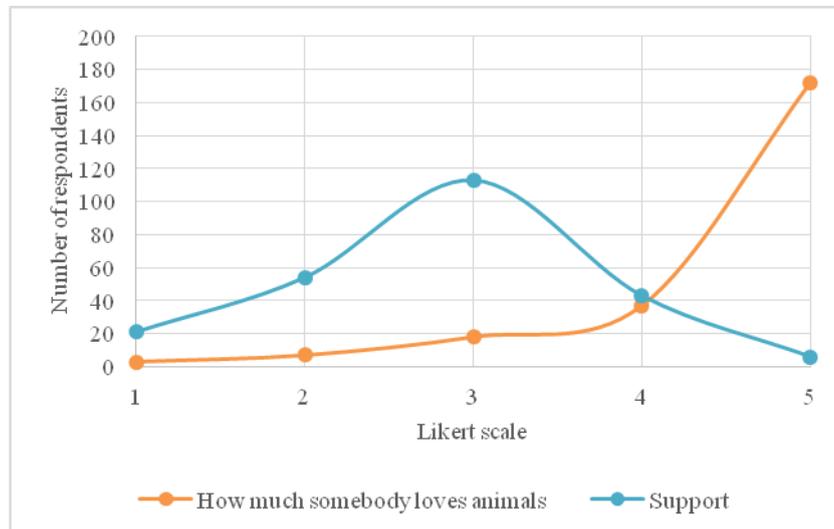
When asked to identify the definition of behavioral euthanasia, the majority (86.81%) correctly recognized it. Of these, 48.49% understood the definition but lacked deeper knowledge, 26.81% could accurately define the concept, 11.06% found it somewhat familiar but uncertain, and 13.19% had never heard of it.

A Chi-square test of independence was performed to examine the relationship between respondents' recognition of behavioral euthanasia as a concept and their ability to identify it based on its definition. The analysis revealed a statistically significant association between the two variables ( $\chi^2(8) = 112, p < .001$ ). This result indicates that individuals who had previously encountered or heard of the term were significantly more likely to recognize and correctly interpret its definition than those unfamiliar with the concept. The strong level of statistical significance suggests that prior exposure to the terminology plays a key role in shaping comprehension, implying that knowledge dissemination through education, professional training, or media exposure substantially improves understanding of behavioral euthanasia.

A separate Chi-square test was conducted to examine the relationship between educational level and knowledge of behavioral euthanasia ( $\chi^2(10) = 11.3, p = 0.338$ ). The analysis revealed no statistically significant association between the two variables, suggesting that awareness of the concept was not influenced by the respondents' level of formal education. This finding indicates that understanding of behavioral euthanasia is not necessarily linked to general educational attainment, but rather may depend on exposure to specific fields—such as veterinary medicine, animal welfare, or behavioral sciences—where the term and its ethical implications are more commonly discussed. The absence of a significant relationship also underscores the need for broader educational outreach and public communication beyond formal academic contexts. When asked which behavioral disorders could justify euthanasia, aggression was identified most frequently (49.15%), followed by compulsive, destructive, and inappropriate elimination behaviors. A minority (14.49%) believed that no behavioral disorder should result in euthanasia, while two respondents (0.8%) were unable to answer.

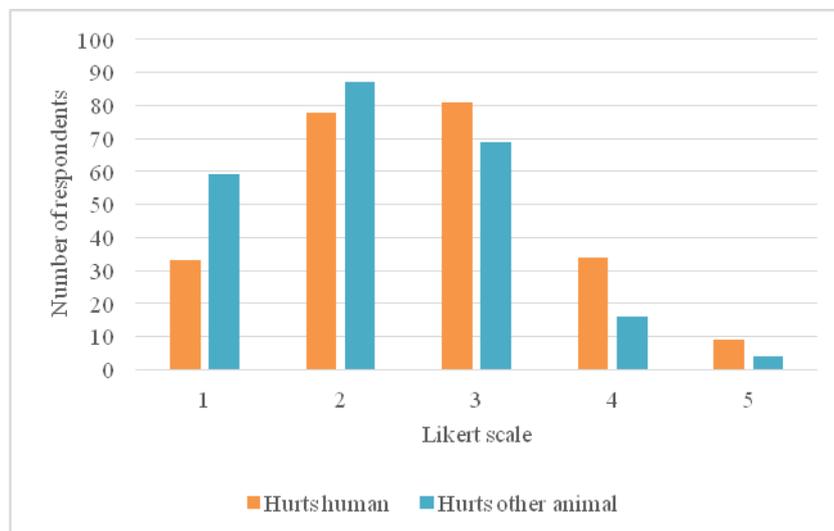
Support for behavioral euthanasia was generally neutral, with 47.33% (n = 111) expressing neutrality, 31.91% opposing it, and 20.85% supporting it to some extent. 72.77% of respondents were unaware of the legal status of behavioral euthanasia in Serbia; 16.37% believed it to be legal, while 11.06% thought it was illegal.

To explore potential differences in attitudes, a Mann–Whitney U test was conducted to compare levels of support for behavioral euthanasia between respondents with and without a health-related educational background. The analysis revealed a statistically significant difference between the two groups ( $U = 4175$ ,  $p = 0.033$ ,  $r_b = 0.176$ ). Although the effect size was small, participants with medical or veterinary training tended to show slightly higher levels of support for behavioral euthanasia than those without such education, suggesting that professional familiarity with animal health and welfare issues may influence attitudes toward this ethically sensitive practice.



**Figure 2.** Correlation between loving animals and supporting behavioral euthanasia

When asked whether euthanasia was justified if an animal had injured a human, 47.23% opposed it, 34.47% said it depended on the situation, and 18.3% supported it. However, when the same question referred to animals that had harmed another animal, opposition increased significantly to 62.13%, while only 8.51% expressed support.



**Figure 3.** Difference between respondents' answers when behavioral euthanasia should be used if the animal hurts a human or another animal

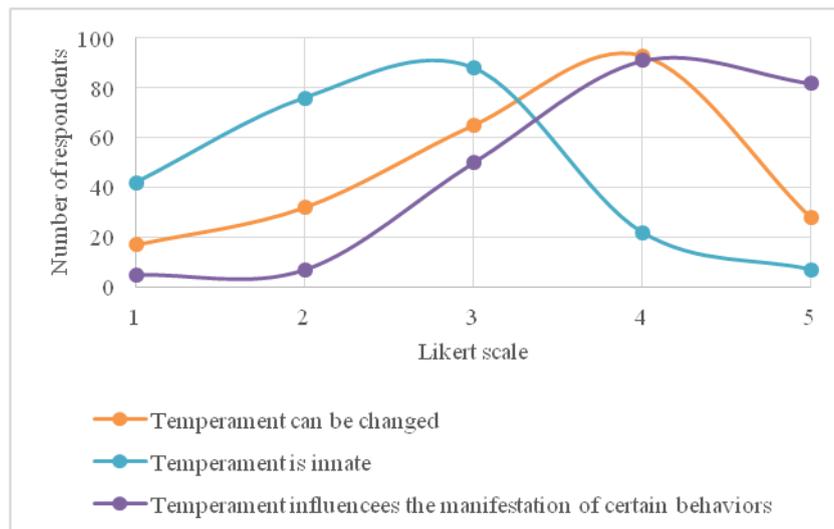
When asked whom behavioral euthanasia benefits, one-third (32.77%) believed it helps both humans and animals, 26.81% thought it helps humans primarily, 8.09% said it benefits animals primarily, and 16.6% believed it benefits no one and should not be performed. The remaining 15.74% could not decide.

More than half of respondents (55.32%) believed that animal behavior depends equally on genetics and experience, while 33.19% attributed it mainly to life experience, and 4.68% to genetics. A small proportion

(3.4%) believed behavior is determined solely by the environment, and only 1.28% believed it is determined exclusively by genetics.

The majority (88.09%) understood the concept of temperament, showing higher familiarity than with euthanasia itself. 4.68% had heard of the term but were uncertain of its meaning, while 7.23% had never encountered it.

When asked whether temperament is innate (1 = does not agree with the statement at all; 5 = agrees with the statement completely), most respondents were neutral (37.44%), though the remainder leaned toward viewing temperament as an inborn rather than an acquired trait. Regarding temperament’s modifiability, 39.57% believed it to be mostly changeable, with fewer neutral responses compared to the previous item. A majority also agreed that temperament influences the expression of behavioral patterns (38.72% “mostly agree,” 34.89% “strongly agree”).



**Figure 4.** Answers to different statements about temperament

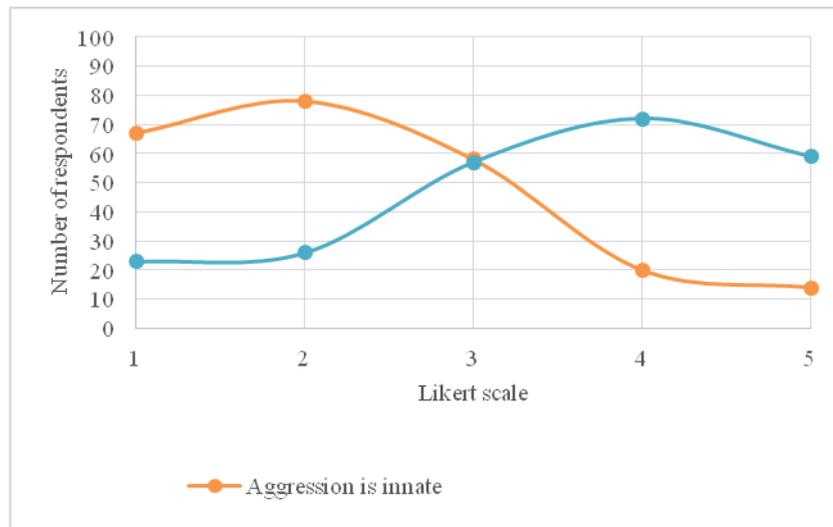
Most respondents (61.27%) believed that aggression is not innate, with 28.51% completely and 32.76% mostly rejecting the idea of inborn aggression. However, a notable inconsistency emerged in responses to the final statement: while most believed aggression is environmentally shaped, a large portion (30.64% mostly agree; 25.11% strongly agree) also believed that some breeds are more predisposed to aggression than others. This contradiction suggests limited understanding of the interplay between genetics, environment, and behavioral expression. Participants who believe aggression isn’t innate are also significantly more likely to believe that some breeds are predisposed to aggression. This suggests a consistent tendency to attribute aggression to genetic or inherent factors rather than environmental or experiential ones.

The findings demonstrate that public knowledge of behavioral euthanasia in Serbia is limited, and the concept remains ethically and emotionally controversial. Although general familiarity with euthanasia is high, understanding of behavioral euthanasia is weak, reflecting the absence of legal and educational frameworks.

Respondents displayed high concern for animal welfare, prioritizing physical needs and safety, yet showing uncertainty regarding behavioral freedoms. Attitudes toward euthanasia were generally neutral or negative, indicating emotional resistance and possibly lack of understanding of situations in which behavioral euthanasia may be justified.

While participants exhibited good awareness of animal behavior concepts, contradictory views on aggression and temperament highlight incomplete behavioral literacy. Many acknowledged both environmental and genetic influences but failed to integrate these perspectives coherently.

Overall, the data underscore the urgent need for educational initiatives in Serbia focusing on animal behavior, behavioral disorders, and humane decision-making, including the ethical and practical considerations surrounding behavioral euthanasia.



**Figure 5.** Answers to 2 different statements about aggression

### CONCLUSION

The results of this study demonstrate that public awareness and understanding of behavioral euthanasia in Serbia remain limited. While most respondents were familiar with the general concept of euthanasia, few had encountered or correctly defined behavioral euthanasia. The findings reveal a predominantly neutral or negative attitude toward its application, reflecting emotional resistance and the absence of clear legal and ethical guidelines within the country.

Participants showed a relatively good level of knowledge about animal behavior and expressed strong concern for animal welfare, particularly regarding the prevention of hunger, pain, and fear. However, inconsistencies in responses – especially those relating to aggression and its innateness – suggest that understanding of behavioral causes and their management remains fragmentary and often contradictory.

The results highlight the importance of education and public engagement on animal behavior, behavioral disorders, and welfare ethics. It is essential to introduce professional standards and legal frameworks that would guide veterinarians and animal owners in making informed, humane decisions regarding behavioral euthanasia. Addressing these gaps would contribute not only to animal welfare but also to greater social awareness and responsible animal care in Serbia.

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## **EVOLUTION OF PRODUCTIONS OBTAINED ON A FARM IN WESTERN ROMANIA, OVER A PERIOD OF THREE YEARS, DEPENDING ON METEOROLOGICAL RISK FACTORS**

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*In this paper, we wanted to highlight the evolution of production of the main crops grown in the area of Seleus commune, Arad county, in the western part of Romania, over a period of three years, between 2022 and 2024. To present this study, we took into account the meteorological data provided by the Banat Crisana Regional Meteorological Center in Timisoara, data that were correlated with the productions obtained.*

**Key words:** temperate climate, multiannual average, rainfall regime, absolute maximum temperature

### **INTRODUCTION**

The western part of Romania, like the entire country, is located in the temperate zone due to its geographical position, being exposed to a wide range of risk weather and climate phenomena with the potential to occur throughout the year (1, 2, 7). The multiannual average air temperature is 10.6°C, the warmest month is July, with a value of 20.7°C, and the coldest is January, with a value of -1.1°C. Winters are generally moderate, without severe frosts due to the western climatic influence and due to the fact that the area has a more sheltered position from the invasions of polar-continental air from the east and northeast. In spring, under the influence of the western circulation and the extension of the Azores anticyclone ridges over southern Europe, spring and summer occur faster than in the eastern part of the country. In the summer months, the air temperature is not too high, due to the western influences. In autumn, the multiannual averages of these months vary between 16.2°C in September and 6.2°C in November. This temperature drop is due to the advection of cold air under the action of the Euro-Asian anticyclone ridge (8). The area is frequently subjected to invasions of warm air from the southwestern sector, which makes the temperature 1°-2° higher than in the eastern part of the country in this season. Summer days occur from April to October inclusive, with the highest frequency in July and August (8,9,10,11). Tropical days occur more in the summer months, with an average annual number of approx. 30 to 40 days in conditions of atmospheric drought, during these days damage to agricultural crops can occur, and in recent years this number of days has increased significantly (12,13,14).

### **MATERIAL AND METHODS**

The experiments of this work were carried out in the Seleuş commune, a locality located on a relief whose morphological characteristics are similar to the Banat Plain. The locality is located at about 110 m altitude and is included in the Criş Plain, which was born by the deposition of sediments transported by the Crişul Alb River, on the edge of the old Pannonian lake. In order to correlate the production data with certain meteorological risk indicators, we took into account the meteorological data obtained from the Banat Crişana Regional Meteorological Center, in Timisoara, for the meteorological station in Chisineu Criş, Arad county (5, 6, 7, 15, 16). Following the analysis of these risk aspects that occurred during the three years presented, in the interval 2022-2024, the production results obtained for the crops specific to the analyzed area were also presented (3, 4, 14, 15, 16).

## RESULTS AND DISCUSSION

The climate in this western region of Romania is moderate continental, with weak Mediterranean influences. The altitude in this relatively small area, on average 110 m, is climatically distinguished by more attenuated differences in temperatures in the succession of seasons, in a uniform annual distribution of dynamic elements and in a homogeneous distribution of solar radiation. As a result of the increase in the intensity of solar radiation in the warm period of the year and the predominance of clear weather, the air temperature registers high values, the monthly average exceeding 21°C, while the annual temperature value is 12.4°C. The absolute maximum temperature recorded in this area of Romania was 37.4°C; while the absolute minimum temperature recorded was -16.5°C.



**Figure 1.** Geographical position of the town of Seleuș in Arad County

For the climatic characterization of the studied area, local records of the meteorological station in Arad were used, and the table below shows the rainfall regime over a period of 60 years, and table 2 shows the wind regime.

**Table 1.** Rainfall regime (l/mp)

Months	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Total
	29.5	42.1	32.9	49.8	72.1	94.3	57.7	61.1	55.3	37.5	41.4	52.0	625.6

On the ground, the highest frequency is the winds from the southeast and the north, and the winds from the west also have a fairly high frequency, which are more than twice as frequent as those from the east.

**Table 2.** Aeolian regime

	N	NE	E	SE	S	SV	V	NV	Calm
Average speed (m/s)	4.4	3.33	2.7	6.5	3.7	3.4	4.0	4.1	-
Frequency %	12.4	7.0	3.8	13.7	13.0	10.1	8.0	10.7	21.3

Our family has been involved in agriculture for more than twenty years and we work 200 ha, of which 30 ha are our property, the difference of 170 ha being rented. On our farm we have three tractors of different power, a combine, a straw seeder, precision seeders, three plows, a disc, a combine and a vegetable waste shredder. The land I work is located in the Seleus area, and as a credit rating on a scale from 1 to 10, the worked land is at level 6. We also own an area of 0.3 ha of solariums that we cultivate intensively, with the following crops: green onions, zucchini, celery, and in early spring I cultivate an area with lettuce and tomatoes.

The structure of the crops on the 200 ha cultivated is as follows: wheat 70 ha, sunflower 30 ha, corn 40 ha and rapeseed 60 ha. I have been cultivating sunflower since 2022, with the other three crops being present every other year since the farm was founded. Of the three years considered, between 2022 and 2024, 2024 was the driest, while excess precipitation was in the first five months of 2023. In 2022, in September we had a record excess of 148.9 l/m<sup>2</sup>, but also a dry month during the summer, in June only 3.5 l/m<sup>2</sup> were recorded. The year 2022 was characterized by normal values in terms of temperature, with small exceptions being positive

temperatures in winter, with an average of 0.4°C recorded in January, and the summer was very hot, with an average of 24.8°C, in July, while in August a monthly temperature value of 23.8°C was recorded. In July 2022, values of 33°C to 34°C were reported. We must point out that this year late spring frosts were reported, in the last decade of March, more precisely between 23.03.2022- 27.03.2022.



**Figure 2.** Crops in July and August 2023

The structure of crops on our farm, during the study period of 2022 - 2024, is illustrated in the table below, which also presents the productions obtained during this int Tab.3 Production obtained from the main crops grown in the period 2022-2024erval.

**Table 3.** Production obtained from the main crops grown in the period 2022-2024

<b>Crop/production</b>	<b>2022</b>	<b>2023</b>	<b>2024</b>
corn	6000 kg/ha	8800kg/ha	3500 kg/ha
rapeseed	3400 kg/ha	3480 kg/ha	3300 kg/ha
wheat	6700 kg/ha	5200 kg/ha	5500 kg/ha
sunflower	3200kg/ha	1600kg/ha	2500 kg/ha

In 2023, it rained enough in the first five months, the hectoliter weight of wheat decreased and that is why we had a lower production compared to 2022. In the first four months, over 400 l/m<sup>2</sup> were recorded, which was a pluviometric excess for this period, a record value in the last ten years. There were days when it rained 30 - 35 l/m<sup>2</sup>, in five days, in March and over 30l/m<sup>2</sup> were recorded in 4 days in April. On July 21, 2023, a strong storm was recorded in our area. It rained 39.l/m<sup>2</sup>, but this rain was followed by the entry into our region of a front accompanied by clouds with high vertical extension, of the Cumulonimbus type, clouds accompanied by heavy hail. This phenomenon has compromised certain productions, especially in the sunflower crop, where we obtained a production between 1.500 kg/ha and 1.600 kg/ha. It is worth noting that I have only been cultivating sunflowers since 2022, when I achieved an average production of 3.200 kg/ha, and in rapeseed 3.400 kg/ha.

In the last year presented in the paper, we can state that it was the most unfavorable in terms of corn production, only an average of 3.500 kg/ha, compared to 8.800 kg/ha, a value obtained in 2023, and 6000 kg/ha, in 2022. This difference was affected by the drought in the spring months and unfavorable conditions. Due to the rainfall regime recorded in the last months of 2023, the wheat and rapeseed production in 2024 was similar to that obtained in 2023.

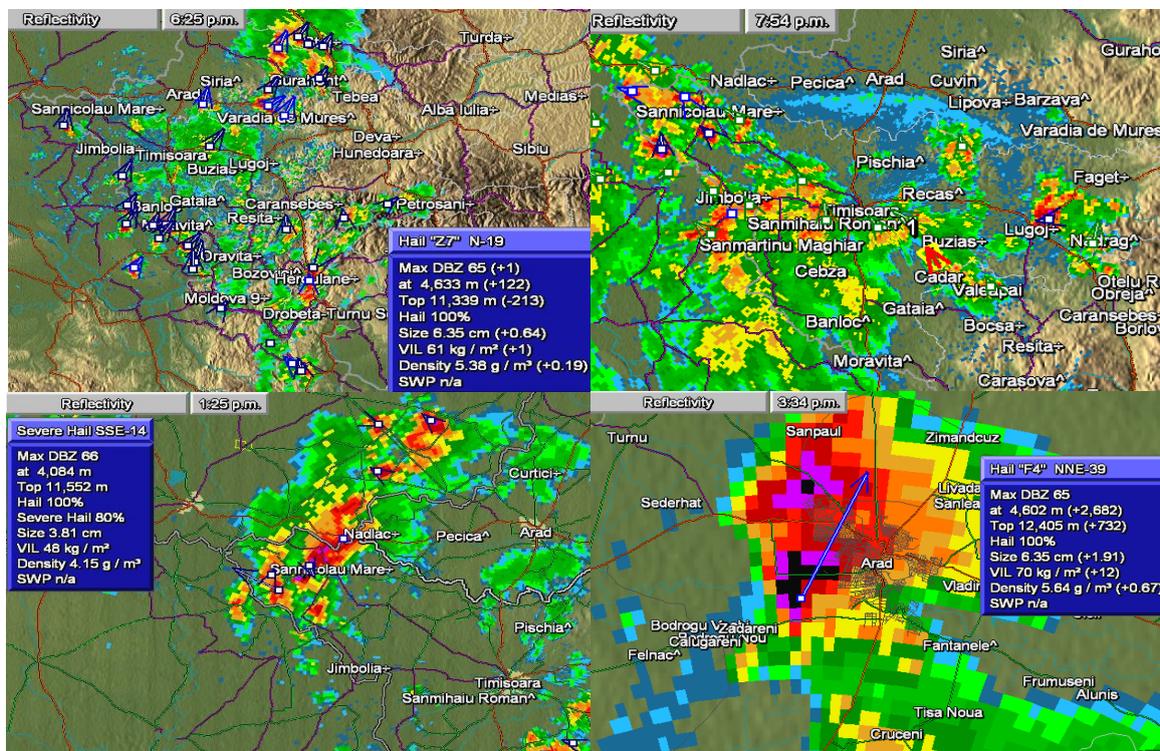
The production obtained in the solar did not fluctuate much during the three years, because we have adequate irrigation conditions to ensure the plants the amount of water. For green onions, we obtained an average of 75.000-80.000 /bundles of five pieces, for tomatoes, we obtained an average of 8.000 kg for each solar, that is, on an area of 10 ares, in 2023. In the last year studied, tomato production decreased to 6.500 kg, due to the temperature differences recorded in May, conditions that affected the plant from the beginning of planting and stressful development.



**Figure 3.** Images from 2023 (wheat crop) and 2024 (sunflower crop)

With the help of the meteorological radar located within the Banat Crisana Regional Meteorological Center, the main direction of evolution of the frontal systems affecting the west of Arad County was identified. The first is the western atmospheric circulation and which corresponds to the general circulation at the level of Romania. Such circulation usually affects the entire western area of the country, from west to east.

The second component in importance is the southern atmospheric circulation which, although initiated south of the western region of Romania, tends to develop in our area of interest. The amplification of convection can be attributed to the relative humidity input coming from the Mureș River and the forests to the south, through the evapotranspiration process.



**Figure 4.** Convective systems with south-north movement and their amplification in the western area of Romania (direction of movement with white arrow)

The wind rotates in the atmosphere on a vertical axis, being correlated with the convection movements of the air; it is accompanied by black storm clouds, Cumulus and Cumulonimbus clouds. The air vortex rises from

the earth's surface to the level of the clouds, this definition by Alfred Wegener, from 1917, is still valid today. Tornadoes produced in the temperate zone have a weaker intensity and are less frequent, with approximately 10 tornadoes/year occurring annually in Romania, due to the decrease in centrifugal force and the increase in the Coriolis force. Following detailed meteorological studies related to the climatology of tornadoes, without specifying their intensity, the presence of tornadoes was also found in the western part of Romania, even in Timis and Arad counties, the study interval being over a period of more than a hundred years.

## CONCLUSION

In 2023 it rained a lot and the hectoliter weight decreased and that is why we had a lower wheat production. In the first four months, over 400 l/m<sup>2</sup> were recorded, which was a pluviometric excess for this period, in the last ten years. There were days when it rained 30 - 35 l/m<sup>2</sup>, in five days. On July 21, 2023, it rained 39 l/m<sup>2</sup>, but this rain was followed by the entry into our area by a front accompanied by clouds with high vertical extension, of the Cumulonimbus type, clouds accompanied by heavy hail, a phenomenon that compromised certain productions, in particular in the sunflower crop, where we obtained a production of 1.400 kg/ha and 1.500 kg/ha.

In the last year presented in the paper, we can state that it was the most unfavorable in terms of corn production, only an average of 3.500 kg/ha, compared to 8.800 kg/ha, a value obtained in 2023, and 6.000 kg/ha, in 2022. This difference was affected by the drought in the spring months and unfavorable conditions. Due to the rainfall regime recorded in the last months of 2023, the wheat and rapeseed production in 2024 was similar to that obtained in 2023.

Regarding the production obtained in the solar for the three years, we did not obtain representative differences, for green onions this being 75.000 -80.000 / bundles of five pieces. For tomatoes, for each plot we obtained a quantity of 8.000 kg, only in 2024 the quantity was 6.500 kg, the cause being the unfavorable thermal conditions from the beginning of planting.

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# ADAPTATION OF ORNAMENTAL SPECIES TO SPECIFIC PEDO-CLIMATIC CONDITIONS OF CARAȘ-SEVERIN COUNTY, ROMANIA

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*This study investigates the adaptation of ornamental plant species to the pedo-climatic conditions of Vărădia locality, Caraș-Severin County, Romania. The research focuses on soil resources, climatic factors, and the adaptability of selected ornamental species under local conditions. Soil samples were analyzed for texture, pH, humus content, and nutrient availability, while observations were made regarding plant growth, health, and morphological adaptations. The results show that soil properties, including slightly acidic to neutral pH, medium humus content, and adequate drainage, significantly influence species performance. Climatic factors, such as mean annual temperature, precipitation patterns, and seasonal variations, also play a critical role in the survival and ornamental value of these plants. Species such as *Rosa* spp., *Lavandula angustifolia*, and *Acer platanoides* exhibited differential responses to soil fertility and microclimatic variations, with morphological adaptations such as leaf thickness, flowering period adjustment, and root system modifications enhancing their survival. The study provides practical insights for urban and peri-urban landscaping, emphasizing the importance of matching species to site-specific pedo-climatic conditions. These findings contribute to sustainable ornamental horticulture practices in southwestern Romania and may serve as a model for similar ecological contexts in Europe.*

**Keywords:** ornamental species; adaptability; soil type; pedo-climatic conditions; fertility; PCA; Caraș-Severin

## INTRODUCERE

Soil is one of the most important natural resources of terrestrial ecosystems, providing support for plant life and contributing to environmental stability (Lal, 2020). The physicochemical properties of the soil, such as texture, structure, pH, humus content and degree of fertility, directly influence the growth, development and adaptability of plant species (Brady & Weil, 2019; Roberts, 2017). In the current context of climate change, the adaptation of ornamental plants to local pedo-climatic conditions has become a major priority in urban and peri-urban landscaping (Smith et al., 2015; Müller & Klein, 2016).

At the European level, numerous studies have highlighted the importance of correlating soil type and climatic factors with the ornamental performance of cultivated species (Brown & Davis, 2018; Turner & Wright, 2014; White & Black, 2017). The results show that variations in pH, organic matter content, and nutrient availability cause significant differences in phenology, leaf color intensity, and flowering capacity of decorative plants (Thomas, 2016; Popa, 2018).

In Romania, studies on the adaptability of ornamental species to local soil and climatic conditions have undergone significant development in the last two decades (Nicolae et al., 2018; Popescu, 2017; Ionescu et al., 2020). The regions in the south-west of the country, especially Caraș-Severin County, are characterized by a great pedological diversity from clayey, well-drained soils to vertosols and gleiosols with temporary excess moisture which requires careful selection of species for green spaces (Stan, 2016; Ionescu & Nicolae, 2018).

Numerous studies have shown that the morphological and physiological adaptation of ornamental species is directly related to their ability to make efficient use of available soil resources (Davis & Green, 2015; Green & Brown, 2015; Popa & Stan, 2017). For example, the species *Lavandula angustifolia* and *Buxus sempervirens* show good resistance to slightly alkaline soils and short periods of drought, while *Hydrangea macrophylla* and

*Betula pendula* prefer slightly acidic soils rich in organic matter (Miller & Jackson, 2014; Gheorghe & Petrescu, 2020).

In Caraș-Severin, the diversity of soils, influenced by the varied relief (hills, meadows and hilly areas), favors the formation of several types of soil: preluvosols, vertosols, cambisols and alluvial soils (Ionescu et al., 2020). These determine, together with the climatic particularities (average annual temperatures of about 11.5°C and precipitation of 680 mm), a mosaic of favorable conditions for numerous horticultural and ornamental species (Popescu, 2017; Nicolae et al., 2018).

The purpose of this paper is to evaluate the degree of adaptability of ten representative ornamental species (*Rosa spp.*, *Lavandula angustifolia*, *Acer platanoides*, *Buxus sempervirens*, *Lonicera japonica*, *Forsythia suspensa*, *Hydrangea macrophylla*, *Betula pendula*, *Tulipa spp.* and *Chrysanthemum spp.*) depending on the type of soil predominant in Vărădia, Caraș-Severin county.

The main objectives of the study are: characterization of the physicochemical properties of the main soil types; evaluation of the ornamental performance and adaptability of the selected species; determining the correlations between soil factors and growth parameters; statistical analysis of species suitability through ANOVA, Pearson and PCA correlations; formulating recommendations on the selection of ornamental species suitable for different pedo-climatic conditions in south-western Romania.

Through these results, the research contributes to the foundation of a sustainable approach to local landscaping, based on the knowledge of soil resources and the correct adaptation of plant species to their natural environment.

## MATERIAL AND METHODS

The study was carried out in Vărădia, Caraș-Severin county, located in southwestern Romania, within the Oravița Depression, a relief unit characterized by an alternation of meadow, plain and low hill areas. The altitudes vary between 125 and 365 m, and the land presents a slight fragmentation, favorable to a significant pedological diversity.

The climate has a moderate temperate-continental character, with an average annual temperature of 11.5°C and average annual rainfall of about 680 mm. Winters are relatively mild, and summers are hot, with moderate periods of drought. From a phytogeographical point of view, the area falls within the subarea of oak forests (*Quercus spp.*), with the natural presence of the species *Fagus sylvatica*, *Tilia tomentosa*, *Fraxinus excelsior*, *Acer campestre* and *Rosa canina*, which indicates a good natural fertility of the soils.

### 1. Soil characterisation and sampling

At the level of the administrative territory of Vărădia commune, six dominant soil types have been identified:

Preluvosol (25.4%)

Alluviosol (18.3%)

Vertosol (19,3%)

Gleiosol (5.0%)

Eutricambosol (3.1%)

Stagnosol (3,0%)

For each type of soil, samples were taken from the 0–20 cm layer, corresponding to the active area of development of the root system of the ornamental species.

The laboratory determinations were carried out according to the standard methodologies used by OSPA Caraș-Severin and in the specialized literature (Florea & Munteanu, 2012; Ionescu et al., 2020). Soil drainage was evaluated in the field, based on the observation of groundwater depth, texture and structure of the soil profile.

### 2. Species analysed and biological observations

Ten ornamental species with high decorative value were selected, widespread in urban and peri-urban green spaces in the south-western region of Romania: *Rosa spp.*, *Lavandula angustifolia*, *Acer platanoides*, *Buxus sempervirens*, *Lonicera japonica*, *Forsythia suspensa*, *Hydrangea macrophylla*, *Betula pendula*, *Tulipa spp.* and *Chrysanthemum spp.*

For each species, observations were made regarding: increase in height and diameter, foliage density, the degree of flowering, general vigor and plant health, morphological and phenological adaptations (adjustment of the flowering period, leaf color, drought resistance).

### 3. Statistical data processing

The statistical analysis aimed to identify the relationships between soil properties and the degree of adaptability of each species.

For each species-soil type combination, five replications were performed, and the values obtained (suitability scores 1–5) were analyzed as follows:

Analysis of variance (ANOVA) was used to test for significant differences between soils for each species;

Standard deviation (SD) was calculated for each experimental group, to highlight the variability of plant response;

Pearson(r) correlation was determined between the main soil properties (pH, humus, N, P, K, drainage) and the mean adaptability scores;

Principal component analysis (PCA) was applied to the average species–soil matrix to identify ecological groupings and soil preference gradients.

The results were summarized in the form of tables and graphs, presenting the mean values, standard deviations, statistical meanings ( $p < 0.05$ ), correlations between variables and the representation of the PCA.

All analyses were performed using dedicated statistical packages (Python 3.11 – *scipy*, *pandas*, *scikit-learn*, *matplotlib*), ensuring the accuracy of the calculation and the correct interpretation of the relationships between the parameters.

## RESULTS AND DISCUSSIONS

### 3.1. Soil properties of the studied area

The territory of Vărădia commune in Caraș-Severin county is characterized by a high pedological diversity, determined by variations in relief, texture and water regime. The soil analyses carried out highlighted the following dominant types of soil: Preluvsol, Aluviosol, Vertosol, Gleiosol, Cambisol and Stagnosol, which occupy different proportions of the agricultural area of the locality.

The average values determined for the chemical and physical parameters of the soil are in favorable intervals for the growth of ornamental species (Table 1). The reaction of soils varies between slightly acidic and weakly alkaline (pH 6.0–7.8), which gives good ecological plasticity. The humus content is moderate (2.6–3.8%), and the levels of total nitrogen (850–1100 mg/kg), mobile phosphorus (35–55 mg/kg) and mobile potassium (160–200 mg/kg) ensure medium to good fertility.

The Preluvsol and Eutricambisol soils have the most balanced physicochemical composition, with moderate to good drainage and a clayey texture, favorable to the development of deep root systems. In contrast, Vertosols and Gleiosols have poor drainage, heavy texture, and variable clay content, which limits soil aeration and can reduce the adaptability of species sensitive to excess moisture.

Such pedological differences explain the variations observed in the behavior of ornamental species cultivated on the studied lands, confirming the results reported by Popescu (2017), Nicolae et al. (2018) and Ionescu et al. (2020) regarding the direct influence of soil properties on the decorative performance of plants.

### 3.2. Adaptability of ornamental species to soil types

The results obtained from the comparative analysis of the ten ornamental species on the six soil types are presented in Table 1 (*Mean suitability*  $\pm$  *SD*; *ANOVA p-value*). The values of the suitability scores vary between 2.6 and 4.8, which reflects good adaptability for most of the species analyzed.

**Table 1.** Mean suitability ( $\pm$ SD) of ornamental species across soil types (ANOVA  $p < 0.05$ ).

Species	Aluviosol (mean)	Cambisol (mean)	Gleiosol (mean)	Preluvsol (mean)	Stagnosol (mean)	Vertosol (mean)
Acer platanoides	3.293	4.292	3.537	4.469	3.900	3.010
Betula pendula	1.042	2.468	3.229	2.166	3.171	1.150
Buxus sempervirens	4.084	3.990	1.891	3.973	1.696	1.809
Chrysanthemum spp.	3.428	4.666	2.809	4.879	3.302	2.343
Forsythia suspensa	3.691	4.664	3.263	4.966	3.656	2.581
Hydrangea macrophylla	1.059	2.795	2.992	2.292	3.368	1.062
Lavandula angustifolia	4.279	2.558	1.070	2.983	1.112	1.859
Lonicera japonica	3.737	4.802	3.069	4.857	3.282	2.287
Rosa spp.	3.634	4.659	2.929	4.981	3.261	2.100

Tulipa spp.	3.962	4.109	2.480	4.739	2.650	2.530
Species	Aluviosol (SD)	Cambisol (SD)	Gleiosol (SD)	Preluvosol (SD)	Stagnosol (SD)	Vertosol (SD)
Acer platanoides	0.201	0.226	0.262	0.204	0.160	0.427
Betula pendula	0.083	0.290	0.228	0.140	0.293	0.251
Buxus sempervirens	0.181	0.391	0.194	0.162	0.221	0.125
Chrysanthemum spp.	0.109	0.152	0.249	0.170	0.162	0.329
Forsythia suspensa	0.302	0.147	0.255	0.076	0.300	0.258
Hydrangea macrophylla	0.084	0.252	0.170	0.191	0.443	0.088
Lavandula angustifolia	0.244	0.176	0.115	0.290	0.120	0.204
Lonicera japonica	0.315	0.227	0.288	0.200	0.252	0.210
Rosa spp.	0.205	0.236	0.163	0.027	0.150	0.230
Tulipa spp.	0.248	0.317	0.209	0.216	0.309	0.395

The highest adaptability values were recorded on Preluvosol and Aluviosol soils, where most species showed vigorous growth, dense foliage and a quality ornamental appearance. These soils, characterized by a clayey texture and a weak acidic to neutral reaction, provided optimal conditions for *Rosa spp.*, *Lavandula angustifolia*, *Acer platanoides* and *Buxus sempervirens*.

In contrast, Vertosol and Stagnosol soils, with poorer drainage and poor alkaline reaction, recorded the lowest suitability scores, especially for *Hydrangea macrophylla*, *Betula pendula* and *Tulipa spp.*, species sensitive to excess water and poor aeration.

The analysis of variance (ANOVA) indicated significant differences ( $p < 0.05$ ) between soil types for all species, demonstrating the existence of clear ecological preferences. In particular, *Lavandula angustifolia* and *Buxus sempervirens* have been shown to be more ecologically stable with low standard deviations ( $SD < 0.25$ ), adapting well to soils with neutral to low alkaline pH.

Based on the average values and the observed variations, three categories of ecological behavior were outlined:

Species with high adaptability (*Rosa spp.*, *Lavandula angustifolia*, *Buxus sempervirens*, *Acer platanoides*) – tolerated on most soils, preferring neutral conditions and good drainage;

Species with moderate adaptability (*Lonicera japonica*, *Forsythia suspensa*, *Chrysanthemum spp.*, *Tulipa spp.*) – adapted to clay and alluvial soils, but sensitive to excessive moisture;

Species with low adaptability (*Hydrangea macrophylla*, *Betula pendula*) – require acidic, humus-rich soils with constant moisture.

These results confirm the conclusions reached by Miller & Jackson (2014) and Gheorghe & Petrescu (2020), which emphasize the importance of selecting ornamental species according to soil texture and reaction to ensure the stability and aesthetic value of plantations.



Lavandula angustifolia



Buxus sempervirens



Acer platanoides



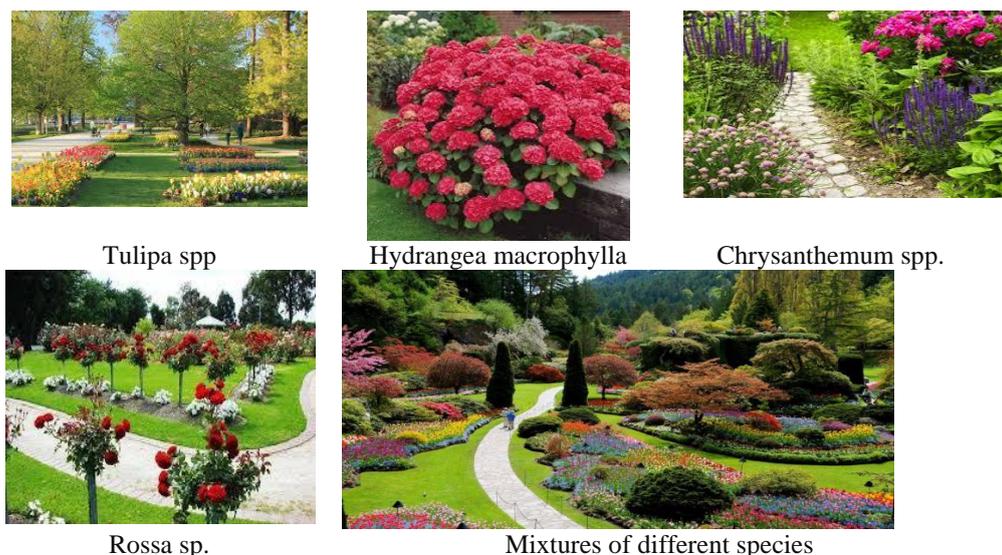
Forsythia suspensa



Betula pendula



Lonicera japonica

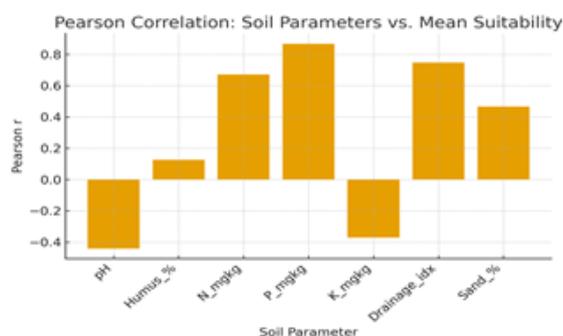


### 3.3. Correlations between soil parameters and species adaptability

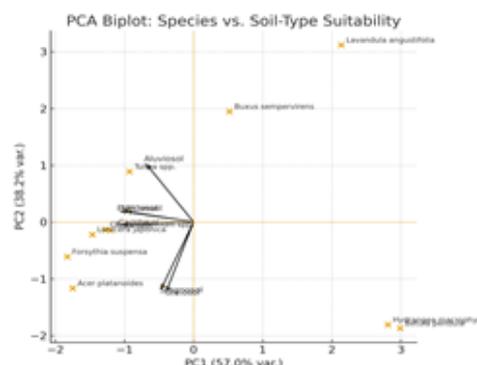
Pearson's correlation analysis (Figure 1) revealed a significant positive relationship ( $r = 0.71$ ;  $p < 0.05$ ) between soil fertility (N, P, K, humus content) and mean adaptability scores. Also, moderate correlations were identified between soil drainage and species performance ( $r = 0.56$ ), which confirms the importance of the water regime in the development of ornamental species.

The parameters with a negative influence were the high pH ( $>7.5$ ) and the too heavy (clayey) texture, which reduce the absorption of nutrients and limit root growth, an aspect observed in *Hydrangea macrophylla* and *Betula pendula*.

Principal component analysis (PCA) allowed species to be ordered according to their ecopedological preferences (Figure 2). The first two main components explain 68.8% of the total variability (PC1 = 41.2%; PC2 = 27.6%).



**Figure 1.** Pearson correlation between soil parameters and mean suitability



**Figure 2.** PCA biplot (Species × Soils) – distribution of species by ecological preference.

PC1 is positively correlated with drainage and humus content, representing a gradient of soil fertility and aeration.

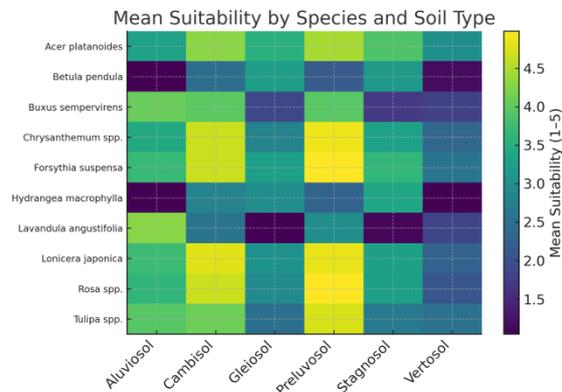
PC2 reflects the variation of soil reaction (acid–alkaline) and the availability of phosphorus and potassium.

The species *Lavandula angustifolia* and *Buxus sempervirens* are located in the positive quadrant of PC1 and PC2, associated with well-drained and slightly alkaline soils (Aluviosol, Cambisol). In opposition, *Hydrangea macrophylla* and *Betula pendula* are positioned on the negative axes, associated with acidic soils rich in organic matter (Preluviosol, Gleiosol).

The PCA representation confirms the results obtained by ANOVA and Pearson's correlations, providing a synthetic picture of species-specific soil preferences.

The heat map (Figure 3) also highlighted the average adaptability values (1–5), visually illustrating the grouping of species by soil type and ecological stability.

The results are in line with the conclusions of other European studies (Smith et al., 2015; Brown & Davis, 2018; Ionescu et al., 2020), which shows that the correct match of ornamental species with local pedoclimatic conditions contributes to increasing the sustainability of green spaces and reducing the need for maintenance.



**Figure 3.** Heatmap of mean suitability by species and soil type.

## CONCLUSIONS

The study carried out in Vărădia, Caraș-Severin county, highlights the importance of detailed knowledge of the local soil and climatic conditions in the selection of ornamental species used in the arrangement of green spaces. The analyses carried out on the six dominant soil types (Preluvosol, Aluviosol, Vertosol, Gleiosol, Cambisol and Stagnosol) and on the ten ornamental species demonstrated a significant ecological variability, influenced by soil reaction, humus content and drainage.

The results obtained can be summarized as follows:

Preluvosol and Aluviosol soils offer optimal conditions for most ornamental species, due to their slightly acidic to neutral pH, clayey texture and high fertility.

Vertosols and Stagnosols limit the development of species sensitive to excess moisture and poor soil aeration, requiring breeding works (loosening, drainage, addition of organic matter).

The analysis of variance (ANOVA) indicated significant differences between soils for all species ( $p < 0.05$ ), confirming the decisive influence of pedological factors on ornamental performance.

Pearson correlations highlighted strong positive relationships between soil fertility (N, P, K, humus) and species adaptability ( $r = 0.71$ ), while high pH and heavy texture were limiting factors.

The PCA analysis showed that *Lavandula angustifolia*, *Buxus sempervirens* and *Rosa spp.* it is associated with well-drained and slightly alkaline soils, and *Hydrangea macrophylla* and *Betula pendula* prefer acidic soils rich in humus.

Therefore, the appropriate selection of ornamental species, depending on the local pedo-climatic characteristics, contributes to increasing the sustainability and aesthetics of green spaces, reducing maintenance costs and making the most of the ecological potential of the soils in southwestern Romania.

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# STUDIES ON THE IMPLEMENTATION OF CONSERVATIVE TILLAGE SYSTEMS FOR CROPS TRITICUM AESTIVUM

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*The main objective of the studies on the implementation of conservative tillage systems for wheat is to evaluate and optimize the impact of these systems (such as no-till or mini-till) on: soil quality and fertility; wheat production and profitability; sustainability and ecological impact. Therefore, the studies aim to demonstrate that conservative systems represent a viable and superior alternative to conventional ones, while ensuring stable productivity and long-term protection of soil resources. The studies in this paper were carried out in the SW part of Caraș Severin County in the fall of 2024. The studies refer to the technology of mechanization of minimum works for wheat cultivation. The following mechanized works were carried out: scarification, preparation of the germination bed and sowing of wheat. In essence, although the implementation of conservative tillage systems requires a considerable technological and logistical transition, these practices represent the optimal agrotechnical solution for stabilizing *Triticum Aestivum* (Wheat) production and improving the resilience of the agroecosystem to water and thermal stress, direct consequences of contemporary climate change.*

**Key words:** soil, wheat, conservation system, fertility, production

## INTRODUCTION

The purpose of this study is the scientific evaluation and demonstration of the efficiency of new soil tillage technologies as an alternative to conventional plowing. (Mihuț et al 2022).

A retrospective look highlights that the last century will remain in the memory of mankind primarily as a period of rapid development of science and technology and, related to these, the development of agriculture. (Mihuț et al 2023).

In recent years, Romanian agriculture has undergone numerous transformations, one of which is the transition from state to private agriculture.

As in more developed countries, in our country, the aim is to increase labor productivity by mechanizing agriculture and practicing it scientifically, resulting in an agriculture that is both ecological and efficient in obtaining large productions. (Mircov et.al. 2022; Mircov et al. 2024)

Agriculture has been, since ancient times, and will remain the essential source of food and clothing production for the entire population of the globe. (Okros et.al. 2024)

The intensive use of agricultural machinery ensures the increase in labor productivity in agriculture, making it possible that, in parallel with the expansion of mechanization, each agricultural producer can feed, through the products obtained, an increasing number of people. (MIHUȚ, & NIȚĂ 2018)

For Romania, which has a great agricultural potential due to the land area per capita and the favorable pedoclimatic conditions, it is important to increase labor productivity by increasing the degree of mechanization in agricultural holdings.

The development of alternative soil cultivation technologies, which ensure the conservation and maintenance of its productive capacity as well as the reduction of energy consumption per unit of cultivated area, is today a necessity for the development of sustainable agriculture. (Okros et. al. 2023).

Conservative tillage is a modern and sustainable agricultural practice, designed to optimize crop productivity and protect the environment at the same time. (Ilea R., et.al., 2017). In the context of *Triticum aestivum* (wheat), one of the most important cereals worldwide, the implementation of conservative tillage systems becomes essential to ensure a balance between yield and sustainability. (M.A Drăgan, et. al., 2024).

Studies on these systems analyze how minimal tillage or reduced tillage techniques influence soil structure and fertility, moisture conservation, erosion reduction and increased crop resistance to stress factors. In this paper, we will explore the principles and methodologies used in the implementation of conservative tillage systems for wheat, as well as their impact on the environment and agricultural productivity. Thus, the importance of adopting sustainable practices to ensure food security and protect natural resources in the context of climate change and increasing societal demands is highlighted. (Popa D., et. al., 2015.)

## MATERIAL AND METHODS

The implementation of the field work was carried out by a farm from Oravița, Caraș-Severin County, using the following specialized mechanical aggregates:

Through the use of a high-power aggregate, composed of the Challenger MT-765B tractor coupled with the Horsch Tiger 8AS scarifier, deep loosening (scarification) was carried out.

Minimal soil tillage excludes plowing from mechanized technology. For deep soil loosening, scarification is practiced. The soil is deeply mobilized for water and air penetration, and is not turned over.

For soil scarification, an agricultural aggregate consisting of a Challenger MT-865E tractor and a Horsch 6 MT deep loosening machine was used (Figure 3.1).



**Figure 1.** Horsch 6 MT deep tillage machine

Secondary Preparation of the Germination Bed: - the operation aimed at refining and leveling the soil, being carried out with the same Challenger MT-765B tractor that towed a Swifter ST 17000 combine.

The Swifter ST 17000 combine (figure 2) is an agricultural machine towed by the Challenger MT-865E tractor, which performs the work of total cultivation (crushing, leveling, superficial loosening of the land) in order to sow straw cereals.



**Figure 2.** Swifter ST 17000 combiner

Sowing - the introduction of the seed material was carried out with a Horsch Pronto 6AS precision seeder, ensuring uniform distribution in the minimally worked land, this being towed by the Fendt-820 Vario tractor.

The Horsch 6AS pneumatic seeder (figure 3) is an agricultural machine towed and driven by the Fendt 820 Vario agricultural tractor. This agricultural machine performs the sowing of straw cereals.



**Figure 3.** Horsch 6AS seed drill

The system of minimum tillage or reduced tillage (mini-Till)

Includes the basic tillage without turning the furrow, keeping the plant residues in a proportion of 15-30% on the soil surface or superficially incorporated by the works carried out, performing the role of mulch.

The "no-tillage" system or direct sowing (no-tillage, direct drill)

Direct sowing in stubble is considered the most conservative tillage system, as it is closest to the natural state of the soil, under the perennial plants.

This is the system that combines, in the most suggestive way, the oldest practice of sowing, of introducing seeds into a soil as close as possible to its natural state.

This technological practice is the result of permanent changes in agricultural production systems, in soil cultivation methods, in the modernization and improvement of the machinery system and, at the same time, it is the consequence of the intensification of the processes of soil degradation and other environmental resources, specific to conventional technologies.

## RESULTS AND DISCUSSION

The results obtained using the mentioned aggregates (Horsch Tiger scarifier, Swifter combiner, Horsch Pronto seeder) highlight the following: compaction and loosening: the scarification work (with the Horsch Tiger) was essential for breaking the "plough sole" and reducing the resistance to penetration in the deep layers (below 25 cm). This improves porosity and allows better development of the wheat root system.

A trend of increasing the accessible water reserve in the 0-30 cm soil layer was observed in the variants with minimal tillage, especially in years with poor rainfall.

This phenomenon is due to the preservation of part of the plant residues that reduce evaporation and increase the infiltration of rainwater.

The secondary preparation carried out with the Swifter ST 17000 combiner ensured a fine germination bed on the surface, favorable for precision sowing with the Horsch Pronto 6AS seeder, even in conditions of eliminating plowing.

Comparison of wheat production between the minimal tillage system and the conventional system

Wheat Production:

-short term - wheat production in the Mini-Till system (scarified + combined) can be comparable to or slightly below that obtained by plowing, as the soil adapts.

-medium/long term: the minimum tillage system tends to stabilize production and can record production increases in dry years, due to superior soil water management.

Quality indices (protein, gluten) are not directly and significantly influenced by the type of tillage, but rather by the nitrogen fertilization regime.

Although the Mini-Till system involves three passes (scarified, combined, sowing), compared to the No-Till system which has a single pass, it is superior to classic plowing (which usually involves four or more passes) in terms of reducing soil disturbance and preserving its structure.

Economic efficiency is the major argument for adopting conservative technologies:

The implementation of minimum tillage (Mini-Till) has led to a significant reduction in specific fuel consumption per hectare and to a shortening of the total working time compared to classic ploughing.

Although scarification is an energy-consuming work, the elimination of the plough and other preparatory work (additional discs) ensures considerable savings.

It has been demonstrated that the use of high-performance aggregates (Challenger, Fendt, Horsch) ensures the necessary working capacity and the quality of the execution of operations (uniform scarification depth, precision in sowing) even in conditions of higher plant residues.

## CONCLUSION

Preventing soil degradation and other environmental resources, as well as improving degraded soils through conventional technologies, reducing energy consumption, increasing soil productive potential, and increasing water use efficiency were decisive reasons for implementing and expanding the technology of direct sowing in stubble in modern agriculture.

Direct sowing is the most advanced technology developed in agriculture at the current level of knowledge.

This system involves sowing in uncultivated land, which remains so until harvest, without mechanical maintenance and weed control.

Since the soil remains covered with the plant residues of the previous crop and cultivation is carried out only on the sowing strips, adequate control against water and wind erosion is ensured, and labor and fuel savings are achieved.

The need to transition from classical intensive tillage methods to conservative systems is dictated by the urgency of combating soil erosion and degradation, while ensuring fertility stability (ecological) and reducing energy costs (economic), thus underpinning a sustainable agriculture essential for future food security.

The results confirm the hypothesis that the minimum tillage system, well calibrated and appropriately equipped, represents an efficient compromise between the need to break the compacted layer (by scarification) and the need to protect the soil (by avoiding plowing).

The adoption of this technology improves ecological sustainability and economic efficiency in wheat cultivation, especially in regions where soil compaction and water stress are limiting factors.

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## ANALYSIS OF MORPHOGENESIS IN AROMATIC PLANTS FROM THE GENUS LEVISTICUM

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*This study evaluated the early growth of Levisticum officinale genotypes under controlled conditions. Plant height, leaf number, and root dimensions were measured. ANOVA showed no significant differences in height and leaf number, but root size differed among genotypes. The Banat population had the largest roots. Results highlight genotypic variability in root development.*

**Key words:** *Levisticum officinale, genotypes, root development, plant height, leaf number*

### INTRODUCTION

*Levisticum officinale*, commonly known as lovage, is a perennial edible plant used as a culinary herb in soups and other dishes (Spréa et al., 2020; Santos et al., 2005). This herbaceous perennial develops a taproot and a dense aerial rosette of leaves (Marchyshyn et al., 2019; Kemzuraite et al., 2014). Its stem is thick, tubular, and can reach a height of two to three meters (Marchyshyn et al., 2019). The leaves are compound, bipinnate, with finely toothed leaflet margins, long petioles, and a strong aromatic fragrance, arranged in rosettes (Marchyshyn et al., 2019; Spréa et al., 2020).

Lovage flowers in summer, producing yellow flowers organized in umbel-shaped inflorescences, from which seeds develop (Marchyshyn et al., 2019; Kemzuraite et al., 2014). Although native to the Mediterranean region and first used as a food source in Italy, *L. officinale* has adapted successfully to the pedoclimatic conditions of Romania (Ivancheva & Stantcheva, 2000). The species is undemanding, tolerating both high temperatures and frost (Spréa et al., 2020; El-Din & Hendawy, 2010).

As a perennial garden plant, lovage has an average lifespan of 7–10 years (Santos et al., 2005). It grows well on heavy and cold soils (Spréa et al., 2020). Leaves can be harvested during the summer, while in autumn, bunches are formed that can be dried and stored throughout the winter (Spréa et al., 2020; Mohamadi et al., 2017). Lovage exhibits low requirements regarding soil and environmental factors, being highly resistant and adaptable (Khodashenas et al., 2015; Spréa et al., 2020). It prefers deep soils with high humus content (Santos et al., 2005; Kemzuraite et al., 2014). Its tolerance to low winter temperatures and drought resistance is supported by its taproot, which can penetrate the soil up to 1 meter in depth (Santos et al., 2005; Khodashenas et al., 2015).

Lovage cultivation can be established either by sowing seeds or vegetatively through division of the clumps (Spréa et al., 2020; El-Din & Hendawy, 2010).

### MATERIAL AND METHODS

#### Description of Experimental Material

The study on the growth of *Levisticum officinale* (lovage) plants was conducted using four genotypes as experimental variants, each representing distinct morphological and aromatic characteristics:

Control – Populație de Banat: A perennial local population characterized by tall, robust stems, large bright green aromatic leaves, and yellow-green umbels. This landrace is traditionally cultivated in gardens and small farms across Timiș, Caraș-Severin, and Arad counties. Leaves are harvested throughout spring and summer, while roots are collected in autumn. Owing to its climatic adaptability and authentic flavor, this population is highly appreciated for both culinary and medicinal use.

V1 – Leuștean de Buzău (Topaz cultivar): Developed at the Research and Development Station for Vegetable Growing (SCDL) Buzău, this vigorous perennial cultivar exhibits a high production potential and a

strong, characteristic aroma, particularly pronounced in May. It is suitable for both protected cultivation and open-field conditions and is valued for its dual culinary and therapeutic properties.

V2 – Deutsche Riesen (Giant German Lovage): A robust, high-yielding perennial genotype reaching up to 2.5 m in height. It produces large, intensely aromatic leaves and is primarily cultivated for culinary purposes.

V3 – Maggikraut (German Lovage): A compact perennial plant with an intense, celery-like aroma, frequently used as a key flavoring ingredient in soups, broths, and sauces.

The experiment was organized in two developmental stages — early growth and the vegetative growth period — to evaluate morphological differences among genotypes. The main objective was the determination of the number and length of roots formed in the analyzed *Levisticum officinale* genotypes under controlled environmental conditions.

## Germplasm maintenance

*Levisticum officinale* (lovage) is a perennial aromatic and medicinal plant valued for its adaptability to temperate climates and its high morphological and biochemical variability. In the present study, all genotypes were cultivated under controlled environmental conditions to ensure uniform growth and reliable comparison.

Seed material and vegetative propagules were obtained from certified collections and local populations. The experimental plants were established in protected spaces (greenhouse and seedbeds) at a constant temperature of approximately 20–22°C and relative humidity of 60–70%. After germination, seedlings were transplanted into pots containing a standardized soil mixture (peat:sand:perlite, 2:1:1) to maintain consistent nutrient and moisture availability.

Regular irrigation was applied to preserve optimal soil moisture, and plants were exposed to a 14-hour photoperiod under natural and supplemental light sources. No chemical fertilizers or growth regulators were used, in order to maintain the genotypes' natural morphological expression.

Throughout the vegetation period, plants were monitored for uniformity and health status. Leaves were harvested during the active growth phase (spring–summer), while root samples were collected in the late vegetative stage (autumn) to evaluate root system development. This maintenance protocol ensured the preservation of genetic integrity and the accurate assessment of phenotypic variability among the studied *Levisticum officinale* genotypes.

## Experimental Design

The experiment was conducted under controlled environmental conditions to evaluate the morphological variability and early growth dynamics among four *Levisticum officinale* genotypes. A completely randomized design (CRD) was applied, ensuring identical cultivation conditions and uniform replication for all variants (Control – Populație de Banat, V1 – Leuștean de Buzău, V2 – Deutsche Riesen, and V3 – Maggikraut).

Morphological observations were carried out during three consecutive growth periods to capture the dynamics of plant development:

Stage I – 7 days after germination, corresponding to primary root initiation and early shoot emergence;

Stage II – 13 days after germination, representing active shoot elongation and initial leaf expansion;

Stage III – 18 days after germination, marking the stabilization of root and aerial organ development prior to transplanting.

The following parameters were analyzed for each genotype:

Plant height (cm) – measured from the soil surface to the apex of the main stem;

Number of leaves per plant – total number of fully developed leaves per individual;

Number and length of roots (cm) – determined after gentle washing and measurement of the entire root system.

Descriptive statistical analysis was performed for all measured variables. The mean, standard deviation, and range values (minimum–maximum) were calculated to characterize the growth pattern and inter-genotypic variability across the three observation periods.

## Results objective

The present study aimed to evaluate the early vegetative development of different *Levisticum officinale* genotypes under controlled environmental conditions. The specific objectives were to:

Determine the number and length of roots formed in each genotype to assess root system development;

Evaluate plant height dynamics across the studied genotypes at distinct developmental stages (7, 13, and 18 days after germination);

Quantify the number of leaves per plant to characterize aerial growth performance;

Perform a descriptive statistical analysis of all measured parameters, calculating the mean, standard deviation, and standard error to assess inter-genotypic variation and phenotypic stability.

### Statistical analysis

Data were analyzed using Microsoft Excel and R Studio to evaluate morphological variability among *Levisticum officinale* genotypes. Descriptive statistics (mean, standard deviation, and range) were calculated for all measured traits. Analysis of variance (ANOVA) was performed within a completely randomized design to determine significant differences among genotypes, considering the significance level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Study on Plant Height Growth in *Levisticum officinale*

A one-way analysis of variance (ANOVA) was performed to evaluate the effect of genotype on plant height in *Levisticum officinale* during the initial growth stage. The analysis revealed no statistically significant differences ( $p > 0.05$ ) among the genotypes, indicating a relatively uniform growth pattern at the beginning of development (table 1).

**Table 1.** ANOVA – Plant Height in *Levisticum officinale* at the Beginning of the Growth Period

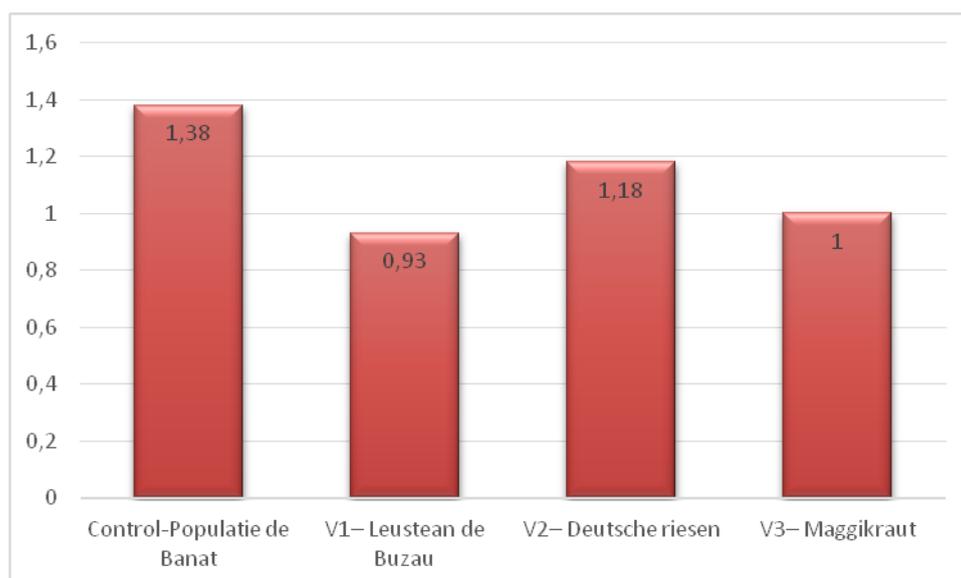
Source of variation	SS	df	MS	F	p-value
Between groups	0.48	3	0.16	0.41	0.74
Within groups	4.70	12	0.39		
Total	5.18	15			

In the first stage of the experiment, plant height growth in *Levisticum officinale* was analyzed by comparing the results obtained for the control variant, representing the Banat population, with the three other variants corresponding to different cultivars. During the initial growth period, mean plant heights were similar across variants, with only minor variations in size (Table 2).

**Table 2.** Statistical values of plant height in *Levisticum officinale* at the beginning of the growth period

Variante	Media	Abaterea standard	Maximul	Minimul	Varianta
Control-Populatie de Banat	1,38	0,40	2,00	1,00	0,16
V1– Leustean de Buzau	0,93	0,52	1,60	0,50	0,27
V2– Deutsche riesen	1,18	0,90	3,00	0,70	0,81
V3– Maggikraut	1,00	0,55	2,00	0,50	0,30

In the second stage of the experiment, assessing the growth capacity of *Levisticum officinale*, differences between the cultivars (V1, V2, and V3) and the control population became evident. The highest values were recorded in the control variant, followed by the experimental variants V1, V2, and V3, where smaller differences in growth were observed. The mean plant height for the control variant was 1.38 cm, with a standard deviation of 0.4 cm, a maximum of 2 cm, and a minimum of 1 cm. For variant V1, the mean height was 0.93 cm, with a standard deviation of 0.52 cm, a maximum of 1.6 cm, and a minimum of 0.5 cm. In variant V2, the mean height was 1.18 cm, with a standard deviation of 0.90 cm, a maximum of 3 cm, and a minimum of 0.70 cm. For variant V3, the mean height was 1 cm, with a standard deviation of 0.55 cm, a maximum of 2 cm, and a minimum of 0.50 cm (Table 1 and Figure 1).



**Figure 1.** Plant height growth in *Levisticum officinale*

### Study on Leaf Number Formation in *Levisticum officinale*

A one-way analysis of variance (ANOVA) was performed to determine whether the mean number of leaves differed significantly among the four *Levisticum officinale* genotypes at the beginning of the growth period. The analysis revealed no statistically significant differences ( $p > 0.05$ ) among genotypes, suggesting a relatively uniform leaf formation pattern during early development (Table 3).

**Table 3.** Anova on leaf number in *Levisticum officinale* at the beginning of the growth period.

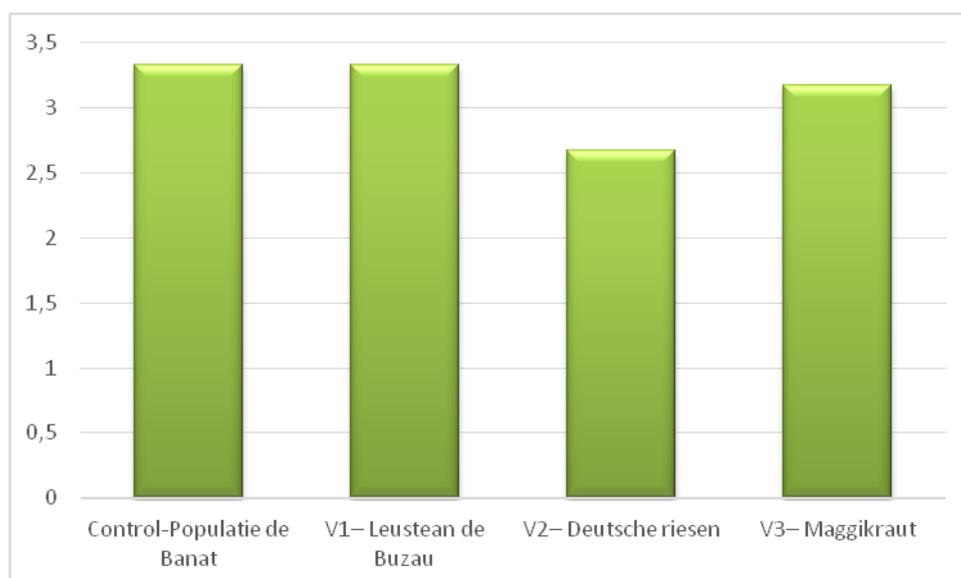
Source of variation	SS	df	MS	F	p-value
Between groups	1.05	3	0.35	0.46	0.72
Within groups	9.00	12	0.75		
Total	10.05	15			

The control variant exhibited a mean leaf number of 3.33, with a standard deviation of 0.52 leaves, a maximum of 4.00 leaves, and a minimum of 3.00 leaves. For variant V1, the mean was similar, 3.33 leaves, but with a higher standard deviation of 1.03 leaves, a maximum of 4.00 leaves, and a minimum of 2.00 leaves. Variant V2 had a mean of 2.67 leaves, with a standard deviation of 0.52 leaves, a maximum of 3.00 leaves, and a minimum of 2.00 leaves. In variant V3, the mean leaf number was 3.17, with a standard deviation of 0.75 leaves, a maximum of 4.00 leaves, and a minimum of 2.00 leaves.

**Table 4.** Variation in leaf number in *Levisticum officinale* at the beginning of the growth period.

Variante	Media	Abaterea Standard	Maximul	Minimul	Varianta
Control-Populatie de Banat	3,33	0,52	4,00	3,00	0,27
V1- Leustean de Buzau	3,33	1,03	4,00	2,00	1,07
V2- Deutsche riesen	2,67	0,52	3,00	2,00	0,27
V3- Maggikraut	3,17	0,75	4,00	2,00	0,57

The results indicate that, in the control genotype, the number of leaves remains relatively constant, similar to observations in genotype V1, whereas genotype V2 shows a significant decrease. The control variant exhibits a more pronounced increase in leaf number compared to the other experimental variants (Figure 2).



**Figure 2.** Leaf number results in *Levisticum officinale* genotypes during the growth period

A one-way analysis of variance (ANOVA) was conducted to assess the effect of genotype on root dimensions in *Levisticum officinale* during the growth period. The results showed a statistically significant effect of genotype ( $F_{3,12} = 6.74$ ,  $p = 0.006$ ), indicating that root size differed among the genotypes (Table 5).

**Table 5.** ANOVA root dimension in *Levisticum officinale* during the growth period.

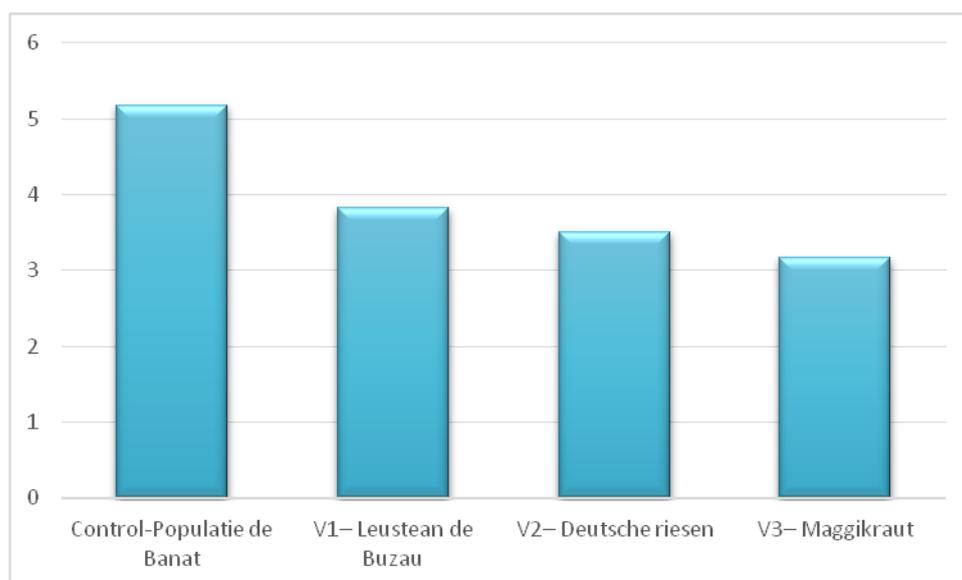
Source of variation	SS	df	MS	F	p-value
Between groups	6.37	3	2.12	6.74	0.006
Within groups	3.77	12	0.31		
Total	10.14	15			

**Table 6.** Variation in root dimension in *Levisticum officinale* during the growth period.

Variante	Media	Abaterea Standard	Maximul	Minimul	Varianta
Control-Populație de Banat	5,17	0,75	6,00	4,00	0,57
V1- Leuștean de Buzău	3,83	0,75	5,00	3,00	0,57
V2- Deutsche riesen	3,50	0,55	4,00	3,00	0,30
V3- Maggikraut	3,17	0,41	4,00	3,00	0,17

The ANOVA results revealed that genotype had a significant influence on root length, demonstrating genetic variability among *Levisticum officinale* populations. The Control – Populație de Banat recorded the highest mean root length (5.17 cm), significantly exceeding that of Maggikraut (3.17 cm), which exhibited the lowest values. Intermediate results were observed in Leuștean de Buzău and Deutsche Riesen, suggesting a moderate root development potential.

These findings indicate that local genotypes, particularly Populația de Banat, possess superior root growth characteristics under the given environmental conditions, highlighting their adaptive advantage and potential for selection in breeding programs (Figure 3).



**Figure 3.** Root length results in *Levisticum officinale* genotypes during the growth period

## CONCLUSION

The comparative analysis of growth parameters in *Levisticum officinale* revealed distinct genotypic responses during early development. Although differences in plant height and leaf number were not statistically significant ( $p > 0.05$ ), indicating uniform initial growth among genotypes, significant variability was observed. The Control – Populație de Banat demonstrated superior root growth, suggesting enhanced adaptability to local environmental conditions and greater developmental vigor compared to the introduced cultivars (*Leustean de Buzău*, *Deutsche Riesen*, and *Maggikraut*). These findings highlight the importance of genetic background in determining belowground biomass allocation and suggest that native genotypes, such as the Banat population, may be valuable resources for future breeding programs aimed at improving growth stability and adaptability in *Levisticum officinale*.

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# ASPECTS OF MORPHOLOGICAL DEVELOPMENT IN DIFFERENT VARIETIES OF OCIMUM BASILICUM

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*This study evaluated morphological variability in four Ocimum basilicum genotypes under controlled conditions. Traits analyzed included root number and length, plant height, and leaf number. ANOVA showed significant genotype-dependent differences. The control had the highest root and plant growth, V3 the lowest, and V2 stood out for high anthocyanin content. Results highlight genetic influence on vegetative traits and inform breeding and selection.*

**Key words:** *Ocimum basilicum*, Genotypic variability, Root formation, Morphological traits

## INTRODUCTION

Plants belonging to the mint family, Lamiaceae (Labiatae), constitute one of the richest sources of bioactive compounds and essential oils, recognized for their characteristic aromas (Švecová & Neugebauerová, 2010; Mothana & Lindequist, 2005; Nguefack et al., 2004). Among the genera in this family, *Ocimum*, *Lavandula*, *Mentha*, *Thymus*, *Rosmarinus*, and *Pogostemon* stand out for their economic and medicinal importance (Maisuthisakul et al., 2008; Teliban et al., 2022). The genus *Ocimum* L., commonly known as basil, holds significant cultural, religious, and therapeutic value in many regions of the world, particularly in India and other South Asian countries, where it is revered as *Tulsi* – “The Incomparable” or “Queen of Herbs” (Khair-ul-Bariyah et al., 2012). All parts of this plant, including the leaves, inflorescences, seeds, and oils, are used for medicinal purposes (Khair-ul-Bariyah et al., 2012; Bora et al., 2011).

Etymologically, the name *Ocimum* derives from the Greek term *Okimon*, meaning “smell,” and was used by ancient botanists Theophrastus and Dioscorides to describe the aromatic properties of the plant (PraKash & Gupta, 2020). The genus exhibits a pantropical distribution, being predominantly found in warm regions of the globe (Harley et al., 2004; Teliban et al., 2022). Many species are widely cultivated for medicinal and culinary purposes, as well as for cultural and ritual contexts (Švecová & Neugebauerová, 2010; Mulugeta et al., 2023).

Over time, the genus *Ocimum* has attracted significant scientific interest due to its high content of bioactive secondary metabolites, responsible for antioxidant, antimicrobial, and anti-inflammatory properties (Maisuthisakul et al., 2008; Mothana & Lindequist, 2005; Nguefack et al., 2004). These compounds give basil substantial value in the pharmaceutical, food, and nutraceutical sectors and are also used in formulations aimed at promoting health. Natural antioxidants extracted from plants are preferred over synthetic ones due to their lower toxicity and additional beneficial biological effects (Maisuthisakul et al., 2008).

Several varieties of *Ocimum basilicum* L. are recognized for their specific properties and uses (Švecová & Neugebauerová, 2010; Varga et al., 2017; Yaldiz et al., 2021). For instance, *O. basilicum* var. *basilicum*, var. *minimum*, and var. *purpurascens*, commonly known as sweet basil, dwarf basil, and red basil, respectively, have been traditionally cultivated in various regions, including northeastern Brazil, since the 17th century, for their antibacterial, antifungal, and insecticidal activities, as well as for ritual and therapeutic uses (Nguefack et al., 2004; Mothana & Lindequist, 2005; Khair-ul-Bariyah et al., 2012).

The variety *O. basilicum* var. *thyrsiflora* (Thai basil) is widespread in Asia, Africa, and the Americas, and is characterized by sweet leaves with an anise-like aroma and spicy notes. In traditional medicine, it is used to relieve cough, fever, headaches, diabetes, and various inflammatory conditions (Bora et al., 2011; Sajjadi, 2006). Recent studies have confirmed its antioxidant and antimicrobial activity, highlighting the potential of this variety as a natural source of bioactive compounds (Maisuthisakul et al., 2008; Mulugeta et al., 2023).

The variety *O. basilicum* var. *purpurascens* (red basil) is distinguished by its high anthocyanin content, which gives the leaves a deep violet color and provides remarkable antioxidant and protective properties (Varga et al., 2017; Švecová & Neugebauerová, 2010). Anthocyanins, classified as flavonoids, are recognized for their

role in reducing oxidative stress, preventing cardiovascular diseases, and ameliorating metabolic disorders (Maisuthisakul et al., 2008). Additionally, they are used worldwide as natural food colorants (E163 in the European Union), due to their safety and biological functionality (Maisuthisakul et al., 2008).

Despite the long-standing use of basil in culinary and medicinal traditions, contemporary research continues to explore its nutritional, chemical, and biological potential. Studies have highlighted the presence of proteins, fatty acids, tocopherols, phenolic compounds, and organic acids, all contributing to the functional, antimicrobial, and health-promoting properties of the plant (Mulugeta & Tesfaye, 2024; Juškevičienė et al., 2022; Branca et al., 2024).

Therefore, *Ocimum basilicum* and its varieties represent a multifunctional group of plants that combine traditional significance with vast modern biomedical and nutraceutical potential, representing a major area of interest for current scientific research (Teliban & Stoleru, 2022; Dinu & Pop, 2023).

## MATERIAL AND METHODS

### Germplasm selection

As part of the germplasm selection and characterization work, we analyzed several *O. basilicum* genotypes collected and maintained in our experimental collection. The selected genotypes were evaluated for their morphological and agronomic traits, with the following variants included in the experiment:

Control – *Ocimum basilicum* var. *basilicum*

V1 – *Ocimum basilicum* var. *minimum*

V2 – *Ocimum basilicum* var. *purpurascens*

V3 – *Ocimum basilicum* var. *thyrsiflora*

Control – *Ocimum basilicum* var. *basilicum* (common basil) is an annual herbaceous plant reaching 30–60 cm in height, with oval, bright green leaves and a sweet, slightly spicy aroma. It is widely used in gastronomy, especially in Italian cuisine (pesto sauce, pasta, salads), as well as in traditional medicine due to its antioxidant, anti-inflammatory, and digestive properties.

V1 – *Ocimum basilicum* var. *minimum* (Greek basil) has a compact, spherical bushy growth, with small, rounded leaves and a height of 15–50 cm. Its aroma is intense, sweet-spicy, with anise-like notes. Because of its compact size and decorative appearance, this variety is ideal for pot cultivation and is used both as an ornamental plant and as a versatile culinary herb for salads, sauces, meat or fish dishes, and even infusions.

V2 – *Ocimum basilicum* var. *purpurascens* (red basil) is characterized by its deep purple leaves, rich in anthocyanins, and a sweet aroma with hints of cinnamon and cloves. It is a dense, erect annual plant that prefers warm climates, full sun exposure, and well-drained soil. This variety is suitable for both culinary use (salads, sauces, pesto) and ornamental purposes (gardens or pots).

V3 – *Ocimum basilicum* var. *thyrsiflora* (Thai basil) is an aromatic plant native to Southeast Asia and a member of the mint family (*Lamiaceae*). It features narrow, glossy leaves, purple stems, and a distinctive aroma reminiscent of anise and cinnamon. Due to its intense flavor and heat tolerance, it is commonly used in Asian cuisine, particularly in curries, soups, and various dishes.

### Germplasm maintenance

Common basil (*Ocimum basilicum* L.) is an annual aromatic and medicinal species appreciated for its high adaptability, morphological variability, and ease of cultivation under both open-field and controlled conditions. The species originates from India and subsequently spread to tropical and subtropical regions worldwide. In our study, we established *O. basilicum* crops under controlled environmental conditions, following standard horticultural practices. Seeds were sown in spring in protected spaces (seedbeds), and seedlings were transplanted into open soil approximately 45 days after germination. Indoor cultivation was also maintained between February and November, under constant temperature ( $\approx 20^{\circ}\text{C}$ ) and adequate light conditions.

### Experimental Design

The experiment was conducted under controlled environmental conditions to evaluate the morphological variability among selected *Ocimum basilicum* L. genotypes. A completely randomized design was applied, ensuring uniform replication and identical growth conditions for all variants.

Morphological assessments focused on key vegetative parameters relevant to early growth performance and root system development. The analyzed traits included: (1) the number of roots per plant, (2) mean root length (cm), (3) plant height (cm), and (4) number of leaves per plant.

Measurements were performed at the same developmental stage using standardized procedures to ensure accuracy and reproducibility. Descriptive statistical analysis was applied to each variable, calculating the mean, standard deviation, and range values (minimum–maximum) for each genotype.

Measurements were performed at three consecutive observation periods to capture the dynamics of early vegetative growth:

Stage I – 7 days after germination, corresponding to primary root initiation;

Stage II – 13 days after germination, representing the onset of active shoot and leaf growth;

Stage III – 18 days after germination, marking the stabilization of root and aerial development prior to transplanting.

For each parameter, the mean values were calculated based on data collected across the three observation periods, in order to obtain representative estimates of genotype performance. All determinations were carried out using standardized morphometric procedures to ensure accuracy and reproducibility. This experimental framework enabled a comparative evaluation of genotype-specific growth patterns and provided insights into the phenotypic variability within the analyzed *O. basilicum* germplasm.

## Research objectives

The present study aimed to evaluate the morphological variability among different *Ocimum basilicum* L. genotypes under controlled growth conditions. The specific objectives were to:

Determine the number and length of roots formed in each genotype;

Assess plant height variation across genotypes;

Quantify the number of leaves per plant for each analyzed genotype.

Descriptive statistical analyses were performed for all measured parameters, including the calculation of mean values and standard errors, to characterize inter-genotypic differences and assess phenotypic variability within the studied basil germplasm.

## Statistical Analyses

Descriptive statistical analysis and one-way ANOVA were performed to assess morphological variability among *Ocimum basilicum* L. genotypes. The mean, standard deviation, standard error, and range (minimum–maximum) were calculated for each parameter. Data from the three observation periods (7, 13, and 18 days after germination) were averaged to obtain representative genotype values. All analyses were conducted using R software (version 4.x, R Core Team, Vienna, Austria) with the psych, dplyr, and ggplot2 packages. Results were expressed as mean ± standard error, and significant differences among genotypes were determined at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Determination of the Number of Roots Formed in *Ocimum basilicum* Plants

The one-way analysis of variance (ANOVA) showed a highly significant effect of genotype on root formation ( $p < 0.001$ ). This indicates that the mean number of roots differed significantly among the four *Ocimum basilicum* variants (Table 1.).

**Table 1.** ANOVA – Determination of the Number of Roots Formed in *Ocimum basilicum*.

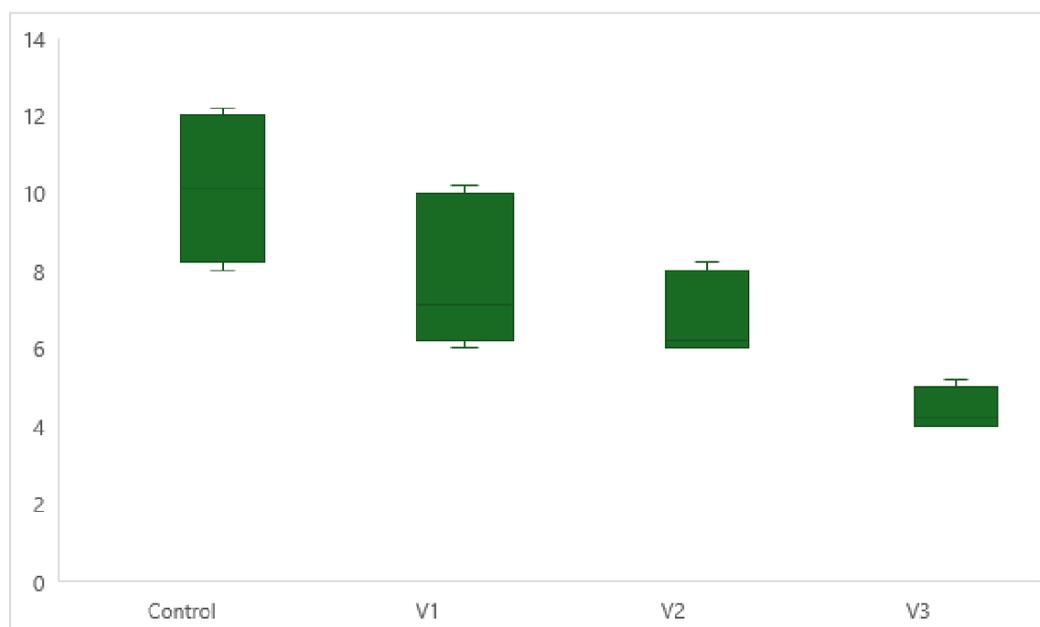
Source of variation	SS	df	MS	F	p-value
Between groups	80.25	3	26.75	16.45	< 0.001
Within groups	19.52	12	1.63		
Total	99.77	15			

The analysis of root formation across different *Ocimum basilicum* genotypes revealed significant variation among the experimental variants. For the control genotype (*Ocimum basilicum* var. *basilicum*), the mean number of roots formed during the entire observation period was 10.10, with a variation range between 8.00 and 12.20. The standard deviation value (1.71) indicates a moderate degree of variability, suggesting relatively uniform root development within this group. In the case of variant V1 (*O. basilicum* var. *minimum*), a significant reduction in the mean number of roots was observed, with an average value of 7.77 and a variation range between 6.00 and 10.20. For variant V2 (*O. basilicum* var. *purpurascens*), the mean number of roots

formed was 6.77, with values ranging from 6.00 to 8.20, indicating a lower root formation capacity compared to the control. The lowest mean number of roots was recorded in variant V3 (*O. basilicum* var. *thyrsiflora*), with a value of 4.43, as shown in Table 1. These results highlight notable differences among genotypes, suggesting a significant genetic influence on root formation in the studied basil plants.

**Table 2.** Statistical values of the number of roots formed in *Ocimum basilicum*.

Variants	Mean	Standard deviation	Maximum	Minimum
Control- <i>Ocimum basilicum</i> var. <i>basilicum</i>	10,10	1,71	12,20	8,00
V1- <i>Ocimum basilicum</i> var. <i>minimum</i>	7,77	1,78	10,20	6,00
V2- <i>Ocimum basilicum</i> var. <i>purpurascens</i>	6,77	0,99	8,20	6,00
V3- <i>Ocimum basilicum</i> var. <i>thyrsiflora</i>	4,43	0,50	5,20	4,00



**Figure 1.** Variation in the number of roots among *Ocimum basilicum* genotypes

### Evaluation of Root Length in *Ocimum basilicum* Genotypes

The one-way analysis of variance (ANOVA) revealed a highly significant effect of genotype on root formation in *Ocimum basilicum* ( $p = 0.0004$ ). This indicates that the mean number of roots differed significantly among the four *O. basilicum* variants (Table 3).

**Table 3.** Anova - Statistical values of the number of roots formed in *Ocimum basilicum*

Source of Variation	DF	SS	MS	F	p-value
Between groups	3	24.45	8.15	20.63	0.0004
Within groups	8	3.16	0.39		
Total	11	27.61			

Table 2 presents the differences in root length among the four *Ocimum basilicum* genotypes analyzed.

For the control variant, the mean root length was 5.73 cm, with a variation range between 5.4 and 6.2 cm, and a standard deviation of 0.29 cm, indicating low variability for this parameter.

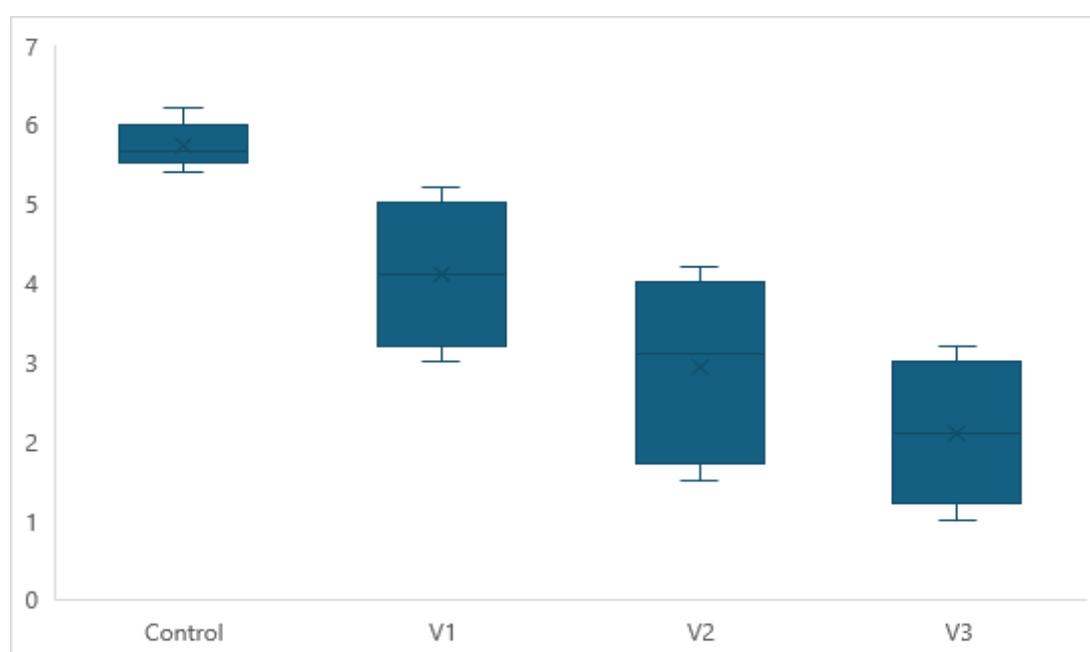
In the case of V1 (*O. basilicum* var. *minimum*), the mean root length decreased to 4.10 cm, with a variation range between 3.0 and 5.2 cm. The standard deviation of 0.41 cm suggests a higher variability compared to the control.

For V2 (*O. basilicum* var. *purpurascens*), the highest variability was observed, with a standard deviation of 1.08 cm. The mean root length for this variant was 2.93 cm, with values ranging from 1.5 to 4.2 cm.

In V3 (*O. basilicum* var. *thyrsiflora*), the mean root length further decreased to 2.10 cm, with a variation range between 1.0 and 3.2 cm, and a standard deviation of 0.86 cm, similar to that observed in variant V1.

**Table 4.** Statistical values of the number of roots formed in *Ocimum basilicum*

Variants	Mean	Standard deviation	Maximum	Minimum
Control- <i>Ocimum basilicum</i> var. <i>Basilicum</i>	5,73	0,29	6,20	5,40
V1- <i>Ocimum basilicum</i> var. <i>Minimum</i>	4,10	0,86	5,20	3,00
V2- <i>Ocimum basilicum</i> var. <i>purpurascens</i>	2,93	1,08	4,20	1,50
V3- <i>Ocimum basilicum</i> var. <i>Thyrsiflora</i>	2,10	0,86	3,20	1,00



**Figure 2.** Variation in the number of roots among *Ocimum basilicum* genotypes

### 3. Determination of Plant Height Growth in *Ocimum basilicum*

The one-way analysis of variance (ANOVA) showed a highly significant effect of genotype on root formation in *Ocimum basilicum* ( $p < 0.001$ ). This indicates that the mean number of roots differed significantly among the four *O. basilicum* variants (Table 3).

**Table 5.** Anova analysis for value of plant hight

Source of variation	SS	df	MS	F	p-value
Between groups	80.25	3	26.75	16.45	< 0.001
Within groups	19.52	12	1.63		
Total	99.77	15			

Plant height growth in *Ocimum basilicum* varied significantly depending on the genotype.

Plants in the control variant exhibited the greatest mean height (6.93 cm), with moderate variability indicated by a standard deviation of 1.62 cm, and a range between 5.0 and 8.2 cm, suggesting optimal growth conditions.

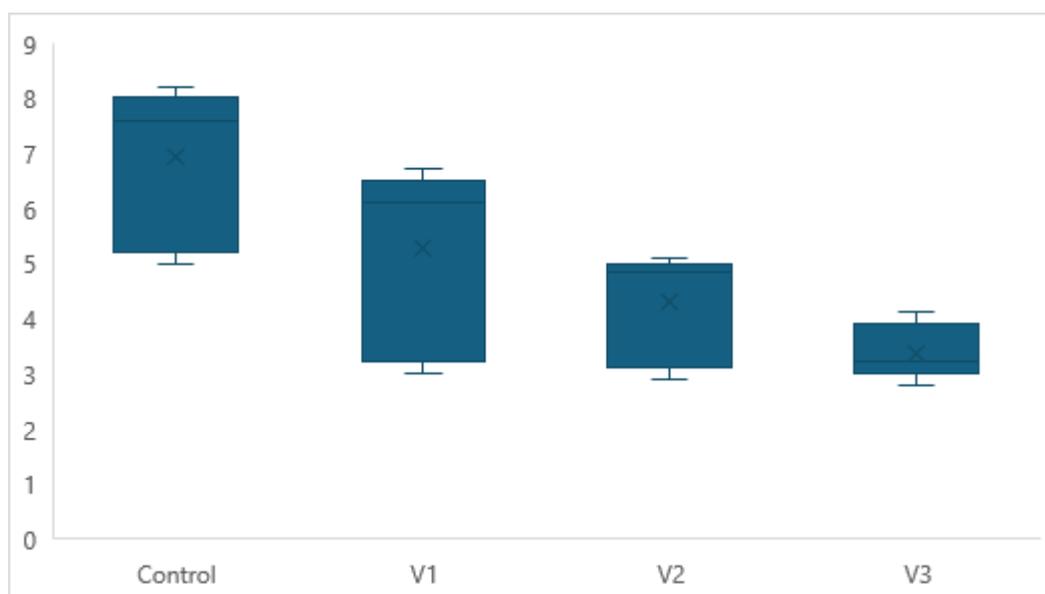
In variant V1 (*O. basilicum* var. *minimum*), the mean plant height decreased to 5.27 cm, with higher variability reflected by a standard deviation of 1.87 cm. Minimum and maximum values ranged from 3.0 to 6.7 cm.

For variant V2 (*O. basilicum* var. *purpurascens*), the mean plant height was 4.30 cm, with a standard deviation of 1.11 cm, and values ranging between 2.9 and 5.1 cm, indicating reduced growth compared to the control.

In variant V3 (*O. basilicum* var. *thyrsiflora*), variability was similar (1.11 cm), with a mean plant height of 4.30 cm, and minimum and maximum values ranging from 2.8 to 4.1 cm, as presented in Table 3.

**Table 6.** Statistical values of plant height growth in *Ocimum basilicum*

Variants	Mean	Standard deviation	Maximum	Minimum
Control- <i>Ocimum basilicum</i> var. <i>basilicum</i>	6,93	1,62	8,20	5,00
V1- <i>Ocimum basilicum</i> var. <i>minimum</i>	5,27	1,87	6,70	3,00
V2- <i>Ocimum basilicum</i> var. <i>purpurascens</i>	4,30	1,11	5,10	2,90
V3- <i>Ocimum basilicum</i> var. <i>thyrsiflora</i>	3,37	0,65	4,10	2,80



**Figure 3.** Variation in plant height among *Ocimum basilicum* genotypes

#### 4. Determination of the Number of Leaves per Plant in *Ocimum basilicum*

The one-way analysis of variance (ANOVA) indicated a significant effect of genotype on the number of leaves per plant in *Ocimum basilicum* ( $p = 0.011$ ). This suggests that the mean number of leaves differed significantly among the four *O. basilicum* variants (Table 7).

**Table 7.** Anova analysis for leaves per plant

Source of variation	SS	df	MS	F	p-value
Between groups	12.58	3	4.19	5.74	0.011
Within groups	8.76	12	0.73		
Total	21.34	15			

The number of leaves per plant varied significantly among the *Ocimum basilicum* genotypes.

In the control variant, the mean number of leaves was 6.07, with a range of 4 to 12 leaves, and a standard deviation of 3.03, indicating moderate variability for this genotype.

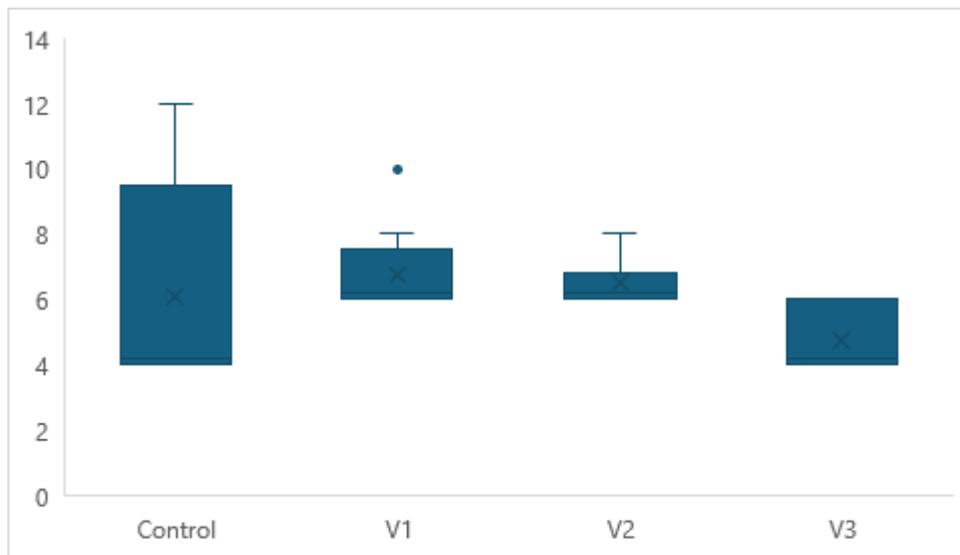
Variant V1 (*O. basilicum* var. *minimum*) exhibited lower variability compared to the control, with a mean leaf number of 6.73, a range between 6 and 10 leaves, and a standard deviation of 1.27.

For variant V2 (*O. basilicum* var. *purpurascens*), the mean number of leaves was 6.48, with a range of 6 to 8 leaves and a standard deviation of 0.76, indicating variability similar to that observed in V1.

Variant V3 (*O. basilicum* var. *thyrsiflora*) showed a mean number of leaves of 4.73, with a range between 4 and 6 leaves, and a standard deviation of 0.94, indicating moderate variability, as presented in Table 4 and Figure 4a–b.

**Table 8.** Statistical values of leaf number formation per plant in *Ocimum basilicum*

Variants	Mean	Standard deviation	Maximum	Minimum
Control- <i>Ocimum basilicum</i> var. <i>basilicum</i>	6,07	3,03	12,00	4,00
V1- <i>Ocimum basilicum</i> var. <i>minimum</i>	6,73	1,27	10,00	6,00
V2- <i>Ocimum basilicum</i> var. <i>purpurascens</i>	6,48	0,76	8,00	6,00
V3- <i>Ocimum basilicum</i> var. <i>thyrsiflora</i>	4,73	0,94	6,00	4,00



**Figure 4.** Variation in leaf number formation per plant among *Ocimum basilicum* genotypes

## CONCLUSION

The number and length of roots decreased significantly from one genotype to another. Regarding aerial traits, plant height in *Ocimum basilicum* was highest in the control variant compared to the other genotypes, in which the mean plant height decreased significantly. The largest differences were observed between the control and V1, while the smallest variations occurred in V3, highlighting the genetic differences among the studied genotypes.

Leaf number formation varied significantly depending on the genotype. Variants **V1** and **V2** exhibited lower variability in leaf number, whereas the **control** and **V3** variants showed higher variability, indicating a genotype-dependent influence on leaf development.

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# MATEMATICAL MODELING AND OPTIMIZATION OF FRUIT DEVELOPMENT IN PRUNUS PERSICA VAR. NUCIPERSICA

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*This study modeled fruit growth of two Romanian peach cultivars, Flacăra and Raluca, using nonlinear regression. Fruit weight, diameter, and height were measured, revealing significant morphological differences: Flacăra had larger, heavier fruits than Raluca. The models provide insights for orchard management and cultivar selection.*

**Keywords:** Peach, fruit growth, nonlinear regression, morphometrics, Flacăra, Raluca

## INTRODUCTION

The peach (*Prunus persica*) is a species belonging to the family Rosaceae, genus *Prunus* (Policarpo M., 2002; Pantelidis G., 2023). Peach is a valuable fruit species in Romania due to its climatic adaptability, distinctive flavor, and economic importance. Peaches are among the most highly valued seasonal fruits, exhibiting remarkable beneficial properties. They can be considered important sources of vitamins, minerals, and other bioactive compounds (Mihaylova, Popova, Desseva, Manolov et al., 2021).

The growth and development of peach trees are strongly influenced by meteorological conditions during early spring. During the active growth phase, seasonal factors such as temperature, humidity, precipitation, and active orchard management are critical for achieving optimal fruit quality (Jiang and He, 2021). Numerous studies have demonstrated that fruit size at harvest is not significantly influenced by external factors during the early stages of fruit growth. In contrast, fruit size can be affected by prolonged drought periods during the main fruit growth phase (Chalmers et al., 1985; Li et al., 1989). It has been reported that peach fruit development may follow a double-sigmoid growth pattern (Silva et al., 2019).

However, this assertion is not entirely consistent, as peach cultivars exhibit variation in their phenological cycles. Specifically, short-cycle cultivars tend to display a simple sigmoid growth curve, whereas intermediate- and long-cycle cultivars generally follow a double-sigmoid growth pattern (Della Bruna, 2007; Della Bruna and Moreto, 2011). To characterize peach fruit growth, various morphometric parameters—such as fruit height, weight, and diameter—have been widely employed, most often analyzed through regression modeling (Casierra-Posada et al., 2004; Quevedo et al., 2017). Nonlinear regression models, however, offer distinct advantages over linear models, particularly in simple nonlinear cases, as they allow for more efficient resource utilization and provide a direct biological interpretation of the estimated parameters.

Nonlinear regression models are frequently applied in the analysis of agricultural data (Archontoulis and Míguez, 2015) due to their methodological simplicity and superior interpretability compared to alternative approaches, even though their specific objectives and applications may vary. The selection between asymptotic and non-asymptotic forms depends partly on the response variable and the duration of the experimental period. Asymptotic models are particularly suitable for analyses encompassing the entire life cycle of organisms or biological processes, whereas non-asymptotic models assume continuous, unrestricted growth over time (Paine et al., 2012).

Growth curves are employed to illustrate the temporal dynamics of change occurring during experimental exposure (Szabelska et al., 2010). Time is considered a continuous rather than a discrete variable, representing a fundamental condition for the generation and analysis of growth curves (Hanusz et al., 2008). Typically, such curves exhibit a sigmoidal (S-shaped) form, which can be modeled using nonlinear regression techniques, thereby providing a practical and biologically meaningful representation of the developmental process (Leal do Prado et al., 2013; Sari et al., 2019; Menezes da Silva et al., 2020).

Sigmoid models are commonly employed to describe variables that evolve over time, including plant height, weight, leaf area index, and seed germination percentage (Míguez et al., 2018). Several mathematical formulations, such as the Gompertz, Richards, and Weibull models, generate sigmoidal-type growth curves (Míguez et al., 2018). These equations define a growth rate that accelerates until reaching an inflection point and subsequently declines. Owing to this behavior, sigmoid models are particularly well-suited for analyzing biological processes characterized by two distinct phases of growth (Shabani et al., 2018).

Previous studies have investigated the growth and development of peach fruits under varying temperature conditions and growth dynamics (Fischer et al., 2011). In the present study, fruit growth dynamics were monitored throughout the entire experimental period in relation to temperature for the *Flacăra* and *Raluca* cultivars.

## MATERIAL AND METHODS

### Germplasm Description

The experimental material consisted of fruits from two Romanian *Prunus persica* (L.) Batsch cultivars — Flacăra and Raluca — selected for their agronomic value and contrasting morphometric traits.

Flacăra is an early-ripening cultivar developed at the Bistrița Research Station for Fruit Growing. It produces medium-sized fruits with an intense red skin and yellow, juicy, and aromatic flesh. The variety is well adapted to local climatic conditions and suitable for both fresh consumption and processing.

Raluca, bred at the Constanța Research and Development Station for Fruit Growing, is also an early-ripening cultivar characterized by large, yellow fruits with firm, sweet, and highly aromatic pulp. It is appreciated for its excellent quality and storage capacity, being suitable for fresh market and industrial use.

Both cultivars were grown under similar orchard conditions, in uniform seven-year-old trees spaced 3 m between plants and 2.5 m between rows. Fruits were harvested manually in the afternoon, placed in labeled paper bags, and analyzed the following morning to minimize post-harvest variation.

### Experimental Design

The study was conducted under uniform orchard conditions using a completely randomized design (CRD). Two *Prunus persica* cultivars (Flacăra and Raluca) were analyzed, with 25 fruits per tree collected for each cultivar.

Measurements included fruit weight, polar diameter, and fruit height, determined on samples of 10 fruits per replicate. All measurements were performed under controlled laboratory conditions.

Data were processed using Excel and R Studio. Descriptive statistics (mean, standard deviation, and standard error) and regression analyses were applied to describe growth patterns and model fruit development dynamics.

## RESULTS AND DISCUSSION

### Analysis of Fruit Weight Using Linear Regression

The one-way analysis of variance (ANOVA) presented in Table 1 indicates a highly significant effect of genotype on fruit weight in *Prunus persica* cultivars ( $p < 0.001$ ). This result demonstrates that the variation in fruit mass is not due to random error, but is strongly associated with genetic differences between the two studied cultivars, *Flacăra* and *Raluca*.

**Table 1.** ANOVA comparison of mean fruit weight between *Flacăra* and *Raluca* cultivars.

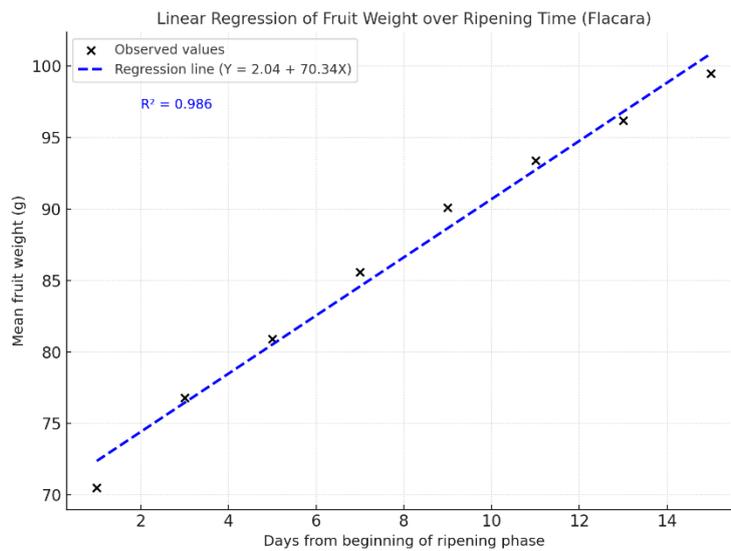
Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	p-value	Significance
Between Cultivars	1	10567.35	10567.35	216.57	< 0.001	***
Within Cultivars (Error)	48	2345.12	48.86			
Total	49	12912.47				

According to Table 2, the Flacăra cultivar recorded a mean fruit weight of 90.65 g, with a standard deviation of 10.14 g, indicating moderate intra-cultivar variability and relatively homogeneous fruit development. The range of values (80.15 – 100.40 g) and the median value (91.42 g) reveal a balanced distribution, confirming uniform ripening and size stability among individual fruits. The narrow confidence interval (65.45 – 115.86 g) further suggests that Flacăra maintains a consistent weight pattern under field conditions.

The higher mean weight observed in Flacăra compared to Raluca emphasizes its superior sink strength, reflecting more active photosynthate allocation to fruit tissues and greater cell expansion during maturation. This trait is particularly relevant for breeding and orchard management, as it confers advantages in both commercial yield potential and market quality standards.

**Table 2.** Statistical values of fruit weight (g) for the *Flacăra* cultivar.

Descriptive	Statistic	Standard Error
Mean	90.65	5.85
Lower Limit	65.45	-
Upper Limit	115.86	-
Median	91.42	-
Standard Deviation	10.14	-
Minimum	80.15	-
Maximum	100.40	-



**Figure 1.** Linear regression of mean fruit weight over ripening time for the *Flacăra* cultivar (*Prunus persica* L. Batsch).

Linear regression analysis shows a strong positive relationship between ripening time and fruit weight in the *Flacăra* cultivar ( $R^2 = 0.986$ ). The model indicates a consistent daily weight increase and uniform fruit development, confirming the cultivar’s efficient ripening and high biomass accumulation capacity (**Figure 1**).

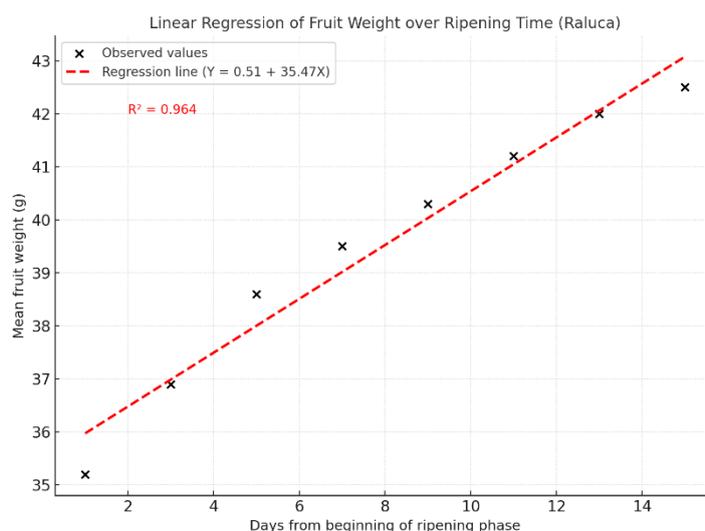
The *Raluca* cultivar recorded a mean fruit weight of 40.28 g, showing lower values than *Flacăra* and a narrower variation range (38.07–42.56 g).

**Table 3.** Statistical values of fruit weight (g) for the *Raluca* cultivar.

Descriptive	Statistic	Standard Error
Mean	40.28	1.30
Lower Limit	34.70	-

Upper Limit	45.86	-
Median	40.20	-
Standard Deviation	2.25	-
Minimum	38.07	-
Maximum	42.56	-

The regression analysis revealed a strong linear relationship ( $R^2 = 0.962$ ) between ripening time and fruit weight, indicating gradual and uniform biomass accumulation. Despite slower growth dynamics, Raluca exhibited stable and consistent fruit development, reflecting its balanced physiological behavior during ripening.



**Figure 2.** Linear regression of mean fruit weight over ripening time for the *Raluca* cultivar (*Prunus persica* L. Batsch).

### Fruit height in the Flacăra and Raluca Cultivars

The ANOVA revealed a highly significant difference ( $p < 0.001$ ) in mean fruit height between the Flacăra and Raluca cultivars. This demonstrates a strong genotypic effect on vertical fruit growth, with Flacăra showing consistently greater height, reflecting its enhanced elongation capacity and distinct morphometric profile.

**Table 4.** ANOVA comparison of mean fruit *high* between *Flacăra* and *Raluca* cultivars.

Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	p-value	Significance
Between Cultivars	1	418.72	418.72	92.64	<0.001	***
Within Cultivars (Error)	48	217.02	4.52			
Total	49	635.74				

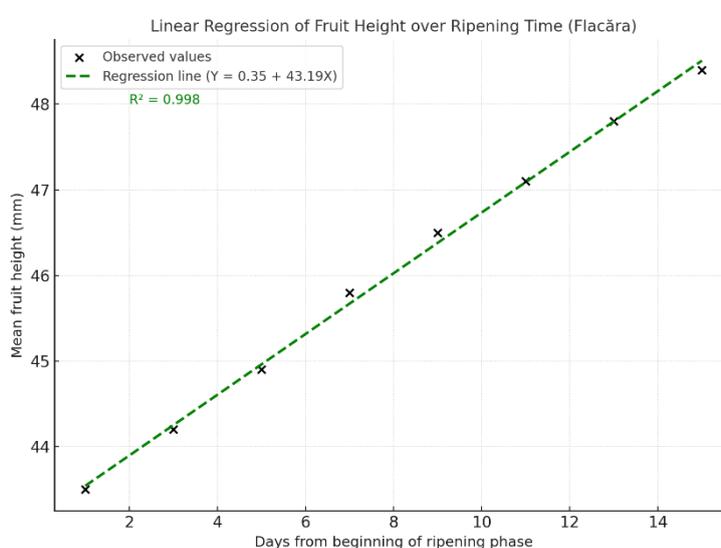
The *Flacăra* cultivar recorded a mean fruit height of 45.82 mm, with a low standard deviation (1.28 mm), indicating uniform fruit size. The narrow range (44.90–47.29 mm) and small variance (1.64) reflect high morphological stability and consistent vertical growth across samples.

**Table 5.** Variation in fruit height (mm) for the *Flacăra* cultivar.

Statistic	Value	Standard Error
Mean	45.82	0.74028

Lower Limit	42.64	-
Upper Limit	49.01	-
Median	45.29	-
Variance	1.64	-
Standard Deviation	1.28	-
Minimum	44.90	-
Maximum	47.29	-

The regression analysis for the Flacăra cultivar shows a very strong linear relationship ( $R^2 = 0.998$ ) between ripening time and fruit height. The model indicates a steady daily increase of 0.35 mm, confirming the cultivar's uniform and predictable vertical growth throughout the ripening phase (Figure 3).

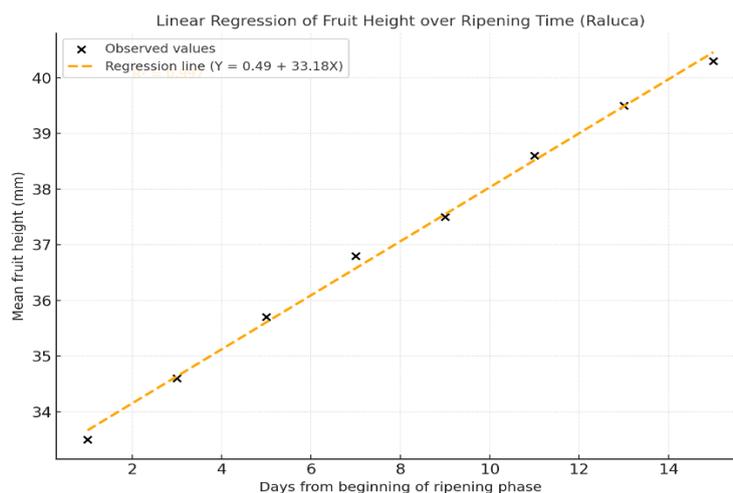


**Figure 3.** Linear regression of mean fruit height over ripening time for the *Flacăra* cultivar (*Prunus persica* L. Batsch).

The Raluca cultivar showed a mean fruit height of 37.75 mm, with a standard deviation of 4.28 mm, indicating moderate variability among samples (Table 5).

**Table 6.** Regression of fruit weight (g) for the Raluca cultivation.

Statistic	Value	Standard Error
Mean	37.75	2.46854
Lower Limit	27.13	-
Upper Limit	48.37	-
Median	37.16	-
Standard Deviation	4.28	-
Minimum	33.80	-
Maximum	42.29	-



**Figure 4.** Linear regression of mean fruit height over ripening time for the *Raluca* cultivar (*Prunus persica* L. Batsch).

The regression model revealed a strong linear correlation ( $R^2 = 0.982$ ) between ripening time and fruit height, reflecting gradual and consistent vertical growth, though at a slower rate than in the Flacăra cultivar (Figure 4).

#### Fruit Diameter in the Flacăra and Raluca Cultivars

The ANOVA revealed a highly significant difference ( $p < 0.001$ ) between cultivars in fruit diameter. The *Flacăra* cultivar produced significantly larger fruits than *Raluca*, confirming a strong genotypic effect on fruit size development (Table 7).

**Table 7.** ANOVA comparison of mean fruit diameter between Flacăra and Raluca cultivars.

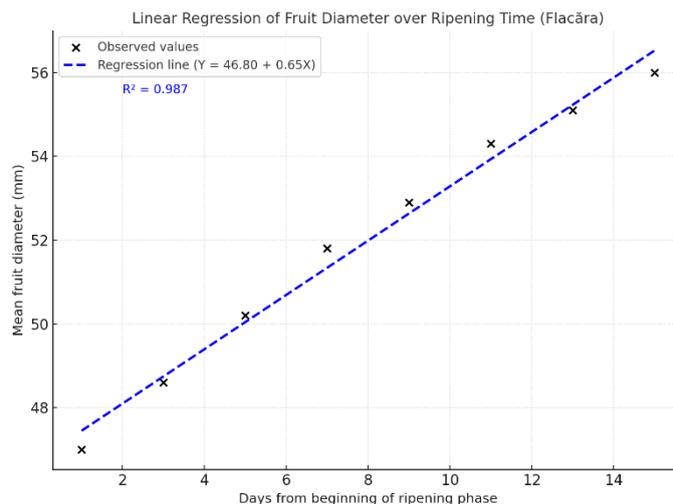
Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	p-value	Significance
Between Cultivars	1	1392.42	1392.42	105.73	< 0.001	***
Within Cultivars (Error)	48	632.24	13.17			
Total	49	2024.66				

Fruit diameter (*Prunus persica*) was evaluated for the Flacăra and Raluca cultivars to characterize their growth.

For the Flacăra cultivar, the mean fruit diameter was  $53.03 \pm 2.17$  mm, with a range between 43.66 mm and 62.40 mm. The median diameter of 54.67 mm indicates a balanced distribution of values, while the standard deviation of 3.77 % reflects moderate dispersion around the mean. The fruit diameter ranged from 48.72 mm to 55.71 mm (Table 8).

**Table 8.** Variation in fruit diameter (mm) for the *Flacăra* cultivation.

Statistic	Value	Standard Error
Mean	53.03	2.17
Lower Limit	43.66	-
Upper Limit	62.40	-
Median	54.67	-
Standard Deviation	3.77	-
Minimum	48.72	-
Maximum	55.71	-



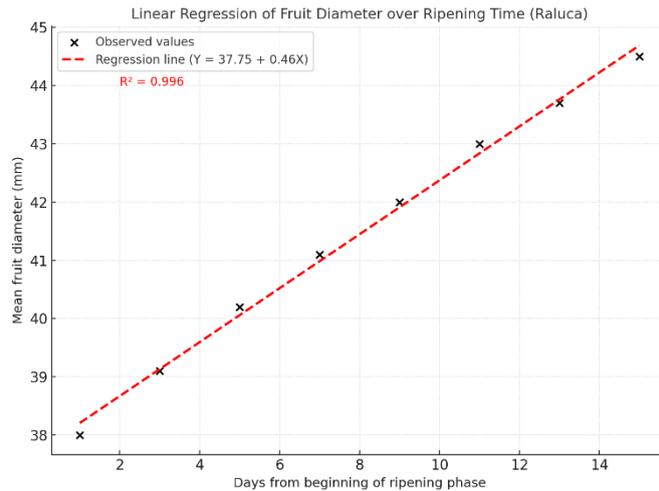
**Figure 5.** Linear regression of mean fruit diameter over ripening time for the *Flacăra* cultivar (*Prunus persica* L. Batsch).

The regression model for the Flacăra cultivar shows a strong linear relationship ( $R^2 = 0.987$ ) between fruit diameter and ripening time. The steady slope indicates a consistent and uniform increase in fruit size throughout maturation, confirming the cultivar's stable growth dynamics during the ripening phase (Figure 5).

For the Raluca cultivar, the mean fruit diameter was  $42.06 \pm 2.46$  mm, with a range between 31.45 mm and 52.66 mm. The median of 41.12 mm suggests a relatively balanced distribution, and the standard deviation of 4.26 % indicates moderate dispersion around the mean (Table 9).

**Table 9.** Variation in fruit diameter (mm) for the *Raluca* cultivation.

Statistic	Value	Standard Error
Mean	42.06	2.46
Lower Limit	31.45	-
Upper Limit	52.66	-
Median	41.12	-
Standard Deviation	4.26	-
Minimum	38.34	-
Maximum	46.72	-



**Figure 6.** Linear regression of mean fruit diameter over ripening time for the *Raluca* cultivar (*Prunus persica* L. Batsch).

The regression model for the *Raluca* cultivar displays a very strong linear correlation ( $R^2 = 0.996$ ) between fruit diameter and ripening time. The gentler slope compared to *Flacăra* indicates a slower but steady fruit enlargement, reflecting this cultivar's more moderate growth dynamics during maturation (Figure 6).

## CONCLUSION

The comparative morphometric analysis of *Prunus persica* cultivars *Flacăra* and *Raluca* demonstrated clear genotypic differences in fruit development dynamics. Across all evaluated parameters—fruit weight, height, and diameter—*Flacăra* consistently exhibited superior values, indicating enhanced growth potential and more efficient assimilate partitioning during maturation. The high coefficients of determination ( $R^2 > 0.98$ ) in regression models confirm strong linear relationships between ripening time and fruit size, evidencing uniform and predictable growth patterns.

ANOVA results ( $p < 0.001$ ) further highlight the significant influence of genotype on morphometric traits, with *Flacăra* showing more pronounced enlargement and stability, while *Raluca* displayed moderate but consistent growth rates. These findings underline the importance of cultivar selection in breeding and orchard management, with *Flacăra* emerging as a promising genotype for achieving superior fruit quality and yield under local agro-climatic conditions.

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## AGRICULTURE OF LIEBLING, TIMIȘ COUNTY - ROMANIA

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*The aim of this paper is to carry out an in-depth analysis of the agricultural system practiced in Liebling, Timiș County, Romania, an area characterized by fertile soils (mainly chernozems) and a temperate continental climate. The study focuses on assessing the structure of the main crops, quantifying the yields obtained in the reference period 2021-2023, and detailing the use of agricultural inputs (amounts of fertilizers and pesticides applied). Also, an important objective is to identify and analyze agricultural areas affected by natural disasters, especially drought, which had a significant impact in 2022 and 2023. The contribution of the study lies in understanding the agronomic particularities of southwestern Romania and providing essential benchmarks for adapting agricultural practices in order to achieve the Sustainable Development Goals.*

**Keywords:** agriculture, soil, crops, production, Liebling

### INTRODUCTION

Agriculture, one of the oldest human activities, remains the foundation of global food security and an essential pillar of economic development in many regions. In the current context, the transition from subsistence to commercial farming poses major challenges related to sustainability, resource management and adaptation to climate change. Recent studies underline the need to integrate modern and efficient agricultural practices that mitigate environmental risks and ensure the stability of production. Timiș County, located in western Romania, stands out for its high agricultural potential, due to the favorable pedoclimatic conditions. Liebling is located in a well-defined natural setting, benefiting from fertile soils, predominantly chernozems, and a temperate continental climate, with an average annual temperature of 10.5°C and a multiannual average rainfall of 630 mm. These characteristics support a wide range of crops, from cereals (wheat, corn, barley) and technical plants (rapeseed, sunflower) to fodder plants (alfalfa, permanent pastures). In the literature, the importance of monitoring the impact of pedoclimatic conditions on agricultural performance is a central topic. The work of Pejić et al. (2009) and Vučić (1976) has already explored the relationship between water balance and irrigation regime, while Payero et al. (2006) have focused on the response of maize crops to poor irrigation in semi-arid areas. At local and regional level, research such as that of Okros et al. (2019) analyzed intensive agricultural management in Banat, and Mihuț et al. (2018) evaluated the production capacity of agricultural land in the perimeter of Periam. Mircov et al. (2022) also conducted a climate risk assessment in the Western and North-West region of Romania. The main objective of this research is to present a detailed analysis of agriculture in Liebling, Timiș, in the period 2021-2023, with a particular focus on:

- Identification and ranking of the main crops and the productions obtained.
- Quantifying the use of chemical fertilizers and pesticides.
- Assessing the impact of extreme weather events, especially drought, on disaster agricultural areas.

### MATERIAL AND METHODS

The study was conducted in Liebling, Timiș County, over a period of three agricultural years: 2021, 2022 and 2023. The primary data concerned the harvested areas (ha), the yields obtained (tonnes), and the quantities of agricultural inputs (chemical fertilizers, herbicides, insecticides, fungicides) applied. Information on pedoclimatic conditions shows that the area benefits from fertile chernozem-type soils and a temperate continental climate.

The methodology was based on the collection and analysis of agronomic data at the level of Liebling, in accordance with the requirements of a descriptive case study. In carrying out the present study, primary

agronomic data were used on the main crops (cereals, technical plants, fodder plants) and on the application of chemicals during the three years of the study. Data were also collected on areas affected by natural risk factors such as drought. The data were structured in separate tables for each agricultural year, allowing a direct comparison of the harvested areas and the yields obtained for the main crop groups (grain cereals, oily plants and fodder plants). When evaluating the Use of Inputs, the total amounts of chemical fertilizers (nitrogen, phosphate, potassium) and pesticides (herbicides, insecticides, fungicides) applied, correlated with the treated area, were analyzed.

The data processing included methods of descriptive analysis, comparison of means and annual variations.

## RESULTS AND DISCUSSION

The results obtained from the analysis of agronomic data for the years 2021, 2022 and 2023 are presented briefly, illustrating the dynamics of crops and inputs used in Liebling.

### Crop dynamics (2021-2023)

The three-year analysis indicates a crop structure dominated by grain cereals and oily plants (Table 1, 2, 3, 5, 6, 7, 9, 10, 11).

**Table 1.** Grain crops in 2021

Crops	Harvested areas	Productions obtained
Grain cereals - total	534	2026.45
Common Autumn Wheat	452.25	1868.16
Barley	39.58	147.40
Corn for grains	42.17	10.89

For grain cereals, in 2021, the total harvested areas were 534 ha, with a total production of 2026.45 tons. Common autumn wheat dominated, having the largest harvested area (452.25 ha) and the highest production (1868.16 tons). Grain corn had the lowest production (10.89 tons).

**Table 2.** Oil crops in 2021

Crops	Harvested areas	Productions obtained
Oily plants – total	560.09	935.71
Sunflower	192.64	189.61
Rape	345.65	746.10
Soy	21.75	0

In oilseeds, rapeseed was the main oilseed crop in all the years studied. In 2021, rapeseed recorded the largest harvested area (345.65 ha).

**Table 3.** Fodder crops harvested since 2021

Crops	Harvested areas	Productions obtained
Forage plants-total	97.47	366.19
Alfalfa for hay and green table	97.47	366.19

In 2021, the alfalfa crop between hay and green mass was harvested from an area of 97.47 hectares.

As for the production obtained at the alfalfa crop for hay and green mass in 2021, it was 366.19 tons.

### Use of Agricultural Inputs (2021-2023)

Consistent application of chemical fertilizers was observed throughout all three years (Table 4, 8, 13).

**Table 4.** Application of fertilisers, amendments and pesticides in the calendar year 2021

Indicator name	Supra.	Singing.
Chemical fertilizers - total	1191.56	246.54
Nitrogen	1191.56	198.88
Phosphate	1191.56	23.83
Potassium	1191.56	23.83
Herbicides - total, of which for:	1191.56	237.30
Wheat	452.25	904.50

In 2021, 246.54 tons} of total chemical fertilizers applied on 1191.56 ha, of which 198.88 tons were nitrogen.

#### Liebling 2022

**Table 5.** Grain cereal crops in 2022

Crops	Harvested areas	Productions obtained
Grain cereals - total	651.11	3042.43
Common Autumn Wheat	491.22	2112.25
Barley	107.40	909.36
Corn for grains	46.06	16.12
Forage plants-total	91.38	341.78
Alfalfa for hay and green table	89.95	339.28
Other perennials for hay and green table	1.43	2.5

The year 2022 recorded the largest harvested areas for grain cereals (651.11 ha), with a total production of 3042.43 tons. Common autumn wheat maintained the supremacy in area (491.22 ha) and production (2112.25 tons).

**Table 6.** Oily plant crops in 2022

Crops	Harvested areas	Productions obtained
Oily plants – total	432.92	1059.49
Sunflower	50	31.50
Rape	380.74	1027.99
Soy	2.18	0

In 2022, rapeseed production was the highest (1027.99 tons) on an area of 380.74 ha.

**Table 7.** Fodder crops in 2022

Crops	Harvested areas	Productions obtained
Forage plants-total	91.38	341.78
Alfalfa for hay and green table	89.95	339.28
Other perennials for hay and green table	1.43	2.5

**Table 8.** Application of fertilizers, amendments and pesticides within the calendar year 2022

Indicator name	Supra.	Singing.
Chemical fertilizers - total	1084.03	238.49
Nitrogen	1084.03	195.13
Phosphate	1084.03	21.68
Potassium	1084.03	21.68
Herbicides - total, of which for:	1084.03	21680.60
Wheat	491.22	9824.40

In 2022, nitrogen fertilizers had the highest share of chemical fertilizers applied, with 195.13 tons, while potassium and phosphatic fertilizers had the same amount applied, with 21.68 tons.

## Impact of natural disasters

The data confirm an increased vulnerability of agriculture in Liebling to drought, a risk also highlighted in regional climate assessment work (Table 9, 11, 14).

**Table 9.** Agricultural areas affected by disasters in 2022

Natural risk factors	Total agricultural (ha)	Arabil (ha)
Drought	31.35	31.35
Total	31.35	31.35

In 2022, a calamity area of 31.35 ha (arable) was recorded due to drought.

## Liebling 2023

**Table 10.** Grain crops from 2023

Crops	Harvested areas	Productions obtained
Grain cereals - total	641.83	2260.30
Common Autumn Wheat	534	1990.30
Autumn barley	69.85	270
Corn for grains	37.68	0

In 2023, the total area remained high (641.83 ha), but the production decreased to 2260.30 tons. Grain corn again had the smallest harvested area (37.68 ha) and a production of 0 tons.

Common autumn wheat was constantly, in all three years analyzed, the crop with the highest productions. The corn grain crop in 2023 has harvested areas of 37.68 hectares, being the crop with the lowest harvested area within grain crops.

**Table 11.** Oil crops in 2023

Crops	Harvested areas	Productions obtained
Oily plants – total	438.07	1258
Sunflower	5.80	0
Rape	432.27	1258

In 2023, rapeseed reached the largest harvested area in the three years (432.27 ha) and the highest production (1258 tons) in the oilseed group. The sunflower had a small area (5.8 ha).

**Table 12.** Oily plant crops in 2023

Crops	Harvested areas	Productions obtained
Forage plants-total	62.24	234.60
Alfalfa for hay and green table	62.24	234.60

Alfalfa for hay and green mass was the only fodder crop in 2021 (97.47 ha and 366.19 tons) and the main one in 2022 and 2023.

**Table 13.** Application of fertilizers, amendments and pesticides within the calendar year 2023

Indicator name	Supra.	Singing.
Chemical fertilizers - total	1036.42	228.02
Nitrogen	1036.42	186.56
Phosphate	1036.42	20.73
Potassium	1036.42	20.73
Insecticides	1036.42	293.74
Fungicides	1036.42	621.85
Herbicides - total, of which for:	1074.10	21.48
Wheat	534.30	10.64
Corn	37.68	0.75

In 2023, 228.02 tons of total chemical fertilizers, with 186.56 tons of nitrogen.

Pesticides, a wide range of pesticides were used. In 2023, applications of Insecticides (293.74) and Fungicides (621.85) were recorded on 1036.42ha, in addition to herbicides.

**Table 14.** Agricultural areas affected by calamity in 2023

Natural risk factors	Total agricultural (ha)	Arabil (ha)
Drought	470	470
Total	470	470

In the year, the calamity area reached the maximum value of the studied period, reaching 470 ha (arable), entirely due to drought.

## CONCLUSION

The analysis of agriculture in Liebling during the period 2021-2023 revealed a series of agronomic particularities essential for understanding and adapting local agricultural practices.

In the crop structure, common autumn wheat has consistently held the crop with the highest yields over the three years. Similarly, rapeseed has established itself as the main oil crop.

The year 2022 recorded the largest harvested areas for grain cereals. However, productions were strongly influenced by environmental conditions.

A constant application of chemical fertilizers was confirmed in all three years, with a major share of nitrogen fertilizers.

The most significant climate risk is represented by drought. The year 2023 recorded the highest share of areas affected by drought, reaching 470 ha. This reality underlines the need for more effective adaptation strategies, such as irrigation practices (see the relationship between yield and water consumption illustrated for maize) and conservative water management.

The study provides valuable baseline data for future decisions on resource management and agricultural sustainability in Liebling, Timiș.

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## ANTIFUNGAL ACTIVITY OF *BACILLUS* SP. 12/R ISOLATED FROM VINEYARD RHIZOSPHERE AGAINST *BOTRYTIS* SPP.

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*Botrytis cinerea* represents one of the major causative agents of severe losses in many economically important agricultural crops worldwide. Considering the negative effects of conventional chemical pesticides, there is a growing need to develop alternative approaches in which bioagents play a crucial role. *Bacillus* species represent some of the most potent microorganisms in this field due to their broad-spectrum antimicrobial activity. On the other hand, wastewater from various food industries, which represents a major environmental problem, could be utilized as a nutrient-rich medium for the production of microbial agents. The aim of this study was to investigate the antifungal activity of the strain *Bacillus* sp. 12/R, isolated from the vineyard rhizosphere, when cultivated on winery flotation wastewater (WFW) against *Botrytis* spp., the causative agents of grey mold on many economically important crops. The cultivation of *Bacillus* sp. 12/R was carried out for 96 h in WFW and in nutrient broth as the control. Biomass content was monitored during cultivation and determined using the drop plate method. Antifungal activity of the isolate against *Botrytis* spp. was determined by the dual-culture method. After 72 h of cultivation, the biomass content increased by approximately 2 log units, which was only slightly lower than in the nutrient broth. *Botrytis* sp. A107 was suppressed by 82.35% in the presence of *Bacillus* sp. 12/R in the growth medium compared to the control, where *Botrytis* sp. A107 was incubated on agarized WFW. Inhibition of *Botrytis* sp. A89/1 by *Bacillus* sp. 12/R reached 92.94%. These results suggest the potential use of WFW as a cultivation medium for the production of a biocontrol agent based on *Bacillus* sp. 12/R as an active component against *Botrytis* spp.

**Key words:** biocontrol, *Bacillus*, winery wastewater, grey mold, *Botrytis* spp.

### INTRODUCTION

Most crop losses in agriculture are caused by diseases resulting from various pathogenic organisms (Roca-Couso et al., 2021). Among these, the fungal pathogen *Botrytis cinerea* is one of the most destructive, with economic losses estimated to range from USD 10 to 100 billion per year (Spada et al., 2024). *Botrytis* is a genus of plant-pathogenic fungi, with *B. cinerea* being the most extensively studied species. It causes pre- and postharvest grey mold on more than 1,400 plant species worldwide (Orozco-Mosqueda et al., 2023). Although *B. cinerea* predominantly infects dicotyledonous plants, certain monocotyledonous species can also serve as hosts. The fungus is capable of colonizing root, stem, and leaf tissues and most frequently affects tomato, grape, and strawberry crops (Zheng et al., 2021). Upon penetrating host tissues, it may remain latent until environmental or physiological changes trigger rapid tissue decay. Typical symptoms on leaves and soft fruits include soft rot accompanied by tissue collapse and water-soaking of parenchymatous cells, followed by the formation of gray conidial masses. This phytopathogenic fungus remains particularly challenging to control due to its exceptionally broad host range, diverse infection strategies, and the presence of both sexual and asexual reproductive cycles, which enable its persistence under variable environmental conditions (Chen et al., 2023; Dujković et al., 2023).

Chemical pesticides are still the most commonly used method for controlling pre- and postharvest crop diseases. Fungicides used strictly to control *Botrytis cinerea* represent about 10% of the fungicide market worldwide (Orozco-Mosqueda et al., 2023). Considering the negative environmental impact of conventional fungicides, there is an urgent need to develop new, environmentally friendly strategies for controlling such pathogens. One of the most promising approaches involves the use of biocontrol agents containing microorganisms as active components (Khan et al., 2022). Among them, *Bacillus* species stand out as particularly effective due to their broad-spectrum antimicrobial activity against various phytopathogens. The

biocontrol mechanisms of *Bacillus* species include the production of antimicrobial compounds classified into three main groups—ribosomal peptides (RPs), non-ribosomal lipopeptides and peptides (NRPs), and polyketides (PKs)—as well as the synthesis of cyclic enzymes, siderophores, volatile compounds, and the induction of systemic resistance. In addition, *Bacillus* species can compete for nutrients and space, further limiting pathogen growth (Karačić et al., 2024). In addition to this biocontrol potential *Bacillus* species have the ability to replicate rapidly, survive in diverse environmental conditions and are able to produce endospores, which make them suitable for the development of bioprocess solutions (Vlajkov et al., 2022).

Food industry effluents represent one of the major environmental concerns, but on the other hand, are of special interest as nutrient-rich media for bioconversion. The high organic and inorganic load of these effluents makes their pretreatment before disposal complicated and expensive, which is why their utilization as raw materials for other production processes is particularly attractive (Dmitrović et al., 2022). The wine industry is considered one of the most socio-economically important activities in Europe (Rodrigues et al., 2022). It is estimated that during the production of 750 L of wine, about 1650 L of wastewater and 200 kg of solid waste are generated. This presents a significant environmental threat considering the high content of organic and inorganic matter in these effluents (Oliveira and Duarte, 2016). The utilization of winery wastewater as a medium for the cultivation of *Bacillus* sp. 12/R provides an opportunity to reduce environmental pollution while simultaneously producing biocontrol agents, aligning with the principles of the circular economy (Dmitrović et al., 2022; Dujković et al., 2025).

The aim of this study was to investigate the antifungal activity of the strain *Bacillus* sp. 12/R, isolated from the vineyard rhizosphere, when cultivated on winery flotation wastewater against *Botrytis* spp., the causative agents of grey mold on many economically important crops.

## MATERIAL AND METHODS

### Microorganisms

*Bacillus* sp. 12/R, isolated from the vineyard rhizosphere, was used as the producing microorganism. Selective isolation was performed by resuspending 1 g of rhizosphere soil in 9 mL of sterile saline solution and homogenizing for 10 min on a rotary shaker at 150 rpm. The suspension was then subjected to heat treatment at 100 °C for 7 min to eliminate all non-sporogenic species. Tenfold, hundredfold, and thousandfold serial dilutions of the suspension were prepared and inoculated onto HiChrome *Bacillus* agar (HiMedia Laboratories, India) plates, followed by incubation at 28 °C for 48 h. Subculturing was performed on fresh medium until a visually pure culture was obtained.

*Botrytis* sp. A107 and A89/1 isolates, obtained from infected grape berries, were used as test microorganisms.

### Medium and cultivation conditions

Winery flotation wastewater (WFW) was mixed with distilled water at a ratio of 4:1 and sterilized by autoclaving at 121 °C and 2.1 bar for 20 min. Nutrient broth (NB) (HiMedia Laboratories, India) was used as the control medium and sterilized in the same manner.

The inoculum of the producing microorganism was prepared by transferring a loopful of *Bacillus* sp. 12/R into sterile nutrient broth and incubating for 24 h on a rotary shaker at 28 °C and 150 rpm. The prepared media were then inoculated with 10% (v/v) inoculum and cultivated for 96 h on a rotary shaker under the same conditions.

### Biomass content determination

During 96 h of cultivation, samples were taken every 24 h to determine biomass content in both WFW and NB media. Biomass content was determined using the drop plate method.

### Antifungal activity of *Bacillus* sp. 12/R

The dual culture method was used to evaluate the antifungal activity of the *Bacillus* isolate. At the end of cultivation, 1 mL of cultivation broth was added to 15 mL of melted agarized WFW (prepared by adding 2% agar to wastewater) and tempered to 50 ± 1 °C and poured into Petri dishes. After solidification, an agar plug (6 mm diameter) containing the test microorganism was placed in the center of the plate, and incubation was carried out at 28 °C for 96 h. After incubation, the growth diameter of the test microorganism was measured.

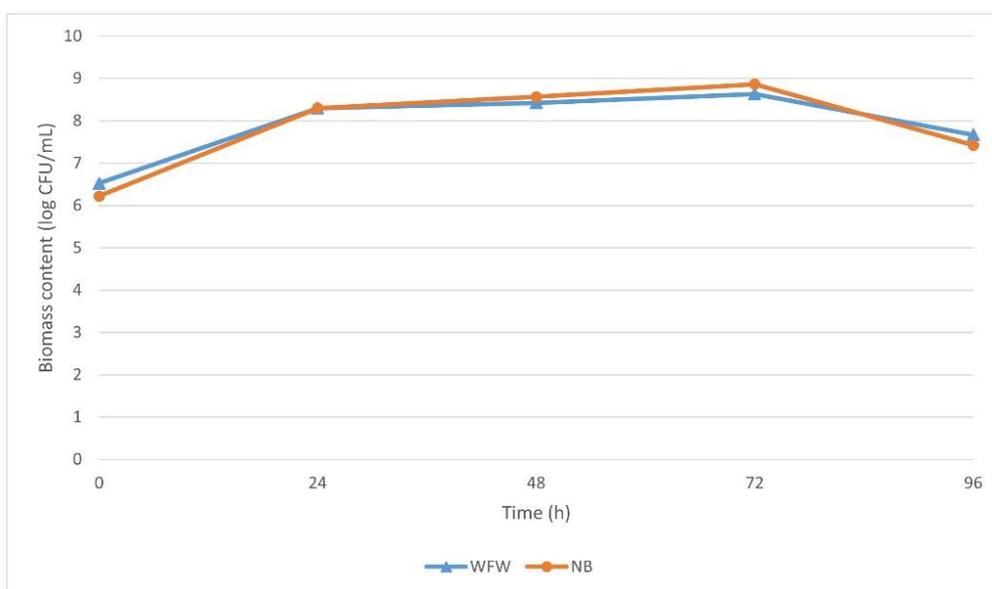
The percentage of inhibition (PI) was calculated using the following formula:

$$PI = \frac{(K - R)}{K} \times 100\%$$

where  $K$  is the growth diameter of the test isolate on agarized WFW, and  $R$  is the growth diameter of the test microorganism on agarized WFW containing the culture broth of *Bacillus* sp. 12/R. Additionally, *Botrytis cinerea* was also cultivated on PDA as a control, but no differences in growth were observed between PDA and agarized WFW, indicating that WFW had no inhibitory effect on fungal growth.

## RESULTS AND DISCUSSION

During 96 h of cultivation, biomass content increased by approximately 2 log units. As shown in Figure 1, the maximum bacterial content was observed at 72 h of cultivation, after which it started to decline. Comparison of biomass content in WFW and NB media indicates that bacterial growth in nutrient broth was slightly higher than in WFW, suggesting that WFW is also suitable for supporting bacterial growth.



**Figure 1.** Growth dynamics of *Bacillus* sp. 12/R in WFW-based and NB medium during 96 h of cultivation.

Agroindustry, which can be defined as a set of economic activities including the production, processing or industrialization, and commercialization of agricultural and forestry products for food or non-food purposes, represents one of the world's largest sources of pollution (Jorge et al., 2022). Agro-industrial waste has gained significant attention over the last decade as a low-cost and sustainable medium for microbial bioconversion. Considering that these byproducts can vary in nature and composition, there is a wide range of possible applications in terms of products, yield, and usage (Astudillo et al., 2023).

Liquid agro-industrial wastes can be generated at all stages of processing and represent a potent source of nutrients for microbial cultivation. In a study conducted by Nguyen et al. (2025), beverage wastewater, pulp and paper mill activated sludge, food industry wastewater, and starch industry wastewater were evaluated as media to produce protease, amylase, and lipase using *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, and *Bacillus megaterium*. The results indicated the potential of these waste streams as suitable media for enzyme production (Nguyen et al., 2025).

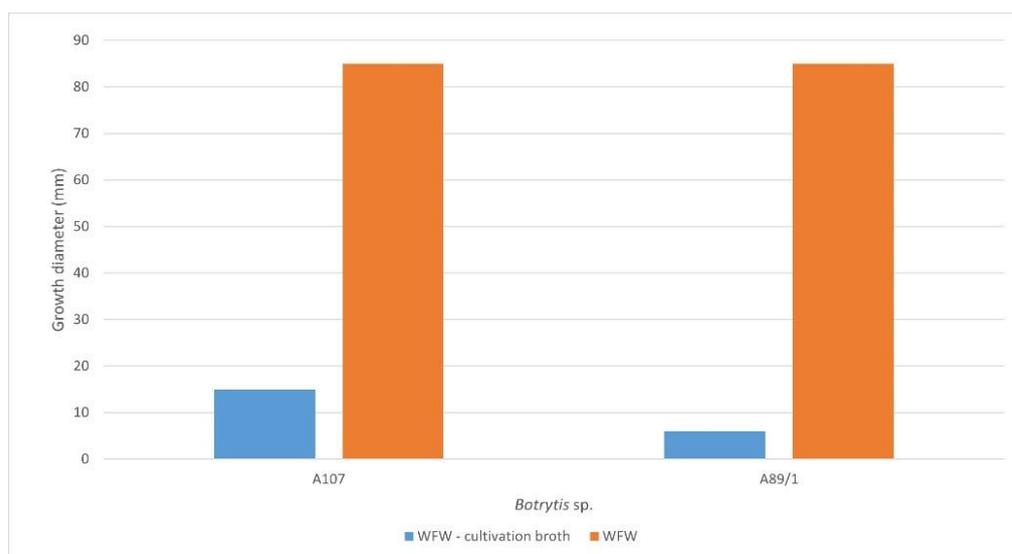
Different agroindustrial wastewaters, including sugarcane vinasse, palm oil mill effluent, cassava processing wastewater, abattoir wastewater, dairy processing wastewater, and aquaculture wastewater, have been investigated for microalgae production (De Carvalho et al., 2022). Starch industry wastewater has also been explored as a medium to produce bioinsecticides based on *Bacillus thuringiensis* var. *kurstaki* HD1 (Ndao et al., 2021).

Winery wastewater as a major effluent of the winemaking industry, exhibits high volumes, pronounced seasonal variability, acidic pH, and elevated concentrations of biodegradable organic compounds, suspended solids, and polyphenols, along with moderate nitrogen, phosphorus, and salt levels. The nutrient-rich and highly biodegradable nature of this waste makes it a suitable substrate for microbial bioconversion processes, such as anaerobic digestion, enabling the production of bioenergy and other value-added products, including biofertilizers (Dujković et al., 2025). Winery wastewater has been considered as a growth medium for microalgae production, either for winery waste treatment (Marchão et al., 2021), to produce agro-industrial

additives (Sousa et al., 2025), or for edible microalgae as a valuable protein product (Spannati et al., 2022). In a study conducted by Dmitrović et al. (2022), winery flotation wastewater was used as a medium to produce a biocontrol agent based on *Bacillus* sp. BioSol021, while in the study by Pajčin et al. (2022), the same isolate was cultivated on barrel-washing winery wastewater.

The observation that maximum bacterial growth occurred at 72 h of cultivation suggests that the bioprocess could potentially be shortened without compromising biomass yield. This reduction in cultivation time could offer significant economic advantages by decreasing operational costs, improving production efficiency, and increasing overall process sustainability.

After 96 h of incubation, the growth diameter of *Botrytis* spp. mycelia on WFW agar containing *Bacillus* sp. 12/R was measured, and the inhibition percentages were calculated.



**Figure 2.** Effect of *Bacillus* sp. 12/R cultivation broth on the growth of *Botrytis* sp. A107 and *Botrytis* sp. A89/1 compared to the growth of these isolates on agarized WFW medium.

As shown in Figure 2, the diameter of *Botrytis* spp. mycelia were significantly reduced in the presence of the cultivation broth of *Bacillus* sp. 12/R. The growth of *Botrytis* sp. A107 was inhibited by 82.35% compared to the control, where the fungus was incubated on WFW agar and reached a maximum diameter of 85.00 mm. Inhibition of *Botrytis* sp. A89/1 by *Bacillus* sp. 12/R was recorded at 92.94%. The control assay demonstrated that WFW alone did not inhibit the growth of *Botrytis cinerea*, confirming that the antifungal effect observed in the dual culture assay resulted from the metabolic activity of *Bacillus* sp. 12/R.

*Bacillus* spp. are recognized as effective biocontrol agents capable of producing a broad spectrum of antifungal metabolites, including lipopeptides, antagonistic proteins, and various secondary metabolites. These bioactive compounds inhibit the growth and sporulation of phytopathogenic fungi, enhance plant resistance, and contribute to sustainable plant protection strategies (Ren et al., 2022). Several *Bacillus subtilis* strains isolated from *Zea mays* were reported by Bolivar-Anillo et al. (2021) to inhibit *Botrytis cinerea* growth by more than 40% in vitro. Similarly, *Bacillus pumilus* strain PTB180 and *B. subtilis* strain PTB185, previously recognized as effective biocontrol agents against various plant pathogens, exhibited strong antifungal activity against *B. cinerea*. The study demonstrated that PTB185 produces lipopeptides of the surfactin, iturin, and fengycin families, while PTB180 secretes surfactins exclusively (Bouchard-Rochette et al., 2022). Furthermore, *Bacillus mojavensis* D50, isolated from the tomato rhizosphere, showed the highest inhibitory effect among eight isolates tested against *B. cinerea*, with an inhibition rate of 69.88% (Zheng et al., 2023).

## CONCLUSION

This study demonstrated that winery flotation wastewater can serve as an efficient and sustainable medium for the cultivation of *Bacillus* sp. 12/R, supporting substantial bacterial growth over 96 h of incubation. The isolate exhibited strong antifungal activity against *Botrytis* spp., with inhibition rates exceeding 80%, indicating its high potential as a biocontrol agent for economically important crops such as tomato, grape, and strawberry.

The results demonstrate that the use of agro-industrial effluents can simultaneously mitigate environmental pollution and serve as a cost-effective, nutrient-rich medium for microbial cultivation. Future research could

focus on optimizing the cultivation conditions of *Bacillus* sp. 12/R in winery wastewater, evaluating its field efficacy against *Botrytis* spp., and exploring its potential application in integrated pest management strategies.

## ACKNOWLEDGEMENTS

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## ANALYSIS OF FACTORS AFFECTING EVAPORATION FROM THE WATER SURFACE OF RESERVOIRS

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*The demand for water resources in countries worldwide is increasing day by day, with the main reasons being the depletion of resources and the growing need for water. The rational use of available water resources and accurate accounting of water in reservoirs are directly linked to the economies of countries specializing in agriculture. This article addresses issues such as scientifically substantiating effective operational regimes for reservoirs, improving the accuracy of water accounting in reservoir reserves, identifying water losses, and developing and enhancing measures to reduce them for a specific object. This article analyzes the patterns of factors affecting evaporation from the water surface of reservoirs.*

**Keywords:** Reservoir, weather station, nature, observation, wind speed, air temperature, evaporation, evaporator, anemometer, thermometer, psychrometer, tacheometer, burette, tube, comparison, object, synchronous, experimental.

### INTRODUCTION

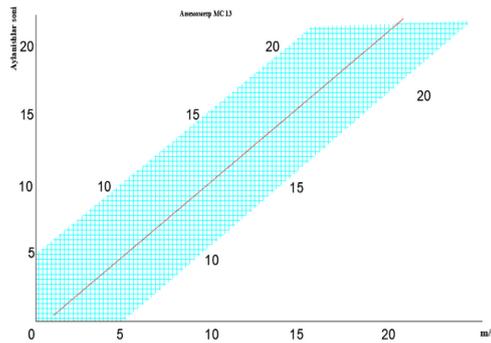
The study was conducted at the Hisorak Reservoir, located in the mountainous region of Kashkadarya province. Due to the absence of permanent meteorological stations at reservoirs, on-site observations and data from the nearest meteorological stations were used. For this reservoir, data from the Shahrissabz meteorological station were utilized [1; 2; 3].

### MATERIAL AND METHODS

The following devices were used to measure wind speed, air temperature, and evaporation in the reservoir:

- GGI 3000 evaporimeter installed on the ground to determine the amount of evaporation;
- MS 13 anemometer, installed at a height of 200 cm above the water surface to measure wind speed;
- TM-6 thermometer to measure water surface and air temperatures;
- MV-4M aspiration psychrometer to determine air humidity;
- AUPNT Tacheometer M3 No. 330101 to accurately mark the evaporimeter installation location.

Research methodology. To measure wind speed, an Anemometer MS 13 handheld measuring device was used.



**Figure 1.** The measurement process using the MS 13 anemometer

MS-13 anemometer (measurement range: 1-20 m/s, sensitivity not exceeding 0.8 m/s, permissible error limit  $\pm (0.1)$ ) does not exceed (Figure 1).

For measuring air humidity, an MV-4-2M aspiration psychrometer was used.

The readings of dry and wet thermometers are taken with an error level of up to  $0.1^{\circ}\text{C}$ , and measurement processes are carried out without removing the device from the stand or touching it by hand (Table 1, Figure 2).

**Table 1.** Absolute humidity of air at a height of 200 cm above the water surface according to the measurement results

Months	1	2	3	4	5	6	7	8	9	10	11	12
$I_{200}$ , gPA	6,11	6,15	8,38	11,13	12,55	13,75	15,44	14,32	11,72	8,61	7,34	6,58



**Figure 2.** The process of taking measurements with an aspiration psychrometer

Evaporimeter GGI 3000: The Evaporimeter GGI-3000 is designed to measure evaporation from water surfaces in open reservoirs and is used for observations at air temperatures ranging from  $+1$  to  $+50^{\circ}\text{C}$ . The operating principle is based on measurements using burettes. It consists of two metal vessels, each 30 cm in diameter, cylindrical in shape with a conical bottom. In the center of one of the water-filled vessels, there is a tube with a measuring scale, ending with a needle that also has a measuring scale. A burette for collecting water is placed on top of this. The amount of evaporated water is determined by calculating the difference in water volumes collected by the burette during two consecutive observation periods.

Technical specifications: Receiving area  $300 \pm 19 \text{ cm}^2$ , overall dimensions  $757 \times 685 \text{ mm}$ , weight should not exceed 17 kg (Figure 3).



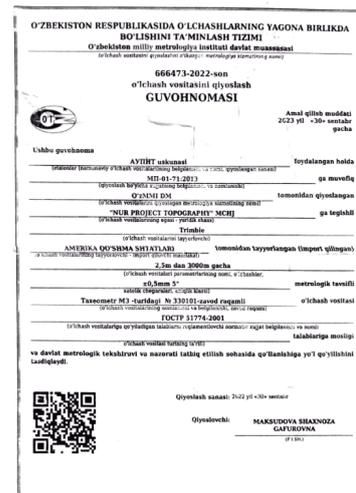
**Figure 3.** Installation and measurement processes of the GGI-3000 evaporator

We installed an evaporator at a point 100 meters from the shore (determined using a tacheometer) relative to the PK102+28 marker on the bank of the Talimarjon Reservoir. At this location, we recorded the wind speed (using an MS 13 anemometer), wind direction (using a wind vane), air temperature (using a thermometer), and atmospheric pressure (using a barometer). Similar measurements and observations were also conducted at designated points on the water surface [4; p. 5].

The Tacheometer M3 device has a verification certificate for measuring instruments No. 666473-2023 issued by the UzNMI DM (Date of verification: September 30, 2022). The AUPNT equipment was operated using the Tacheometer M3 No. 330101, MP-01-71:2013 and the measuring instrument GOSTR51774-2001, radio receivers No. 3293723, 3294066, and GPS receivers. This modern geodetic measuring instrument is capable of performing measurements at distances ranging from 2.5 to 3000 m [6; pp. 17-21]. The maximum error margin of this modern measuring instrument is  $\pm 0.5\text{mm}$ . This modern measuring instrument and its verification certificate are presented, with a validity period until September 30, 2024 (Figures 4 and 4.1) [7; pp. 25-27, 8.].

Data sources. Data from 1975, 2002, and 2019 were compared with the results of our survey work conducted in December 2023.

To determine the relationship between wind speeds at the research site and at the meteorological station, synchronously measured data were analyzed, and a correlation between temperature and wind speed was established [75; pp. 140-143]. Simultaneous measurements were carried out to determine the relationship between wind speeds at the research sites and wind speeds at the meteorological station.



**Figure 4.** M3 Tacheometer



**Figure 4.1:** The Measurement Process

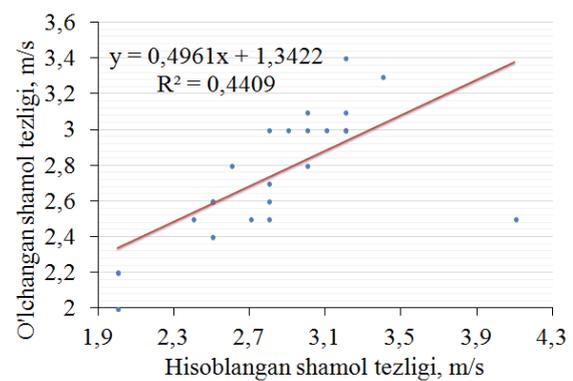
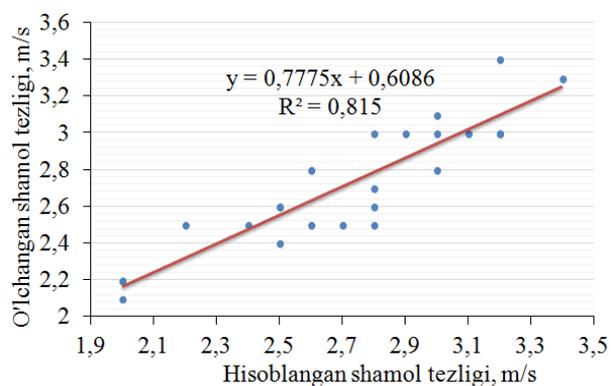
## RESULTS AND DISCUSSION

The following correlations were obtained by analyzing wind speeds and air temperatures measured directly at the Hisorak reservoir, along with data simultaneously recorded at the Karshi meteorological station (Table 2; Figures 5 and 6).

The measurement results justifying the use of data from the Shakhrisabz meteorological station for the Hisorak reservoir are presented in the research table below (Table 2). The analyzed meteorological data were obtained through synchronous measurements conducted simultaneously at the research site and the meteorological station.

**Table 2.** Analysis of wind speeds at the Hisorak reservoir compared to wind speeds at the meteorological station

Wind speed at the meteorological station, in meters per second	Measured wind speed in meters per second	Wind speed at the meteorological station, in meters per second	Measured wind speed in meters per second
3,2	3,4	2	2,2
3,1	3	2,4	2,5
3	2,8	2	2,2
3,4	3,3	2,6	2,8
3,2	3	2,8	2,7
2,5	2,6	2,5	2,6
2,6	2,5	2,8	2,6
2,2	2,5	2,7	2,5
2,8	2,5	2,8	3
3	3	3	3,1
2	2,1	2,9	3
2,5	2,4	3,2	3



**Figure 5.** The relationship between wind speeds measured at Hisorak reservoirs and the Shahrissabz meteorological station

**Figure 6.** The relationship between wind speeds measured at Hisorak reservoirs and those recorded at the Kitab meteorological station

We used the Thermometer TM-6 to measure air temperature: Following the GGI instructions [10; p. 11], we conducted synchronous measurements of air temperature at the research site and the weather station simultaneously.

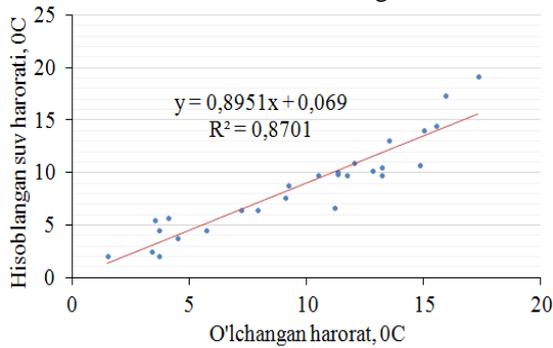
Based on GGI guidelines, correlations were obtained between the measured air temperature in the Hisorak reservoirs and the air temperature at the weather station, recorded simultaneously (Table 3; Figures 7, 8).

The obtained data were compared with the data from the weather station and experimental observations.

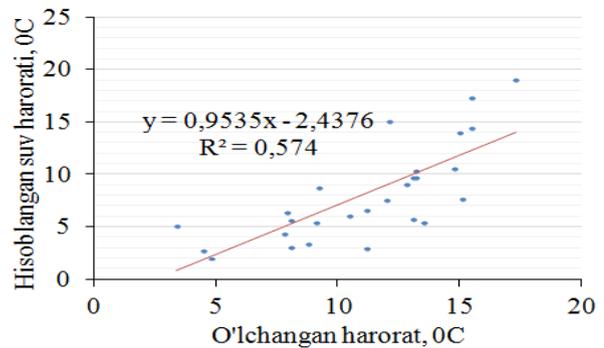
**Table 3.** Results of simultaneous synchronous measurements of air temperature in Hisorak Reservoir and at the weather station

Temperature at the meteorological station, 0°C	Measured temperature, °C	Meteostansiyadagi suv harorati, 0°C	Measured temperature, 0°C
1,5	2,1	11,2	6,7
3,4	2,5	11,3	9,9
3,7	2,1	11,3	10,1
4,5	3,8	12	11
3,5	5,5	13,2	10,5
3,7	4,5	12,8	10,2
4,1	5,7	11,7	9,8
5,7	4,6	13,2	9,8
7,2	6,5	14,8	10,7
7,9	6,5	13,5	13,1
9,1	7,6	15	14,1
9,2	8,8	15,5	14,5
10,5	9,8	15,9	17,4

For the Talimarjon Reservoir, data from the Guzar and Karshi meteorological stations were used. According to these data, wind speeds showed a correlation of  $R^2=0.325$  for the Guzar meteorological station and  $R^2=0.829$  for the Karshi meteorological station. Air temperatures demonstrated a correlation of  $R^2=0.361$  for the Guzar meteorological station and  $R^2=0.851$  for the Karshi meteorological station. Therefore, based on the stronger correlation between the meteorological data of the Talimarjon Reservoir and the Karshi meteorological station, the data from the Karshi meteorological station were used for the reservoir.



**Figure 7.** Correlation between the measured water surface temperature in the Hisorak reservoir and the air temperature at the Shahrissabz meteorological station



**Figure 8.** Relationship between the measured surface water temperature in Hisorak reservoirs and the air temperature at the Kitab meteorological station

## CONCLUSION

Data from the Kitob and Shahrisabz meteorological stations were used for the Hisorak reservoir. The analysis showed that wind speeds correlated with  $R^2=0.441$  for the Kitob meteorological station and  $R^2=0.815$  for the Shahrisabz meteorological station. Air temperatures correlated with  $R^2=0.574$  for the Kitob meteorological station and  $R^2=0.870$  for the Shahrisabz meteorological station. Consequently, based on the stronger correlation between the Hisorak reservoir data and the Shahrisabz meteorological station data, it is justified to use the Shahrisabz meteorological station data for the reservoir (Figures 7 and 8).

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# COMPARISON OF DIFFERENT TYPES OF CONSTRUCTED WETLANDS FOR WASTEWATER TREATMENT

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*This paper investigates the efficiency of wastewater treatment using various types of constructed wetlands (CWs), including horizontal and vertical systems with and without active aeration and planted vegetation. The analysis is based on measurements of parameters such as biochemical oxygen demand (BOD<sub>5</sub>), total organic carbon (TOC), total nitrogen (TN), and ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N). Statistical analysis (ANOVA) and post hoc comparison (Tukey HSD test) revealed that aerated wetlands significantly outperform non-aerated systems, while planting vegetation improved efficiency in all cases. Vertical flow wetlands with active aeration demonstrated greater efficiency compared to horizontal systems in nitrogen removal, particularly ammonium nitrogen. It was concluded that wetlands with active aeration provide optimal conditions for wastewater purification, especially for nitrogen removal, while depth and substrate type also influence overall performance. This study contributes to the advancement of environmentally sustainable wastewater treatment technologies.*

**Key words:** wastewater treatment, constructed wetlands, horizontal and vertical flow, vegetation, purification parameters

## INTRODUCTION

Plant-based wastewater treatment plants are a recognized and widely used alternative wastewater treatment solution. These technologies mimic the processes of self-cleaning of natural ecosystems. In the case of alternative technologies, one of the current proposals stands out, which is the treatment of wastewater using water plants in specially built wetlands, wetlands. This is a biological method of wastewater treatment that is based on a complex combination of the following elements: water, substrate, microorganisms and plants (Davis, 1995). The system mimics the natural wastewater treatment process that exists in natural wetlands. This means that these are built ecosystem services that are similar to natural ecosystem services. Thus, built wetlands represent a system that has certain ecological and economic values. These values can be expressed in the form of direct or indirect values that are ultimately important to human well-being. Moreover, wetlands provide vital goods and services such as gas regulation, groundwater recharge, water runoff, are habitat for different species and have scientific and educational value (Yang et al., 2008).

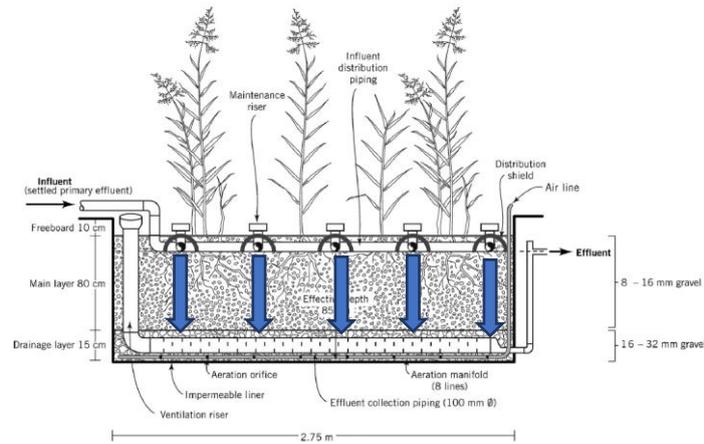
## TYPES OF CONSTRUCTED WETLANDS

There are different types of wetlands for wastewater treatment. There are several types of built wetlands that are used for wastewater treatment. Built-up wetlands can be classified according to the life form of the dominant macrophyte (aquatic plants) at (Brix, 1993):

- 1) Free-floating macrophytes
- 2) Submerged macrophytes
- 3) Rooted emergent macrophytes

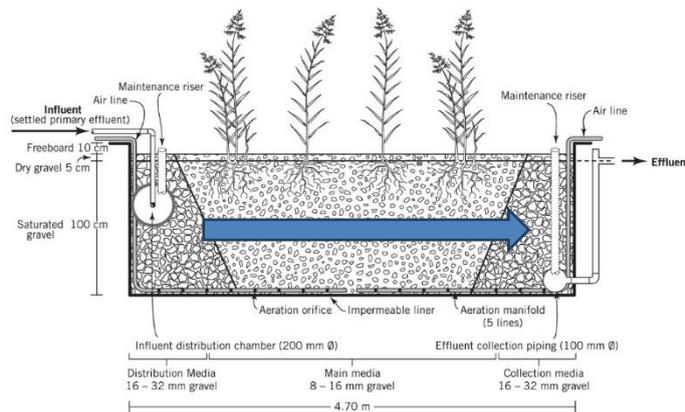
CWs can also be classified based on their hydrology, i.e. the way water flows in these systems (Vymazal, 2010). Therefore, we can distinguish between CWs with free surface water flow and CWs with underground flow. There is a horizontal with underground flow system and due to the significant need to remove ammonia, the development and use of CWs with vertical flow has been developed (Vymazal, 2008). There are also hybrid CWs, i.e. they are usually a combination of horizontal and vertical flow. In this paper, CWs with underground horizontal and vertical water flow were taken into consideration.

The first type of CW refers to a CW in which wastewater moves vertically with active aeration and planted plants (Figure 1). The depth of the substrate is 0.85 cm and the area of the plant is 6.20 m<sup>2</sup>. The substrate that used is gravel, with the hydraulic load of 95 L/m<sup>2</sup> per day, and dosing period of wastewater is 1 hour.



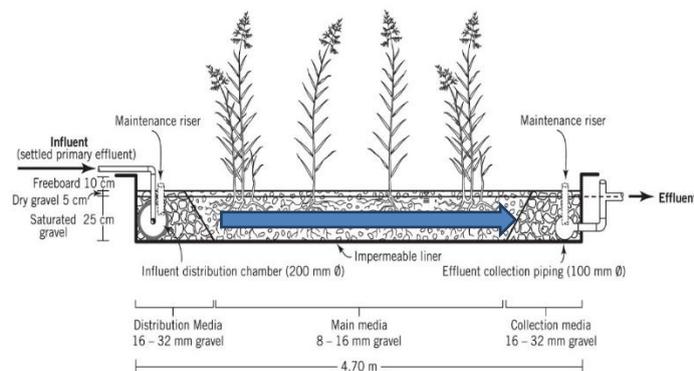
**Figure 1.** Graphical representation of a CW with a vertical flow of wastewater (Nivala et al., 2013)

The second type of CWs refers to a CW in which the wastewater moves horizontally and it has active aeration (Figure 2). It has an substrate depth of 100 cm, and planted plants. As a substrate gravel is used and it has the hydraulic loading is 130 L/m<sup>2</sup> per day. The dosing interval is 0.5 h with the area of the plant of 5.64 m<sup>2</sup>.



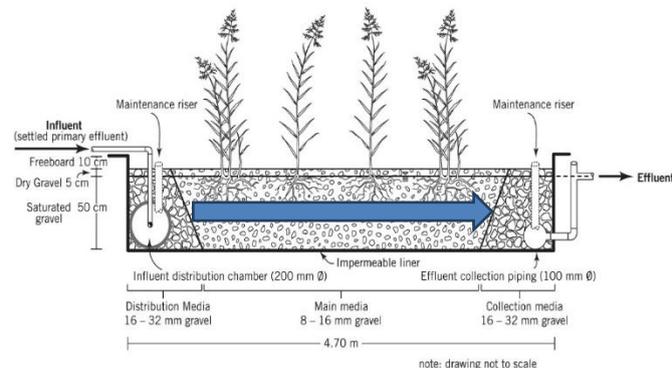
**Figure 2.** Graphical representation of a CW with a horizontal flow of wastewater (Nivala et al., 2013)

In the third type of CW wastewater moves horizontally, and the depth of substrate is 25 cm (Figure 3). It has planted plants or no planted plants and gravel is used as a substrate. The hydraulic load is 18 L/m<sup>2</sup> per day, with dosing interval of 0.5 h, and the area of the plant is 5.64 m<sup>2</sup>.



**Figure 3.** Graphical representation of CW with horizontal movement of wastewater (25 cm depth, with and without plants) (Nivala et al., 2013)

In the fourth type of CW wastewater moves horizontally and the depth of active substrate is 50 cm (Figure 4). It has planted plants or no planted plants and gravel is used as a substrate. The hydraulic load is 36 L/m<sup>2</sup> per day, with dosing interval of 0.5 h, and the area of the plant is 5.64 m<sup>2</sup>.



**Figure 4.** Graphical representation of CW with horizontal movement of wastewater (50 cm depth, with and without plants) (Nivala et al., 2013)

## GOALS AND HYPOTHESES

The aim of this paper is to compare different types of CWs in terms of eliminating different pollutant parameters in order to determine whether there are differences between different types of CWs. The following pollutant parameters are used for comparison: biochemical oxygen demand (BOD<sub>5</sub>), total organic carbon (TOC), total nitrogen (TN), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N). The systems are exposed to different hydraulic loads, have different depths and therefore have different volumes. Therefore, the comparison will be based on the relative removal of pollutants. They are statistically summarized and then compared using hypothesis tests.

The null hypothesis is: Different types of CWs do not show a significant difference in the removal efficiency of different pollutant parameters.

Hypothesis 1: CWs with aeration achieve higher relative pollutant removal than other systems

Hypothesis 2: Planted systems (with plants) have higher relative removal of pollutants than those that are not planted (without plants).

Hypothesis 3: Shallow, non-aerated wetlands have a higher relative removal of contaminants than deeper, non-aerated ones.

## DATA AND METHOD

Measurement data from CWs in the period from September 2010 to July 2014 were analyzed. A total of six constructed wetlands of the following structure were examined.

1. Vertical CW with planted plants for wastewater treatment with active aeration - VAp
2. Horizontal CW with planted plants and active aeration - HAp
3. Horizontal CW with planted plants with a depth of 25 cm - H25p
4. Horizontal CW with a depth of 25 cm without plants - H25
5. Horizontal CW with a depth of 50 cm - H50p
6. Horizontal CW with a depth of 50 cm without plants - H50

For comparison, statistical hypothesis tests were conducted in R Studio software. Analysis of variance (ANOVA) was used for this purpose. Variance analysis compares the variance between different groups (type of plant treatment) with the variance within each of the groups and thus shows whether there are significant differences between the means of the individual groups (Pallant, 2007). A post hoc comparison with the Tukey HSD test was used to clarify which groups have significant differences in the mean. The differences were considered significant if the p-value was less than 0.05. Analysis of variance was performed for the following parameters: biochemical oxygen demand (BOD<sub>5</sub>), total organic carbon (TOC), total nitrogen (TN), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N).

Calculation equation for relative removal of pollutants:

$$PMR (\%) = \left( \frac{Q_i * C_i - Q_o * C_o}{Q_i C_i} \right) * 100$$

$Q_i * C_i$   $\left( \frac{g}{m^2} * d \right)$  is the load of pollutants in the inflow

$Q_o * C_o$   $\left( \frac{g}{m^2} * d \right)$  is the load of pollutants in the outflow

**Table 1.** The data analysed in the paper

Percentage of mass removal of pollutants PMR (%)			
Biochemical oxygen demand - BOD <sub>5</sub>	Total organic carbon - TOC	Total nitrogen – TN	Ammonium Nitrogen - NH <sub>4</sub> <sup>+</sup> -N

## RESULTS

All systems show relative removal of pollutants, biochemical oxygen demand of more than 76%, and total organic carbon more than 77.0% (Table 2). Systems with active aeration VAp and HAp achieve the highest average values for both parameters. In the case of total nitrogen, VAp is the most efficient system, with HAp and H25p achieving a purification performance of 40-48%. It can be seen that planted H50p and H25p systems achieve significantly higher relative removal of pollutant loads than unplanted H50 and H25. Table 2 also shows that systems with aeration of VAp and HAp achieve a purification level of almost 99% in the removal of ammonium nitrogen. Planted systems H25p (28.8%) and H50p (14.6%) are well below this level. In contrast, systems that are not planted have a negative average cleaning performance, which is equivalent to the production of ammonium nitrogen.

**Table 2.** Summary of the percentage of pollutant removal in different CWs, shown as means, including standard deviation in parentheses

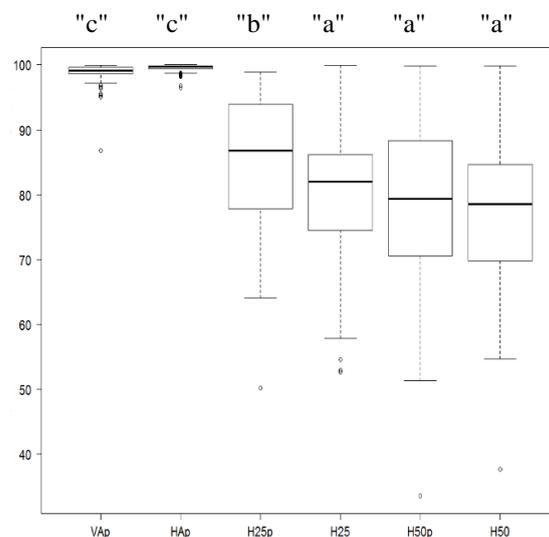
Parameter/Type of CW	VAp	HAp	H25p	H25	H50p	H50
BOD <sub>5</sub>	98.8 (1.5)	99.5 (0.6)	85.3 (9.5)	79.3 (9.3)	78.8 (10.9)	76.6 (10.2)
TOC	90.3 (3.8)	89.8 (5.3)	82.2 (9.0)	77.0 (9.2)	78.5 (9.9)	77.1 (9.1)
TN	53.3 (15.0)	48.8 (13.0)	40.5 (23.1)	13.8 (12.1)	28.0 (16.9)	13.7 (12.3)
NH <sub>4</sub> <sup>+</sup> -N	98.6 (4.1)	98.9 (8.7)	28.8 (29.1)	-4.9 (23.3)	14.6 (23.5)	-3.4 (21.6)

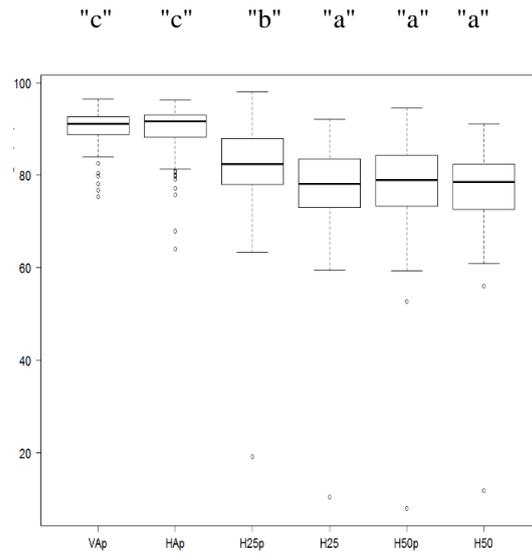
The results of the corresponding analyses of variance for the different parameters of the pollutants show that there is a statistically significant difference between the systems, since the p-value [Pr (>F)] is in each case below 0.05 (Table 3). The null hypothesis can therefore be rejected.

**Table 3.** Summary, results of analysis of variance (ANOVA) of relative pollutant removal

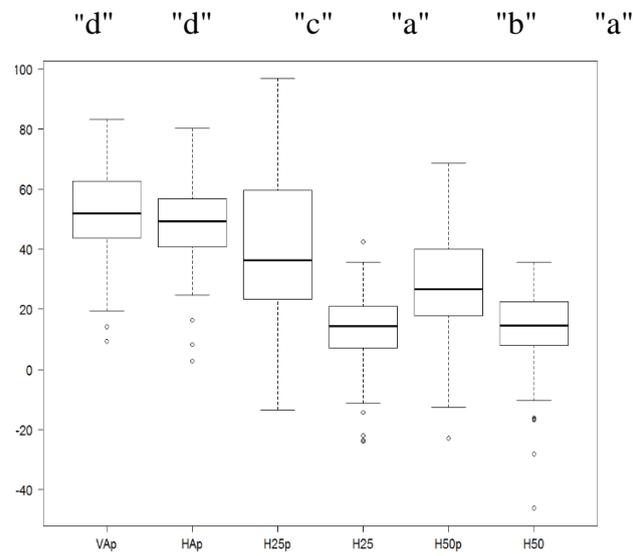
Parameter		Degree of freedom	SSKE	MSKE	F-Value	Pr (>F)
BOD <sub>5</sub>	System	5	69498	13900	203.9	<2e-16 ***
	Remains	794	54132	68		
TOC	System	5	24542	4908	73.8	<2e-16 ***
	Remains	793	52745	67		
TN	System	5	199684	39937	158.2	<2e-16 ***
	Remains	809	204173	252		
NH <sub>4</sub> <sup>+</sup> -N	System	5	1533536	306707	722.5	<2e-16 ***
	Remains	805	341730	425		

The relative removal of pollutants of individual systems for different parameters are shown on box plots (Figures 5 - 8). The results of the Tukey HSD test are marked with letters in the corresponding boxes. These letters ("c" "c" "b" "a" "a" "a") provide information about whether the means of the individual systems differ significantly from each other. If two or more systems have the same letters, their mean values do not differ significantly. If they have different letters, the mean values are significantly different from each other. Post hoc comparisons using the Tukey HSD test show that the mean biochemical oxygen demand and total organic carbon between HAp and H50, VAp and H50, HAp and H50p, VAp and H50p, HAp and H25, VAp and H25, H50 and H25p, H50p and H25p, HAp and H25p, VAp and H25p, H25p and H25 differ significantly. Moreover, the mean between VAp and HAp, H50p and H50, H25 and H50p, H50 and H25 does not differ significantly (Figures 1 and 2). In the case of total nitrogen and ammonium nitrogen removal, post hoc comparisons with the Tukey HSD test show that the mean value between H50p and H25, HAp and H25, VAp and H25, H50 and H25p, H50p and H25p, HAp and H25p, VAp and H25p, H50p and H50, HAp and H50, VAp and H50, HAp and H50p, VAp and H50p differ significantly. Moreover, the mean value between VAp and HAp, H50 and H25 does not differ significantly. This is shown in Figures 3 and 4 and these letters ("d" "d" "c" "a" "b" "a"). The results show that aerated wetlands have the best purification performance. In addition, boxing plots show that non-aerated systems with plants have better performance compared to non-aerated systems without plants. According to the results, hypotheses 1 and 2 can be accepted. Hypothesis 3 can be partially accepted. Within the non-aeration system, the H25p system has the best removal of contaminants. However, in the case of removing total nitrogen and ammonium nitrogen, H50p performs better than H25 and H50. That is why there is a mutual effect of plants and depth on the process of removing pollutants.

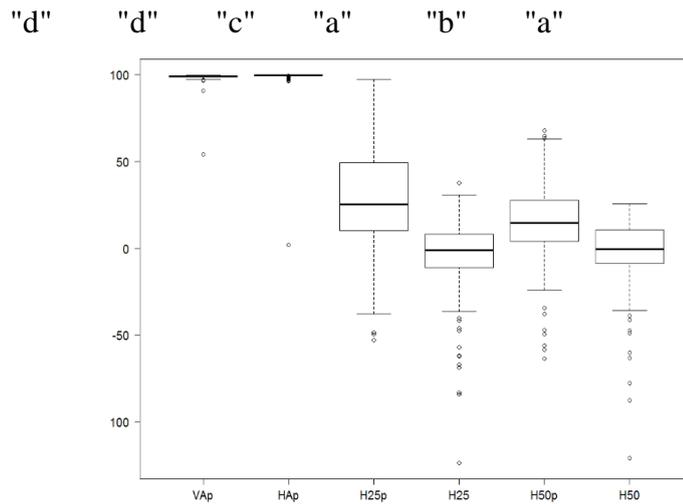
**Figure 5.** Comparison of the relative removal of biochemical oxygen demand (BOD<sub>5</sub>)



**Figure 6.** Comparison of relative removal of total organic carbon (TOC)



**Figure 7.** Comparison of relative removal of total nitrogen (TN)



**Figure 8.** Comparison of the relative removal of ammonium nitrogen ( $\text{NH}_{4+}\text{-N}$ )

## DISCUSSION

The purification efficiency for biochemical oxygen demand and total organic carbon analyzed in this paper is between 76.6 to 99.5% and 77.0 to 90.3%, respectively. In the literature, it is commonly reported that the removal of organic pollutants in wetlands is between 80-90% (Lee et al., 2009; Shutes, 2001). The results obtained in this study are comparable. Generally speaking, wet fields can be designed to remove more than 90% of biochemical demand for oxygen. However, there are differences between the different types of wet fields. The results show that aerated wet fields (VAp and HAp) provide the greatest removal of biochemical oxygen demand, total organic carbon, total nitrogen and ammonium nitrogen. Aerated systems do not achieve complete removal of total nitrogen (48.8 - 53.3%), but significantly higher than non-aerated systems (13.7 - 40.5%). The H25p system has the best performance in non-aerated wet fields. However, plants allow for a significant increase in this in aerial systems. The wet fields used in this paper are planted with reeds (*Phragmites australis*). An important factor that could affect the performance of wet fields is the type of plants and their ability to supply the root zone with oxygen, which is very important for the metabolism of microorganisms. Species such as reed (*Phragmites australis*) and cattail (*Typha latifolia*) have good contaminant removal properties (Shutes, 2001). Therefore, species such as reeds achieve greater oxygen transport to the root region via internal convective oxygen transport than species whose internal oxygen transport is diffuse (Brix, 1994; Vymazal and Kroepfelova, 2008).

The average efficiency of elimination of total nitrogen in this work ranges from 13.7% to 53.3%. In the literature, nitrogen removal values are given as 25 to 85% (USEPA, 1988). In the case of removal of ammonium nitrogen, non-aerated systems show negative values, which means that they do not remove the pollutant but produce it. In this paper, this is the case for the H25 and H50 systems. One of the reasons is the occurrence of ammonification of organic nitrogen contained in influent and the lack of oxygen in conventional, horizontally wet fields, and therefore the ability of the system to nitrify the ammonium contained in influent and formed in the system (Vymazal, 2007). This lack of oxygen is caused by its consumption in the removal of organic matter and thus significantly reduces nitrification and, therefore, the removal of ammonium. One of the most important processes for further nitrogen removal is denitrification. This describes the reductive removal of nitrate nitrogen and therefore depends on prior nitrification during municipal wastewater treatment. Unlike nitrification, denitrification usually takes place only in the absence of oxygen. Both processes therefore have different requirements for the respective environment (Vymazal, 2001).

## CONCLUSION

From the results obtained, it can be concluded that the different wet fields presented in this paper allow good purification performance in the case of organic carbon compounds. However, there is a clear difference between the different systems. The results of the analysis of variance (ANOVA) show that there is a statistically significant difference between the systems for all pollutant parameters ( $p < 0.05$ ), which means that different types of wetlands have different efficiency in removing pollutants, so the null hypothesis is refuted. Aerated systems achieve the highest purification performance in terms of organic carbon and nitrogen. This confirms

hypothesis 1 because the active aeration systems (VAp and HAp) showed the highest average values in the removal of biochemical oxygen demand, total organic carbon, total nitrogen and ammonium nitrogen. Non-aeration systems have significantly lower purification performance when it comes to removing nitrogen, especially ammonium, although plants are increasingly influencing this. Planted systems without aeration H50p and H25p achieve significantly higher relative removal of contaminants compared to unplanted systems without aeration H50 and H25, thus confirming hypothesis 2. In non-aerial systems, the shallow H25p system had the best results in removing contaminants compared to the deeper H50p system. As for the shallow H25 system without bilges and the deeper H50 system, the results for ANOVA show that there is no statistically significant difference between the two systems. With this, hypothesis 3 is partially confirmed, indicating a complex interaction between depth and the presence of plants. Finally, the results show that the constructed wastewater treatment plant with active aeration (VAp and HAp) is a suitable technology for all four wastewater parameters.

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# THE INFLUENCE OF CALCIUM DEFICIENCY ON TOMATO DISEASE, IN THE CLIMATIC CONDITIONS OF THE WESTERN PART OF ROMANIA

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*The study analyzes the influence of calcium deficiency on the occurrence and severity of tomato diseases under the specific climatic conditions of western Romania, correlating plant health status with the pedological characteristics of the cultivated area. A detailed soil survey identified a typical Eutric Gleysol, developed on fluvial deposits with medium to fine textures, moderately acidic reaction in the upper horizons, reduced aeration, diminished microbiological activity, and low availability of mobile phosphorus and potassium. These characteristics, combined with hydric stress and high atmospheric temperatures, favoured the appearance of several physiological and pathogenic disorders in tomato crops, such as *Leveillula taurica* (powdery mildew), *Botrytis cinerea* (grey mould), and sunscald associated with mildew. Soil physical and chemical parameters were analyzed across five diagnostic horizons, providing essential insights into water retention, porosity, pH evolution, humus content, and nutrient supply. Ameliorative strategies, including drainage works, deep ploughing, soil loosening, organic and mineral fertilization, and calcium amendments, were applied to improve soil structure and nutrient balance. The results confirm the strong interaction between soil constraints, climatic conditions, and plant nutritional status. The integration of pedo-hydro-ameliorative measures with adaptive technological practices is essential for restoring soil fertility and reducing crop vulnerability to abiotic and biotic stress factors.*

**Key words:** gleysol; calcium; tomatoes; diseases; pedology; fertility; western Romania

## INTRODUCTION

The soil is one of the main factors that determine the development and productivity of horticultural crops, directly influencing the nutritional status of plants, sensitivity to stress and the appearance of diseases. In the agricultural areas of western Romania, characterized by large variations in the water regime and frequent episodes of heat, nutritional imbalances – especially calcium deficiency – are becoming more and more common, significantly affecting tomato crops. The relationship between edaphic properties, calcium availability, and plant susceptibility to disease has been intensively studied in recent decades, both internationally (Simon, 1978; Kirkby and Pilbeam, 1984; White & Broadley, 2003), and in Romania (MIHUȚ et al., 2024; Gerard Jitareanu et al., 2007).

Calcium is an indispensable nutrient with a structural role in stabilizing cell walls and membranes, cell division, and nutrient translocation (Kirkby & Pilbeam, 1984; White and Broadley, 2003). Its deficiency causes a number of physiological disorders characterized by tissue collapse and increased susceptibility to disease (Chao et al., 2008; De Freitas et al., 2016), being reported in numerous horticultural species, including spinach, tomatoes or lettuce (Story et al., 2010; Khan et al., 2017). Although calcium deficiency is frequently attributed to reduced soil supply, recent studies suggest that symptoms such as tomato apical rot may also be caused by other physiological factors, not just reduced availability of the element (Nonami et al., 1994; Torres et al., 2024).

Tomatoes are particularly sensitive to water oscillations, extreme temperatures and reduced mobility of calcium in tissues, which favors the appearance of diseases such as apical burn, *Botrytis cinerea*, *Leveillula taurica* or sunburn (Kalra, 1956; Morard et al., 1996; John and Brandenberger, 2014; Olle & Bender, 2009). The international literature highlights the fact that fungal and viral pathogens manifest themselves with a greater amplitude in crops exposed to nutrient or water stress (Lukyanenko, 1991; Hanssen et al., 2010), and this sensitivity is directly influenced by the health of the soil and its fertility.

At the soil level, the availability of calcium directly depends on a number of edaphic properties, such as soil reaction, texture, cation exchange capacity, presence of salts, degree of glaciation or humus level. Studies

carried out in western Romania show that soils with acid reaction, periodic water stagnation, low porosity and weak microbiological activity favor the appearance of nutrient deficiencies and reduce calcium mobility in the soil-plant system (Gerard Jitareanu et al., 2007; MIHUȚ et al., 2024). This category also includes gleisols, which are widespread in lowland areas, where excess moisture, settlement and high clay content limit aeration and ion exchange.

In parallel, numerous studies highlight the correlation between abiotic stress and disease dynamics in tomatoes, including the occurrence of manna, gray rot, or emerging viral diseases (Hanssen et al., 2010; Minghua Zhang et al., 2003). Modern methods such as hyperspectral sensors, computer vision or digital analysis of plant traits allow the early detection of calcium deficiency-induced stress, confirming its major impact on crop health (Story et al., 2010; Minghua Zhang et al., 2003).

In this context, the pedological analysis of an agricultural area and the correlation of soil properties with the symptoms of calcium deficiency and the occurrence of diseases in tomatoes become essential for the development of sustainable cultivation technologies. The present study aims to highlight the characteristics of the soil in the investigated perimeter, to identify the limiting factors of fertility and to analyze how they influence the occurrence of diseases associated with calcium deficiency in tomatoes grown in the climatic conditions specific to the western part of Romania (Sânandrei commune).

## **MATERIAL AND METHOD**

The study was carried out on the territory of Sânandrei commune (Timiș county), located in the Tisa plain, about 15 km north of Timișoara. The area is characterized by two main forms of mesorelief: the high plain of subhillary glacia and the low plain of subsidence.

The methodology includes: detailed morphological description of the soil profile according to the Field Description Guide sampling on diagnostic horizons (Ap, Ao, ABGo, BtGo, Gr); physical analysis: texture, density, porosity, field capacity, useful water, according to Mihut & Lațo (2007); chemical analysis: pH, humus, macroelements, cation exchange capacity assessment of diseases in tomatoes in correlation with nutritional status and climatic regime; application of foliar treatments and calcareous soil amendments (Stroia, 2009).

**Table 1.** Chemical and physical properties of typical gleiosol (after OSPA Timișoara 1997)

HORIZONS	Ap	Ao	ABGo	BtGo	Gr
Depths	0-12	12-26	26-41	41-62	62-120
Size (2.0-0.2 mm) %	4,3	3,1	2,5	2,5	3,8
Fine sand (0.2-0.02 mm) %	26,9	25,7	27,5	25,6	29,2
Dust (0.02-0.002 mm) %	29,5	29,8	32,6	32,0	26,2
Clay 2 (less than 0.002 mm) %	39,3	41,4	37,4	39,9	40,8
Physical clay (under 0,01 mm) %	56,3	58,7	57,8	59,5	54,5
TEXTURE	TT	TT	TT	TT	TT
Density (g/cm <sup>3</sup> )		2,50	2,51	2,54	
Bulk density (DA g/cm <sup>3</sup> )		1,32	1,42	1,28	
Total porosity (PT %)		47,2	43,42	49,60	
Aeration porosity (PA %)		-6,52	-8,83	-6,92	
Degree of settlement (GT %)		8,78	15,01	3,68	
Coef. de higroscopicitate (CH %)		9,9	9,9	9,6	
Coef. by ofilire (CO %)		13,2	11,85	14,4	
Field capacity (CC %)		40,70	36,80	44,16	
Total Capacity (CT %)		35,75	30,58	38,75	
Useful water capacity (CU %)		27,50	24,95	29,76	
pH in H <sub>2</sub> O	5,45	5,55	6,05	6,15	7,00
Humus (%)	2,97	2,42	1,67	1,31	
P min.		51,74	51,09	51,50	
Nitrogen index (IN)	2,3	1,9			
N total (%)	0,147	0,120	0,080		
P mobile (ppm)	8,0	7,0	4,0		
K mobile (ppm)	104	10	84		
Exchange bases (SB m.e./100 g sol)	23,15	23,54	25,90	27,07	28,84
Exchangeable hydrogen (SH m.e./100 g sol)	6,84	6,22	5,52	4,73	4,48
Capac. de schimb cationic (T m.e./100 g sol)	29,99	29,76	31,42	31,80	32,32
Degree of saturation, in bases (V, %)	77,19	79,09	82,43	85,15	89,23

The bulk density (1.28–1.42 g/cm<sup>3</sup>) and total porosity (43.42–49.60%) indicate a high degree of compaction, specific to gleiosols (Rusu et al., 2002).

The soil reaction is acidic in the upper horizons (pH 5.45–5.55) and weakly acidic to neutral in the lower horizons (pH 7.00). The humus content is moderate in the Ap horizon (2.97%), reducing towards the base (1.31%) — characteristic of soils with periodic water stagnation.

The supply of mobile P and K is low, which limits microbiological activity and the availability of other nutrients.

### Fertility limiting factors

Based on the analyzed data, the major limiting factors (Figure 1, 2, 3) are: excess moisture in the cold season and stagnation of water; compaction of the upper horizons; acid reaction; low P and K intake; relatively low aeration capacity.

These are consistent with the conclusions of other regional studies.



**Figure 1.** Effect of drought on tomato crops



**Figure 2.** Efectul excesului de apă asupra culturii de tomate



**Figure 3.** Manifestarea carenței de Calciu la fructele de tomate

### Effects of calcium deficiency on tomatoes

In the analyzed perimeter, calcium deficiency, associated with atmospheric drought, caused the appearance of the following diseases (Figure 4 and 5): *Leveillula taurica* (manna); *Botrytis cinerea* (gray rot); sunburn associated with manna.

Calcium plays a role in membrane stability, nutrient translocation and mechanical resistance of the cell wall, and its deficiency increases the vulnerability of plants to pathogens.

The treatments applied (Foliq Calmax, Humitex, Champ, calcareous amendments) reduced the effects of diseases and improved the general condition of the plants, confirming the importance of nutritional management.



**Figure 4.** Manifestarea Botrytis cinerea la tomate



**Figure 5.** Manifestarea Leveillula taurica la tomate

***Proposed ameliorative measures***

drainage and dewatering;  
deep ploughing and loosening in a direction perpendicular to the drains;  
application of calcareous amendments;  
organic fertilization;  
adaptation of cultural technologies to local conditions.

## CONCLUSIONS

The analyzed agricultural perimeter is characterized by a typical Gleiosol with low natural fertility, influenced by excess moisture, compaction, moderate acidity and reduced nutrient supply. Calcium deficiency, correlated with water and heat stress, favors the appearance of important diseases in tomatoes. Soil improvement, through hydroameliorative and pedomeliorative measures, associated with adapted technological management, can contribute considerably to increasing productivity and reducing the vulnerability of horticultural crops.

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## WATER QUALITY OF RODIC BARA POND – PRELIMINARY RESEARCH

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*Wetlands function as part of the landscape with or without the presence of humans. They have value because many of their functions have proved to be useful to human societies. Wetlands provide a range of critically important ecosystem services including fresh water, nutrient cycling, food and fibre production, carbon fixation and storage, flood mitigation and water storage, water treatment and purification and habitats for biodiversity. Despite their importance, the extent and quality of wetlands continue to decline worldwide. This study assessed the physico-chemical water quality of the Rodic Bara pond. The results were evaluated and compared with the national legislative framework — Regulation on Parameters of Ecological and Chemical Status of Surface Waters and Parameters of Chemical and Quantitative Status of Groundwaters (Official Gazette of the Republic of Serbia, No. 74/2011). Water samples were collected from four different pond points from the shore, including one from an anthropogenically impacted part. Based on the analyzed parameters (DO,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , COD,  $\text{BOD}_5$ , suspended solids, pH, and EC), the findings indicate that the water of „Rodic Bara“ pond classifies to Class IV quality, which reflect a poor ecological status under national classification standards. Preliminary results from this study highlight the impact of anthropogenic pressures on wetland water quality and to emphasise importance of these ecosystems for biodiversity, threats and conservation of these critically important ecosystems.*

**Key words:** Wetlands, biodiversity, water quality, anthropogenic pressure, conservation

### INTRODUCTION

Wetlands have been defined as areas that have free water at or on the surface for at least the major part of the growing season. Wetlands have value because their functions have proved to be useful to humans (Mitsch and Gosselink, 2000). The water is sufficiently shallow to allow the growth of a wetland crop or natural vegetation rooted in the soil (Brinkman and Blokhuis, 1986). Wetlands are irreplaceable ecosystems due to the numerous benefits, or ecosystem services, they provide to humanity — including freshwater supply, food and building materials, biodiversity conservation, flood control, groundwater recharge, and climate change mitigation (Ramsar, 2025 and 2023). Wetlands support unique and high levels of biodiversity, not only obligate organisms but also many terrestrial and marine organisms indirectly, through wetland productivity, nursery habitats and fresh water (Kingsford et al. 2016). Despite their importance, numerous studies indicate that the area and quality of wetlands continue to decline in most parts of the world. As a result, the ecosystem services that wetlands provide to humans are increasingly being degraded. There has been a much (3.7 times) faster rate of wetland loss during the 20th and early 21st centuries (Davidson, 2014). A realistic estimate is that 50 % of the world's wetlands have been lost (Verhoeven and Setter, 2010). Wetland loss and degradation is an indisputable reality, and human activities are chiefly to blame as the main factors (Shengjie et al., 2017). Anthropogenic pressures, such as urbanization, agricultural runoff, wastewater discharge, and land-use changes, are among the main causes of wetland degradation. Hoffmann (1964) concluded that in temperate regions drainage of wetlands is proceeding at an increased rate and without reference to their diverse values, and recommended the establishment of an international convention on wetlands. This led, in 1971, to the global in scope establishment of the ‘Ramsar Convention on Wetlands’ which recognised the great value of wetlands (Carp, 1972). Within the territory of the Republic of Serbia, most wetland areas are situated in the north of the state (Grabic, 2024). There are 11 Ramsar sites located in the Serbia and one of them, Koviljsko-Petrovaradinski Rit, is mostly located on the territory of Novi Sad. Furthermore, wetlands in urban regions may take on human-related values that they lack in non-urban areas, as they provide some contact with nature, and some

opportunities for recreations that are otherwise rare in the urban landscape (Ehrenfeld, 2000). The aim of this preliminary study was to assess the impact of anthropogenic factors on the degradation of the physical and chemical water quality of Rodic Bara pond which can directly influence the biodiversity (Birn, 2025). Aim of this preliminary research is especially interesting since Novi Sad has been named an official Wetland City, one of 31 new cities to receive this title in January 2025.

## MATERIAL AND METHODS

Official documents of the City of Novi Sad describe examined water body as an elliptical wetland area of lake type, without direct contact with watercourses. Its maximum length is about 700 meters, and its maximum width about 650 meters. At this site, 104 bird species have been recorded, 89 of which are strictly protected. Among various avian species, Great cormorant *Phalacrocorax carbo* is widely present and well known as a main piscivorous species in Novi Sad area. This water body was formed in the past as a result of sand excavation (NSA 10a,b) and nowadays represent important habitat refugia for migratory birds. An additional threat is posed by the proximity of the city landfill, and deposition of debris from various sources (Figure 1).

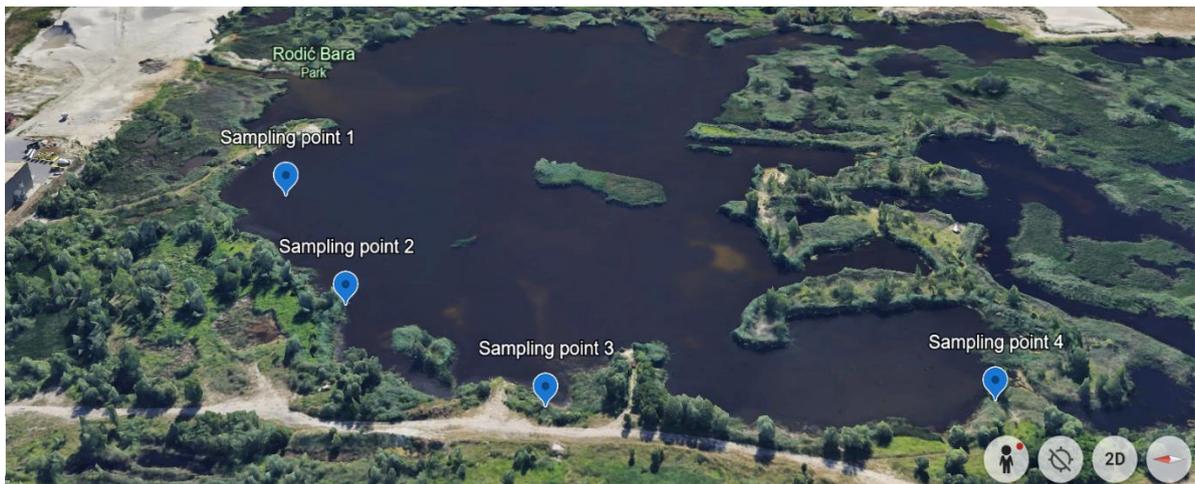
Water sampling was conducted on October 4, 2025. For the purpose of analyzing the physico-chemical parameters of water quality in the studied aquatic body, a total of four samples were collected from different sampling points (Figure 2). The water samples were taken from the shoreline and, after *in situ* measurement of selected parameters, were carefully stored in clean plastic bottles intended for transport to the laboratory.



**Figure 1.** Filling of the northern shores of the water body (left), and construction debris deposited along the shoreline of the water body (right). (Photo: Marko Lazovic)

The following physico-chemical parameters were measured on site: Temperature of water and air ( $^{\circ}\text{C}$ ), Electrical Conductivity ( $\mu\text{S}/\text{cm}$ ), pH value, and Dissolved Oxygen ( $\text{mgO}_2/\text{L}$ ).

Under laboratory conditions, the following parameters were determined: Ammonium concentration  $\text{NH}_4^+$  ( $\text{mgN}/\text{L}$ ), Nitrate concentration  $\text{NO}_3^-$  ( $\text{mgN}/\text{L}$ ), Nitrite concentration  $\text{NO}_2^-$  ( $\text{mgN}/\text{L}$ ), Chemical Oxygen Demand (COD,  $\text{mgO}_2/\text{L}$ ), Biochemical Oxygen Demand ( $\text{BOD}_5$ ,  $\text{mgO}_2/\text{L}$ ), Orthophosphate concentration ( $\text{mgPO}_4/\text{L}$ ), and Suspended Solids concentration ( $\text{mg}/\text{L}$ ).



**Figure 2.** Sampling points from different parts of the water body. (Source: Google Earth <https://earth.google.com/>)

## RESULTS AND DISCUSSION

After completing all field and laboratory analyses of the basic physico-chemical parameters of water quality sampled from Rodić Bara pond, the obtained data were grouped and presented in tabular form for the purpose of evaluating the results and comparing them with the current national legislation (Regulation, 74/11) (Table 1).

Regarding the legal framework related to surface and groundwater quality, the Regulation (74/2011) is currently in force in Serbia. The research results were compared with the table “Lakes at altitudes up to 200 m a.s.l., all shallow lakes (up to 10 m depth), all marsh and wetland ecosystems” within the aforementioned Regulation.

Based on this comparison, conclusions were drawn regarding the anthropogenic pressures contributing to the deterioration of the physico-chemical parameters of water quality in the studied aquatic body and their potential impact on the aquatic biota. The analysis revealed that the water quality of Rodić Bara pond corresponds to Class IV, indicating a poor ecological status. Elevated values of certain parameters, such as ammonium ( $\text{NH}_4^+$ ), and orthophosphates ( $\text{PO}_4^{3-}$ ), and decreased value of Dissolved oxygen (DO) in anthropogenically degraded part of the water body suggest nutrient enrichment and the potential onset of eutrophication, which is consistent with findings from previous studies of wetland systems in the region (Grabice et al., 2016).

The Report on the State of Nature in the City of Novi Sad (2013–2017) (PSUPEP, 2019) highlights similar trends, emphasizing that wetland ecosystems in the wider Novi Sad area are under significant anthropogenic pressure. Intensive agriculture, wastewater inflow, and land conversion for construction and infrastructure have led to fragmentation and degradation of wetland habitats, particularly in low-lying areas and floodplains. Drainage activities and modifications of the natural water regime have been identified as key drivers of wetland degradation, resulting in loss of biodiversity and alteration of water quality.

Additionally, the report points out that pollution from agricultural runoff, rich in nitrogen and phosphorus, accelerates eutrophication processes and leads to the dominance of nitrophilous species, thereby reducing floristic and faunal diversity. These patterns align with the conditions observed in „Rodić Bara“ pond, where increased concentrations of biogenic elements correspond to signs of anthropogenic influence on water quality.

Urban expansion and industrial activities in the Novi Sad region have also been recognized as contributing factors to the chemical and structural degradation of aquatic ecosystems, with cumulative effects on small wetland bodies such as Rodić Bara pond. Despite these pressures, maintaining water flow and periodic sediment removal are considered beneficial management measures, helping to sustain hydrological connectivity and ecological function. Possible impacts include the presence of construction waste depots and the proximity of the landfill, which contribute to the spread of pollution by wind and through groundwater.

Obtained results confirm that Rodić Bara pond reflects the broader regional trends of wetland degradation and poor ecological quality under human influence.

**Table 1.** Comparison of the results with water quality class according to the Regulation (74/11)

Parameter	Unit	SP 1	WQ class for SP1	SP 2	WQ class for SP2	SP3	WQ class for SP3	SP 4	WQ class for SP4
Dissolved Oxygen (DO)	mgO <sub>2</sub> /L	3.84	IV	7.82	II	6.6	II	7.88	II
Ammonium Ion (NH <sub>4</sub> <sup>+</sup> )	mg/L	0.42	III	0.37	III	0.32	III	0.33	III
Nitrites (NO <sub>2</sub> <sup>-</sup> )	mg/L	<0.01		<0.01		<0.01		<0.01	
Five-day Biochemical Oxygen Demand (BOD <sub>5</sub> )	mgO <sub>2</sub> /L	1.0	I	4.3	II	3.2	II	4.9	II
		1.6	I	5.4	III	3.8	II	6.0	III
		2.1	II	5.4	III	4.3	II	6.0	III
		2.7	II	6.5	III	4.9	II	6.5	III
		3.2	II	7.1	III	5.4	III	7.1	III
Chemical Oxygen Demand (COD)	mgO <sub>2</sub> /L	72		69		66		35	
Nitrates (NO <sub>3</sub> <sup>-</sup> )	mg/L	<1	I	<1	I	<1	I	<1	I
Suspended Solids	mg/L	18		19		19		15	
Orthophosphates (o-PO <sub>4</sub> <sup>3-</sup> )	mg/L P	0.16	III	0.24	IV	0.29	IV	0.16	III
pH	—	8.3	I	8.3	I	8.3	I	8.3	I

## CONCLUSION

This study highlights the ecological importance of wetlands as key providers of ecosystem services, including water purification, nutrient cycling, and biodiversity maintenance. The physico-chemical analysis of water from Rodic Bara pond indicates Class IV quality, reflecting a poor ecological status and evident anthropogenic influence. Obtained nutrient concentrations (NH<sub>4</sub><sup>+</sup>, Dissolved Oxygen and PO<sub>4</sub><sup>3-</sup>) suggest eutrophication driven by agricultural runoff and urban pressures.

Findings are consistent with the Report on the State of Nature in the City of Novi Sad (2013–2017), which identifies habitat fragmentation, drainage, and pollution as major drivers of wetland degradation. These pressures collectively reduce biodiversity and ecosystem functionality.

To ensure the long-term stability of Rodic Bara pond and similar wetlands, integrated management, continuous water quality monitoring, and stricter pollution control are essential. Strengthening conservation actions and sustainable land use practices will be key to preserving the ecological integrity of wetland ecosystems.

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# FERMENTATION POTENTIAL OF FIELD PEA (*PISUM SATIVUM*): A NUTRITIONAL PERSPECTIVE

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*This research explored the compositional characteristics of several pea (*Pisum sativum*) varieties to assess their potential for use in fermentation processes. The analyses revealed noticeable differences in macronutrient composition among the varieties, particularly in protein, sugar, and carbohydrate content, while fat levels, typical of legumes, remained consistently low. These variations suggest that certain pea types may be more favorable for supporting microbial growth and shaping fermentation dynamics. Two contrasting varieties were identified as promising candidates for further trials aimed at understanding how their composition affects fermentation performance and final product attributes. Overall, the study emphasizes the importance of selecting suitable legume varieties to enhance the efficiency, nutritional value, and sensory quality of plant-based fermented foods.*

**Key words:** *Pisum sativum, nutritional analysis, protein, sugar, fermentation*

## INTRODUCTION

The aim of this study was to determine the physicochemical properties of five physiologically ripe *Pisum sativum* (pea) varieties – “Dunav”, “Tamiš”, “Kelvedon”, “Mali Provansalac” and “Čudo Amerike”- in order to evaluate their potential as substrates in the fermentation process. The analysis focused on key nutritional parameters with critical influence in microbial growth, fermentation kinetics, and development of desirable sensory and nutritional profiles in fermented products.

## MATERIAL AND METHODS

Pea seeds obtained from the Institute of Field and Vegetable Crops in Novi Sad, were milled into fine flour using a laboratory mill (Knife mill KN 295 Knifetec, FOSS Analytical, Denmark) and stored at 4°C until analysis. Each variety was analyzed in duplicate, and mean values calculated per dry weight were used for comparison followed by statistical analysis. Moisture (926.5), ash (930.22), protein (950.36), fat (935.38) and dietary fibre (991.43) content of were determined by standard methods defined by the Association Official of Analytical Chemists (AOAC, 2000). Total carbohydrates (CHO) were calculated by difference in percentages:  $100\% - (\text{moisture} + \text{ash} + \text{crude protein} + \text{lipids}) \%$ .

## RESULTS AND DISCUSSION

Results showed considerable variations among the five varieties, with protein content ranging from 18.85% (“Dunav”) to 23.84% (“Mali Provansalac”) and total sugar content from 1.25% (“Dunav”) to 4.20% (“Tamiš”). Beyond simple sugars, total carbohydrate content - including starch and dietary fiber - also varied among the samples. Fat content was relatively low across all samples (1.06% - 1.79%), consistent with typical legume profiles. Based on a comparative assessment, two varieties, one with the highest fermentable sugar content and balanced carbohydrate profile (“Tamiš”) and the other with the lowest sugar and comparatively lower protein levels (“Dunav”), were selected for further fermentation trials. Both varieties were selected for additional studies to evaluate how compositional extremes influence microbial activity and product quality in fermentation process.

**Table 1.** Nutrient composition of five pea varieties based on fresh weight (g/100g)

	Dunav	Tamiš	Kelvedon	Mali Provansalac	Čudo Amerike
Moisture content	10.85	10.51	10.84	11.09	11.53
Ash content	2.19	2.82	2.89	2.96	2.63
Total crude fat	1.21	1.79	1.13	1.06	1.18
Sugar	1.11	3.76	2.80	1.39	2.56
Protein content	18.85	22.08	22.14	23.84	21.44
Total carbohydrate content	66.92	62.82	63.02	61.06	63.23

## CONCLUSION

The obtained nutritional composition values were consistent with previously reported data for *Pisum sativum* (Maninder et al., 2007; Ma et al., 2011; Talens et al., 2025), confirming the reliability of the applied analytical methods and the representativeness of the analyzed samples. Minor variations observed among the varieties likely reflect differences in genotype, cultivation conditions, and environmental factors such as soil composition and climate. These compositional distinctions are particularly relevant for fermentation applications, as the balance of macronutrients, especially fermentable sugars and proteins, directly influences microbial metabolism and the formation of bioactive compounds. Overall, the findings provide a basis for further optimization of legume-based fermentation systems and contribute to the development of sustainable, plant-derived substrates with improved nutritional quality, functional properties, and potential for diverse food innovations.

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# MICROPLASTICS AS EMERGING STRESSORS: EFFECTS ON GREEN WORM (*ALLOLOBOPHORA CHLOROTICA*, LUMBRICIDAE) BIOMASS AND MORTALITY

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Recently, soil has been recognized as one of the main reservoirs of microplastics, which may negatively affect biota and ecosystem function. The aim of this study was to examine effects of microplastics on *Allolobophora chlorotica* biomass and mortality under controlled laboratory conditions. Average initial biomass was 3.12 g with no group differences ( $p=0.594$ ). Exposure caused dose-dependent mortality (up to 88.89%) and biomass loss (1.827 g in average), with significant statistical differences between groups ( $p=0.0036$ ). Microplastics act as stressors, reducing earthworm vitality.

**Key words:** microplastics, *Allolobophora chlorotica*, biomass, mortality, soil ecology

## INTRODUCTION

Microplastics, defined as plastic particles smaller than 5 mm, have become one of the most pervasive and persistent pollutants in terrestrial ecosystems. Although initially studied in marine environments, studies conducted in recent years have revealed that principally soils act as major microplastic reservoirs, primarily due to agricultural activities such as plastic mulching, wastewater irrigation, sewage sludge application, and atmospheric deposition (Bläsing and Amelung, 2018; Corradini et al., 2019). Once deposited, these particles can alter soil structure, porosity, water-holding capacity, and pH, while also influencing microbial diversity and enzymatic activity (de Souza Machado et al., 2018; Zhang et al., 2020). Consequently, such changes can disrupt soil ecological balance and directly affect organisms that live and/or depend on the soil environment.

Earthworms, particularly species within the family Lumbricidae, are considered key bioindicators of soil health due to their crucial role in organic matter decomposition, nutrient cycling, and soil aeration (Edwards and Bohlen, 1996). Their continuous contact with soil particles during feeding and burrowing makes them particularly sensitive to contaminants, including heavy metals, pesticides, and plastics. Several studies have demonstrated the negative impacts of microplastics on earthworms. Rodriguez-Sejjo et al. (2017) reported tissue inflammation and digestive damage in *Eisenia andrei* Bouché, 1972 exposed to polyethylene microplastics. Huerta Lwanga et al. (2017) found that *Lumbricus terrestris* Linnaeus, 1758 exhibited reduced growth and altered burrowing behavior under high exposure levels, while Cao et al. (2017) and Ding et al. (2021) showed significant inhibition of growth, reproduction, and survival in *Eisenia fetida* (Savigny, 1826) when exposed to polystyrene or mixed polymer particles. Yu et al. (2022) observed that exposure to polyethylene (PE) and polylactic acid (PLA) microplastics decreased antioxidant enzyme activity and induced oxidative stress in *E. fetida*, effects which persisted despite differences in polymer type.

Li et al. (2021) further demonstrated that *E. fetida* can ingest high-density polyethylene (HDPE) and polypropylene (PP) particles, leading to enzyme disruption, oxidative stress, and neurodegenerative changes. Baeza et al. (2020) observed physical abrasions and mucus loss in *L. terrestris* exposed to high microplastic concentrations, indicating strong physiological stress responses. Additionally, Rillig et al. (2017) documented that earthworms facilitate vertical transport of microplastics within the soil profile, showing their ecological role in redistribution of pollutants.

Despite these findings, data concerning the effects of polypropylene (PP), one of the most widely used and chemically stable thermoplastic polymers remain scarce. Given its persistence and potential for accumulation, PP represents a significant but understudied source of soil contamination.

The aim of this study was to determine the influence of different concentrations of polypropylene microplastics on the biomass and mortality rate of the green worm *Allolobophora chlorotica* (Savigny, 1826) under controlled laboratory conditions.

## MATERIAL AND METHODS

The test organism used in this study was the green worm, *Allolobophora chlorotica*. Only adult individuals with a fully developed clitellum were selected for the experiment. Polypropylene (PP) was used as the test microplastic pollutant, obtained by shredding common PP materials in microplastic particles (<5 mm), irregular in shape, in order to mimic the possible habitat contamination.

Four experimental groups were established: a control group (C0) without microplastic, and three treatment groups (C1, C2 and C3) containing PP microplastics at concentrations of 0.05%, 0.10%, and 0.15%, respectively. The total soil mass per container was 200 g, with 0.1 g, 0.2 g and 0.3 g of microplastic particles per treatment group respectively, vigorously mixed in. Each group had three replicates with three adult individuals. The soil substrate had a defined nutrient composition presented in Table 1.

**Table 1.** Nutrient content of the soil used in the experiment

Parameter	Value
Nitrogen (N) – easily available in KCl (mg/l)	0-50
Nitrogen (N) – nitrate form (mg/l)	0-50
Nitrogen (N) – ammonium form (mg/l)	0-50
Phosphorus – easily available in CAI (P <sub>2</sub> O <sub>5</sub> ) (mg/l)	100-500
Potassium – easily available in CAI (K <sub>2</sub> O) (mg/l)	300-1000
Salt content (g/l)	<3
pH value (in KCl)	4.0-7.0

Before exposure, the biomass of all earthworms was measured using a Kern ADB 200-4 analytical balance to allow later comparison. The experiment lasted seven days under controlled laboratory condition where relative humidity and temperature were continuously monitored using Thermo-hygrometer Micro-Temp® TH-812E. After exposure, all individuals were weighed again using the same balance, following calibration and taring procedures to ensure precision. Prior to weighing, earthworms were rinsed with distilled water and dried with filter paper to remove excess soil and moisture.

Mortality was assessed on the second, fifth, and seventh day by observing earthworm movement, tactile response, and body tonus. The corrected percentage of mortality was calculated using Schneider-Orelli's formula (Baloš et al., 2024). Data on biomass and mortality rate were statistically analyzed using software Statistica 14.0.0.15 (TIBCO Software Inc., University license). Statistical analyses included analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test. Statistical significance were observed for  $p < 0.05$  (interpreted as a high significance) and  $p < 0.01$  (interpreted as a very high significance).

## RESULTS AND DISCUSSION

The results of changes in *A. chlorotica* biomass are presented in Table 2.

The total biomass at the beginning of the experiment ranged from 2.0124 g to 3.8756 g, while the average mass per individual ranged from 0.6708 g to 1.2919 g. At the end of the experiment, significant changes in total biomass were observed depending on the experimental group and microplastic concentration. In certain samples (C2-1, C3-1, and C3-3) with medium and high microplastic concentrations, the total earthworm biomass at the end of the experiment could not be measured, as 100% mortality in those replicates were observed. This result could be interpreted as an effect of high microplastic concentrations acting as a stress factor for earthworms. Conversely, in samples such as C0-1 and C1-1, smaller increases or decreases in biomass were recorded (differences ranging from 0.4307 g to 0.1588 g), suggesting relatively stable physiological worm activity under those conditions. The average mass per surviving individual showed an increase in certain samples (e.g., C0-1), possibly indicating that surviving worms had access to a greater amount of nutrients due to the reduced competition or that their body mass increased as a result of adaptation to experimental conditions. Overall, the results suggested that different microplastic concentrations can have a significant impact on both biomass and mortality rate of earthworms. Complete biomass loss in certain samples indicates toxic or stress-induced effects of microplastics, whereas moderate increases or decreases in others may reflect adaptation or individual resistance. These findings demonstrate that microplastic concentration directly influenced the physiological condition of these soil organisms as well as biomass dynamics under controlled conditions.

**Table 2.** *A. chlorotica* biomass measurement results

Treatment	MP Concentration (%)	Initial biomass (g)	Total biomass (g) at the end of the experiment	Biomass difference (g)	Initial average individual mass (g)	Average mass per survived individual
C0-1	0.00	3.5345	3.9652	-0.4307	1.1782	1.3217
C0-2	0.00	2.0124	3.4950	-1.4826	0.6708	1.1650
C0-3	0.00	2.9599	3.3542	-0.3943	0.9866	1.1181
C1-1	0.05	2.7622	2.6034	0.1588	0.9207	1.3017
C1-2	0.05	3.7130	2.5513	1.1617	1.2377	1.2757
C1-3	0.05	3.1585	2.7137	0.4448	1.0528	0.9046
C2-1	0.10	2.8803	0.0000	2.8803	0.9601	-
C2-2	0.10	3.0268	1.9718	1.0550	1.0089	0.9859
C2-3	0.10	3.1802	0.7189	2.4613	1.0601	0.7189
C3-1	0.15	3.4447	0.0000	3.4447	1.1482	-
C3-2	0.15	3.8756	0.5564	3.3192	1.2919	0.5564
C3-3	0.15	2.9041	0.0000	2.9041	0.9680	-

At the beginning of the experiment, differences in earthworm biomass were not statistically significant between replicates or microplastic concentrations, confirming an appropriate experimental design ( $p=0.594183$  for  $p<0.05$ ). Nevertheless, after the final measurement, the ANOVA revealed statistically high significant differences ( $p=0.000224$  for  $p<0.01$ ) between measured biomasses regarding the microplastics concentration in the soil. Fisher's LSD test highlighted statistically high significant differences between biomasses measured at microplastic concentrations of 0.10% and 0.15% compared with the control group (without microplastic) and the group containing 0.05% microplastic.

The results of *A. chlorotica* mortality are presented in Table 3.

Earthworm survival as a function of microplastic concentration showed a clear positive correlation between increased concentration and mortality. Control groups (C0) maintained 100% survival throughout the entire experimental period, whereas groups with low microplastic concentration (C1, 0.05%) exhibited partial mortality, with survival rates of 33.33%. As concentrations increased (C2, 0.10% and C3, 0.15%), mortality rose noticeably, and in some replicates, all individuals died by the fifth day. Corrected mortality values calculated using the Schneider-Orelli's formula confirmed the statistical accuracy of mortality estimates for small samples, with correction values ranging from 66.67% to 88.89% in groups with total mortality rate. These findings clearly indicate that increasing microplastic concentration in soil negatively affects earthworm survival, suggesting toxic effects.

Correspondingly, ANOVA demonstrated statistically high significant differences ( $p=0.003608$  for  $p<0.01$ ) between the measured mortality rates (dependent variable) and microplastic concentrations in the soil samples (independent variable). Fisher's test again confirmed statistically high significant differences between mortality rates recorded at 0.10% and 0.15% microplastic concentrations compared with the control group (without microplastic) and the group with the lowest concentration. Numerous studies have confirmed that non-degradable plastics can adversely affect earthworms and their physiological activities. Although limited data are available on the effects of microplastics on *A. chlorotica*, other Lumbricidae species have been extensively studied. For instance, experiments on *E. andrei* showed digestive tract damage at microplastic concentrations of 125 mg/kg<sup>-1</sup> soil, accompanied by an immune stress response (Rodriguez-Seijo et al., 2017).

**Table 3.** *A. chlorotica* mortality rates

Treatment	MP Concentration (%)	Survival rate				Mortality (%)	Corrected Mortality (%)
		0 day	2 <sup>nd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day		
C0-1	0	3	3	3	3	0	0.00
C0-2	0	3	3	3	3	0	
C0-3	0	3	3	3	3	0	
C1-1	0.05	3	2	2	2	33.33	22.22
C1-2	0.05	3	2	2	2	33.33	
C1-3	0.05	3	3	3	3	0	
C2-1	0.1	3	2	0	0	100	66.67
C2-2	0.1	3	3	2	2	33.33	
C2-3	0.1	3	2	2	1	66.67	
C3-1	0.15	3	0	0	0	100	88.89
C3-2	0.15	3	3	2	1	66.67	
C3-3	0.15	3	1	0	0	100	

In *L. terrestris*, reduced growth rates were observed at extremely high exposure levels (280 g/kg<sup>-1</sup>) (Huerta Lwanga et al., 2017). Cao et al. (2017) demonstrated that polystyrene particles (~58 µm) at concentrations of 10-20 g/kg<sup>-1</sup> significantly inhibited growth and increased mortality in *E. fetida*, while Jiang et al. (2020) reported intestinal epithelial damage in the same species.

Ding et al. (2021) found that microplastic concentrations above 40 g/kg<sup>-1</sup> negatively affected earthworm biomass and reproduction. Given that microplastic concentrations in certain soil types have already exceeded 67 g/kg<sup>-1</sup>, these results suggest that microplastics in agroecosystems are beginning to threaten earthworm populations and degrade soil biodiversity. The authors emphasized that microplastic concentration is the dominant factor influencing earthworm biomass and reproduction, whereas polymer type played a lesser role. A similar study was conducted by Baeza et al. (2020) on *L. terrestris*, investigating the effects of different microplastic concentrations (2.5%, 5%, and 7% w/w). This species was unable to distinguish microplastic particles from soil particles, and high concentrations led to physical lesions on the body surface and loss of protective mucus, resulting in physiological stress.

Yu et al. (2022) studied the effects of different concentrations of microplastics on *E. fetida* in two soil types, using polyethylene (PE) and biodegradable polylactic acid (PLA) at concentrations ranging from 0.5% to 14% (w/w). After 14 days of exposure, activities of SOD, CAT, POD, GST, and AChE enzymes decreased, while malondialdehyde (MDA) levels increased, indicating oxidative stress. After 28 days, enzyme activities increased and MDA levels decreased, suggesting that toxic effects depend primarily on microplastic concentration rather than polymer type or soil composition. PE exhibited stronger toxic effects than PLA during the first 14 days, although the difference diminished after 28 days. Changes were also observed in gut microbiota composition (Actinobacteriota, Bacteroidota, Ascomycota, Rozellomycota). The results indicate that both conventional and biodegradable microplastics can equally induce oxidative stress in *E. fetida*.

Rillig et al. (2017) conducted one of the first studies on vertical transport of microplastics in soil, showing that earthworms can move PE particles from the surface down to depths of about 10 cm. The number of transported particles depended on their size, with smaller particles penetrating deeper.

Li et al. (2021) demonstrated that *E. fetida* can ingest HDPE and PP particles of various sizes, which altered activities of SOD, CAT, and GST enzymes and induced oxidative stress. Exposure to these polymers also caused neurodegeneration, inflammation, and other physiological disorders in earthworms. Cao et al. (2022) analyzed microplastic particles and tris(2-chloroethyl) phosphate contamination at agricultural sites, detecting polyethylene and phosphates at all locations. Histological damage, oxidative stress, and biomass loss (3.42-14.96%) were observed in earthworms, indicating inhibited growth and feeding activity.

The increasing accumulation of plastic and plastic waste in agricultural soils inevitably disrupts soil ecosystems. The presence of microplastics interferes with nutrient intake in rhizospheric fauna, causing

imbalances in digestion and assimilation processes, leading to reduced growth and reproduction, organ damage, and disturbances in immune and metabolic functions of soil organisms (Lahive et al., 2019; Wang et al., 2019).

## CONCLUSION

Based on the results obtained, the green worm *Allolobophora chlorotica* can be considered as a sensitive bioindicator of soil contamination by microplastics. Reduction in biomass and increased mortality has significant ecological consequences, as they affect soil fertility and the natural nutrient cycling processes. Long-term studies should investigate different types of microplastics, their accumulation, potential synergistic effects with other contaminants, and the mechanisms of toxic effects on worm physiology. The conducted experiment clearly demonstrated that microplastics affect the survival rate and physiology of *A. chlorotica*, highlighting the necessity of microplastic monitoring in agricultural and natural habitats to preserve ecosystems and biodiversity.

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# QPCR DETECTION OF RALSTONIA SOLANACEARUM IN POTATO TUBERS USING DIFFERENT DNA ISOLATION TECHNIQUES

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*The study aimed to detect Ralstonia solanacearum in potato tubers using qPCR and two DNA isolation techniques (CTAB and DNeasy Plant Mini Kit). The results confirmed that both methods ensured reliable detection of the pathogen in artificially inoculated tubers, with a detection threshold value of 0.03, validating the qPCR protocol for phytopathological diagnostics.*

**Key words:** *Ralstonia solanacearum, qPCR, potato, DNA isolation, CTAB, DNeasy*

## INTRODUCTION

The pathogen *Ralstonia solanacearum* is an aerobic, gram-negative, rod-shaped bacterium with two membrane structures and is considered one of the most harmful plant pathogens in the world. *R. solanacearum* is the causal agent of vascular wilt disease affecting more than 200 plant species, including economically important crops such as potato, tomato, tobacco, pepper, and banana.

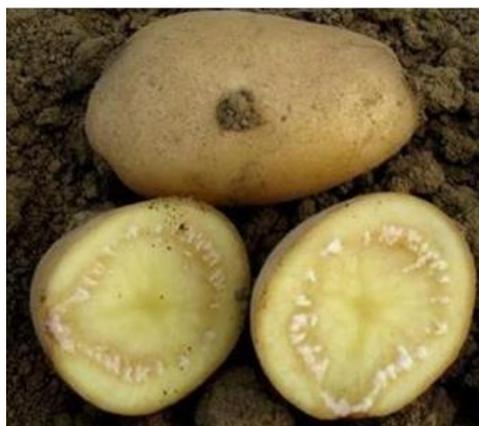
Infection by *R. solanacearum* causes xylem blockage due to the accumulation of bacterial extracellular polysaccharides, resulting in plant wilting, decay, and yield reduction. *R. solanacearum* race 3 biovar 2 is a soil- and water-borne pathogen; the bacterium can survive and spread over various time periods in infested soil or water, creating a reservoir of inoculum for further infections (Hayward, 1991).

Although it is best known for its destructive effects, *R. solanacearum* also represents an important model organism for studying plant–pathogen interactions, horizontal gene transfer, and the evolution of virulence. It is used in scientific research to understand pathogenicity mechanisms as well as to develop new strategies for biological and genetic control of plant diseases. Additionally, some studies indicate the potential role of this bacterium in the biodegradation of organic compounds, which may have ecological and biotechnological implications (Genin, 2010).

Early detection of *R. solanacearum*, especially in the latent infection phase when symptoms are not yet visible on the plant, is crucial for preventing the spread of this dangerous phytopathogenic bacterium (Elphinstone et al., 1998). Latently infected material, such as asymptomatic tubers or planting material, represents the main source of disease dissemination to new regions (Fegan and Prior, 2005).

The quantitative PCR method (qPCR) has proven to be a highly sensitive and specific tool for detecting *R. solanacearum* in both latent and active stages of infection (Weller et al., 2000). This method enables precise and rapid detection of even a small number of bacterial cells in different sample types, which is essential for phytosanitary monitoring, control, and epidemic prevention (Lopez et al., 2003; Opina et al., 1997).

The results show that *R. solanacearum* has been present in Serbia since 2011, with the first confirmed infected samples originating from Vojvodina, and it has been classified as a quarantine pest in Serbia. In addition, legal frameworks have been established (Regulation, 2014) governing detection, restriction, and suppression, all in accordance with national and international phytosanitary policy (Milijasevic-Marcic et al., 2013).



**Figure 1.** infected potato tuber

## **MATERIAL AND METHODS**

### **BACTERIAL STRAIN**

The study used a reference strain of *R. solanacearum* race 3 biovar 2 (CFBP 4958), which, according to the prescribed standards for a quarantine species, is maintained in the microorganism collection of the Faculty of Agriculture, University of Novi Sad, Laboratory for Biological Research and Pesticides.

The bacterial strain was revitalized and grown on Yeast Peptone Glucose Agar (YPGA) medium (Lelliott & Stead, 1987) in a thermostat at 28°C for 72 hours.

For the experiment, a bacterial suspension was prepared by resuspending the bacterial culture in sterile distilled water to achieve a concentration of  $10^6$  CFU/ml.

### **INOCULATION OF POTATO TUBERS**

Potato tubers without visible damage or signs of disease intended for inoculation were first washed with warm water, left to dry, and then wiped with 70% ethanol. The prepared tubers were placed in plastic containers previously disinfected with 70% ethanol. Inoculation of tubers was performed in two ways: by pipetting 500  $\mu$ l of bacterial suspension with a concentration of  $10^6$  CFU/ml into previously made holes in the vascular tissue zone (tuber bud), and by the stab method using a sterile toothpick previously dipped into a 72-hour bacterial culture.



**Figure 2.** Inoculation of potato tubers

### **DNA EXTRACTION**

DNA extraction directly from plant tissue was performed 48 hours after artificial inoculation of potato tubers. Before extraction, a vascular tissue extract was prepared at a distance of 0.5 cm from the inoculation site.

Slices of vascular tissue from the inoculated potato tubers were homogenized using a rubber mallet inside a maceration bag. The homogenate was then covered with sterile 50 mM phosphate buffer (PB), pH 7.0, in a volume just sufficient to submerge the tissue. After homogenization, the supernatant was carefully pipetted off, while the remaining solid material and maceration bags were discarded. The extract was centrifuged at 180

(250) × g for 10 minutes at 4–10°C, and the resulting supernatant was transferred to a new Eppendorf tube, with the pellet being discarded. A second centrifugation was then performed at 10,000 × g for 10 minutes at 4–10°C, after which the supernatant was again removed and discarded. The remaining pellet was resuspended in 1.5 ml of 10 mM phosphate buffer (PB), pH 7.2, and the suspension was briefly vortexed to ensure complete homogenization.

DNA was isolated from the prepared vascular tissue extract of artificially inoculated potato tubers using two different methods: the CTAB method and the DNeasy Plant Mini Kit (Qiagen).

The isolated DNA from samples was used for further analysis using the REAL-TIME TAQMAN PCR method according to the protocol by Weller et al. (2000). This test is used for detection and identification of *Ralstonia solanacearum*, *Ralstonia pseudosolanacearum*, and *Ralstonia syzygii* (phylotypes I–IV), as well as *R. solanacearum* phylotype IIB, sequevar 1.

The reaction included the following reagents:

- Forward primer RS-I-FmAK 5'-CATGCCTTACACATGCAAGTC-3'
- Reverse primer RS-II-RmAK 5'-CACGTTCCGATGTATTACTCA-3'
- Probe: RSP-55T 5'-[FAM]-AGCTTGCTACCTGCCGG-[NFQ-MGB]-3'

The working mixture used for detection consisted of the above primers and probe, the Luna Universal qPCR Master Mix (New England Biolabs), isolated DNA, and DNA-free water, with a total reaction volume of 25 µl (23 µl + 2 µl DNA = 25 µl), as shown in Table 1.

**Table 1.** Reaction Working Mixture

Molecularly pure water	8,5 µl
Luna® Universal qPCR Master Mix	12,5 µl
Forward Primer (10 µM)	0,75 µl
Reverse Primer (10 µM)	0,75 µl
Probe (5 µM)	0,5 µl
DNA	2 µl

In this study, two tubers inoculated with bacterial suspension and two tubers inoculated with a pure bacterial culture were used. Isolated DNA from each sample was analyzed using a PCR machine in duplicate. This resulted in 20 variants, which are presented in the following chapter.

The following variants were analyzed in the qPCR reaction:

1. DNA sample isolated from the artificially inoculated potato tuber with bacterial suspension (II), using the CTAB method (C) - IIC;
2. DNA sample isolated from the artificially inoculated potato tuber with pure bacterial culture (I), using the CTAB method (C) - IC;
3. DNA sample isolated from the artificially inoculated potato tuber with bacterial suspension (II), using the DNeasy Plant Mini Kit (Qiagen) isolation kit (Q) - IIQ;
4. DNA sample isolated from the artificially inoculated potato tuber with pure bacterial culture (I), using the DNeasy Plant Mini Kit (Qiagen) isolation kit (Q) - IQ;
5. Positive control - DNA molecule isolated directly from the bacterial culture of *R. solanacearum* grown on Yeast Peptone Glucose Agar (YPGA) nutrient medium – K+;
6. Negative control - sterile water without traces of DNA molecules – K-.

The prepared samples were analyzed using a PCR machine (Qiagen: Rotor-Gene Q 5plex HRM Platform, serial no. R1020222/5CG0342WJ2) with the application of appropriate reagent quantities and reaction condition settings according to the protocol for detection and identification OEPP/EPPO (2018) 48 (1), 32–63 PM 7/21 (2) *Ralstonia solanacearum*, *R. pseudosolanacearum*, and *R. syzygii* (*Ralstonia solanacearum* species complex), provided in Table 2.

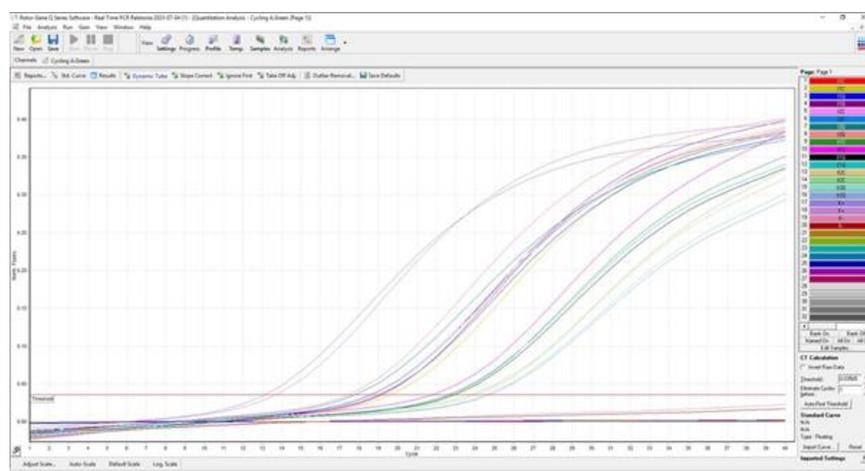
**Table 2.** Recommended Reaction Conditions

1.	10 min	95 °C
2. 40 cycles	15 s	95 °C
	60 s	60 °C



**Figure 3.** DNA extraction

## RESULTS



**Figure 3.** Diagram of Amplification Curves of Tested Samples in the qPCR Reaction

Based on the obtained qPCR results (Figure 5, Table 3), all tested samples, except negative controls, formed exponential amplification curves of proper shape. These fluorescence profiles indicate the presence of specific amplification of the target *R. solanacearum* DNA sequence.

Negative controls showed no fluorescence increase during cycles, confirming the absence of contamination or nonspecific amplification. In contrast, all positive reactions showed a clearly defined log-phase of amplification, which provides valid evidence that *R. solanacearum* DNA was detected in all tested samples.

It was determined that the threshold value was 0.03. This value represents the fluorescence level above the baseline signal at which nonspecific amplification can be reliably distinguished from actual target sequence amplification. Setting the threshold at 0.03 allows for clear and reproducible identification of the crossing point (Ct value) in all positive samples, ensuring the reliability of obtained results and preventing false positives or negatives.

Analysis of results confirmed that the applied detection and identification protocol successfully enabled detection of *R. solanacearum* in all tested variants, with the threshold value determined to be 0.03.

## CONCLUSION

Both tested DNA extraction techniques (CTAB and DNeasy Plant Mini Kit (Qiagen)) proved sufficiently reliable for isolating *R. solanacearum* DNA from potato tuber tissue, regardless of the inoculation method.

The qPCR technique specified in the OEPP/EPPO (2018) 48(1), 32–63 PM 7/21(2) protocol proved to be a reliable method for detecting *R. solanacearum* DNA in artificially inoculated potato tubers, whether inoculated with bacterial culture or suspension.

The threshold value was determined to be 0.03.

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## DEVELOPMENT OF THE YELLOW MEALWORM (*TENEBRIO MOLITOR* L.) ON DIETS WITH DIFFERENT LEVELS OF PROTEIN AND CARBOHYDRATES

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*Due to the growing need for sustainable protein sources, insects are increasingly recognized as a valuable nutritional resource. This study examined the development of Tenebrio molitor on diets with different protein and carbohydrate ratios. The fastest development occurred on a diet with high protein and high energy, while the greatest larval mass was recorded on a diet rich in protein and with a medium energy level.*

**Key words:** proteins, energy, farming, Tenebrio molitor

### INTRODUCTION

Insects are a major problem in stored agricultural products, but some species, such as yellow mealworm *Tenebrio molitor* (Coleoptera, Tenebrionidae), are considered edible and suitable for industrial production due to their widespread distribution and high nutritional value (Štrbac, 2002; Kröncke et al., 2019). The global demand for animal protein is expected to rise with population growth, making sustainable protein sources increasingly important (Hong et al., 2020; Dragojlović et al., 2022).

*Tenebrio molitor* larvae are rich in protein and fatty acids, easily digestible, and neutral in taste, making them a promising alternative protein source for animal and human consumption (Yoo et al., 2019; Khanal et al., 2023). They are commonly reared on flour-based substrates with added moisture and sometimes protein supplements. Diets with optimized protein, carbohydrate, and lipid content can improve larval growth, biomass, and reduce developmental time (Van Broekhoven et al., 2015; Morales-Ramos et al., 2013; Kröncke et al., 2019).

Optimizing diet composition, particularly the levels of protein and carbohydrates, is crucial for maximizing biomass production and shortening the developmental cycle of *T. molitor*, which is essential for industrial-scale farming.

This research aims to show on which diet insects develop best in terms of number, mass and development time. The goal of this study is to monitor the development of the species *T. molitor* on diets with different chemical composition in order to determine which diet is most favorable for the cultivation of this insect species.

### MATERIALS AND METHODS

For the purpose of this study, nine different diets were prepared: one control and eight experimental diets with varying protein-to-carbohydrate ratios. Wheat bran served as the base for all diets, while pure wheat bran was used as control. To increase protein content, soybean meal, a common protein source in animal feed, was added. Corn was added to increase energy and carbohydrate content. Additionally, rapeseed meal was included as a protein source only, without contributing to the energy content. By varying these components, diets with three protein levels, each combined with three energy levels, were formulated, resulting in a total of nine diets. Protein and carbohydrate levels were classified as low, medium, or high.

The insects used in the experiment were part of a long-term laboratory population maintained in the Laboratory of Entomology at the Department of Phytomedicine and Environmental Protection at the Faculty of Agriculture, University of Novi Sad. The experiment was conducted on nine different diet mixtures, one serving as the control, while the remaining eight represented mixtures with varying protein and energy ratios. Each mixture was set up in four replications. Each replication contained 50 grams of the selected diet mixture. In each replication, 25 insects were placed together with the feed into plastic containers of 1.65 L volume. The

containers were placed in a climate chamber under controlled conditions of 26 °C and 55% relative humidity and kept for 65 days. Pieces of carrot were added three times per week as an additional source of moisture.

After 65 days, the experiment was examined for the presence of different developmental stages of *T. molitor* and the individuals were counted. During counting, the number of live and dead adult insects, the number and mass of larvae, as well as the number and mass of pupae were recorded.

## RESULTS AND DISCUSSION

To better understand the composition of the diets, a chemical analysis was performed. The control diet had low protein and low energy levels, with 13.38% crude protein and 6.04% crude fiber in the dry matter. Diet 1 had medium protein (15.74%) and low carbohydrate content (7.05%), while diet 2 had high protein (18.33%) and low carbohydrate content (7.6%). Moisture content was similar across all diets, around 9–10%.

**Table 1.** Chemical composition of control, diet 1 and diet 2.

	Protein 1 energy 1	Protein 2 energy 1	Protein 3 energy 1
	Control	Diet 1	Diet 2
Dry matter	90.19	90.2	90.04
Moisture	9.81	9.80	9.96
Crude protein	13.38	15.74	18.33
Crude fat	3.24	2.94	2.57
Crude cellulose	6.04	7.05	7.6
Ash	4.74	4.85	4.9

Chemical analysis of diets 3, 4, and 5 is presented in Table 2. Diet 3 had low protein (13.22%) and medium carbohydrate content (5.04%). Diet 4 contained medium protein (15.66%) and medium carbohydrates (5.61%), while diet 5 had high protein (17.74%) and medium carbohydrate content (2.5%). Dry matter content ranged from 89.46–90.37%, with moisture around 9–10%.

**Table 2.** Chemical composition of diet 3, diet 4 and diet 5

	Protein 1 energy 2	Protein 2 energy 2	Protein 3 energy 2
	Diet 3	Diet 4	Diet 5
Dry matter	89.64	90.37	90.16
Moisture	10.36	9.63	9.64
Crude protein	13.22	15.66	17.74
Crude fat	2.83	2.52	2.5
Crude cellulose	5.04	5.61	5.99
Ash	4.17	4.38	4.43

The chemical composition of diets 6, 7, and 8 is shown in Table 3. Diet 6 had low protein (13.59%) and high carbohydrate content (5.27%). Diet 7 contained medium protein (15.79%) and high carbohydrates (5.41%), while diet 8 had high protein (18.06%) and high carbohydrate content (5.69%). Dry matter ranged from 90.19–91.21%, with moisture around 8.8–9.8%.

**Table 3.** Chemical composition of diet 6, diet 7 and diet 8.

	Protein 1 energy 3	Protein 2 energy 3	Protein 3 energy 3
	Diet 6	Diet 7	Diet 8
Dry matter	90.19	90.26	91.21
Moisture	9.81	9.74	8.79
Crude protein	13.59	15.79	18.06
Crude fat	2.58	2.4	2.51
Crude cellulose	5.27	5.41	5.69
Ash	3.67	3.9	3.77

The average number of living adults of *T. molitor* individuals in the control and dietary treatments is shown in Table 4. Mean values ranged from 18.75 (diets 4 and 7) to 22.75 (diet 3). The lowest standard deviation was recorded in diet 5 (1.15), indicating low variability among replicates, while the highest was in diet 8 (2.98), indicating high variability. ANOVA showed no statistically significant differences ( $F = 1.204$ ;  $p = 0.333$ ). According to Duncan's post-hoc test, the treatments were grouped into three homogeneous groups: "a", "b", and "ab". Diets 4 and 8 formed group "a", diet 3 formed group "b", while the control and diets 1, 2, 5, 6, and 7 belonged to group "ab"

**Table 4.** Number of living adults of *Tenebrio molitor* on different diets

	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean value	Standard deviation
Control	20	22	20	18	20.00 ab	1.63
Diet 1	22	20	23	18	20.75 ab	2.21
Diet 2	17	21	24	21	20.75 ab	2.87
Diet 3	21	23	21	24	22.25 b	1.50
Diet 4	17	19	20	19	18.75 a	1.25
Diet 5	20	22	22	20	21.00 ab	1.15
Diet 6	19	18	21	22	20.00 ab	1.82
Diet 7	18	20	21	22	20.25 ab	1.70
Diet 8	22	18	15	20	18.75 a	2.98
ANOVA			F	1.204		
			p	0.333		

The average number of dead adults of *T. molitor* individuals in the control and dietary treatments is presented in Table 5. Mean values ranged from 2.75 in diet 3 to 6.25 in diets 4 and 8. The lowest standard deviation was recorded in diet 5 (1.15), indicating low variability among replicates, while the highest was observed in diet 8 (2.98), indicating high variability. ANOVA revealed statistically significant differences ( $F = 1.204$ ;  $p = 0.333$ ). Based on Duncan's post-hoc test, the treatments were grouped into three homogeneous groups: diet 3 was placed in group "a", diets 4 and 8 in group "b", while the control and diets 1, 2, 5, 6, and 7 formed group "ab".

**Table 5.** Number of dead adults of *Tenebrio molitor* on different diets

	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean value	Standard deviation
Control	5	3	5	7	5.00 ab	1.63
Diet 1	3	5	2	7	4.25 ab	2.21
Diet 2	8	4	1	4	4.25 ab	2.87
Diet 3	4	2	4	1	2.75 a	1.50
Diet 4	8	6	5	6	6.25 b	1.25
Diet 5	5	3	3	5	4.00 ab	1.15
Diet 6	6	7	4	3	5.00 ab	1.82
Diet 7	7	5	4	3	4.75 ab	1.70
Diet 8	3	7	10	5	6.25 b	2.98
ANOVA			F	1.204		
			p	0.333		

The average number of *T. molitor* larvae in the control and dietary treatments is shown in Table 6. The lowest mean was observed in diet 8 (584.25), and the highest in diet 1 (829.00). The lowest standard deviation was recorded in the control group (58.08), and the highest in diet 1 (188.57). ANOVA revealed no statistically significant differences ( $F = 1.761$ ;  $p = 0.130$ ). Duncan's post-hoc test grouped the treatments into three homogeneous groups: diet 8 in group "a", diets 2–7 in group "ab", and control and diet 1 in group "b"

**Table 6.** Number of *Tenebrio molitor* larvae on different diets

	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean value	Standard deviation
Control	865	765	854	754	809.50 b	58.08
Diet 1	924	658	686	1048	829.00 b	188.57
Diet 2	646	888	835	800	792.25 ab	103.99
Diet 3	847	604	637	644	683.00 ab	110.71
Diet 4	659	516	760	850	696.25 ab	143.27
Diet 5	850	738	832	665	768.75 ab	90.34
Diet 6	837	516	643	492	622.00 ab	157.90
Diet 7	517	870	601	587	643.75 ab	155.24
Diet 8	503	750	656	428	584.25 a	145.64
ANOVA			F	1.761		
			p	0.130		

The average larval mass of *T. molitor* in the control and dietary treatments is shown in Table 7. Mean larval mass ranged from 27.46 g in diet 3 to 39.36 g in diet 5 (Figure 4). Standard deviations ranged from 0.47 in diet 1 (low variability) to 5.68 in diet 8 (high variability). ANOVA indicated statistically significant differences among treatments ( $F = 8.307$ ;  $p < 0.001$ ). Duncan's post-hoc test divided the treatments into several homogeneous groups: diet 3 in group "a", diets 4 and 6 in "ab", diet 7 in "abc", diet 8 in "bc", control and diet 2 in "cd", diet 2 in "c", and diet 5 in "d".

**Table 7.** Mass of *Tenebrio molitor* larvae on different diets

	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean value	Standard deviation
Control	35.19	35.17	35.79	36.13	35.57 cd	0.47
Diet 1	35.47	32.62	33.69	38.44	35.12 c	2.49
Diet 2	37.36	34.82	33.36	36.33	35.47 cd	1.75
Diet 3	28.67	25.40	27.56	28.21	27.46 a	1.44
Diet 4	29.90	27.92	30.02	31.25	29.77 ab	1.37
Diet 5	40.01	39.42	37.39	40.65	39.36 d	1.41
Diet 6	32.62	28.46	29.70	24.10	28.72 ab	3.53
Diet 7	30.15	34.55	31.50	30.39	31.65 abc	2.02
Diet 8	30.91	37.92	36.78	25.65	32.82 bc	5.68
ANOVA			F	8.307		
			p	0.003		

The average number of pupae of *T. molitor* in the control and dietary treatments is shown in Table 8. Mean values ranged from 0 in diets 1, 3, 4, and the control to 5.25 in diet 8 (Figure 5). The lowest standard deviation was 0.00 in several treatments, indicating no variability, while the highest was 5.12 in diet 8, indicating high variability. ANOVA revealed statistically significant differences ( $F = 3.443$ ;  $p = 0.007$ ). Duncan's post-hoc test grouped the treatments into two homogeneous groups: "a" (control and diets 1–7) and "b" (diet 8).

**Table 8.** Number of *Tenebrio molitor* pupae on different diets

	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean value	Standard deviation
Control	0	0	0	0	0 a	0.00
Diet 1	0	0	0	0	0 a	0.00
Diet 2	0	2	2	0	1.00 a	1.15
Diet 3	0	0	0	0	0.00 a	0.00
Diet 4	0	0	0	0	0.00 a	0.00
Diet 5	1	0	0	0	0.25 a	0.50
Diet 6	0	0	0	1	0.25 a	0.50

Diet 7	3	0	1	0	1.00 a	1.41
Diet 8	6	3	0	12	5.25 b	5.12
ANOVA			F	3.443		
			p	0.007		

The average pupal mass of *T. molitor* in the control and dietary treatments is shown in Table 9. Mean values ranged from 0.00 g in the control and diets 1, 3, and 4 to 0.49 g in diet 8 (Figure 6). Standard deviations were 0.00 in several treatments, indicating no variability, while the highest was 0.49 in diet 8, indicating notable variability. ANOVA revealed statistically significant differences ( $F = 3.421$ ;  $p = 0.008$ ). Duncan's post-hoc test grouped the treatments into two homogeneous groups: "a" (control and diets 1–7) and "b" (diet 8).

**Table 9.** Mass of *Tenebrio molitor* pupae on different diets

	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean value	Standard deviation
Control	0	0	0	0	0.00 a	0.00
Diet 1	0	0	0	0	0.00 a	0.00
Diet 2	0	0.14	0.16	0	0.07 a	0.08
Diet 3	0	0	0	0	0.00 a	0.00
Diet 4	0	0	0	0	0.00 a	0.00
Diet 5	0.07	0	0	0	0.01 a	0.03
Diet 6	0	0	0	0.10	0.02 a	0.05
Diet 7	0.25	0	0.09	0	0.08 a	0.11
Diet 8	0.60	0.24	0	1.12	0.49 b	0.49
ANOVA			F	3.421		
			p	0.008		

Numerous studies indicate that the nutritional composition of the diet plays a key role in the growth and development of *T. molitor* larvae. While higher protein content is generally assumed to enhance growth, this is not always the case. Mlček et al. (2021) reported that wheat bran supported the fastest larval development and high numbers of pupae and adults, although faster growth was associated with increased mortality. Rumbos et al. (2020) found that cereals with moderate protein content (11.1–14.2%) or complex diets (dairy feed) resulted in the highest larval biomass, while legume flours, despite being high in protein (22.9–42.4%), produced lower growth. Rho and Lee (2016) suggested that an approximately 1:1 protein-to-carbohydrate ratio is optimal for larval growth. Barragan-Fonseca et al. (2019) demonstrated that both protein and carbohydrate levels significantly affect larval mass in *Hermetia illucens*, with carbohydrate content strongly influencing growth rate.

Our results are consistent with these findings: the highest larval biomass of *T. molitor* was observed on diets with high protein and medium carbohydrate levels, while diets with high protein and high carbohydrate accelerated development but did not maximize biomass. This highlights the importance of optimizing the macronutrient ratio for efficient growth and biomass production.

## CONCLUSION

The lowest larval mass of *Tenebrio molitor* was observed in diet 3, with low protein and medium carbohydrate levels (27.46 g; 683 larvae), and no pupae developed. Diet 6 (low protein, high carbohydrate) resulted in a slightly higher mass (28.72 g; 622 larvae) and few pupae (0.25 on average). Diet 4, with medium protein and carbohydrate levels, produced 696 larvae with an average mass of 29.77 g, but no pupae.

Higher larval masses were recorded in diets 7 and 8 (31.65–32.82 g; 584–644 larvae), with pupae development ranging from 1 to 5.25. Control and diets 1–2 yielded high larval mass (35.12–35.57 g; 792–829 larvae), with pupation observed only in diet 2 (approximately 1 pupa). The highest larval mass (39.36 g; 769 larvae) was achieved in diet 5, with high protein and medium carbohydrate levels, accompanied by 0.25 pupae on average.

These results indicate that diets with medium to high protein and medium carbohydrate levels optimize larval growth, while diets high in carbohydrates and medium to high protein shorten the developmental cycle.

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