Universitatea Tehnică a Moldovei
Technical University of Moldova

Institutul de Microbiologie şi Biotehnologie
Institute of Microbiology and Biotechnology

Simpozion Ştiinţific Naţional cu Participare Internaţională
National Scientific Symposium with International Participation

„Biotehnologiile şi Dezvoltarea Durabilă”
“Biotechnologies and Sustainable Development″

Chişinău, 12 mai 2026
Chişinău, Moldova, May 12 2026

Scientific Committee:

The Symposium is organized within the Research Project 25.80013.5107.04 RE ”Nanobiotechnology and Modeling for Climate-Smart Agriculture in the Republic of Moldova”, funded by the NARD (Financing Contract no.131 RE from 02 January 2026).

DESCRIEREA CIP A CAMEREI NAŢIONALE A CĂRŢII DIN REPUBLICA MOLDOVA
“Biotehnologiile şi dezvoltarea durabilă”, simpozion ştiinţific naţional (2026 ; Chişinău). Biotehnologiile şi dezvoltarea durabilă = Biotechnologies and Sustainable Development : Simpozion Ştiinţific Naţional cu Participare Internaţională = National Scientific Symposium with International Participation, Chişinău, 12 mai 2026 / scientific committee: Liliana Cepoi. – Chişinău : [S. n.], 2026 (Artpoligraf). – 142 p. : fig., tab.
Antetit.: Universitatea Tehnică a Moldovei = Technical University of Moldova, Institutul de Microbiologie şi Biotehnologie = Institute of Microbiology and Biotechnology. – Funded by the NARD. – Ref. bibliogr. la sfârşitul art. – [100] ex.

ISBN 978-9975-3711-6-2. – Text : nemediat.
ISBN 978-9975-3711-7-9 (PDF). – Text : electronic.

Tipărit la tipografia „ArtPoligraf”.

CUPRINS

INFLUENCE OF MANNOPROTEIN-BASED REACTIVATION MEDIA ON THE POST-LYOPHILIZATION VIABILITY OF *RHODOTORULA MUCILAGINOSA* CNMN-YS-10

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https://doi.org/10.52757/bsd26.01

ABSTRACT

Preserving the long-term viability of microorganisms is a fundamental objective of the National Collection of Nonpathogenic Microorganisms (NCNM). The aim of the study was to evaluate the influence of mannoprotein preparations from *Lager* beer yeast sediments (MPB) and *Merlot* red wine (MPV) at concentrations of 1%, 5% and 10% on the cell viability of the *Rhodotorula mucilaginosa* CNMN-YS-10 strain after long-term preservation by lyophilization. Reactivation with MPB at concentrations of 1% and 10% reduced cell viability to 78.14 ± 2.8%, whereas at the intermediate concentration, viability remained comparable to the control range. Reactivating the culture with MPV, even at the minimum concentration, reduced viability to 78.44%, whereas at higher concentrations, viability remained comparable to the control.

Keywords: *Rhodotorula mucilaginosa*, mannoproteins, viability, rehydratation

1. INTRODUCTION

The preservation of cell viability during long storage is essential to ensure the genetic and functional integrity of strains, as the decline in viability can irreversibly compromise biological resources of significant value. Preservation of viability, morpho-cultural and biochemical characteristics of microorganisms involves the use of effective methods and continuous monitoring of their effectiveness [1, 2].

Pigmented yeasts of the genus *Rhodotorula*, and especially *Rhodotorula mucilaginosa*, are of particular interest due to their ability to synthesize significant carotenoids and to adapt to diverse environmental conditions, which places them among the most valuable microbial sourse of natural pigments [9]. This species demonstrates a remarkable capacity to accumulate lipids, carotenoids and possesses diverse enzymatic activity, including lipase and protease activity, properties that recommend it for biotechnological applications on an industrial scale [12, 13].

The most effective long-term preservation method used within the CNMN is lyophilization, which involves dehydrating the culture by sublimation under vacuum conditions and low temperatures, significantly reducing metabolic activity and allowing for long-term preservation of the strains [1]. However, the lyophilization process exposes cells to intense thermal and osmotic stress, which can affect the integrity of cell membranes and reduce viability after reactivation. The quality of the reactivation medium is a determining factor for the recovery of viability and biosynthetic potential of strains [8, 11].

Mannoproteins are the major protein components of the external cell wall of yeasts, consisting of a central protein structure heavily glycosylated with mannose chains, the glycosidic fraction exceeding 90% of the total molecular mass [12]. They are released into the environment following cell autolysis and can be recovered from the sediments remaining after beer or wine fermentation [4]. Their unique structure endows them with excellent biological activities, such as: antioxidant activity, immunostimulatory properties, cell membrane stabilization capacity and cryoprotective potential [11]. Research has demonstrated that mannoproteins isolated from conventional and unconventional yeasts exhibit antimicrobial activity and probiotic effects [6], and their inclusion in microbial inoculation systems contributes to improving microbiological stability [13].

Considering the importance of mannoproteins as biopreparations with antioxidant properties and cryoprotective potential, the aim of this study is to evaluate the bioprotective potential of mannoproteins from brewer's yeast (MPB) and wine yeast (MPV) at concentrations of 1%, 5% and 10% on the viability of the *R. mucilaginosa* strain CNMN-YS-10 after long-term preservation.

2. MATERIALS AND METHODS

The pigmented yeast strain *Rhodotorula mucilaginosa* CNMN-YS-10, preserved in the National Collection of Non-pathogenic Microorganisms at the Institute of Microbiology and Biotechnology of the Technical University of Moldova, was used as the study object.

Mannoprotein extracts were obtained in the Laboratory of Fungal Biotechnology from yeast sediments remaining after production of Lager beer (MPB) and fermentation of Merlot red wine (MPV) [10]. Both extracts were standardized to 25 mg/mL dry matter.

Culture reactivation after lyophilization was performed with 1 mL distilled water (control) or MPB and MPV extracts at 1%, 5%, and 10% (v/v) for 16 h at 28 ± 1°C.

Viability was determined by cultivating reactivated strains on YPD agar at 28 ± 1°C for 5 days, followed by counting colony-forming units (CFU mL^{-1}) according to Uzunova-Doneva and Donev. Viability was calculated as $c\% = (\lg CFU\ mL^{-1}fin / \lg CFU\ mL^{-1}in) \times 100\%$, where lg CFU mL^{-1}in represents the control culture and lg CFU mL^{-1}fin the culture reactivated with MPB or MPV [15].

Statistical analysis was performed using Microsoft Excel and Statistica 9.0. Results are presented as mean ± standard deviation of three biological replicates. Differences were considered significant at $p \leq 0.05$.

3. RESULTS

The results obtained demonstrated that the *Rhodotorula mucilaginosa* CNMN-YS-10 strain remains viable after prolonged storage in a lyophilized state and can be recovered for further research, confirming the efficiency of the lyophilization as a long-term preservation method [12].

The effect of mannoprotein preparations in the reactivation medium revealed a concentration-dependent behavior, distinct depending on the source of the mannoproteins. The viability results are illustrated in Fig. 1.

The use of MPB extract at a concentration of 1% and 10% led to a reduction in cell viability compared to the control, recording a value of 78.14 ± 2.8% (Fig. 1). At a 5% concentration, MPB did not affect cell viability, with survival rates remaining comparable to the control group. This profile, with the maximum at 5%, suggests the existence of an optimal concentration for MPB extract at which the bioprotective effect of beer mannoproteins is maximized.

Mannoprotein extract from wine yeast (MPV) reduces the viability of the *Rhodotorula mucilaginosa* CNMN-YS-10 strain to 78.14 ± 2.8% even at a concentration of just 1%, while at the higher concentrations studied (5% and 10%), it maintains viability at the level of the control sample.

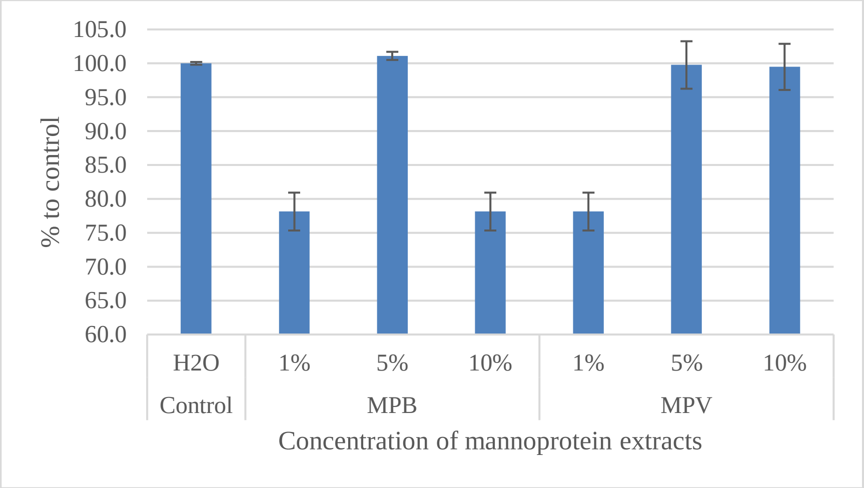

Fig. 1. Viability of *Rhodotorula mucilaginosa* CNMN-YS-10 strain after reactivation with mannoprotein extracts

4. DISCUSSION

The effects observed for the MPB extract may be explained by the antioxidant properties of mannoproteins, which at moderate concentrations protect cell membranes from oxidative stress accumulated during long-term storage [11]. At higher concentrations, the inhibitory effect may result from changes in the osmotic pressure of the reactivation medium or non-specific interactions with cellular components, as reported for yeast extracts used as bioprotective agents [4, 8].

The different behavior of MPV and MPB at higher concentrations is likely related to their distinct biochemical composition and structure. *Saccharomyces cerevisiae* strains are adapted to wine fermentation conditions through specific cell wall composition. Mannoproteins from different yeast sources vary in glycosylation patterns, affecting their biological properties and interactions with target cells [6, 13]. In addition, wine yeast sediments differ from beer sediments in protein and polysaccharide content [10]. A comparative analysis of the two preparations showed that MPB mannoproteins at 5% maintained cell viability at a level comparable to the control. Likewise, MPV at 5% and 10% effectively preserved viability without statistically significant changes. These findings are consistent with the functional properties of yeast mannoproteins reported in the literature [9, 14].

Previous studies on *Rhodotorula gracilis* CNMN-YS-03 and *Rhodotorula glutinis* CNMN-YS-08 showed that, although mannoproteins did not significantly affect cell viability, they markedly influenced biosynthetic activity, increasing the synthesis of protective compounds such as carotenoids and altering the accumulation of reserve carbohydrates [3].This metabolic reorientation can be explained by stress response mechanisms which involve the regulation of gene expression and enzymatic activity to enhance cellular resistance to external factors. Thus, even without directly affecting cell viability, mannoproteins can indirectly enhance cellular adaptability and metabolic performance, with significant implications for biotechnological processes and the optimization of cultivation conditions. A limitation of this study is that the exact molecular mechanism by which mannoproteins confers protection was not investigated, representing an important direction for future research.

The obtained data open important perspectives for further research on the effect of MPB and MPV preparations on the carotenoid synthesis potential of the *Rhodotorula mucilaginosa* CNMN-YS-10 strain in the regeneration process after long-term preservation.

5. CONCLUSIONS

The results obtained confirm that the *Rhodotorula mucilaginosa* strain CNMN-YS-10 retains a high level of viability after long-term preservation by lyophilization, confirming the effectiveness of this method for yeast preservation of biotechnological importance. At the same time, the study highlights the crucial role of the reactivation medium's composition in restoring cellular capacity after storage.

Among the tested concentrations of mannoprotein extract from beer (MPB), 5% demonstrated the highest efficacy, with a cell viability of 101.10 ± 0.6%, slightly above the control. At concentrations of 1% and 10%, viability decreased to 78.14 ± 2.8%, indicating the existence of an optimal range of action. Mannoprotein extract from wine (MPV) showed a more stable behavior, concentrations of 5% and 10% maintained viability at levels comparable to the control, 99.76 ± 3.5% and 99.48 ± 3.4%, respectively, without significant inhibitory effects.

The experimental data obtained support the inclusion of the mannoprotein preparations MPB and MPV in the composition of the reactivation media for yeasts of the genus *Rhodotorula*, the optimal concentrations - 5% for MPB and 5–10% for MPV were validated as essential parameters for the efficient recovery of cell viability after lyophilization.

ACKNOWLEDGMENTS

This study was supported by the research project 25.80012.7007.16TC „Viability and productive potential of pigmented yeasts of the genus *Rhodotorula* – carotenoid producers, after long-term preservation", funded by National Agency for Research and Development.

DECLARATIONS

Conflict of interest: The authors declare that they have no conflict of interest.

Author Contributions: B.L. designed the study; B.L. and B.G.N. performed experiments; B.L. analyzed data; T.E. and R.A. writing—original draft preparation; B.G.N. writing – review & editing; all authors reviewed and approved the final version.

*Data Availability Statement***:** The datasets generated during the current study are available from the corresponding author upon reasonable request.

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

REFERENCES

1. Arellano-Ayala, K., Lim, J., Yeo, S., Bucheli, J. E. V., Todorov, S. D., Ji, Y., & Holzapfel, W. H. (2021). Rehydration before application improves functional properties of lyophilized *Lactiplantibacillus plantarum* HAC03. Microorganisms, 9(5), 1013. https://doi.org/10.3390/microorganisms9051013
2. Balan, L., & Slanina, V. (2021). Modificarea conținutului de proteine și carbohidrați în biomasa levurilor liofilizate în prezența extractelor din spirulină. In: *Biotehnologii moderne — soluții pentru provocările lumii contemporane*, 20–21 mai 2021 (p. 129). Chişinău. ISBN 978-9975-3498-7-1. https://doi.org/10.52757/imb21.071
3. Balan, L., Bogdan-Golubi, N., Rozlovan, A., & Țîbîrnac, E. (2025) Influența manoproteinelor asupra viabilității tulpinilor de *Rhodotorula* după păstrare îndelungată. Revista de proprietate intelectuală, știință și educație „Intellectus", 2, 183-190. ISSN 1810-7087 https://doi.org/10.56329/1810-7087.25.2.17
4. Beşliu, A., Chiseliță, O., Chiselita, N., Efremova, N., Tofan, E., & Lozan, A. (2020). Compoziția biochimică a sedimentelor levurilor de bere la diferite procedee de autoliză. Studia Universitatis Moldaviae (Seria Științe Reale și ale Naturii), 6(136), 54–59. ISSN 1814-3237. https://doi.org/10.5281/zenodo.4431557

5. Burțeva, S., Chiseliță, O., Bîrsa, M., & Mașcenco, N. (2021). Viabilitatea tulpinii *Streptomyces canosus* CNMN-AC-02 după liofilizare în prezența glicozidelor flavonoide. In: *Biotehnologii moderne — soluții pentru provocările lumii contemporane*, 20–21 mai 2021 (p. 134). Chișinău. ISBN 978-9975-3498-7-1. https://doi.org/10.52757/imb21.076
6. Bzducha-Wróbel, A., Farkaš, P., Chraniuk, P., Popielarz, D., Synowiec, A., Pobiega, K., & Janowicz, M. (2022). Antimicrobial and prebiotic activity of mannoproteins isolated from conventional and nonconventional yeast species. World Journal of Microbiology and Biotechnology, 38(12), 256. https://doi.org/10.1007/s11274-022-03441-2
7. Chiseliță, O., Darie, Gr., Rotari, D., Chiselița, N., Beșliu, A., Efremova, N., & Caraman, M. (2023). Brewer yeast mannoproteins as an efficient supplement for preservation of ram sperm by refrigeration. Scientific Papers. Series D. Animal Science, LXVI (1), 184–194.
8. Chiselita, O., Burtseva, S., Byrsa, M., & Rudik, V. (2021). Liofilizarea *Streptomyces canosus* CNMN-Ac-02 în prezența polizaharidelor sulfatate cu Zn din spirulina. Buletinul Academiei de Științe a Moldovei. Științele vieții, 1(343), 99–105. ISSN 1857-064X. https://doi.org/10.52388/1857-064X.2021.1.13
9. Chraniuk, P., & Bzducha-Wróbel, A. (2025). Functional properties of yeast mannoproteins — current knowledge and future perspectives. Fermentation, 11(7), 374. https://doi.org/10.3390/fermentation11070374
10. Efremova, N., Besliu, A., Chiselita, N., Chiselita, O., Tofan, E., & Danilis, M. (2024). Biochemical characterization of the yeast biomass resulting from the winemaking processes. Journal of Experimental and Molecular Biology, 25(3), 141–150. https://doi.org/10.47743/jemb-2024-179
11. Jaehrig, S. C., Rohn, S., Kroh, L. W., Fleischer, L.-G., & Kurz, T. (2007). In vitro potential antioxidant activity of (1→3),(1→6)-β-d-glucan and protein fractions from *Saccharomyces cerevisiae* cell walls. Journal of Agricultural and Food Chemistry, 55(12), 4710–4716. https://doi.org/10.1021/jf070041t
12. Sîrbu, T., & Slanina, V. (2022). Evaluarea viabilității tulpinilor de drojdii după 15 ani de conservare. One Health and Risk Management, 3(3), 18–25. ISSN 2587-3458. https://doi.org/10.38045/ohrm.2022.3.03
13. Toraño, P., Gombau, J., Mejías, I., Bordons, A., Rozès, N., & Reguant, C. (2024). Evaluation of the addition of yeast mannoprotein to *Oenococcus oeni* starter cultures to improve wine malolactic fermentation. Fermentation, 10(1), 52. https://doi.org/10.3390/fermentation10010052
14. Utama, G. L., Oktaviani, L., Balia, R. L., & Rialita, T. (2023). Potential application of yeast cell wall biopolymers as probiotic encapsulants. Polymers, 15(16), 3481. https://doi.org/10.3390/polym15163481
15. Uzunova-Doneva, T., & Donev, T. (2002). Influence of the freezing rate on the survival of strains *Saccharomyces cerevisiae* after cryogenic preservation. Journal of Culture Collections, 3, 78–83.

IMPACT OF A CARBOHYDRATE-RICH DIET ON GUT MICROBIOTA COMPOSITION AND ENTEROCOCCAL DYNAMICS

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https://doi.org/10.52757/bsd26.02

ABSTRACT

Excessive consumption of refined carbohydrates from processed foods is increasingly associated with intestinal dysbiosis and metabolic disturbances. The present study aimed to evaluate the impact of a carbohydrate-rich diet, using processed white bread as the main source, on the dynamics of enterococci in comparison with beneficial bacteria (bifidobacteria and lactobacilli) and opportunistic *Escherichia coli* in an experimental animal model. Laboratory rats were divided into control (standard diet) and experimental groups (high-carbohydrate diet), and intestinal bacterial counts were assessed at baseline, after 5, 10, and 15 days of dietary intervention, and following a 7-day recovery period. Microbial populations were quantified and expressed as log CFU/g. The high-carbohydrate diet induced a significant reduction in enterococci (up to 21%) and bifidobacteria (up to 22.2%), accompanied by a moderate decrease in lactobacilli and an increase in *E. coli* populations during the early phase of intervention. Restoration of the standard diet partially or completely reestablished microbial balance. These findings suggest that processed carbohydrate excess disrupts intestinal microbial homeostasis, highlighting the sensitivity of enterococci to dietary carbohydrate shifts and supporting their potential commensal role in gut ecosystem stability.

Keywords: intestinal dysbiosis, dietary intervention, microbial balance, commensal bacteria, Escherichia coli, bifidobacteria, lactobacilli

1. INTRODUCTION

In recent scientific literature, the term "industrialized gut microbiota" is increasingly used to describe a bacterial community characterized by a reduced capacity to degrade dietary fiber and by shifts in microbial composition associated with modern lifestyle and dietary changes (Adolph et al., 2022). Increased consumption of simple carbohydrates and processed foods is considered an important factor contributing to the development of this type of microbiota. Studies indicate that high intake of glucose and fructose is associated with alterations in gut microbial composition and with the development of metabolic disorders, including non-alcoholic fatty liver disease (Garcia et al., 2022; Zhang et al., 2025).

A diet rich in refined carbohydrates may promote intestinal dysbiosis, characterized by a reduction in beneficial bacteria and proliferation of potentially pathogenic microorganisms (Yang & Kweon, 2016). Frequent consumption of processed products, such as bread made from refined flour, has been associated with changes in gut microbiota composition and impairment of intestinal barrier function (Wang et al., 2023).

The physiological role of enterococci under conditions of excessive carbohydrate intake remains insufficiently elucidated, although their ability to metabolize a wide range of carbohydrates provides them with an ecological advantage in the gastrointestinal tract (Ramsey et al., 2014).

In this context, the aim of the present study was to evaluate the effect of processed foods, represented by bread, on enterococcal populations in comparison with beneficial bacteria (lactobacilli and bifidobacteria) and with *Escherichia coli*.

2. MATERIALS AND METHODS

The experiment evaluated the effect of a carbohydrate-rich diet on the animal organism. In this dietary model, carbohydrates were administered in proportions exceeding physiological requirements. The percentage composition of the principal nutrients in the experimental diets is presented in Table 1.

Table 1. Percentage composition of nutrients in the experimental diets

Principal nutrients	***Standard diet (%)***	***High-carbohydrate diet (%)***
Proteins	14,88	13,4
Lipids	2,9	2,11
Carbohydrates	82,2	84,5

Due to the exploratory nature of the study, a limited number of animals was used; all animals were housed individually in separate cages. The animals were divided into two groups: control animals (Lots I and II), receiving the standard diet, and experimental animals (Lots III and IV), receiving the high-carbohydrate diet. The experimental diet was based primarily on processed white bread as the main carbohydrate source.

Intestinal microbiota dynamics were evaluated at baseline (prior to diet administration), after 5, 10, and 15 days of dietary intervention, and after a 7-day recovery period following the return to the standard diet.

Microbiological analyses were performed using selective culture media: Bile-Esculin Azide Agar (Himedia, M4931) for *Enterococcus spp.*, Bifidobacterium Agar (Himedia, M1396) for bifidobacteria, MRS Agar (Himedia, M641) for lactobacilli, and Endo Agar (Himedia, M029R) for *Escherichia coli*. All determinations were carried out in triplicate according to the SM EN ISO 4833-1:2014 standard. Microbial counts were calculated per gram of intestinal content and expressed as decimal logarithms (log CFU/g).

3. RESULTS AND DISCUSSION

Processed white bread was used as the primary source of dietary carbohydrates in the experimental diet (Figures 1–4).

The high-carbohydrate diet exerted an inhibitory effect on enterococcal populations. After 5 days of diet administration, enterococci decreased by approximately 14.6–16.2%. The most pronounced reduction was observed after 10 days, when the enterococcal population declined by 19.7–21% compared with baseline values. After 15 days, the decrease became less pronounced (11.1–14.9%), suggesting partial microbial adaptation to the dietary conditions (Figure 1).

These observations indicate that enterococci are sensitive to changes in carbohydrate intake, particularly during the initial stages of dietary intervention. Restoration of the standard diet resulted in the recovery of enterococcal populations to levels comparable to those recorded before the experiment.

To better understand the role of enterococci in intestinal microbial balance, changes in the populations of bifidobacteria and lactobacilli (beneficial bacteria) and *Escherichia coli* (representing opportunistic microorganisms) were also analyzed.

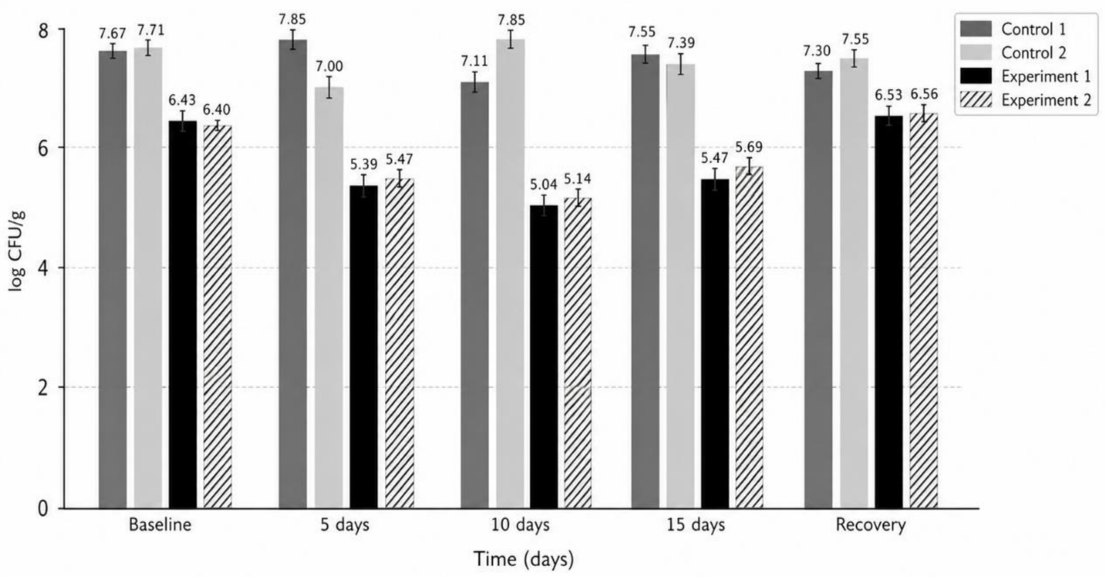

Figure 1. Dynamics of *Enterococcus* spp. (log CFU/g) in intestinal content during a high-carbohydrate diet and recovery period.

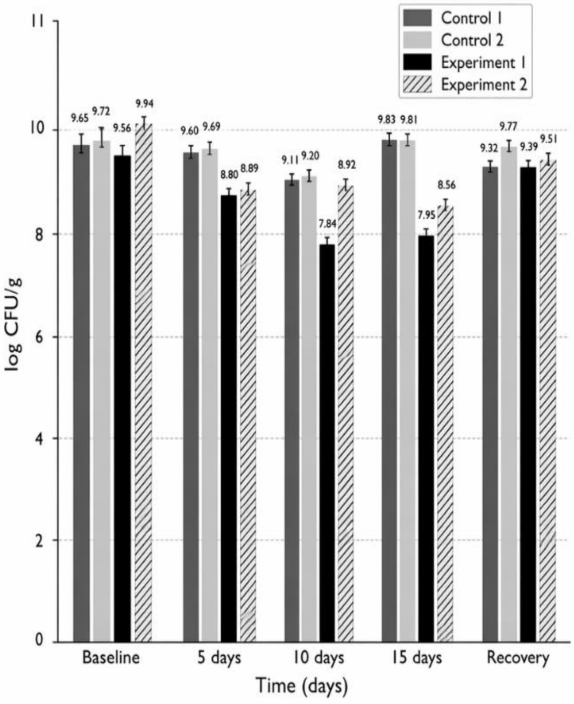

Figure 2. Dynamics of ***Lactobacillus* spp.** (log CFU/g) in intestinal content during a high-carbohydrate diet and recovery period.

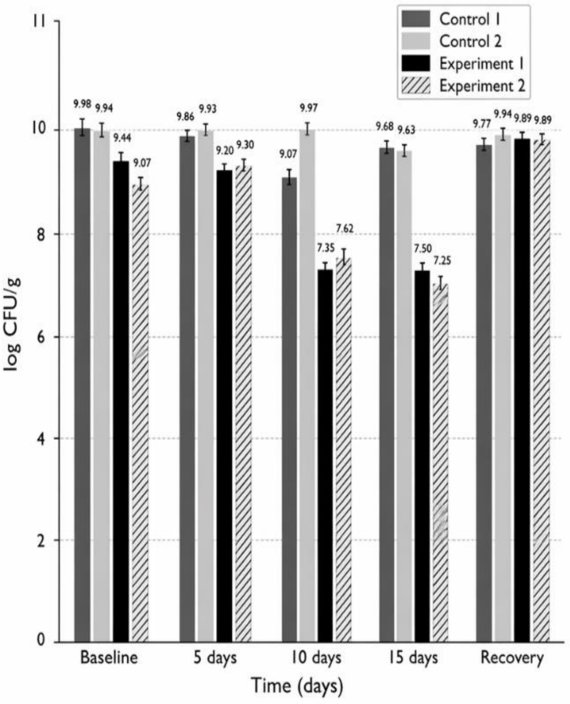

Figure 3. Dynamics of ***Bifidobacterium*** spp. (log CFU/g) in intestinal content during a high-carbohydrate diet and recovery period.

Bifidobacteria and lactobacilli appeared less sensitive than enterococci during the early phase of the experiment. After 5 days of carbohydrate-rich feeding, bifidobacteria decreased by 2.6–2.7%, while lactobacilli decreased by 7.9–10.6%. However, after 10 days, a more pronounced reduction in bifidobacteria was observed (21.4–22.2%), while lactobacilli decreased by 10.3–18.0% compared with baseline values (Figures 2-3).

After 15 days of dietary intervention, bifidobacteria remained approximately 20% lower than the initial levels, while lactobacilli showed a reduction of 13.9–16.8%. Return to the standard diet led to restoration of bifidobacterial populations to levels comparable to the control group, while lactobacilli also returned to values similar to those recorded before the experimental diet.

In contrast, the carbohydrate-rich diet promoted an increase in *Escherichia coli* populations. After 5 days, the number of *E. coli* increased by 10.6–10.9%, followed by smaller increases after 10 and 15 days (1.9–3.9% and 5.5–6.7%, respectively) (Figure 3).

The restoration of the standard diet resulted in a reduction of *E. coli* populations by 6.0–9.4% compared with baseline values and lower levels than those observed in the control group.

Overall, the results demonstrate that excessive intake of refined carbohydrates derived from processed bread alters the composition of intestinal microbiota by decreasing beneficial bacterial populations and promoting the proliferation of opportunistic microorganisms. These findings are consistent with previous studies indicating that diets rich in refined carbohydrates contribute to intestinal dysbiosis and metabolic disturbances.

The synchronous variation observed between enterococci and beneficial bacteria suggests a commensal ecological role of enterococci within the intestinal microbiota, supporting their potential involvement in maintaining microbial balance under normal dietary conditions.

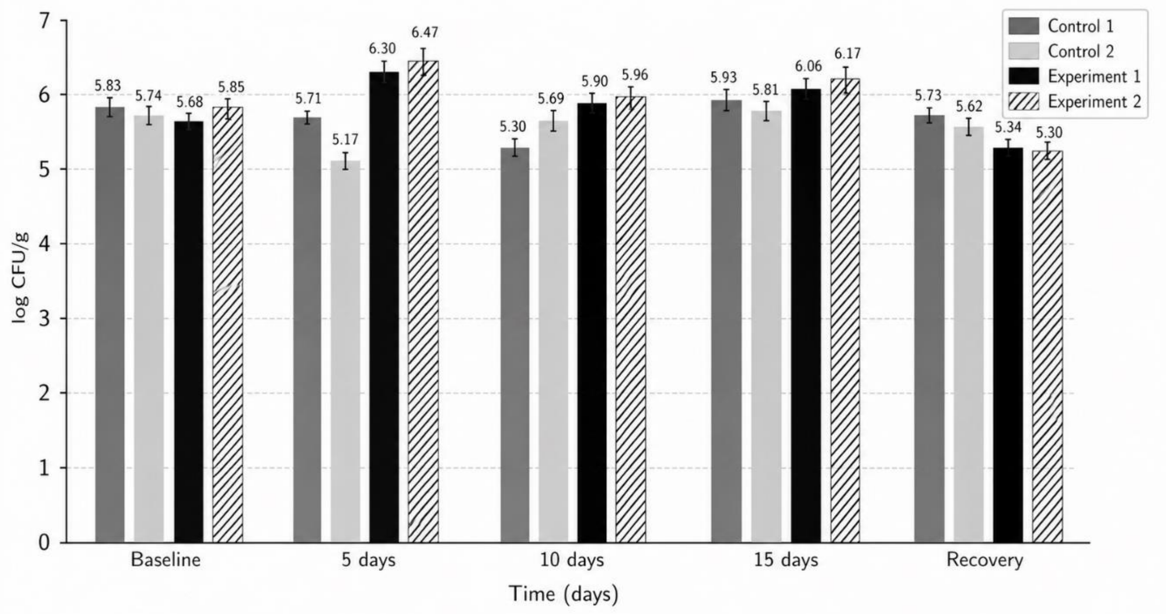

Figure 4. Dynamics of *Escherichia coli* (log CFU/g) in intestinal content during a high-carbohydrate diet and recovery period.

4. CONCLUSIONS

Excessive intake of refined carbohydrates from processed bread leads to a reduction in enterococci and beneficial bacteria (bifidobacteria and lactobacilli), along with an increase in *Escherichia coli*, indicating disruption of intestinal microbial balance.

Enterococci demonstrated sensitivity to changes in dietary carbohydrate intake, supporting their potential commensal role in maintaining gut microbiota stability.

Although beneficial bacteria exhibit a certain degree of resilience, prolonged exposure to excessive carbohydrates reduces their populations and may promote intestinal dysbiosis.

Restoration of a standard diet partially or completely reestablishes microbial balance, demonstrating the capacity of the gut microbiota to recover after diet-induced disturbances.

DECLARATIONS

Ethics Statement: The experiment was conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Confirmation of originality: The author declares that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere.

Data Availability Statement : The datasets generated during the current study are available from the corresponding author upon reasonable request.

REFERENCES:

1. Adolph, T. E., Meyer, M., Schwärzler, J., Mayr, L., Grabherr, F., & Tilg, H. (2022). The metabolic nature of inflammatory bowel diseases. *Nature Reviews Gastroenterology & Hepatology 2022 19:12*, *19*(12), 753–767. https://doi.org/10.1038/s41575-022-00658-y
2. Garcia, K., Ferreira, G., Reis, F., & Viana, S. (2022). Impact of Dietary Sugars on Gut Microbiota and Metabolic Health. *Diabetology*, *3*(4), 549–560. https://doi.org/10.3390/DIABETOLOGY3040042
3. Ramsey, M., Hartke, A., & Huycke, M. (2014). The Physiology and Metabolism of Enterococci. *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*. https://www.ncbi.nlm.nih.gov/books/NBK190432/
4. Wang, Y., Jian, C., Salonen, A., Dong, M., & Yang, Z. (2023). Designing healthier bread through the lens of the gut microbiota. *Trends in Food Science & Technology*, *134*, 13–28. https://doi.org/10.1016/J.TIFS.2023.02.007
5. Yang, J. Y., & Kweon, M. N. (2016). The gut microbiota: a key regulator of metabolic diseases. *BMB Reports*, *49*(10), 536. https://doi.org/10.5483/BMBREP.2016.49.10.144
6. Zhang, Y., Walker, R. W., Kaplan, R. C., & Qi, Q. (2025). Added sugars, gut microbiota, and host health. *Gut Microbes*, *17*(1), 2592431. https://doi.org/10.1080/19490976.2025.2592431

INFLUENCE OF AGAR MEDIA ON THE ANTIBACTERIAL ACTIVITY ASSESSMENT OF *LACTOCOCCUS LACTIS* BY DIFFUSION ASSAY

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https://doi.org/10.52757/bsd26.03

ABSTRACT

Successful application of techniques for quantitative or qualitative antibacterial determination (screening and effectiveness) relies not only on the sensitivity of the test-microorganisms, but also on the agar-medium used. The composition of agar media can significantly influence the outcome of diffusion-based antibacterial assays, affecting the interpretation of inhibition zone measurements. The aim of this study was to evaluate the influence of different agar media on the assessment of antibacterial activity of *Lactococcus lactis* strains using a diffusion assay. Four *L. lactis* strains were tested against selected *Bacillus* and *Pseudomonas* indicator strains using Nutrient Agar (NA), Mueller - Hinton Agar (MHA), and Luria - Bertani (LB) agar. The results demonstrated that inhibition zone diameters varied considerably depending on the medium used, with NA generally providing larger inhibition zones compared to MHA and LB. These differences highlight the strong dependence of antibacterial activity assessment on medium composition and emphasize the need for careful selection and standardization of assay conditions. The findings underline that comparisons of antibacterial activity data across studies should consider methodological differences, particularly the type of agar medium employed.

Keywords: culture media, diffusion assay, *Lactococcus lactis*, antibacterial activity, inhibition zones.

1. INTRODUCTION

Lactic acid bacteria (LAB) are among the most important microbial groups in the food industry, particularly *Lactococcus lactis*, a mesophilic LAB widely used as an active acidifier in the production of sour cream, fresh cheese, and brined cheese. It also contributes to the characteristic flavor and aroma of cheeses such as Cheddar and Camembert through diacetyl production by *Lactococcus lactis subsp. lactis* biovar *diacetylactis* [2].

Lactic acid and other metabolites produced by LAB improve organoleptic and textural properties and extend product shelf life. In addition, lactic acid and bacteriocins produced by some *Lactococcus* strains inhibit the growth of spoilage, pathogenic, and phytopathogenic Gram-positive and Gram-negative bacteria. Food spoilage caused by pathogenic or non-pathogenic microorganisms results in substantial food losses and potential health risks [5,8,14].

Pseudomonas fluorescens is a Gram-negative bacterium that causes food spoilage through the production of proteases, lecithinase, and lipases. It can grow over a wide temperature range, including in refrigerated foods [10, 17].

Bacillus species are Gram-positive bacteria that may contaminate products at all stages of storage. Their hydrolytic enzymes can cause rope spoilage in bread and deterioration of dairy products, leading to economic losses [4, 11, 16]. In addition, *Bacillus* spp. are wheat grain endophytes, and their heat-resistant spores can survive baking in the crumb core, representing a major concern as demand for preservative-free bread increases [15].

Excessive use of antibiotics to control food-spoilage and pathogenic bacteria has promoted microbial resistance, creating new risks to human health [9]. Consequently, the search for new antibacterial compounds, such as bacteriocins, has attracted increasing scientific interest. Various methods are used to evaluate the antimicrobial activity of lactic acid bacteria, mainly through inhibition of pathogenic microorganisms by bacteriocins, organic acids, and hydrogen peroxide [18]. Antibacterial assays for lactic acid bacteria commonly employ agar plate and agar diffusion methods, which are effective for identifying probiotic strains with antimicrobial potential. These techniques assess bacteriocin production and organic acid secretion by measuring clear inhibition zones against indicator pathogens [12].

Dependence on different factors, including media composition and duration of cultivation has an important role and can affect the growth of microorganism, metabolic activity and the diffusion and activity of substances with antibacterial properties [7]. Most studies use standard, nutrient-rich media which provides enabling fast growth of indicator pathogens, and superior diffusion of antimicrobial compounds. The possibility of unspecific reaction between the active substance present in the tested culture and the agar medium should also be considered [3].

The aim of this study was to evaluate the influence of different agar media on the assessment of antibacterial activity of *Lactococcus lactis* strains using a diffusion assay, by analyzing variations in inhibition zone measurements against selected *Bacillus* and *Pseudomonas* indicator strains.

2. MATERIALS AND METHODS

The study included *Lactococcus lactis* CNMN-LB-06, *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* CNMN-LB-07, *Lactococcus lactis* CNMN-LB-09, *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* CNMN-LB-14 lactic acid bacteria strains stored in National Collection of Non-pathogenic Microorganisms at the Institute of Microbiology and Biotechnology of TUM. LAB strains were maintained in MRS medium, cultivation was carried out at 30±1°C for 24 hours. The inoculum concentration was adjusted to 0.5 McFarland standard.

The antibacterial activities of LAB were tested against microorganisms *Bacillus subtilis* CNMN-BB-06, *Bacillus subtilis* CNMN-BB-09, *Bacillus cereus* var. *fluorescens* CNMN-BB-07, *Pseudomonas fluorescens* CNMN-PsB-02, *Pseudomonas fluorescens* CNMN-PsB-12, using the agar well diffusion method. Test cultures were maintained on nutrient agar (NA) and King B media. Cultivation was carried out at a temperature of 35±1°C for a period of 24-48 hours. The growth media used were: (1) Nutrient Agar (NA), containing (g/L): meat extract 1.0, yeast extract 2.0, peptone 5.0, sodium chloride 5.0, and agar 15.0; (2) Mueller–Hinton Agar (MHA), containing (g/L): meat infusion 2.0, casein hydrolysate 17.5, starch 1.5, and agar 13.0; and (3) Luria–Bertani agar (LB), containing (g/L): casein peptone 10.0, yeast extract 5.0, and sodium chloride 10.0. Test bacterial cultures were inoculated by the pour plate method to obtain a uniform bacterial lawn, and agar wells of 8.0 mm diameter were prepared using a cork borer. To ensure proper diffusion of the LAB suspension into the agar medium, plates were kept at room temperature for 1 h before incubation. Growth inhibition zones were measured after 24 h of incubation [2].

Statistical analysis of data was performed using Microsoft Excel 2016, there were three biological replications for each test.

3. RESULTS

The results obtained on the inhibitory effects demonstrated that *L. lactis* strains showed activity against *Bacillus* and *Pseudomonas* bacteria, being cultivated on the agarized media LB, MH or NA, the inhibition zones varying between 11,2 - 16,0 mm, 11,2 - 19,2 mm and 14,0 - 22,3 mm, respectively, the zones of inhibition being dependent on the strain or tested medium. (Table 1).

Table 1. Antibacterial activity of lactic acid bacteria strains at different culture media against *Bacillus* and *Pseudomonas* strains

Strain	Media	*B. subtilis* CNMN-BB-06	*B. cereus* var. *fluorescens* CNMN-BB-07	*B. subtilis* CNMN-BB-09	*P. fluorescens* CNMN-PsB-02	*P. fluorescens* CNMN-PsB-12
Zone of inhibition (mm)						
L. lactis ssp. *lactis* CNMN-LB-06	LB	12,0±1,1	16,0±1,1	13,3±0,7	15,3±0,7	13,7±0,7
	MHA	11,7±1,7	17,3±0,7	17,3±0,7	15,5±0,6	19,2±0,3
	NA	19,3±0,7	21,0±1,1	21,7±0,7	22,3±0,7	15,7±0,7
L. lactis ssp. *lactis* bv.*diacetylactis* CNMN-LB-07	LB	Ni	12,3±1,7	Ni	11,3±0,7	11,2±0,3
	MHA	11,2±0,3	12,3±1,3	Ni	Ni	12,7±1,3
	NA	18,7±0,7	18,7±0,7	19,3±0,7	22,0±1,1	16,0±1,1
L. lactis ssp. *lactis* CNMN-LB-09	LB	13,3±0,7	11,3±0,7	14,3±0,7	12,2±0,3	14,7±0,7
	MHA	Ni	17,0±1,1	Ni	17,3±0,7	16,3±1,7
	NA	17,3±0,7	18,0±1,1	18,7±0,7	22,0±1,1	17,0±0,0
L. lactis ssp. *lactis* bv.*diacetylactis* CNMN-LB-14	LB	11,7±0,7	11,2±0,3	13,3±1,7	11,2±0,3	14,3±0,7
	MHA	Ni	14,3±0,7	Ni	16,7±0,7	12,7±1,3
	NA	14,0±1,1	14,0±1,1	14,7±1,7	14,0±0,0	15,0±0,0

*Ni – no inhibition

The results showed that the tested *B. subtilis*, *B. cereus* var. *fluorescens* and *P. fluorescens* strains were more sensitive to LAB bioactive compounds when cultivated on Nutrient Agar (NA) medium. The most sensitive strain was *P. fluorescens* CNMN-PsB-02, with inhibition zones ranging from 14.0 to 22.3 mm. Selection of the growth agar medium for antibacterial assay is important, as it directly influences bacterial growth and the measured inhibition effect. As expected, both the appearance and diameter of inhibition halos varied according to the agar medium used. Among the four LAB strains tested, only *L. lactis* ssp. *lactis* CNMN-LB-06 showed activity against all four *Bacillus* and *Pseudomonas* strains, regardless of cultivation medium. *L. lactis* biovar *diacetylactis* CNMN-LB-07 inhibited all bacterial strains on NA medium, with zones up to 22.0 mm. *L. lactis* ssp. *lactis* biovar *diacetylactis* CNMN-LB-14 showed antibacterial activity against tested strains cultivated on LB and NA media, with inhibition zones of 11.2–14.3 mm and 14.0–15.0 mm, respectively. For *L. lactis* ssp. *lactis* CNMN-LB-09, higher antagonistic activity was recorded on the same media, with inhibition zones of 11.3–14.7 mm and 17.0–22.0 mm, respectively. On Mueller–Hinton Agar, *B. subtilis* CNMN-BB-06 and *B. subtilis* CNMN-BB-09 did not show sensitivity to *L. lactis* ssp. *lactis* CNMN-LB-09 and *L. lactis* ssp. *lactis* biovar. *diacetylactis* CNMN-LB-14.

4. DISCUSSION

According to the literature, the antimicrobial activity of exometabolites synthesized by lactic acid bacteria may vary considerably. Differences in culture media directly influence LAB growth, metabolic activity, and antimicrobial effectiveness. However, the inhibition zone is only a qualitative indicator and is also affected by factors such as agar depth, well size, and compound solubility. Analysis of media composition has shown major differences in the content of organic and inorganic compounds among commonly used media [3]. Similar effects of salts and nutrient concentration on inhibition zones were reported by Bhattacharjee et al. for rhubarb stalk extracts tested against pathogens [1].

Analysis of the obtained results demonstrated that studied *L. lactis* CNMN-LB-06, *L. lactis* ssp. *lactis* biovar. *diacetylactis* CNMN-LB-07 and *L. lactis* CNMN-LB-09 possessed capacity to produce antibacterial metabolites active against *B. subtilis*, *B. cereus* var. *fluorescens* and *P.*

fluorescens bacteria cultivated on NA, being more efficient for the growth of all tested strains. For testing the antibacterial activity of lactic acid bacteria against Gram-positive and Gram-negative microorganisms, Nutrient agar supports growth and can be used, but in contrast to Mueller-Hinton Agar, is not standardized for diffusion assays. Nutrient agar, Mueller-Hinton and Luria-Bertani are an enriched culture media, are widely used by researchers, supports fast growth of many bacterial species, including *Pseudomonas* and *Bacillus*, but differ through nutrient composition and concentration which influence the growth of microbial strains. High nutrient level in LB, due to peptone and yeast extract, interfere with diffusion of antimicrobial compounds, and are recommended better for maintaining strains before testing, while nutrient level in MHA and NA is more consistent for diffusion of antimicrobial compounds, more preferred for antibacterial assay [6].

5. CONCLUSIONS

The results of this study demonstrate that the composition of culture media significantly influences the assessment of antibacterial activity of *Lactococcus lactis* when evaluated by diffusion assay. The diameter of inhibition zones varied considerably depending on the medium used, confirming that the measured antibacterial effect is strongly dependent on assay conditions.

Among the tested media, Nutrient Agar generally supported the largest inhibition zones, followed by Mueller - Hinton Agar, while Luria - Bertani medium showed lower sensitivity for detecting antibacterial effects. These differences are likely related to variations in nutrient composition and their impact on both microbial growth and diffusion of bioactive compounds. The findings highlight that inhibition zone measurements should be interpreted with caution, as they reflect not only the intrinsic antibacterial potential of the tested strains but also the properties of the culture medium. Therefore, the selection and standardization of agar media are critical for obtaining reliable and comparable results in antibacterial assays.

ACKNOWLEDGMENTS

This research was funded by Government of Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 „InBioS – Innovative biotechnological solutions for agriculture, medicine and environment”.

DECLARATIONS

The author confirms that this manuscript is original, has not been published previously, and is not under consideration elsewhere. Author contributed to research, result analysis, and manuscript writing.

REFERENCES

1. Bhattacharjee, M. K., Bommareddy, P. K., DePass, A. L. (2021). A water-soluble antibiotic in rhubarb stalk shows an unusual pattern of multiple zones of inhibition and preferentially kills slow-growing bacteria. *Antibiotics*, 10(8), 951. https://doi.org/10.3390/antibiotics10080951
2. Bogdan-Golubi, N., Slanina, V., Balan, L. (2025). Antagonistic activity of *Lactococcus lactis* ssp. and *Streptococcus thermophilus* against *Bacillus* and *Pseudomonas* species. *Analele Universității din Oradea, Fascicula Biologie*, 2025, Tom XXXII, Issue 1, pp. 67-72.
3. de Azevedo, P. O. de S., Molinari, F., & de Souza Oliveira, R. P. (2018). Importance of the agar-media in the evaluation of bacteriocin activity against the same test-microorganisms. *Brazilian Journal of Pharmaceutical Sciences*, 54(1), 17533. https://doi.org/10.1590/s2175-97902018000117533, 2-s2.0-85048544800.
4. Elegbeleye, J., Buys, E. (2021). Potential spoilage of extended shelf-life (ESL) milk by *Bacillus subtilis* and *Bacillus velezensis*. *LWT - Food Science and Technology*, 153(9): 112487. https://doi.org/10.1016/j.lwt.2021.112487

5. Fusieger, A., Perin, L.M., Teixeira, C.G., de Carvalho, A.F., Nero L.A., (2020): The ability of *Lactococcus lactis* subsp. *lactis* bv. *diacetylactis* strains in producing nisin. *Antonie Van Leeuwenhoek*, 113(5): 651-662. https://doi.org/10.1007/s10482-019-01373-6
6. Hamdaoui, N., Benkirane, C., Bouaamali, H., Azghar, A., Mouncif, M., Maleb, A., Hammouti, B., Al-Anazi, K.M., Kumar, P., Yadav, K.K., Choi, J.R., Meziane, M. (2024). Investigating lactic acid bacteria genus *Lactococcus lactis* properties: Antioxidant activity, antibiotic resistance, and antibacterial activity against multidrug-resistant bacteria *Staphylococcus aureus*. *Heliyon*, 10(11):e31957. https://doi.org/10.1016/j.heliyon.2024.e31957
7. Huys G., D'Haene K., Swings J. (2002). Influence of the culture medium on antibiotic susceptibility testing of food-associated lactic acid bacteria with the agar overlay disc diffusion method. *Letters in Applied Microbiology*, 34(6), 402–406. https://doi.org/10.1046/j.1472-765x.2002.01109.x
8. Ibrahim, S.A., Ayivi, R.D., Zimmerman, T., Siddiqui, S.A., Altemimi, A.B., Fidan, H., Esatbeyoglu, T., Bakhshayesh, R.V., (2021): Lactic acid bacteria as antimicrobial agents: food safety and microbial food spoilage prevention. *Foods*, 10(12): 3131. https://doi.org/10.3390/foods10123131
9. Ignatova-Ivanova, T., Ibryamova, S., Bachvarova, D., Salim, S., Valkova, S., Simeonova, Y., Dimitrov, D., Ivanov, R., Chipev, N., Natchev, N. (2022). Determination of the antimicrobial activity of lactic acid bacteria isolated from the Black Sea mussel *Mytilus galloprovincialis* Lamarck, 1819. *Pharmacia*, 69(3), 637–644. https://doi.org/10.3897/pharmacia69.e84850
10. Kumar, H., Franzetti, L., Kaushal, A., Kumar, D. (2019). *Pseudomonas fluorescens*: a potential food spoiler and challenges and advances in its detection. *Annals of Microbiology*, 69(5-6), 873–883. https://doi.org/10.1007/s13213-019-01501-7
11. Marangoz, B., Kahraman, S., Bostan, K. (2018). *Bacillus* spp. responsible for spoilage of dairy products. *International Journal of Food Engineering Research*, 4(1), 43–46.
12. Putri, D.A., Lei, J., Rossiana, N., Syaputri, Y. (2024). Biopreservation of food using bacteriocins from lactic acid bacteria: classification, mechanisms and commercial applications. *International Journal of Microbiology*, 2024:8723968. https://doi.org/10.1155/ijm/8723968
13. Sanam, M.U.E., Detha, A.I.R., Rohi, N.K. (2022). Detection of antibacterial activity of lactic acid bacteria, isolated from Sumba mare's milk, against Bacillus cereus, Staphylococcus aureus, and Escherichia coli. *Journal of Advanced Veterinary and Animal Research*, 15;9(1):53-58. https://doi.org/10.5455/javar.2022.i568
14. Sîrbu, I., Vassu, T., Stoica, I., Chifiriuc, C., Bucur, M., Rusu, E., Ionescu, R., Pelinescu, D., (2015). Analysis on the antimicrobial activity of some lactic acid bacteria strains. *Romanian Journal of Infectious Diseases*, 18(2): 53-57. https://doi.org/10.37897/RJID.2015.2-3.6
15. Thompson, J.M., Waites, W., Dodd, C. (2002). Detection of rope spoilage in bread caused by *Bacillus* species. *Journal of Applied Microbiology*, 85, 481–486. https://doi.org/10.1046/j.1365-2672.1998.853512.x
16. Tirloni, E., Stella, S., Celandroni, F., Mazzantini, D., Bernardi, C., Ghelardi, E. (2022). *Bacillus cereus* in dairy products and production plants. *Foods*, 11(17): 2572. https://doi.org/10.3390/foods11172572
17. Xie, J., Zhang, Z., Yang, S.P., Cheng, Y., Qian, Y.F. (2018). Study on the spoilage potential of *Pseudomonas fluorescens* on salmon stored at different temperatures. *Journal of Food Science and Technology*, 55(1), 217–225. https://doi.org/10.1007/s13197-017-2916-x
18. Yadav, M.K., Tiwari, S.K. (2023). Methods for determination of antimicrobial activity of bacteriocins of lactic acid bacteria. *Microbiology*, 92, 745–765. https://doi.org/10.1134/S0026261723600520

USE OF *CHLORELLA VULGARIS* SUSPENSION AS BIOFERTILIZER IN SUNFLOWER CULTIVATION IN THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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https://doi.org/10.52757/bsd26.04

ABSTRACT

The present study evaluates the effect of *Chlorella vulgaris* suspension used as a biofertilizer in sunflower (*Helianthus annuus L.*) cultivation under field conditions in the Republic of Moldova during the 2025 agricultural year. The experiment included pre-sowing seed treatment, soil application, and foliar treatments with algal suspension, compared with a control treated with distilled water.

Morpho-physiological and biochemical parameters were analyzed, including field emergence, plant height at different ontogenetic stages, yield, and seed quality indicators. Results showed that *Chlorella vulgaris* positively influenced early plant development, increasing field emergence by 4.90% (relative growth of 5.93%).

Treated plants showed improved growth during early ontogenetic stages, while final yield increased by about 10%. Significant improvements were also observed in seed quality, particularly oleic acid content (+29.16%), oil content (+4.14%), and protein content (+2.11%). These findings highlight the potential of Chlorella vulgaris as an effective and sustainable biofertilizer that enhances sunflower productivity and quality while supporting environmentally friendly agricultural practices.

Keywords: *Chlorella vulgaris*, biofertilizers, sunflower, crops.

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops worldwide, ranking after soybean, oil palm, and canola [1]. Its seeds are valued for their proteins, fibers, minerals, and phenolic compounds, which explains its wide cultivation across diverse agroecological regions [5].

Efficient fertilization is essential for sunflower production. Conventional agriculture mainly relies on mineral fertilizers (N, P, K), applied according to soil type and environmental conditions [8, 14]. Although these inputs sustain high yields, long-term use may cause soil degradation, reduced biological activity, lower humus content, and environmental pollution.

Biofertilizers and biostimulants are sustainable alternatives that improve nutrient availability and stimulate plant growth through biological processes [2]. Among them, microalgae have gained attention for their multifunctional role in soil–plant systems, contributing to better soil structure, nutrient cycling, water retention, and growth stimulation through bioactive compounds [4, 6, 10, 12, 15, 16]. One of the most studied microalgae is *Chlorella vulgaris*, widely used as a biostimulant due to its rapid growth, metabolic versatility, and rich biochemical composition. Its biomass provides organic matter and bioactive compounds that may enhance soil fertility and plant development [18]. Therefore, this study aimed to evaluate the effect of *Chlorella vulgaris* suspension on field emergence, growth, and selected quality parameters of sunflower seeds.

2. MATERIALS AND METHODS

The experiments were conducted using a *Chlorella vulgaris* strain maintained in the collection of the Scientific Research Laboratory of Algology "V. Șalaru" at Moldova State University. An algal suspension in the exponential growth phase was applied, with a concentration of 2.98 g/L absolutely dry biomass. Field trials were performed during the 2025 agricultural year, following classical experimental design methods [17], on farmland near Hîrtopul Mic, managed by SRL Schim Agromex. The experimental soils were classified as typical moderately humus-rich chernozems.

Seeds of *Helianthus annuus* L. were treated before sowing by spraying with an algal suspension, while distilled water was used for the control. Seeds were then sown at a depth of 3 cm according to standard agricultural practices. Each experimental variant covered 100 ha.

The algal suspension was applied to the soil immediately after sowing at 100 L/ha. Three additional foliar treatments were performed at key ontogenetic stages, each at 5 L/ha.

In both variants, soil fertilization was identical. In autumn 2024, pea green manure was incorporated into the soil. In spring 2025, 2000 kg/ha of solid fertilizer and 5 L/ha of liquid bioorganomineral fertilizer were applied, with chemical composition described in specialized literature [9].

Field emergence was determined under field conditions using representative sampling plots. In each experimental variant, three randomly selected plots of 1 m^2 were used to record the total number of sown seeds and emerged plants. Field emergence was calculated as the percentage of emerged plants relative to the total number of sown seeds.

Plant morphological changes were evaluated through field measurements at different ontogenetic stages until harvest. The main parameters assessed (in 20 replicates) were plant height, stem diameter, leaf development, and capitulum size.

Seed quality was determined using the Spectra Star XT Analyzer. Data processing included calculation of the arithmetic mean (M), standard error (m), differences compared with the control, relative increase, Student's t-test, degrees of freedom (df), probability of differences (p) and effect size (Cohen's d). All determinations were performed in 5 replicates under comparable conditions for both experimental variants to ensure the reliability and relevance of the results.

3. RESULTS AND DISCUSSION

The results obtained in this study are aligned with the main objective of evaluating the effect of *Chlorella vulgaris* on the early stages of development of *Helianthus annuus* L., particularly on seed field emergence and initial plant establishment under field conditions.

As shown in Table 1, the application of *Chlorella vulgaris* suspension increased the field emergence of sunflower seeds compared with the control. The difference was +4.90%, corresponding to a relative increase of 5.93%. Statistical analysis showed a Student's t-test value of 8.77, with df = 398 and $p < 0.0001$, indicating a highly significant difference between variants. The effect size, expressed as Cohen's d, reached 0.88, reflecting a strong biological effect.

These results demonstrate that *Chlorella vulgaris* suspension positively influenced seed field emergence and improved crop establishment at early growth stages. The data confirm the biostimulant role of algal biomass in enhancing the initial development of sunflower plants.

The morphological analysis of sunflower plant height (Table 2) indicates that the application of *Chlorella vulgaris* had a positive effect on plant growth, particularly in the early ontogenetic stages.

Table 1. Influence of *Chlorella vulgaris* suspension on sunflower field emergence, %

Experimental variants	Field emergence (%) (M±m)	Difference from control (%)	Relative growth (%)	t	df	p	Cohen's d
Control	82,60±0,40	-	-	-	-	-	-
Algal treatment	87,50±0,39	+4,90	5,93	8,77	398	<0,0001	0,88

Table 2. Influence of *Chlorella vulgaris* suspension on sunflower plant height, cm

Growth stage	Control (M±m)	Algal treatment (M±m)	Difference (cm)	t	df	p	Cohen's d
Complete emergence	5,01±0,03	5,50±0,02	0,49	13,59	398	0,0000	1,36
Two pairs of leaves	11,05±0,50	13,08±0,71	2,03	2,34	398	0,0199	0,23
Bud stage	65,20±2,13	68,45±2,45	3,25	1,00	398	0,3174	0,10
Flower	152,35±10,21	157,42±11,21	5,07	0,33	398	0,7383	0,03
Full maturity	165,60±10,14	168,30±10,45	2,70	0,19	398	0,8530	0,02

During complete emergence, plant height in the treated variant (5.50 ± 0.02 cm) was significantly higher than in the control (5.01 ± 0.03 cm), with a difference of 0.49 cm ($t = 13.59$, $p < 0.0001$, Cohen's d = 1.36), indicating a strong effect. At the stage of two pairs of true leaves, treated plants also showed significantly greater height, with a difference of 2.03 cm ($p = 0.0199$), although the effect size was relatively small (Cohen's d = 0.23). In the subsequent stages (bud formation, flowering, and full maturity), the treated variant maintained higher average values, but differences were not statistically significant ($p > 0.05$), and effect sizes were low. This suggests that the stimulatory effect of *Chlorella vulgaris* suspension was more pronounced during early plant development and diminished at later stages, likely under the influence of abiotic factors such as reduced soil moisture.

The application of *Chlorella vulgaris* had a beneficial effect on yield, which increased by approximately 10% (Fig. 1), and modified the biochemical composition of sunflower seeds (Table 3). The most representative changes were recorded in palmitic acid (+3.04%), oleic acid (+29.16%), linoleic acid (+3.16%), protein (+2.11%), and oil content (+4.14%).

These results indicate that the application of *Chlorella vulgaris* suspension improves seed biochemical quality, with positive implications for the production of high-quality sunflower oil.

Table 3. Effect of applying *Chlorella vulgaris* suspension on sunflower seed quality, %

Indicator	Control (M±m)	Algae (M±m)	Absolute difference	Relative difference (%)	t	df	p	Cohen's d
Moisture	5,77±0,17	5,68±0,02	-0,09	-1,56	-0,51	4	0,6363	-0,42
Palmitic acid	7,89±0,30	8,13±0,39	0,24	3,04	0,49	4	0,6512	0,40
Stearic acid	7,93±0,17	7,90±0,28	-0,03	-0,38	-0,09	4	0,9314	-0,07
Oleic acid	17,49±0,31	22,59±0,21	5,10	29,16	13,62	4	0,0002	11,12
Acid linoleic	68,28±2,40	70,44±2,39	2,16	3,16	0,64	4	0,5583	0,52
Protein	14,66±0,06	14,97±0,12	0,31	2,11	2,31	4	0,0820	1,89
Oil	42,56±0,19	44,32±0,20	1,76	4,14	6,38	4	0,0031	5,21

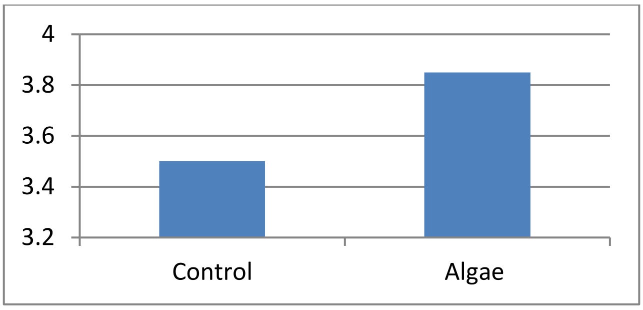

Figure1. Influence of *Chlorella vulgaris* suspension application on sunflower yield, t/ha

According to numerous studies, *Chlorella vulgaris* contains various biologically active compounds, including proteins, enzymes, antioxidants, and phytohormones, which stimulate plant growth, regulate nutrient uptake, enhance plant immunity, and improve tolerance to stress factors [3]. Previous studies reported that its application to *Lactuca sativa* seeds stimulated germination and increased plant growth and biomass [7]. Likewise, in tomato cultivation, *Chlorella vulgaris* treatment promoted plant height and improved biochemical traits, particularly chlorophyll a, b, and carotenoid contents [11]. The results obtained in the present study are consistent with these findings, confirming the positive biostimulant effect of *Chlorella vulgaris*. Similar to previous reports, our research demonstrated enhanced seed germination, improved plant growth, increased yield, and better seed quality, highlighting the broad applicability of this microalga in sustainable crop production.

5. CONCLUSIONS

The results obtained in this study demonstrate that the application of *Chlorella vulgaris* suspension as a biofertilizer has a positive effect on sunflower cultivation under the conditions of the Republic of Moldova. The treatment increased field emergence by 4.90%, indicating improved seed germination and better crop establishment during the early growth stages.

The influence of the algal suspension on plant growth was more pronounced during the initial ontogenetic phases, when significant increases in plant height were observed. In later developmental stages, although treated plants maintained slightly higher values, the differences were not statistically significant, suggesting that environmental factors may have influenced growth dynamics.

The beneficial impact of *Chlorella vulgaris* on sunflower yield highlights the practical importance of this treatment in agricultural production systems. Furthermore, important improvements in seed quality were recorded, particularly in oleic acid, protein, and oil contents, which are essential parameters for the nutritional and industrial value of sunflower.

Overall, the findings confirm that *Chlorella vulgaris* suspension can be successfully used as a sustainable biofertilizer, contributing to enhanced productivity and quality of sunflower crops. Its use represents a promising approach for reducing dependence on chemical fertilizers and promoting environmentally friendly agricultural practices.

ACKNOWLEDGMENTS

This work was supported research subprograms 010102, 010801 funded by the Ministry of Education and Research, Republic of Moldova.

DECLARATIONS

The authors declare that they have no conflict of interest.

Author Contributions: The authors contributed equally to the research presented in the article.

Data Availability Statement: The datasets generated during the current study are available from the corresponding author upon reasonable request.

Ethics Statement: Not applicable,
Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

REFERENCES

1. Adeleke, B,S., Babalola, O.O. (2020). Oilseed crop sunflower (*Helianthus annuus*) as a source of food: Nutritional and health benefits. Food Sci, Nutri, 8, 4666–4684. DOI:10.1002/fsn3.1783.
2. Al-Juheishy, W. (2025). Effect of biofertilizers in increasing sunflower crop productivity: a review. Journal For Agricultural Sciences, 16(1), 67-73. DOI:10.58928/ku25.16108.
3. Ammar, E.E., Aioub, A.A., Elesawy, A.E., Karkour, A.M., Mouhamed, M.S., Amer, A.A., El-Shershaby, N.A. (2022). Algae as bio-fertilizers: Between current situation and future prospective. Saudi Journal of Biological Sciences, 29(5), 3083–3096.
4. Bowker, M.A., Reed, S.C., Maestre, F.T., Eldridge, D.J. (2018). Biocrusts: the living skin of the earth. Plant and Soil, V, 429, 1–7.
5. De Oliveira, Filho, J.G., Egea, M.B. (2021). Sunflower seed byproduct and its fractions for food application: An attempt to improve the sustainability of the oil process. J, Food Sci, 86, 1497–1510. DOI:10.1111/1750-3841.15719.
6. Etesami, Ha, (2025). Resilient pioneers: The ecological role of cyanobacteria in desert ecosystems. Applied Soil Ecology, Vol, 212, https://doi,org/10,1016/j,apsoil,2025,106173.
7. Faheed Fayza, A., FATTAH, Z. A.B. (2008). Effect of *Chlorella vulgaris* as biofertilizer on growth parameters and metabolic aspects of *Lettuce plant*. Journal of agriculture & social sciences, Vol. 4, No. 4, 165-169. http://www.fspublishers.org/published_papers/71170_..pdf.
8. Handayati, W., Sihombing, D. (2019). Study of NPK fertilizer effect on sunflower growth and yield, In AIP Conference Proceedings, 13–14 March 2019, Malang Indonesia; AIP Publishing LLC: Melville, NY, USA, 030-031. https://doi.org/10.1063/1.5115635.
9. Jigău, Gh., Dobrojan, S., Turchin, B., Sprîncean, E., Sprincean, N. (2022). Obţinerea şi utilizarea preparatelor bioorganominerale în tehnologiile modernizate de adaptare a solurilor la schimbările climatice. Ghid practice. Chisinau, CEP: USM, p. 43.
10. Prasad, R,C., Prasad, B,N. (2001). Cyanobacteria as a source biofertilizer for sustainable agriculture in Nepal. J, Plant Sci, Bot, Orient, 1 127–133.
11. Riaz, S., Nadeem, K., Noureen, M. (2025). Effect of *Chlorella vulgaris* as a potential bio-fertilizer on lycopersicon esculentum. Plant Bulletin, 4(1), 31-41. DOI:10.55627/pbulletin.004.01.835
12. Rodríguez, A.A., Stella, A,M., Storni, M,M., Zulpa, G., Zaccaro, M.C. (2006). Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa* L, Saline Syst, 2 (1), 7, https://doi.org/10,1186/1756-1448-2-7.
13. Roger, P.A., Reynaud, P,A. (1982). Free-living blue-green algae in tropical soils. Microbiology of Tropical Soils and Plant Productivity, Springer, Dordrecht, 147–168.
14. Simoes, W,L., Da Silva, J,S., Oliveira, A,R., Regitano Neto, A., Drumond, M,A., Lima, J.A., Nascimento, B.R. (2020). Sunflower cultivation under different irrigation systems and planting spacings in the sub-middle region of Sao Francisco Valley, Semin, Cienc, Agrar, Londrina 41, 2899–2910. http://www.alice.cnptia.embrapa.br/alice/handle/doc/1130148.
15. Subramanian, G., Uma L. (1996). Cyanobacteria in pollution control. J, Sci, Ind, Res, 55, 685–692.
16. XueFeng, Ch., ShiRu, J., Yue, W., Ning, W. (2010). Biological crust of *Nostoc flagelliforme* (cyanobacteria) on sand bed materials. Journal of Applied Phycology, vol, 23, 67–71.
17. Доспехов, Б.А. (1985). Методика полевого опыта. М., 351 с.
18. Шентерова Е.М., Мазиров М.А., Гафурова, Л.А., Джалилова, Г.Т. (2020). Биология и экология почв. Владимир: Изд-во ВлГУ, 217 с.

EFFECT OF *ARTHROSPIRA PLATENSIS* ON ALLEVIATION OF DROUGHT STRESS IN *HELIANTHUS ANNUUS* L.

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https://doi.org/10.52757/bsd26.05

Drought stress is a major constraint limiting sunflower (*Helianthus annuus* L.) productivity, reducing germination, growth, and biomass accumulation. This study evaluated the potential of *Arthrospira platensis* as a biostimulant to enhance drought tolerance in sunflower seedlings. Seed treatments and foliar applications of *A. platensis* were applied under controlled conditions, with drought stress induced by 15% polyethylene glycol (PEG-6000). Drought significantly reduced plant height, root length, and fresh biomass, while dry biomass remained largely unaffected. Treatments with *A. platensis* mitigated these effects, with combined seed and foliar applications increasing plant height to 123% of non-stressed controls and 138% of PEG-stressed controls, root length exceeding 150%, and fresh biomass rising from ~57% in stressed controls to over 300%. Seed priming primarily enhanced root growth, whereas spraying alone improved plant height and fresh biomass. These results indicate that *A. platensis* effectively enhances sunflower seedling growth under water deficit, highlighting its potential as a sustainable biostimulant for improving drought resilience.

Keywords: *Arthrospira platensis*, sunflower, drought stress, biostimulant

1. INTRODUCTION

Actual climate change poses a major challenge by exacerbating drought stress, which can reduce major crop yields by over 50%. Plant adaptations to drought include root elongation to enhance water uptake, leaf rolling to minimize water loss, and upregulation of regulatory proteins involved in stress signaling. Drought induces the expression of stress-responsive genes and transcription factors, along with carbohydrate accumulation, reactive oxygen species (ROS) production, and antioxidant enzyme activity (Adzigbe et al., 2025).

Although sunflower (*Helianthus annuus* L.) is moderately drought-tolerant, its productivity is highly sensitive to water deficit, particularly during critical growth stages such as germination, flowering, and achene filling (Ahmad et al., 2009). Drought stress reduces germination, seedling growth, leaf area, biomass accumulation, and ultimately seed and oil yield (Oraki and Aghaalikhana, 2012). Given these limitations, the use of biostimulants has emerged as a promising strategy to enhance drought resilience.

Cyanobacteria- and microalgae-derived products have gained attention as multifunctional biostimulants in agriculture, improving nutrient uptake, crop performance, and tolerance to abiotic stresses like drought and salinity (Chiaiese et al., 2018; Kusvuran, 2021; Guzmán-Murillo et al., 2013). However, although there is evidence that their bioactive compounds positively influence plant growth, their effects on sunflower remain insufficiently explored. In this context, the aim of this study was to evaluate the effects of *Arthrospira platensis* on the physiological responses of sunflower (*Helianthus annuus*) seedlings under drought stress. Specifically, it investigated the potential of cyanobacteria as a potential biostimulant to enhance drought tolerance by assessing plant growth under PEG 6000-induced water deficit as a controlled drought model.

2. MATERIALS AND METHODS

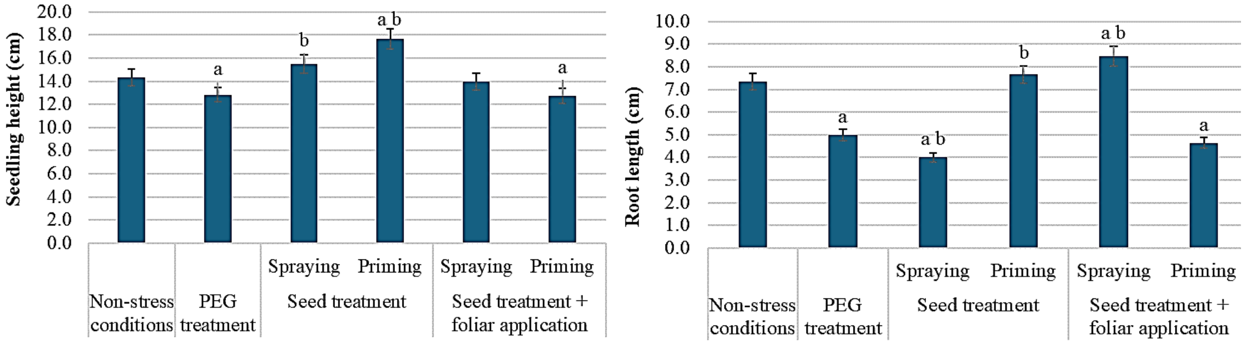
A sunflower (*Helianthus annuus*) hybrid was evaluated to determine the effects of *Arthrospira platensis* on growth and development under drought stress conditions. The experiment was conducted under controlled laboratory conditions (25 ± 3°C) for 22 days, using a three-factor design: water regime, seed treatment, and foliar application.

Seed treatment with *A. platensis* was performed either by soaking the seeds in an algal suspension (exponential growth phase) for 1 hour before sowing or by spraying them prior to sowing. All seeds were sown in soil-filled containers. Drought stress was imposed on day 15 by irrigating seedlings with 15% polyethylene glycol (PEG-6000), while control plants were maintained with distilled water. The stress was sustained for 7 days.

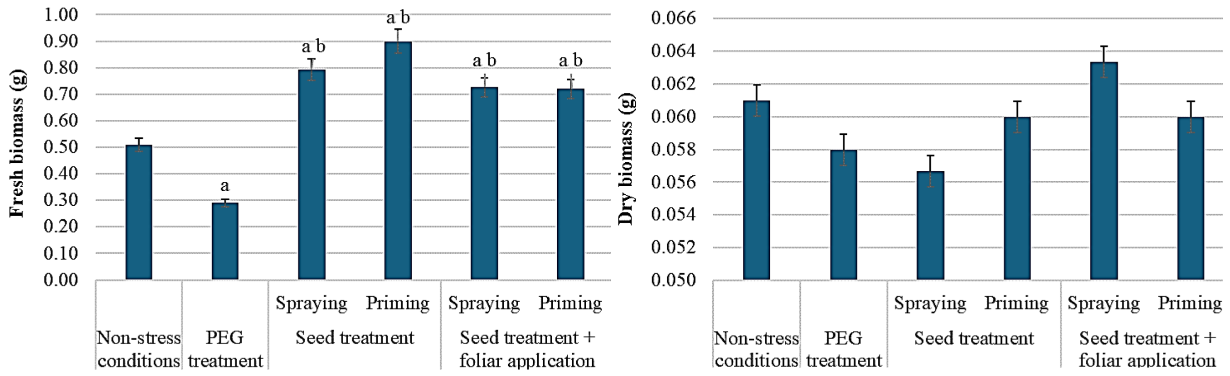
In treatments combining seed and foliar application, the algal suspension was sprayed four times during the early vegetative stage, beginning on the second day of drought stress and continuing until the end of the experiment. At the end of the stress period, growth parameters were measured on five plants per variant, including seedling height, root length, and fresh biomass. Dry biomass was determined by placing plant tissues in paper bags, sealing and labeling them, preheating at 105°C for 20 minutes, and then drying to constant weight at 80°C. All experimental data were statistically analyzed, with significance accepted at $P \leq 0.05$. Data are presented as the mean ± standard deviation (SD), and error bars in figures represent the standard error of the mean.

3. RESULTS

The effects of *Arthrospira platensis* on sunflower seedling growth under drought stress were evaluated through plant height, root length, fresh biomass, and dry biomass (Figure 1). PEG-induced drought significantly reduced growth in untreated seedlings, with plant height, root length, and fresh mass declining from 14.3 to 12.8 cm, 7.3 to 5.0 cm, and 0.51 to 0.29 g, respectively, while dry biomass remained unchanged. *A. platensis* treatments mitigated these effects, increasing plant height to 17.7 cm, root length to 8.5 cm, and fresh biomass to 0.90 g, occasionally exceeding values of the non-stressed control. Dry biomass remained stable, indicating that cyanobacteria primarily enhanced fresh tissue accumulation.



A. **B.**

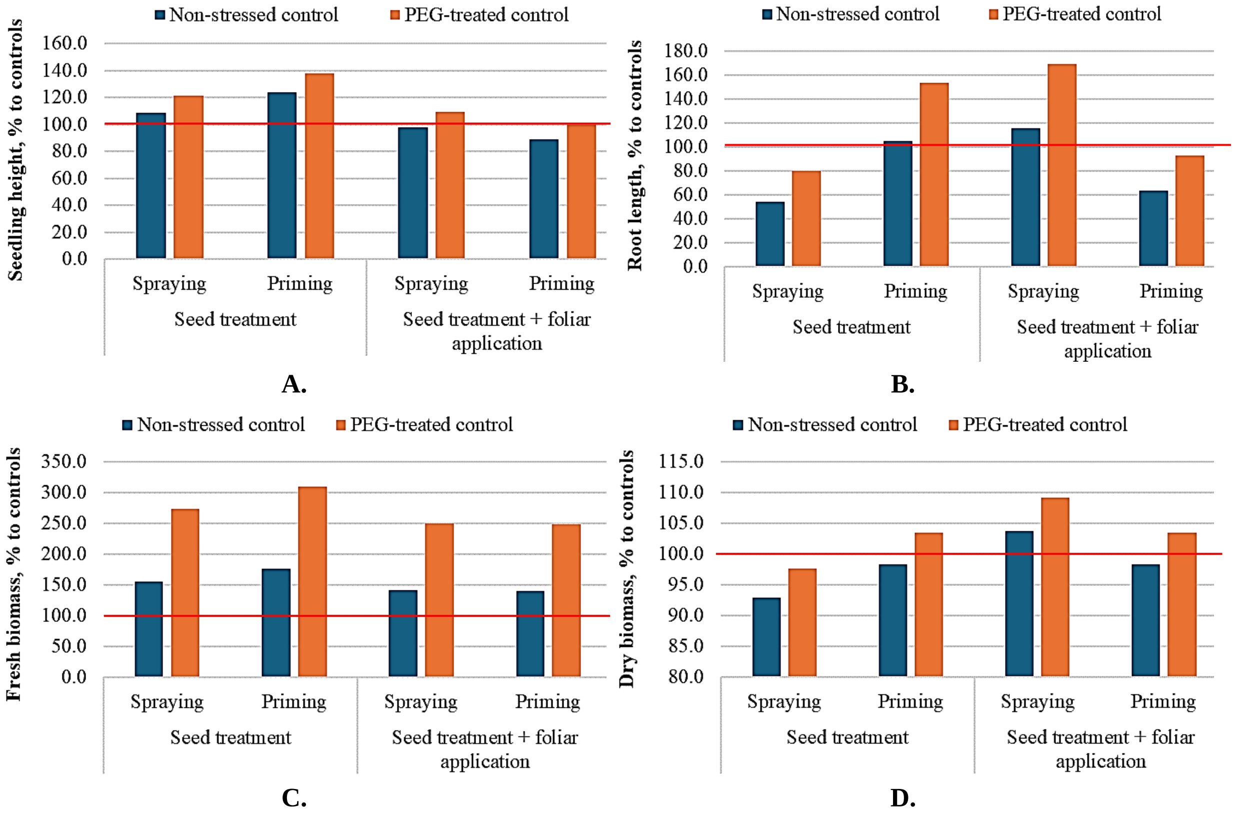


C. D.

Figure 1. Effects of *Arthrospira platensis* application on sunflower (*Helianthus annuus*) growth under drought stress.

Panels show shoot length (A), root length (B), fresh biomass (C), and dry biomass (D). Data are presented as mean ± standard error (n = 3). Different letters indicate statistically significant differences: „a" compared to the non-stressed control, „b" compared to the PEG-induced drought-stressed control ($P \leq 0.05$).

Maximum values for plant height and biomass were observed in plants receiving combined treatments, indicating a synergistic effect on plant development. Thus, sunflower seeds sprayed with *Arthrospira platensis* culture, combined with foliar application, increased plant height to 123% of the non-stressed control and 138% of the PEG-treated control (Figure 2).



A. B.

C. D.

Figure 2. Effects of *Arthrospira platensis* on sunflower (*Helianthus annuus*) growth under drought stress, expressed as a percentage of the non-stressed and PEG-treated controls.

Panels show shoot length (A), root length (B), fresh biomass (C), and dry biomass (D).

Root length exceeded 150%, indicating enhanced root development and potential water uptake. Fresh biomass showed the strongest response, rising from ~57% in stressed controls to over 300% with treatment. Spraying alone also improved plant height and fresh biomass, whereas seed priming mainly enhanced root growth (116% relative to the non-stressed control and 169% relative to the PEG-treated control). The combination of priming and foliar application did not provide additional benefits.

4. DISCUSSIONS

Experimental studies under greenhouse and field conditions have shown that cultures or extracts of microalgae and cyanobacteria enhance seed germination, seedling development, root and shoot biomass, and photosynthetic pigment accumulation across crops such as leafy vegetables, tomato, pepper, wheat, and sugar beet (Abd-El Fattah, 2008; El Arroussi et al., 2018; Aly et al., 2008; Kholssi et al., 2019). Species including *Chlorella vulgaris* and *Spirulina platensis* contain phytohormones, amino acids, vitamins, and metabolites that support growth and stress resilience (Renuka et al., 2018). Their polysaccharides and bioactive compounds promote germination, root and shoot development, and biomass accumulation under challenging conditions, highlighting their value as biofertilizers and biostimulants (du Jardin, 2015; Farid et al., 2019). Consequently, priming sunflower with algal or cyanobacterial extracts offers a sustainable strategy to boost tolerance to drought (Kusvuran, 2021) and salinity (Abd El-Baky et al., 2010), while supporting productivity under stress.

The growth-promoting effects involve multiple mechanisms, including phytohormone production (auxins, cytokinins), exopolysaccharides that improve rhizosphere microbial communities, and the supply of bioavailable nutrients (Jäger et al., 2010; Renuka et al., 2018; Xiao and Zheng, 2016). Together, these factors enhance root architecture, water uptake, and photosynthetic efficiency, mitigating the impacts of water deficit or salinity. For example, foliar applications of *Chlorella vulgaris* (1–5% v/v) on drought-stressed broccoli significantly increased shoot length, fresh and dry weights, leaf area, relative water content, and photosynthetic pigments (chlorophyll-a, chlorophyll-b, and carotenoids) while reducing oxidative damage (Kusvuran, 2021). Polysaccharide extracts from *Spirulina platensis* and *Dunaliella salina* similarly enhanced antioxidant enzymes, secondary metabolites, and stress-related compounds, reducing oxidative damage in plants (Abd El-Baky et al., 2010; El Arroussi et al., 2018; Guzmán-Murillo et al., 2013).

5. CONCLUSIONS

The present study demonstrated that *Arthrospira platensis* treatments alleviated drought-induced reductions in sunflower growth. Seedling height, root length, and fresh biomass were markedly improved relative to PEG-stressed controls. Notably, the combination of seed treatment and foliar application produced the strongest stimulatory effects, suggesting synergistic benefits for water deficit tolerance. These findings support the potential of cyanobacteria-based biostimulants as a sustainable and effective strategy for enhancing crop resilience under drought conditions.

ACKNOWLEDGMENTS: The researches were performed under the subprogramme 011101 Genetic and biotechnological approaches to the management of agroecosystems in the conditions of climate change, funded by the Ministry of Education and Research of the Republic of Moldova.

DECLARATIONS: The authors declare that they have no conflict of interest. The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

M.D. and S.C. designed the study; A.A., R.M., S.D. performed experiments; S.C. and A.A. analyzed data; M.D. and S.C. drafted the manuscript; all authors reviewed and approved the final version.

REFERENCES

1. Abd El-Baky, H. H., El-Baz, F. K., & El Baroty, G. S. (2010). Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. *Journal of the Science of Food and Agriculture*, *90*, 299–303. https://doi.org/10.1002/jsfa.3815
2. Adzigbe, J., Frimpong, F., Danquah, A., et al. (2025). The responses and adaptations of rice (*Oryza sativa* L.) to drought stress: A review. *Climate Smart Agriculture*, *2*(4), 100080. https://doi.org/10.1016/j.csag.2025.100080
3. Ahmad, S., Ahmad, R., Ashraf, M. Y., Ashraf, M., & Waraich, E. A. (2009). Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pakistan Journal of Botany*, *41*(2), 647–654.
4. Aly, M. S., & Esawy, M. A. (2008). Evaluation of *Spirulina platensis* as bio stimulator for organic farming systems. *Journal of General Engineering and Biotechnology*, *6*, 1–7.
5. Chiaiese, P., Corrado, G., Colla, G., Kyriacou, M. C., & Rouphael, Y. (2018). Renewable sources of plant biostimulation: Microalgae as a sustainable means to improve crop performance. *Frontiers in Plant Science*, *9*, 1782. https://doi.org/10.3389/fpls.2018.01782
6. du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, *196*, 3–14. https://doi.org/10.1016/j.scienta.2015.09.021
7. El Arroussi, H., Benhima, R., Elbaouchi, A., et al. (2018). *Dunaliella salina* exopolysaccharides: A promising biostimulant for salt stress tolerance in tomato (*Solanum lycopersicum*). *Journal of Applied Phycology*, *30*, 2929–2941. https://doi.org/10.1007/s10811-017-1382-1
8. Farid, R., Mutale-Joan, C., Redouane, B. et al. (2019). Effect of microalgae polysaccharides on biochemical and metabolomics pathways related to plant defense in *Solanum lycopersicum*. *Applied Biochemistry and Biotechnology*, *188*(1), 225–240. https://doi.org/10.1007/s12010-018-2935-5
9. Guzmán-Murillo, M. A., Ascencio, F., & Larrinaga-Mayoral, J. A. (2013). Germination and ROS detoxification in bell pepper (*Capsicum annuum* L.) under NaCl stress and treatment with microalgae extracts. *Protoplasma*, *250*(1), 33–42. https://doi.org/10.1007/s00709-011-0369-z
10. Kholssi, R., Marks, E. A. N., Miñón, J., et al. (2019). Biofertilizing effect of *Chlorella sorokiniana* suspensions on wheat growth. *Journal of Plant Growth Regulation*, *38*(2), 644–649. https://doi.org/10.1007/s00344-018-9879-7
11. Kusvuran, Ş. (2021). Microalgae (*Chlorella vulgaris* Beijerinck) alleviates drought stress of broccoli plants by improving nutrient uptake, secondary metabolites, and antioxidative defense system. *Horticultural Plant Journal*, *7*(3), 221–231.
12. Oraki, H., & Aghaalikhani, M. (2012). Effect of water deficit stress on proline contents, soluble sugars, chlorophyll, and grain yield of sunflower (*Helianthus annuus* L.) hybrids. *African Journal of Biotechnology*, *11*, 164–168.
13. Renuka, N., Guldhe, A., Prasanna, R., Singh, P., & Bux, F. (2018). Microalgae as multi-functional options in modern agriculture: Current trends, prospects and challenges. *Biotechnology Advances*, *36*, 1255–1273. https://doi.org/10.1016/j.biotechadv.2018.04.004
14. Xiao, R., & Zheng, Y. (2016). Overview of microalgal extracellular polymeric substances (EPS) and their applications. *Biotechnology Advances*, *34*, 1225–1244. https://doi.org/10.1016/j.biotechadv.2016.08.004

EVALUATION OF THE BIOSTIMULATORY AND NEMATICIDAL EFFECTS OF *BACILLUS CEREUS* VAR. *FLUORESCENS* ON *SOLANUM TUBEROSUM*

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https://doi.org/10.52757/bsd26.06

ABSTRACT

This study evaluated the efficacy of bacterial culture liquid as biocontrol agent in potato (*Solanum tuberosum*) cultivation. In vivo application of the culture liquid derived from *Bacillus cereus* var. *fluorescens* CNMN-BB-07 (6×10^8 cells/mL) resulted in a substantial reduction in *Ditylenchus destructor* infestation, decreasing infection levels to 1–4% compared with 40.42% in the untreated control. The treatment also increased potato yield by 1.7-fold relative to untreated seed tubers (control C1), completely prevented tuber rot during storage, and reduced damage caused by *Gryllotalpa gryllotalpa* to 10.4%. Overall, the tested bacterial strain demonstrated pronounced plant growth–promoting and nematode-suppressive effects, indicating their potential as effective and environmentally sustainable tools for integrated potato protection strategies.

Keywords: *Solanum tuberosum*, biocontrol, *Bacillus cereus* var. *fluorescens* CNMN-BB-07, *Ditylenchus destructor*

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide, playing a key role in global food security. However, its productivity is significantly affected by a wide range of soil-borne pathogens and pests, among which phytoparasitic nematodes such as *Ditylenchus destructor* are particularly destructive, causing substantial yield losses and deterioration of tuber quality during storage (Jones et al., 2013).

Conventional control strategies rely heavily on chemical pesticides, which may lead to environmental contamination, development of resistance, and negative impacts on human health (Pimentel & Burgess, 2014). In this context, increasing attention has been directed toward sustainable and environmentally friendly alternatives, such as the use of beneficial microorganisms with plant growth-promoting and biocontrol properties (Compant et al., 2005; Lugtenberg & Kamilova, 2009).

Bacterial species *Bacillus cereus* var. *fluorescens* are known to enhance plant growth through mechanisms including phytohormone production, improved nutrient availability, and suppression of plant pathogens (Vessey, 2003; Kloepper et al., 2004). Moreover, these microorganisms can act as biological control agents against nematodes and other soil-borne pests, reducing infestation levels and improving crop productivity (Siddiqui & Mahmood, 1999).

The objective of this study was to evaluate the nematicidal capacity of the culture liquid (CL) produced by the bacteria *Bacillus cereus* var. *fluorescens* CNMN-BB-07, as well as to monitor their impact on ontogenetic development, productivity, and the phytosanitary status of potato crops.

2. MATERIALS AND METHODS

The experiments were conducted at the Institute of Zoology, Moldova State University (MSU), and at the Institute of Microbiology and Biotechnology, Technical University of Moldova (TUM).

The study was performed on seed potato tubers of the Roko cultivar (*Solanum tuberosum*) and the phytoparasitic nematode *Ditylenchus destructor* Thorne, 1945, corresponding to the second stage of ditylenchosis (Melnic et al., 2014; 2016; 2017).

The experimental biological material was obtained through artificial inoculation of tubers with 30–50 individuals of *D. destructor* (females and males), selected from previously infested potato tubers. The inoculated tubers were then incubated for 2–2.5 months, during which nematode penetration and multiplication occurred concurrently with the initiation of tuber germination processes.

The bacterial strain *Bacillus cereus* var. *fluorescens* CNMN-BB-07 (family Bacillaceae, genus *Bacillus*) was isolated in 2005 from fatty carbonate soil (loamy texture, 3.5% humus content) in the Republic of Moldova and deposited in 2014 in the National Collection of Nonpathogenic Microorganisms (CNMN), Technical University of Moldova. The liquid culture (LC) was obtained by cultivating the strain on King B medium at 27 ± 1 °C for 48 h.

Under in vivo conditions, *B. cereus* var. *fluorescens* CNMN-BB-07 was evaluated on seed potato tubers artificially infested with *D. destructor*. The experimental design included four variants: V1—infested tubers treated with LC diluted 1:200; V2—infested tubers treated with LC diluted 1:400; C1—infested, untreated control; and C2—healthy, nematode-free control. Tubers exhibiting 100% infestation (stage II of ditylenchosis) were used for treatment. All treatments were performed by immersion at room temperature (+15 to +20 °C) for 16 h.

During the growing season, systematic phenological observations were conducted to evaluate potential phytotoxic effects and plant growth responses, including the number of shoots (n), plant height (h). At harvest, yield and health-related parameters were assessed, including the number of tubers per nest, average tuber diameter, tuber weight, total yield per nest, the level of infestation by *D. destructor*, incidence of tuber rot and damage caused by mole crickets. Each experimental variant consisted of 10 plants (tubers), and the experiment was performed in three biological replicates (r = 3) under identical controlled conditions. Data were processed using descriptive statistics and are presented as mean values.

3. RESULTS

Previous multi-annual studies performed under vegetative and field conditions demonstrated that the *B. cereus* var. *fluorescens* CNMN-BB-07 (6×10^8 cells/mL) exhibits strong nematicidal activity against *D. destructor*. Undiluted and low-dilution rates (1:50 and 1:100) ensured complete suppression of nematode infestation (0% at harvest), but also induced phytotoxic effects on plant development and tuber formation.

Based on these findings, further experiments were conducted using higher dilutions of the bacterial culture liquid: V1 (1:200) and V2 (1:400). Two control variants were included: C1 (inoculated, untreated tubers) and C2 (healthy, untreated tubers). All experimental tubers were planted under open-field conditions in soil free from *D. destructor*. Plant development was monitored throughout the entire growing season, with emphasis on vegetative growth, yield parameters, and pest incidence.

The results (Table 1) demonstrate that the effects of the bacterial treatment are strongly dose-dependent. In variant V1 (1:200), a mild phytotoxic effect was observed, expressed by reduced vegetative growth parameters compared to V2. In contrast, variant V2 (1:400) showed a pronounced stimulatory effect on plant development, with superior biometric indices compared to both control variants.

Table 1. Evaluation of the treatment effectiveness of experimental seed potatoes (*Roko* cultivar), infested with *D. destructor*, in contact with the bacteria *B.cereus* var. *fluorescens* CNMN-BB-07

Batch	Dilution/ exposure time (h)	Phase flowering- ripening		Harvest						
		Shoots n	Plant height h (cm)	Tubers / nest n	Avg. tuber diameter d (cm)	Tuber weight m (g)	Yield/ nest m (g)	*D. destructor* (%)	Rots (%)	Mole crickets (%)
V1	1:200/ 16 hours	2.3	47.0	5.0	3.23/4.46	43.4	217.0	0	0	0
V2	1:400/ 16 hours	6.0	67.38	9,6	5.3/4.51	65.3	626.8	1 - 4	0	10.4
C1	inoculated, untreated	3.5	55.6	7,0	3.72/4.63	48.4	377.52	40.42	22.5	40.3
C2	healthy, untreated	4.4	62.36	8,7	5.0 x 3.8	58.3	508.2	0	7.9	12.3

Data are presented as mean values.

Plants from V2 exhibited an average increase in plant height of approximately 12 cm and a two-fold increase in shoot number per plant compared to the infected control (C1). At harvest, the number and mass of tubers per plant were 1.4 times higher in V2 than in C1.

A strong nematicidal effect was recorded in V2, where the infestation level of *D. destructor* decreased from 100% before planting to 1–4% at harvest. In contrast, the infected untreated control (C1) showed a high infestation level of 40.42%.

Additionally, treatment with *B. cereus* CL at 1:400 completely eliminated tuber rot incidence (0%) and significantly reduced damage caused by soil pests (*Gryllotalpa grylotalpa*) to 10.4%, compared to 22.5% in C1 and 40.3% in healthy but untreated control (C2). A proportion of 7.9% of tubers in C2 were affected by bacterial and fungal infections, while 12.3% showed insect damage. At harvest, the number of tubers per nest, as well as the weight of tubers per nest, was on average 1.4 times higher in the experimental batch V2 compared with the control batch C1 (Table 1).

Overall, the results confirm that the biological activity of *B. cereus* var. *fluorescens* CNMN-BB-07 is dose-dependent, with the 1:400 dilution representing the optimal balance between plant growth promotion and nematicidal efficacy (Melnic et al., 2023).

4. DISCUSSION

According to the literature (Avis et al, 2008), rhizosphere-associated bacteria stimulate the synthesis of key secondary metabolites such as phenolic compounds, flavonoids, and phytoalexins. These compounds play an important role in triggering induced systemic resistance (ISR), thereby strengthening plant defense responses against biotic stress. In this context, the presence of such metabolites in the culture liquid may explain not only the direct nematicidal activity against *D. destructor*, but also the improved vegetative performance of potato plants, which may be associated with enhanced physiological status and activation of defense-related pathways.

In vivo experiments demonstrated the biological efficacy of the strain under controlled conditions. The treatment of seed potato tubers (*Roko* cultivar) with culture liquid diluted 1:400 for 16 h resulted in a marked reduction of *D. destructor* infestation, from 100% prior to planting to 1–4% at harvest. In contrast, the untreated infected control (C1) exhibited an infestation level of 40.42%.

Literature data also highlight the antifungal and antimicrobial potential of *Bacillus* spp., including *B. cereus* var. *fluorescens*, suggesting their suitability as biological control agent in sustainable crop protection strategies (Fravel, 2005; Ongena & Jacques, 2008).

Overall, the present data indicate that *B. cereus* var. *fluorescens* CNMN-BB-07 reduces the infestation level of potato tubers by phytoparasitic nematodes while simultaneously promoting plant growth. This multifunctional effect is likely associated with the strain's ability to produce phytohormones and other bioactive metabolites.

5. CONCLUSIONS

The treatment of seed potatoes with culture liquid of *B. cereus* var. *fluorescens* CNMN-BB-07 (1:400, 16 h) significantly reduced *D. destructor* infestation (80–96%) and increased yield by approximately 1.7-fold compared to the untreated control. The strain demonstrated both nematicidal and plant growth–promoting activity, indicating its potential as an environmentally friendly biocontrol agent for sustainable potato protection.

ACKNOWLEDGEMENTS

This work was developed within the institutional projects of the Institute of Zoology, Moldova State University: 01070. Evaluation of the structure and functioning of the animal world and aquatic ecosystems under the influence of biotic and abiotic factors in the context of ensuring ecological security and the population well-being; 20.80009.7007.12. Diversity of hematophagous arthropods, zoo- and phytohelminths, vulnerability, strategies to tolerate climatic factors and the development of innovative procedures for the integrated control of species of socio-economic interest, financed from the state budget

DECLARATIONS

Conflict of interest: The authors declare that they have no conflict of interest.

Author Contributions: S.R. and D.E. designed the study; O.G. and M.M. performed field experiments; V.S. and V.T. performed microbiological cultivation; O.G. and L.B. analyzed data; O.G. and M.M. writing—original draft preparation; D.E.,S.R.,O.G. writing – review & editing; all authors reviewed and approved the final version.

Data Availability Statement: The datasets generated during the current study are available from the corresponding author upon reasonable request.

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

REFERENCES

1. Avis, T. J., Gravel, V., Antoun, H., & Tweddell, R. J. (2008). Multifaceted beneficial effects of rhizosphere microorganisms on plant health. *Plant Science, 175*(3), 237–245.
2. Compant, S., Duffy, B., Nowak, J., Clément, C., & Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases. *Applied and Environmental Microbiology, 71*(9), 4951–4959. https://doi.org/10.1128/AEM.71.9.4951-4959.2005
3. Fravel, D. R. (2005). Commercialization and implementation of biocontrol. *Annual Review of Phytopathology, 43*, 337–359.
4. Jones, J. T., et al (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology, 14*(9), 946–961. https://doi.org/10.1111/mpp.12057
5. Kloepper, J. W., Ryu, C. M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. *Phytopathology, 94*(11), 1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259

6. Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology, 63,* 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918
7. Melnic, M., Toderaș, I., Erhan, D., Rusu, Ș., Onoraș, L., & Todiraș, V. (2014). *Methods of combating and preventing parasitic nematodes in the potato crop: Practical recommendations*. Ed. Știința.
8. Melnic, M., Erhan, D., Rusu, Ș., Gherasim, E., & Chihai, N. (2016). Risk of use of seed potatoes infested with *Ditylenchus destructor* at initial phase of ditylenhosis. In *International Conference of Zoologists IX* (pp. 146–147). Chișinău.
9. Melnic, M., Erhan, D., Rusu, Ș., Batîr, L., Slanina, V., & Rusu, V. (2017). The interrelations of the nematode *Ditylenchus dipsaci* with the bacteria strain *Bacillus cereus var. fluorescens* CNMN-BB-07. In *Actual problems of zoology and parasitology: achievements and prospects* (pp. 190–195). Chișinău.
10. Melnic, M., Erhan, D., Gliga, O., Rusu, Ș., Balan, L., Slanina, V., Onofraș, L., & Todiraș, V. (2023). Method for treating seed potatoes against the nematode *Ditylenchus destructor*. Patent MD 1658 Y.
11. Ongena, M., & Jacques, P. (2008). Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. *Trends in Microbiology, 16*(3), 115–125.
12. Pimentel, D., & Burgess, M. (2014). Environmental and economic costs of the application of pesticides. In D. Pimentel (Ed.), *Integrated Pest Management*. Springer.
13. Siddiqui, Z. A., & Mahmood, I. (1999). Role of bacteria in the management of plant parasitic nematodes. *Bioresource Technology, 69*(2), 167–179. https://doi.org/10.1016/S0960-8524(98)00166-7

IMMUNE DISORDERS IN PULMONARY TUBERCULOSIS AND CONCOMITANT SARS-CoV-2 INFECTION

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https://doi.org/10.52757/bsd26.07

ABSTRACT

The study aimed to analyze immune-metabolic disorders and biomarkers of endogenous intoxication in patients with pulmonary tuberculosis (TB) caused by different *Mycobacterium tuberculosis* strains, including multidrug-resistant TB (MDR-TB), and in cases with concomitant SARS-CoV-2 infection, to assess the impact of coinfection on immune–metabolic homeostasis and systemic inflammation. This prospective study included 191 newly diagnosed TB patients divided into four groups: drug-susceptible TB (n=80), primary MDR-TB (n=40), acquired MDR-TB (n=49), and TB with SARS-CoV-2 coinfection (n=22). A control group comprised 36 conventionally healthy individuals. A more severe disease course was observed in patients with acquired MDR-TB and SARS-CoV-2 coinfection, associated with decreased $CD3^+$, $CD4^+$, $CD16^+$, and $CD8^+$ T lymphocytes. Circulating immune complexes, ESR, acute-phase proteins, and derived hematological indices were elevated in all groups, with the highest levels in coinfected patients. Pro-inflammatory cytokines (TNF-α, IL-6, IL-8) were significantly increased; TNF-α peaked in acquired MDR-TB, while IL-6 and IL-8 were highest in coinfection. Early detection of MDR-TB and SARS-CoV-2 coinfection, combined with timely immunopathogenic interventions, is essential to modulate immune–metabolic disturbances and improve outcomes in severe TB.

Keywords: Tuberculosis, COVID-19, Immune factors.

1. INTRODUCTION

The Republic of Moldova (RM) is among the high tuberculosis (TB) burden countries in Eastern Europe, reporting some of the highest rates of multidrug-resistant tuberculosis (MDR-TB). It is estimated that approximately 33% (30-35%) of newly diagnosed TB cases annually in RM are caused by MDR/rifampicin-resistant (RR-TB) strains, while acquired resistance occurs in nearly 60% (56-64%) of cases [1]. The COVID-19 pandemic further worsened the global MDR-TB situation by disrupting TB detection in high-risk groups and weakening reporting systems, leading to a decrease in newly registered TB cases and an increase in TB-related mortality, especially among patients with MDR-TB and those with concomitant SARS-CoV-2 infection [2]. Early detection of drug resistance and SARS-CoV-2 coinfection, followed by prompt initiation of appropriate treatment, may prevent further resistance and improve treatment outcomes. In addition, early identification of immune-metabolic disturbances and timely initiation of pathogenetic therapy can enhance treatment effectiveness [3, 4].

The study aimed to analyze immune-metabolic disorders in patients with pulmonary tuberculosis (TB) caused by different strains of Mycobacterium tuberculosis (MTB) and concomitant SARS-CoV-2 infection, to assess the impact of coinfection on homeostasis and systemic inflammatory responses.

2. MATERIAL AND METHODS

This prospective study included 191 newly diagnosed patients with pulmonary TB, divided into four groups: the 1st study group (1st SG) comprised 80 patients with drug susceptible TB, the 2nd study group (2nd SG) included 40 patients with primary drug resistant TB (MDR TB), the 3rd study group (3rd SG) consisted of 49 patients with acquired MDR TB, and the 4th study group (4th SG) included 22 patients (11 with drug susceptible TB and 11 with primary MDR TB) co infected with SARS CoV 2. All indices were compared with those of a control group (CG) comprising 36 conventionally healthy individuals. Inclusion criteria for the study were: age> 18 years, signed informed consent, microbiologically confirmed diagnosis of pulmonary TB, and availability of Mycobacterium tuberculosis drug-susceptibility testing results. All patients underwent clinical evaluation, chest radiography, and microbiological investigations, including Ziehl-Neelsen acid-fast staining, culture of Löwenstein-Jensen solid medium and BACTEC liquid medium, and the GeneXpert MTB/RIF assay. Immunological assessments included quantitative flow cytometric immune phenotyping of lymphocyte subsets (CD3+, CD4+, CD8+, CD16+) and measurement of inflammatory and intoxication biomarkers.

3. RESULTS

Patients were similarly distributed across the four study groups by demographic characteristics, with no significant differences in sex distribution. The male to female ratios were 2.1:1 in the 1st SG (54 men [67%] vs. 26 women [32%]), 2.1:1 in the 2nd SG (27 men [67%] vs. 13 women [32%]), 1.5:1 in the 3rd SG (29 men [59%] vs. 20 women [41%]), and 2.0:1 in the 4th SG (14 men [64%] vs. 8 women [36%]). Age distribution revealed a statistically higher proportion of young patients (18–44 years) in the 2nd SG (25 cases [62%]) and the 4th SG (17 cases [77%]), compared with the 1st SG (42 cases [52%]) and the 3rd SG (19 cases [39%]). Patients older than 45 years predominated in the 1st SG (38 cases [48%]) and the 3rd SG (30 cases [61%]), compared with the 2nd SG (15 cases [38%]) and the 4th SG (4 cases [23%]). Evaluation of general symptoms established a significantly higher prevalence of prolonged asthenia in the 3rd SG (49 cases [100%]) and the 4th SG (22 cases [100%]), compared with the 1st SG (62 cases [77%]) and the 2nd SG (21 cases [52%]). Persistent weight loss was more frequent in the 3rd SG (49 cases [100%]) and the 4th SG (22 cases [100%]), compared with the 1st SG (71 cases [87%]) and the 2nd SG (28 cases [71%]). Low-grade fever was present in all patients of the 3rd SG (49 cases [100%]) and in all 22 patients of the 4th SG (100%), compared with 25 cases (31%) in the 1st SG and 8 cases (20%) in the 2nd SG, and was associated with night sweats. Radiological evaluation revealed a statistical higher proportion of patients who met all radiological severity criteria - extensive lung involvement (more than three segments), parenchymal destruction, and disseminated lesions in the 3rd SG (35 cases [71%]) and the 4th SG (16 cases [73%]), compared with half of the 1st SG (44 cases [55%]) and the 2nd SG (23 cases [57%]) ($p<0.05$ for both comparisons). Clinical-radiological severity of pulmonary TB was defined by a pronounced intoxication syndrome (altered general condition, persistent fever, and weight loss), persistent respiratory manifestations (episodes of hemoptysis, dyspnea of MRC grades 2–3), radiological severity criteria, and AFB-positive sputum. Those criteria statistically predominated in the 3rd SG (31 cases [63%]) and the 4th SG (17 cases [78%]) compared with the 1st SG (22 cases [25%]) and the 2nd SG (18 cases [45%]) ($p<0.01$ for both comparisons). To assess cell-mediated immunity, peripheral blood lymphocytes were isolated and their immune phenotypes analyzed, revealing that the proportion of $CD3^+$ T cells, including the T helper subset, was reduced in all SGs compared with the control group (CG) ($p<0.001$), with the lowest levels in the 3rd and 4th SGs compared with the 1st and 2nd SGs ($p<0.001$). The proportion of $CD4^+$ (T helper) cells was also significantly decreased in all SGs vs. the CG ($p<0.001$), statistically more reduced in the 3rd and 4th SGs than in the 1st and 2nd SGs ($p<0.01$). These findings indicated more pronounced depletion of cell-mediated immunity in patients with acquired MDR TB and concomitant SARS-CoV-2 infection. In contrast, CD8^{+} (T suppressor) cells were slightly elevated in the 1st and 2nd SGs, but significantly reduced in the 3rd and 4th SGs compared with the CG ($p<0.001$). The CD4^{+}/CD8^{+} ratio was approximately 1 in the 1st and 2nd SGs and increased to 1.4 in the 3rd SG and 1.5 in the 4th SG, indicating cell-mediated immune hyperactivity in these groups. The proportion of CD16^{+} (natural killer) cells was mildly reduced in the 1st and 2nd SGs and significantly decreased in the 3rd and 4th SGs compared with the CG ($p<0.001$), reflecting impaired innate immunity and diminished cytotoxic function in these groups.

Table 1. Cell-mediated immunity indices (%)

Indices	1st SG	2nd SG	3rd SG	4th SG	CG
CD3+ (%)	63.6±0.9□	66.4±2.5□◊	46.1±1.1□○	37.3±2.1□●◘	67.9±0.5
CD4+ (%)	32.3±0.6□	34.6±1.7□◊	27.5±1.2□○●	22.4±1.13□○◘	38.3±0.6
CD8+ (%)	31.4±0.7□	32.5±1.5□◊	18.6±1.5□○●	15.9±1.2□○◘	29.6±0.7
CD16+ (%)	10.9±1.1 *	11.2±1.6	7.3±2.2□○●	7.5±1.9□	12.1±0.5

Note: □ – statistically significant vs. CG; ◊ 1st vs. 2nd SGs; ○ 1st vs. 3rd SGs; ◌ 1st vs. 4th SGs; ● 2nd vs. 3rd SGs; ♦ 2nd vs. 4th SGs; ◘3rd vs 4th SGs.

Table 2 summarizes the changes in endogenous intoxication and pro-inflammatory biomarkers.

Table 2. Endogenous intoxication and pro-inflammatory biomarkers

Indices	1st SG	2nd SG	3rd SG	4th SG	CG
CIC-2.5%	22.2±4.64 □◊ 300%*	28.2±3.77 □ 400%	47,0±6,56 □● 670%	37.3±5.1□● ◘ 530%	7.2±0.35
CIC-4.2%	45.6±6.79 □◊ 180%*	62.6±5.55 □ 250%	78.3±9.38 □● 300%	52.5±5.73□ ○◘ 200%	25.2±0.84
CIC-8.0%	322±27.4 □◊ 130%	487±39.1 □○ 200%	523±83.7 □ 210%	412.9±1.2□ ○◘ 170%	245.5±7.2
LII$_{\mathrm{Kk}}$	1.3±0.4 130%	1.8±0.2□ 180%	1.9±0.3□○ 190%	2.4±0.5□◌◘ 240%	1.0±0.04
HII	1.4±0.2 120%*	1.5±0.3 130%	2.1±0.4□○ 170%	2.5±0.4□◌◘ 210%	1.2±0.01
ESR mm/h	25±6.1□ 350%*	28±5.6□ 400%	32±6.8□○ 450%	65±5.9□◌◘ 900%	7±2.3
CRP mg/L	36±3.5□ 970%*	39±4.2□ 1030%	64±3.9□○● 1730%	85±5.6□◌◘ 2300%	3,7±1,2
CP mg/L	870±32□ 2310%	892±39□ 2350%	1027±35□○● 2730%	1254±41□◌◘ 3330%	376±23
IL-6 pg/mL	6.3±0.42□ 170%	7.6±0.61□ 200%	8.2±0.81□○ 210%	20.8±0.62□◌◘ 560%	3,7±0,32
IL-8 pg/mL	13.2±0.17□ 1280%	15.4±0.22□ 1320%	16.7±0.27□○● 1430%	19.6±0.24□◌◘ 1670%	1,17±0,23
TNF-α pg/mL	21.1±0.67□ 1850%	27.8±0.64□◊ 4270%	39.8±0.67□○● 6120%	29.6±0.87□◌◘ 4550%	6,5±1,2

Note: □ – statistically significant vs. CG; ◊ 1st vs. 2nd SGs; ○ 1st vs. 3rd SGs; ◌ 1st vs. 4th SGs; ● 2nd vs. 3rd SGs; ♦ 2nd vs. 4th SGs; ◘3rd vs 4th SGs.

Assessment of circulating immune complexes (CICs) revealed that the concentration of high-molecular-weight CICs (2.5%) was significantly higher in all SGs compared with the CG ($p<0.001$), with significantly higher levels observed in 3rd and 4th SGs compared with 1st and 2nd SGs ($p<0.001$). The concentration of medium molecular weight CICs (4.2%) was significantly higher in all SGs compared to the CG ($p<0.001$), with significantly higher levels in the 3rd SG than in the 1st and 4th SGs ($p< 0.01$). Similarly, low molecular weight CICs

(8.0%) were significantly higher in all SGs vs the CG ($p<0.001$), with higher levels in the 2nd and 3rd SGs compared to the 1st and 4th SGs ($p<0.001$). CIC concentrations across all molecular weight fractions were significantly increased in all study groups relative to the CG. Notably, CICs (2.5%) exceeded CICs (4.2%) and (8.0%) only in patients with acquired MDR TB and concomitant SARS-CoV-2 infection, indicating a higher antigenic load in those groups. This pattern may be explained by SARS-CoV-2 coinfection, which promotes the formation of larger CICs through sustained antibody production and complement activation, thereby contributing to persistent systemic inflammation and increased disease severity. Calculation of the hematological indices LIIkk and HII revealed significantly higher values in all SGs compared to the CG ($p < 0.01$ for all comparisons). The erythrocyte sedimentation rate (ESR) was significantly elevated in all SGs versus the CG, with the highest levels observed in the 4th SG compared to the other SGs ($p < 0.001$ for all comparisons). Serum C-reactive protein (CRP) levels were significantly higher in all SGs than in the CG, with significantly greater values in the 3rd and 4th SGs compared to the 1st and 2nd SGs ($p < 0.01$ for both comparisons). Similarly, serum ceruloplasmin (CP), a copper-binding glycoprotein with ferroxidase activity, was significantly elevated in all SGs versus the CG ($p < 0.001$ for all comparisons), with higher concentrations in the 3rd and 4th SGs compared to the 1st and 2nd SGs ($p < 0.001$). Serum concentrations of TNF-α, IL-6, and IL-8 were significantly increased in all SGs relative to the CG ($p < 0.001$ for all comparisons). Among the SGs, TNF-α levels were highest in the 3rd SG ($p < 0.001$), whereas IL-6 and IL-8 concentrations peaked in the 4th SG ($p < 0.001$).

4. DISCUSSION

Our study included TB patients comparably distributed across SGs, with a consistent predominance of males. A higher proportion of younger patients (18-44 years) was observed in the 2nd and 4th SGs, whereas patients aged ≥45 years predominated in the 1st and 3rd SGs. Clinical and radiological assessments indicated a more severe disease course in patients with acquired MDR-TB and concomitant SARS-CoV-2 infection. This was associated with marked depletion of $CD3^+$, $CD4^+$, and $CD16^+$ T cells, as well as reduced $CD8^+$ T-cell counts, findings that are consistent with previous reports [5]. CIC concentrations were elevated across all study groups, reflecting an increased antigenic load and coordinated immune complex formation across all molecular weight fractions. Hematological indices, ESR values, and acute-phase proteins (CRP and ceruloplasmin) were also increased in all SGs, with the highest levels observed in patients with concomitant SARS-CoV-2 infection. Serum concentrations of the pro-inflammatory cytokines TNF-α, IL-6, and IL-8 were significantly elevated in all SGs. Among these, TNF-α reached peak levels in patients with acquired MDR-TB, whereas IL-6 and IL-8 were highest in those with concomitant SARS-CoV-2 infection, in agreement with findings reported in other studies [6,7]. The main limitations of this study include the relatively small sample size, particularly in the 4th SG, which reduces statistical power and limits the generalizability of the findings, especially for comparisons involving concomitant SARS-CoV-2 infection and acquired MDR-TB. In addition, the lack of adjustment for comorbid conditions (such as HIV infection, diabetes, or other immunosuppressive states) may confound the interpretation of immune and inflammatory parameters. The absence of spatial or regional epidemiological data also limits insights into local transmission dynamics and environmental influences [8]. These limitations underscore the need for larger, multicenter studies to better characterize immune-metabolic responses and treatment outcomes in patients with MDR-TB and SARS-CoV-2 coinfection.

5. CONCLUSIONS

Clinical and radiological evaluations revealed a more severe disease course in patients with acquired MDR-TB and concomitant SARS-CoV-2 infection. These patients exhibited marked depletion of $CD3^+$, $CD4^+$, and $CD16^+$ T cells, along with reduced $CD8^+$ T-cell counts, indicating profound impairment of both cell-mediated and innate immunity. Elevated CIC levels across all SGs reflected an increased antigenic load and coordinated immune complex formation across molecular weight fractions. In parallel, hematological indices, ESR, acute-phase proteins, and the cytokines IL-6 and IL-8 were highest in patients with SARS-CoV-2 coinfection, indicating an intensified systemic inflammatory response. Serum TNF-α concentrations were significantly elevated in all groups, with peak levels observed in patients with acquired MDR-TB. Early identification of MDR-TB and concomitant SARS-CoV-2 infection, combined with timely immunopathogenetic interventions, is essential to modulate immune-metabolic disturbances, limit the progression of drug resistance, and improve treatment outcomes in severe forms of TB.

DECLARATIONS

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

Author Contributions: E. L. designed the study; E. L. and S. G. analyzed data; E. L. drafted the manuscript; Both authors have reviewed and approved the final version of the manuscript.

Ethics Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the State Pharmacy and Medicine University ethics committees on 13.11.2017. All participants provided informed consent, and data were anonymized to ensure confidentiality.

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

REFERENCES

1. WHO. List of high TB burden countries. https://www.who.int/news/item/17-06-2021-who-releases-new-global-lists-of-high-burden-countries-for-tb-hiv-associated-tb-and-drug-resistant-tb
2. Compendium of TB/COVID-19 studies. Geneva: World Health Organization; 2022. https://www.who.int/teams/global-tuberculosis-programme/covid-19/compendium
3. McQuaid, C.F., Vassall, A., Cohen, T., Fiekert, K., White, R.G. (2021). The impact of COVID-19 on TB: a review of the data. Int J Tuberc Lung Dis, 25(6),436–446. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8171247
4. World Health Organization. Addressing TB comorbidities and health-related TB factors, https://www.who.int/activities/addressing-tb-comorbidities-and-health-related-risk-factors
5. Venturini, E., Lodi, L., Francolino, I., et al. (2019). CD3, CD4, CD8, CD19, and CD16/CD56 positive cells in tuberculosis infection and disease: Peculiar features in children. Int J Immunopathol Pharmacol, 33, 2058738419840241. https://doi.org/10.1177/2058738419840241
6. Evans, C.A., Jellis, J., Hughes, S.P., et al. (1998). Tumor necrosis factor-alpha, interleukin-6, and interleukin-8 secretion and the acute-phase response in patients with tuberculous osteomyelitis. J Infect Dis, 177(6), 1582-7. https://doi.org/10.1086/515313
7. Kireev, F.D., Lopatnikova, J.A., Alshevskaya, A.A., Sennikov, S.V. (2025). Role of Tumor Necrosis Factor in Tuberculosis. Biomolecules, 15(5), 709. https://doi.org/10.3390/biom15050709.
8. Lan, Y., Crudu, V., Ciobanu, N., et al. (2024). Identifying local foci of tuberculosis transmission in Moldova using a spatial multinomial logistic regression model. EBioMedicine, 102, https://doi.org/10.1016/j.ebiom.2024.105085

PULMONARY TUBERCULOSIS IN THE CONTEXT OF COVID-19. DEMOGRAPHIC CHARACTERISTICS AND RISK FACTORS IN MOLDOVA AND UKRAINE

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https://doi.org/10.52757/bsd26.08

ABSTRACT

The study aimed to analyze the main demographic characteristics and risk factors associated with tuberculosis (TB) before, during, and after the COVID-19 pandemic in the Republic of Moldova (RM) and Ukraine (UA) to identify the key determinants of their trends. A prospective case-control study was conducted between 2018 and 2023, including two cohorts: Moldovan (MD) comprised 1617 and Ukrainian (UA) 896 patients divided into three groups: 1st group corresponding to the pre-COVID-19 period (2018–2019), 2nd group (n=451) corresponding to the COVID-19 period (2020-2021), and 3rd group- post pandemic period (2022–2023). The main determinants influencing TB trends were the combination of social conditions (unemployment, low educational level, deprived housing, institutionalization), associated to malnutrition, harmful behaviors (smoking, alcohol, drug use), and increasing rate of comorbidities such as HIV, diabetes, chronic lung disease, and mental disorders, with each country having a distinct risk profile marked by the pandemic-related conditions. **Conclusions:** TB trends in MD and UA were determined by the complexity of socioeconomic risk factors, malnutrition, harmful behaviors, and rising comorbidities, with each country developing a distinct risk profile influenced by COVID-19-related conditions.

Keywords: Tuberculosis, COVID-19, Risk factors, Republic of Moldova, Ukraine.

1. INTRODUCTION

Socio-demographic determinants and epidemiological risk factors play a crucial role in the development of TB, a classic example of a socially determined disease (SDD), in which onset, progression, and outcomes are influenced primarily by social, economic, and environmental conditions rather than biological factors. The SDDs often disproportionately affect vulnerable and marginalized populations. Their occurrence is strongly influenced by poverty, overcrowded living conditions, limited access to healthcare, and malnutrition, which are deeply associated with social inequality [1]. Comorbid conditions, such as HIV infection, diabetes, chronic lung or kidney disease, undernutrition, and substance use, increase the TB risk by reducing immunity and accelerating progression from infection to active disease, as well as worsening treatment adherence and outcomes [2]. In this context, the implementation of health policies developed for the specific needs of individuals affected by TB, as stated in the WHO End TB Strategy, the Patients' Charter for TB Care, and the TB/HIV collaborative policy, demonstrated that targeted actions are essential to improve TB epidemiology globally and locally [2, 3]. Therefore, analyzing the demographic characteristics and risk factors of TB within different periods and regions is essential to identify the main determinants influencing their trends and elaborate target interventions for improving TB control in vulnerable populations. The study aimed to conduct a comparative assessment of the main demographic characteristics and risk factors associated with TB before, during, and after the COVID-19 pandemic in the Republic of Moldova (MD) and Ukraine (UA) to identify the key determinants influencing their trends.

Hypothesis: The demographic characteristics and risk factors associated with TB changed significantly during the COVID-19 pandemic, with specific demographic, socioeconomic, and health-related determinants.

2. MATERIAL AND METHODS

A prospective case-control study was conducted between 2018 and 2023, including two cohorts. The MD cohort comprised 1617 patients divided into three groups: 1st group (n=664) representing the pre-COVID-19 period (2018–2019), 2nd group (n=451) corresponding to the COVID-19 pandemic (2020–2021), and the 3rd group (n=502) representing the post-pandemic period (2022–2023). Similarly, the UA cohort was divided into three groups: 896 patients in the 1st group, 579 in the 2nd group, and 773 in the 3rd group.

3. RESULTS

The main demographic characteristics, including gender, age, and place of residence, were analyzed. The distribution of MD cohort by gender showed predomination of men in both cohorts ($\chi^2=101$; p=0 in MA; $\chi^2=8$; p=0.01 in UA) with an increasing trend of men over time from 474 (72%) vs 190 women (28%) in the 1st G, 330 (75%) men vs 121 (25%) women in the 2nd G and 376 (80%) men vs 126 (20%) women in the 3rd G. UA cohort showed similar trend: men 527 (69%) vs women 369 (41%) in the 1st G; 433 (75%) men vs 146 (25%) women in the 2nd G, 623 (80%) men vs 150 (20%) women in 3rd G. The increasing male predominance indicated the rise of TB cases, who are more affected by multiple risk factors. The male/female rate was higher in UA cohort - 2.9/1 in the 1st G, 3/1 in the 2nd G, and 4/1 in the 3rd G, compared with MD cohort: 2.5/1 in the 1st G, 2.7/1 in the 2nd G, and 2.9/1 in the 3rd G. The distribution of MD cohort in age subgroups showed that 35–44 years old (y. o.) cases represented the largest proportion and increased over time, accounting for 137 (21%) in the 1st G, 138 (31%) in the 2nd G, then decreased to 138 (27%) in the 3rd G. A similar trend was observed in the 45-54 y. o. subgroup - 156 (23%) in the 1st G, 136 (30%) in the 2nd G, and 135 (28%) in the 3rd G. The 55-64 y. o. Subgroup remained stable: 129 (19%) in the 1st G, 85 (19%) in the 2nd G, and 89 (17%) in the 3rd G. Lower proportions, with a decreasing trend, were observed in the 25–34 y group. o. subgroup, with 121 (18%), 70 (14%), and 77 (15%) across the three groups, also 18-24 y. o. subgroup with 36 (6%), 20 (4%), and 16 (4%) cases, respectively. The proportion of the older subgroup (+65) decreased from 85 (13%) cases in the 1st G to 2 (0.4%) in the 2nd G, then increased to 47 (9%) in the 3 rd G. The distribution of the UA cohort established that the largest subgroups were 35-44 years. o. and 45-54 y. o. which remained stable over time. The 35-44 y. o. Subgroup comprised 241 (27%) in the 1st G, 154 (26%) in the 2nd G, 222 (29%) in the 3rd G, and 45-54 y. o. - 215 (24%) in the 1st G, 136 (23%) in the 2nd G, and 216 (28%) in the 3rd G. Followed 55-64 y. o. Subgroup comprising 162 (18%) in the 1st G, 123 (21%) in the 2nd G, and 122 (16%) in the 3rd G. Lower proportions with an increasing trend were 55-64 y. o. subgroup- 162 (18%) in the 1st G, 123 (21%) in the 2nd G, 122 (16%) in the 3rd G, and 25-34 years. o. 148 (16%) in the 1st G, 79 (14%) in the 2nd G, and 67 (9%) in the 3rd G. In a very low proportion, with an increasing trend, were older adults aged 65 y. o. subgroup - 76 (8%) in the 1st G, 62 (11%) in the 2nd G, 114 (15%) in the 3rd, and a decreasing trend between 18-24 years. o. subgroup-54 (6%) in the 1st G, 25 (4%) in the 2nd G, and 35 (4%) in the 3rd G. The proportions of age subgroups did not differ significantly between the groups and across cohorts. After regrouping into two age categories (18-44 y. o. and ≥45 y. o.), in the MD cohort, the rate of young patients (18-44 y. o. subgroup) did not differ significantly over - 294 (44%) in the 1st G, 228 (45%) in the 2nd G, and 231 (42%) in the 3rd G, also older subgroup (≥45 y. o.) - 370 (56%), 223 (55%), and 271 (58%), respectively. In the UA cohort, 18-44 y. o. Subgroup decreased from 443 (49%) in the 1st group, 258 (45%) in the 2nd group, and 324 (42%) in the 3rd group, whereas older increased 453

(51%), 321 (55%), and 449 (58%) in the respective groups. The mean age did not differ significantly between groups: 46.2±14.7 y.o. in the 1st G and 47.5±12.4 y.o. o. in the 2nd G, and 45.5±13.5 in the 3rd G in MD cohort and 43.5±12.7 y. o. in the 1st G, 46.1±11.5 y. o. in the 2nd G, and 48.4±9.5 y. o. in the 3rd G. Although the majority of TB cases in both cohorts were included in the 35–44 and 45-54 age subgroups, the proportion of older individuals increased over time, while younger subgroups decreased, indicating a shift of TB burden toward older adults.

Urban residence was more common than rural in both cohorts ($\chi^2=46$; p=0 in MD and $\chi^2=57$; p=0 in UA), but its distribution showed different trends. In MD cohort, the rate of urban cases significantly increased over time from 442 (67%) in the 1st G to 378 (83%) in the 2nd G ($\chi^2=41$, p=0) then decreased to 392 (78%) in the 3rd G, whereas the rate of rural residents declined from 222 (33%) in the 1st G to 73 (17%) in the 2nd G, then increased to 109 (24%) in the 3rd G ($\chi^2=5$, p=0.02). In the UA cohort, the proportion of urban residents decreased from 692 (77%) in the 1st G to 421 (73%) in the 2nd G, then increased to 625 (81%) in the 3rd G. The rural residents increased from 204 (23%) in 1st G to 158 (27%) in 2nd G then decreased to 148 (21%) in 3rd G. Homelessness was more frequently established in the MD cohort, with an increasing tendency from 52 (8%) cases in the 1st G to 42 (9%) in 2nd G and 48 (10%) in 3rd G. In contrast, in the UA cohort, it remained at lower levels - 31(3%) in 1st G, 22 (4%) in 2nd G, and 27 (3%) in 3rd G. This pattern demonstrated that homelessness represents a key driver of TB burden, reflecting the intersection of housing instability, social marginalization, and limited access to healthcare.

The socio-economic characteristics assessed included marital status, economic condition, and education level. The distribution of patients by marital status in the MD cohort showed that married or partnered individuals increased from 326 (49%) in the 1st G to 281 (62%) in the 2nd G, then declined to 278 (55%) in the 3rd G. In the UA cohort, married or partnered individuals increased from 521(58%) in the 1st G to 381(66%) in the 2nd G, and decreased to 409 (53%) in the 3rd G, suggesting that partnerships, often associated with prolonged household exposure, contributed to intensified household transmission. In contrast, unpartnered individuals (single, widowed, or divorced) decreased from 375 (42%) in the 1st G to 381 (34%) in the 2nd G (198; 34%), then increased to 364 (47%), suggesting the widening rate of socially isolated TB patients over time. Unemployment was the prevailing economic status in both cohorts. Still, the pattern differed across groups: in MD, it significantly decreased from 465 (70%) in the 1st G to 206 (46%) in the 2nd G ($\chi^2=66$, p=0.2), then rose to 337 (67%) in the 3rd G ($\chi^2=46$, p=0.2). In comparison, professional employment increased from 199 (30%) in the 1st G to 245 (54%) in the 2nd G, then decreased to 165 (33%), respectively. This trend suggested that employment and related working conditions, often involving overcrowded workplaces and prolonged close contact, contributed to the transmission of TB within congregate settings. In the UA cohort, unemployment significantly predominated in all groups: 618 (69%) in the 1st G, 400 (69%) in the 2nd G, and 493 (64%) in the 3rd G, compared with professional employment, which remained lower at 278 (31%), 179 (31%), and 280 (36%), respectively, underscoring persistent economic vulnerability among TB patients ($\chi^2=6.3$, p=0.04). Lower educational level (primary and incomplete secondary) consistently predominated in majority of cases in both cohorts, in MD cohort was common and stable: 459 (69%) in the 1st G, 296 (65%) in the 2nd G, and 325 (65%) in the 3rd G, whereas higher education (secondary and university) moderately increased from 205 (31%) to 157 (35%) and 176 (35%), respectively. In the UA cohort, low education decreased from 502 (56%) patients in the 1st G to 268 (46%) in the 2nd G, then increased to 446 (58%) in the 3rd G, compared with higher education (secondary and high) which increased from 394 (44%) to 311 (54%), then decreased to 327 (42%), respectively. The predominance of a lower educational pattern demonstrated that limited education increased the vulnerability to TB, through socio-economic disadvantage, inadequate health literacy, and reduced access to healthcare.

The assessed risk factors included household characteristics, the presence of a TB source within the household, and behavioral risks such as smoking, substance use, and alcohol consumption, as well as migration characteristics. The assessment of household types revealed that deprived households characterized by the absence of centralized heating, water supply and sanitation increased from 186 (28%) in the 1st G to 162 (36%) in the 2nd G, then declined to 98 (19%) in the 3rd G. Also, in the UA cohort deprivation increased from 128 (14%) cases in the 1st G to 147 (25%) in the 2nd G, and then decreased to 152 (20%) in the 3rd G, indicating moderate but persistent level of inadequate living conditions contributing to TB transmission. The proportion of TB cases from institutional households, such as shelters, residences for older persons, and facilities for people with mental disorders) was higher in the MD cohort compared with the UA cohort. In the MD cohort the rate increased from 81(12%) in 1st G to 92 (20%) in 2nd G then decreased to 78 (15%) in 3rd G, while in UA cohort remained stable low: 18 (2%) patients in the 1st G, 12 (2%) in the 2nd G, and 11 (1%) in the 3rd group, indicating a much smaller proportion of UA patients were exposed to congregate living condition, that facilitate TB transmission, while in MD cohort even increased over time. Patients with prolonged household contact with an active TB, remained at the same level in both cohorts, with an increased tendency in MD cohort from 67 (10%) in the 1st G, 42 (9%) in the 2nd G, to 69 (14%) in the 3rd G, whereas in UA cohort remained stable 82 (9%) in 1st G, 58 (10%) in 2nd G and 81 (10%) in the 3rd G, indicating the persistence of household TB transmission and a continued presence of TB sources in the community. Analysis of migration-related characteristics in the MD cohort revealed that, refugees or migrants represented in a minor proportion, 9 (1%) cases in the 1st G, 5 (1%) in the 2nd G, and 11 (2%) in the 3rd G, while patients returning from abroad within the last year were more frequent and increased over time from 52 (8%) cases in the 1st G to 68 (15%) in the 2nd G, and 62 (12%) in the 3rd G, suggesting that returning migrants constitute a specific subgroup in MD cohort. In the UA cohort, temporarily displaced individuals accounted for 9 (1%) cases in the 1st G, 5 (1%) in the 2nd G, and 11 (2%) in the 3rd G, reflecting the impact of the recent geopolitical context. Behavioral risk factors, particularly active tobacco smoking, showed divergent patterns between cohorts, as it increased in MD cohort from 582 (64%) in 1st G to 403 (70%) in 2nd G and 581 (75%) in 3rd G, whereas in the UA cohort decreased from 551 (83%) in 1st G to 382 (85%) in 2nd G and 348 (69%) in the 3rd G. The high rate of smokers highlighted that it is an important risk factor due to associated comorbid background such as chronic lung disease, cardiovascular conditions, and bronchoplmonary malignancy. Alcohol abuse slightly decreased in both cohorts: in MD from 87 (13%) cases in the 1st G to 49 (11%) in the 2nd G, and 52 (10%) in the 3rd G, and in UA from 101 (12%) cases in the 1st G to 52 (9%) in the 2nd G, and 76 (10%) in the 3rd G. In the MD cohort, the substances or drug use decreased from 19 (3%) cases in the 1st group, 8 (2%) in the 2nd group, and 9 (2%) in the 3rd group, whereas in UA sample was less frequent, accounting for 9 (1%) cases in the 1st G, 8 (1%) in the 2nd G, and 7 (1%) in the 3rd G. Despite low downward trends, alcohol and drug use remained important behavioral risk factors for TB, as they contribute to immune dysfunction, poorer treatment adherence, and higher exposure in high-risk settings.

Biological characteristics analyzed included nutritional status (body mass index, BMI), the presence of comorbidities, and their potential associations. The distribution of underweight patients in the MD cohort showed an increased rate across all groups -119 (13%) cases in the 1st G, 101 (22%) in the 2nd G, and 104 (21%) in the 3rd G, while in UA cohort decreased, from 181 (20%) cases in the 1st G, to 78 (13%) in the 2nd G, and 83 (14%) in the 3rd G. HIV coinfection increased in MD cohort from 52 (6%) cases in 1st G to 49 (11%) in 2nd G, and decreased to 39 (8%) in 3rd G, while in UA cohort significantly increased from 82 (9%) in 1st

G to 121 (21%) in 2nd G (X=x, p=0.05) and decreased to 84 (13%) in 3rd G. Prior TB infection in MD cohort slightly increased from 69 (8%) of 1st G to 78 (17%) in 2nd G, then decreased to 49(10%) in 3rd G, while in UA cohort remained stable across groups - 72(8%) in 1st G1, 44(8%) in 2nd G, and 52 (8%) in 3rd G. Chronic lung diseases (including post-TB lung disease) were diagnosed in 82 (12%) in 1st G, 42 (9%) in 2nd G, and 58 (11%) in 3rd , while in UA cohort remained stable - 72(8%) in 1st G1, 44(8%) in 2nd G, and 52 (8%) in 3rd G. Glucose metabolism disorders, including diabetes, were identified in 61 (9%) cases of 1st G, 48 (11%) in 2nd G, and 31 (6%) cases in 3rd G in MD cohort, while in UA cohort was established a significant increase, rising from 49(5%) in 1st G to 86 (15%) in 2nd G and 97 (17%) in 3rd G (χ^2=6.2, p=0.04). The proportion of gastrointestinal diseases, including viral or toxic hepatitis decreased in MD cohort, from 115 (13%) in 1st G to 78 (17%) in 2nd G, and 52 (10%) in 3rd G, while in UA cohort increased from 128 (14%) patients of 1st G, to 94(16%) in 2nd G, and 103(18%) in 3rd Gr. Chronic renal disease were diagnosed increasingly from 32 (3%) of 1st G to 45 (10%) in 2nd G, and decreased to 12 (2%) in 3rd G of MD cohort, whereas in UA cohort remained in stable low proportion 18 (2%) in 1st G, 12 (2%) in 2nd G, and 21(4%) in 3rd G. Mental health disorders increased in MD cohort from 79 (9%) to 58 (13%), and 39 (8%) of the MD cohort, while in UA cohort in 95(11%) of 1stG, 68(12%) of 2nd G, and 72(12%) of 3rd G.

4. DISCUSSION

Tuberculosis predominantly affected working-age adults, individuals living in urban areas, the unemployed, and those with lower levels of education, findings reported in multiple large cohort studies [2-6]. Comorbidities and poor nutritional status followed divergent trajectories within cohorts but demonstrated a high impact on vulnerability. Particularly, HIV-coinfection increased, highlighting rising TB/HIV in Eastern Europe; also, prior TB infection and chronic lung diseases suggested the persistence of latent TB infection within the population. In contrast, glucose metabolism disorders increased in UA, but declined in MD. At the same time, gastrointestinal disease, chronic renal disease, and mental health disorders showed variable but persistent rates, demonstrating that TB disproportionately affects patients with multiple comorbidities, which worsen prognosis and amplify transmission risk. A potential limitation of the study is that the analysis was restricted to selected regions during specific periods, so results may not reflect national cohorts or fully establish the effect of the pandemic in the context of broader socioeconomic and healthcare system changes.

5. CONCLUSIONS

The analysis of demographic characteristics and risk factors for TB before, during, and after the COVID-19 pandemic in the MD and UA identified several key determinants: TB predominantly affected working-age adults (35–54 years), with an increasing rate of older individuals; urban residence, unemployment, and lower education were associated with higher TB burden. In the MD cohort, homelessness, institutionalization, and malnutrition prevailed, especially during the pandemic period; in contrast, in the UA cohort, diabetes and HIV coinfection prevailed. Prolonged household TB contact and previous TB infection remained stable in both cohorts, indicating persistent transmission.

DECLARATIONS

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

Author Contributions: E. L. designed the study; E. L. and L. T. analyzed data; E. L. drafted the manuscript; Both authors have reviewed and approved the final version of the manuscript.

Ethics Statement: This study was conducted in accordance with the Declaration of Helsinki and was approved by the State Pharmacy and Medicine University ethics committees on

13.11.2017. All participants provided informed consent, and data were anonymized to ensure confidentiality.

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

REFERENCES

1. Lönnroth, K., Jaramillo, E., Williams, B.G., Dye, C., Raviglione, M. (2009). Drivers of tuberculosis epidemics: the role of risk factors and social determinants. Soc Sci Med, 68(12), 2240-6. https://doi.org/10.1016/j.socscimed.2009.03.041
2. World Health Organization. Addressing TB comorbidities and health-related TB factors. https://www.who.int/activities/addressing-tb-comorbidities-and-health-related-risk-factors
3. Wang, Y., Liu, Q., Chen, Z., Liu, M., Chen, B., Zhao, Y., Liu, J. (2025). Disparities in the burden of tuberculosis associated with urbanization across 178 countries and territories: an observational study. Front Public Health, 13, 1658814. https://doi.org/10.3389/fpubh.2025.1658814
4. Krokva, D., Mori, H., Valenti, S., Remez, D., Hadano, Y., Naito, T. (2025). Analysis of the impact of the crises on tuberculosis incidence in Ukraine amid pandemics and war. Sci Rep, 15(1), 17045. https://doi.org/10.1038/s41598-025-01723-7
5. Compendium of TB/COVID-19 studies. Geneva: World Health Organization (2022). https://www.who.int/teams/global-tuberculosis-programme/covid-19/compendium).
6. Public Health Center of Ukraine. https://phc.org.ua/en/diseases-and-information/hivaids/monitoring-and-evaluation/surveillance/tb-statistics
7. McQuaid, C.F., Vassall, A., Cohen, T., Fiekert, K., White, R.G. (2021). The impact of COVID-19 on TB: a review of the data. Int J Tuberc Lung Dis, 25(6), 436–446.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC817

THE IMPACT OF COVID-19 PANDEMIC ON THE DYNAMICS OF TUBERCULOSIS EPIDEMIOLOGICAL INDICATORS BEFORE AND DURING THE COVID-19 PANDEMIC IN THE REPUBLIC OF MOLDOVA AND UKRAINE

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https://doi.org/10.52757/bsd26.09

ABSTRACT

The study aimed to assess the main epidemiological indicators of tuberculosis (TB) before, during, and after the COVID-19 pandemic in the Republic of Moldova and Ukraine, and to identify the key drivers underlying their trends. **Material and methods:** This study is based on the analysis of global WHO tuberculosis reports and national tuberculosis statistical data. **Results:** In RM and UA, the COVID-19 pandemic significantly impacted TB detection and case registrations. TB incidence declined before the COVID-19 pandemic, followed by an increase in subsequent years. In R. Moldova, the incidence decreased from 70.88 to 56.73/100000 population between 2015 and 2019, but rose again to 66.34/100000 by 2024. Similarly, in Ukraine, the incidence declined from 62.3/100000 in 2018 to 42.2/100000 before increasing to 45.1/100000 population in 2022. **Conclusions:** The number of reported tuberculosis (TB) cases decreased during the COVID-19 pandemic in both the Republic of Moldova and Ukraine due to common factors, including mobility restrictions, disruption of TB services, reallocation of healthcare resources, and reduced healthcare-seeking behavior associated with increased stigma and avoidance. A gradual restoration of TB services followed this. In the post-pandemic period (2023–2024), increases in certain indicators, such as global incidence and relapsed cases, may reflect improved case detection.

Keywords: Tuberculosis, COVID-19, epidemiological indicators, Republic of Moldova, Ukraine.

1. INTRODUCTION

WHO reports indicate that the COVID-19 pandemic significantly disrupted TB case detection and treatment outcomes, with globally reported cases declining from about 10 million (130/10,000 population) before the pandemic (2018) to 7.1 million in 2019 and 5.8 million in 2020, followed by a partial recovery to 6.4 million in 2021 and rising again to 10.6 million in 2022, 10.8 million in 2023, and around 10.7 million new cases in 2024, with similar levels estimated for 2025 [1-8]. In 10 high-burden countries (HBC), excluding the Eastern Europe Region, the largest decline (28%) was observed in 2022 compared with 2019 [10]. These reductions in TB notifications during the COVID-19 pandemic led to a significant increase in undiagnosed and untreated TB cases worldwide, especially during the initial phase (2020-2022). In 2022, there was a significant global recovery in the number of patients diagnosed with TB following two years of disruptions caused by the COVID-19 pandemic. In 2022, the global number of newly registered TB cases reached 7.5 million, the highest level recorded since the WHO began global TB surveillance in 1995. The value exceeded the pre-COVID indices (2018-2019) and the previous peak of 7.1 million cases reported in 2019, increasing from 5.8 million in 2020 and 6.4 million in 2021. In 2022, the global incidence indicator included a considerable number of individuals who developed TB during the COVID-19 pandemic. Still, diagnosis and treatment were delayed due to disruptions caused by mobility restrictions, disruptions in the activity of TB services, and avoidance of TB services [2-5]. In the epidemiological context, the Republic of Moldova (RM) and Ukraine (UA) have remained on the WHO list of high MDR/RR-TB burden countries since 2016, which has been a main factor contributing to low treatment success rates [9-10]. UA is also included in the WHO list of high-burden countries for TB, HIV-associated TB, and MDR/RR-TB for the period 2021–2025 [4-8]. Globally, TB remained, from 2020 to 2022, the second-leading cause of death from a single infectious agent, after COVID-19 [3-5]. The study aimed to conduct a comparative assessment of the main epidemiological indicators of tuberculosis before and during the COVID-19 pandemic in the Republic of Moldova and Ukraine, including the trans-border region of Chernivtsi, to identify the key drivers of their trends.

2. MATERIAL AND METHODS

An analysis of global WHO tuberculosis reports and national TB statistical data was performed.

3. RESULTS

Analyzing national statistical reports in the RM, the TB incidence (new cases) declined, since 2015 from 70.88/100.000 (2859 cases), 2016: 70.57/100.000 (2845 cases), 2017: 66.66/100.000 (2682 cases), 2018: 60.99/100.000 (2450 cases), and in 2019: 56.73/100.000 (2279 cases). However, during the period from 2020 to 2024, an increase in indicators was registered, with the incidence rising from 56.68 to 66.34/100.000 population [9]. The upward trend contrasted with the previous 21.7% decrease observed from 2015 to 2020, which indicated a significant shift in TB epidemiology with evident regional disparities. On the Right Bank, the incidence increased from 1421 cases in 2020 to 1649 in 2024, while Transnistria (Left Bank) experienced a decline from 338 to 287 cases over the same period. Significant local variations included Stefan-Voda, which recorded the highest increase of 151% (from 29 to 73 cases), and Donduseni, with the notable decrease of 63% (from 22 to 8 cases). While comparing the global incidence (new cases and relapses) the improving trend with a decrease of 19.1% was noted during 2016-2019, as in 2019 was at 71.6/100.000 population (2877 cases), in 2018: 75.1/100.000 population (3016 cases), in 2017: 83.3/100.000 (3358 cases), in 2016: 88.5/100.000 population (3569 cases). Since 2020, the global incidence has shown a changing trend, rising from 56.68/100.000 population (1759 cases) to 66.34/100.000 (1936 cases) by 2024, representing a 17.1% rise over four years, contrasting with the previous declining trend observed from 2016 to 2019.

The incidence of TB relapses in 2024 was 12,92/100.000 (377 cases), in 2023: 14.87/100.000 (434 cases), while in 2022: 15.08/100.000 (453 cases) compared to 2021: 14.82/100.000 (454 cases), with an increase of 1.7% from 2021 to 2024. In 2020, the incidence of relapses was 12.37/100.000 (384 cases), in 2019: 14.86/100.000 population (597 cases), in 2018: 14.06/100.000 (565 cases), for 2017: 16.68/100.000 (671 cases), in 2016: 17.93/100.000 population (723 cases), and in 2015: 18.52/100.000 population (747 cases). A 18.6% decrease in the rate of TB relapses was observed during the period 2015-2020. Regional variations on the Right Bank included an increase in cases from 302 in 2020 to 328 in 2024, with the incidence rising to 16.4% from 11.46 to 13.34/100.000. In Transnistria (Left Bank), the cases decreased significantly from 82 in 2020 to 49 in 2024, and the relapse incidence rate dropped with 39.2% from 17.51 to 10.64/100.000. The notable changes in specific regions were in Ialoveni with the highest increase, from 16 cases (21.03/100.000) to 23 cases (32.44/100.000), Orhei from 11 cases (12.98/100.000) to 25 cases (32.57/100.000), and Ribnita with a substantial decrease from 20 cases (29.81100.000) to 4 cases (6.11100.000). The gender distribution showed that the rate of male cases decreased from 84% in 2020 to 79% in 2024, while the rate of female cases increased from 16% in 2020 to 21% in 2024. Chisinau (capital) showed a slight increase from 66 cases (9.91100.000) to 71 cases (10.63100.000), while in rural areas, incidence rates increased more than in urban areas.

The analysis of TB prevalence revealed significant changes between the periods 2015-2019 and 2020-2024. During 2015-2019, the total number of TB cases decreased from 4056 in 2015 to 3186 in 2019, with the prevalence rate dropping from 100.55 to 79.31/100.000, and in 2020-2024, the total TB cases further decreased from 2220 in 2020 to 1589 in 2024, with the prevalence rate falling from 71.54 to 54.45/100.000. Regional variations in the Right Bank showed that during 2015-2019, the total number of cases decreased from 3254 to 2533, and during 2020-2024 further decreased from 1718 to 1325. In Transnistria (Left Bank), during 2015-2019, the number of cases decreased from 802 to 653, and from 2020 to 2024, significantly reduced from 502 to 264. Notable changes were observed in Chisinau (capital): in 2015-2019, cases decreased from 782 to 616, and in 2020-2024, further reduced from 425 to 332. During 2015-2019, Stefan Voda determined a decrease from 53 to 44 cases, and during 2020-2024, an increase from 33 to 45 cases, showing a reversal in trend. During 2015-2019, the rate of male cases remained stable at around 77-78%, and during 2020-2024, the rate slightly decreased from 80% to 79%. TB-caused mortality increased due to disruptions in diagnosis and treatment services: 5.2/100000 (207 deaths) in 2022, with an increase of 20% to 6.5/100000 (199 deaths) in 2021. In 2023, a rate of 3.1/100000 (125 cases) was reported, which decreased to 2.2/100000 in 2024 (99 cases). The general decline of indicators suggested a substantial increase in undiagnosed and untreated TB cases during the COVID-19 pandemic. The notification rate of rifampicin-resistant and multidrug-resistant tuberculosis (RR/MDR-TB) decreased from 16.3/100000 population in 2019 to 13.8/100000 in 2024, corresponding to 410 reported cases, while 93.5% of TB patients (new and relapse cases) were tested with WHO-recommended rapid diagnostic tests, with 1,808 of 1,934 patients. The treatment success rate for drug-susceptible TB was 89.9% and for MDR-TB was 76.5% in 2022.

Reviewing national statistical reports in Ukraine (UA), the global incidence (GI) decreased from 62.3/100000 population in 2018 (26321 cases) to 60.1/100000 (25257 cases) in 2019, representing a 2.2% reduction, followed by a further decline of 17.7% to 42.2/100000 (17593 cases). Subsequently, the GI increased to 44.0/100000 (18241 cases) in 2021 and to 45.1/100000 (18510 cases) in 2022, including 4744 women and 13766 men, among them 3909 started the treatment for MDR-TB. Meanwhile, the GI of MDR-TB cases showed a continuous decline, decreasing from 13.7/100000 (5788 cases) in 2018 to 13.2/100000 (5524 cases) in 2019. It further dropped by 4.4% to 8.8/100000 (3675 cases) in 2020, followed by reductions to 8.4/100000 (3489 cases) in 2021 and 7.8/100000 in 2022 [10].

The TB GI in the trans-border Chernivtsi region was 32.2/100000 population in 2022, including 286 new and relapse cases. The GI showed a fluctuating trend over the study pre- and COVID-19 period: from 34.6/100000 (313 cases) in 2018, it increased to 40.6/100000 population (366 cases, +17%) in 2019, followed by a sharp decline of 80% in 2020 to 22.8 per 100,000 population (205 cases). It then slightly increased to 25.3/100000 population (226 cases) in 2021 and further rose to 32.2/100000 population (286 cases) in 2022. Meanwhile, the GI of MDR-RR/TB cases constantly decreased during the study period from 2.9/100000 population (26) in 2018, to 2.2/100000 population (20) in 2019, 2.3/100000 population (21) in 2020, 1.8/100000 population (16) in 2021 and 1.8/100000 population (16) in 2022, with an overall decrease with 60%. The treatment success rate among MDR/RR-TB cases registered in 2020 was 65.2%, with a death rate of 15%, a loss to follow-up rate of 10.6%, and a treatment failure rate of 8.5%. Meanwhile, in the Chernivtsi region, the treatment success rate among MDR/RR-TB cases registered in 2020 was 66.6%, with a death rate of 15.2%, a loss to follow-up rate of 12.1%, and a treatment failure rate of 6.1%.

Reviewing the published reviews and reports was established that the key factors which decreased the TB case detection during the COVID-19 pandemics (2020-2022) were related to several exogenous factors: a) mobility restrictions, lockdowns and other measures which limited patients access to TB diagnosis, treatment, and care services; b) disruption of TB services: national TB programs were reduced as health system prioritized COVID-19 response: c) resource reallocation: human, financial, and technical staff, including laboratory equipment, were diverted from TB to COVID-19 services; d) reduced healthcare seeking as patients which avoided medical facilities due to COVID-19 exposure; c) increased stigma, potentially discouraging patients from seeking TB-related health care [10-14].

5. CONCLUSIONS

The COVID-19 pandemic significantly disrupted tuberculosis detection and case registration in the RM and UA, leading to a temporary decline in reported cases during 2020–2022 due to restricted mobility, reduced healthcare-seeking behavior, and reallocation of healthcare resources. With the gradual restoration of TB services in the post-pandemic period (2023-2024), key epidemiological indicators increased, reflecting improved case detection and the recovery of diagnostic and surveillance activities. In the Republic of Moldova, the incidence of TB cases decreased between 2015 and 2019, from 70.88/100000 population (2859 cases) to 56.73/100000 (2279 cases). However, during the period 2020–2024, the indicator increased again, rising from 56.68 to 66.34/100000 population. In Ukraine, the TB global incidence decreased from 62.3/100000 population in 2018 to 60.1/100000 in 2019, followed by a sharper decline to 42.2/100000, then increased, reaching 44.0/100000 in 2021 and 45.1/100000 population in 2022. Several factors contributed to the disruption of TB control, including mobility restrictions and lockdowns that limited access to diagnosis and treatment, interruptions in TB services as health systems prioritized the COVID-19 response, reallocation of human, financial, and technical resources to COVID-19 care, reduced healthcare-seeking behavior due to fear of infection, and increased stigma.

DECLARATIONS

Conflict of Interest Statement: The authors declare that they have no conflict of interest.
Author Contributions: E. L. designed the study; E. L. and L. T. analyzed data; E. L. drafted the manuscript; Both authors have reviewed and approved the final version of the manuscript.
Ethics Statement: Not applicable.
Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere

REFERENCES

1. WHO Global Tuberculosis Report 2018. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2018
2. WHO Global Tuberculosis Report 20219. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2019
3. WHO Global Tuberculosis Report 2020. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2020
4. WHO Global Tuberculosis Report 2021. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2021
5. WHO Global Tuberculosis Report 2022 https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2022
6. WHO Global Tuberculosis Report 2023. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2023
7. WHO Global Tuberculosis Report 2024. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024

8. WHO Global Tuberculosis Report 2025. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2025
9. Institute of Pneumology of the Republic of Moldova. The report on achievements of the national tuberculosis control program.
10. https://simetb.ifp.md/Download/tbreps.excel/raport_2024.pdf
11. Krokva, D., Mori, H., Valenti, S., Remez, D., Hadano, Y., Naito, T. (2025). Analysis of the impact of the crises on tuberculosis incidence in Ukraine amid pandemics and war. Sci Rep, 15(1), 17045. https://doi.org/10.1038/s41598-025-01723-7
12. Public Health Center of Ukraine. https://phc.org.ua/en/diseases-and-information/hivaids/monitoring-and-evaluation/surveillance/tb-statistics
13. McQuaid, C.F., Vassall, A., Cohen, T., Fiekert, K., White, R.G. (2021). The impact of COVID-19 on TB: a review of the data. Int J Tuberc Lung Dis, 25(6), 436–446. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8171247).
14. Migliori, G.B., Thong, P. M., Alffenaar, J.W., Denholm, J., Tadolini, M., Alyaquobi, F., et al. (2021). Gauging the impact of the COVID-19 pandemic on tuberculosis services: a global study. Eur Respir J, Aug 26. 2101786. https://doi.org/10.1183/13993003.01786-2021)
15. Compendium of TB/COVID-19 studies. Geneva: World Health Organization (2022). https://www.who.int/teams/global-tuberculosis-programme/covid-19/compendium)

CLINICAL AND LABORATORY CHARACTERISTICS OF PULMONARY TUBERCULOSIS IN THE CONTEXT OF THE COVID-19 PANDEMIC IN THE REPUBLIC OF MOLDOVA AND UKRAINE

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https://doi.org/10.52757/bsd26.10

ABSTRACT

The study aimed to analyze the main clinical and laboratory characteristics of tuberculosis and their impact on case management and outcomes before, during, and after the COVID-19 pandemic in the Republic of Moldova (RM) and Ukraine (UA). A prospective case-control study was conducted between 2018 and 2023, including two cohorts: Moldovan (MD) comprised 1617 and Ukrainian (UA) 896 patients divided into three groups: 1st group corresponding to the pre-COVID-19 period (2018–2019), 2nd group (n=451) corresponding to the COVID-19 period (2020–2021), and 3rd group- post pandemic period (2022–2023). Primary healthcare providers played a central role in TB case detection and clinical assessment of the majority of TB cases in both countries, which were clinically symptomatic with TB-related presentations, whereas active screening made a limited contribution. The COVID-19 period impacted the case-management, determining a marked increase in laboratory-diagnosed TB, a higher rate of late-detected cases, and lower outcomes, particularly in drug-resistant TB. The results highlighted the importance of strengthening primary care-based case detection, expanding active screening, and sustaining progress in TB control while adapting to region-specific needs.

Keywords: Tuberculosis, COVID-19, Microbiology, Case management, Republic of Moldova, Ukraine.

1. INTRODUCTION

The COVID-19 pandemic had a profound impact on global tuberculosis (TB) control efforts, significantly disrupting the progress [1]. According to the World Health Organization (WHO) Global Tuberculosis Report, while some improvements have been made, TB case numbers continue to exceed pre-pandemic levels. In 2023, an estimated 10.8 million people developed TB, slightly more than in 2022, 7.5 million, with an incidence rate rising to 134 per 100,000 population [2]. At a rate of 87%, these cases occurred in 30 high-TB burden (HTB) countries, including the Republic of Moldova (MD) and Ukraine (UA). At the same time, India, Indonesia, China, the Philippines, and Pakistan accounted for half of global cases [3]. Although TB-related deaths declined to 1.25 million in 2023, from 1.32 million in 2022, the COVID-19 pandemic reversed the progress, and TB is now the leading global cause of death from a single infectious agent [2]. The COVID-19 pandemic severely disrupted TB diagnostic and treatment services, leading to delayed detection, increased transmission, and undiagnosed 2.7 million cases [4]. It is important to mention that RM continues to advance in the fight against TB, although it did not meet the 2025 global targets set by the WHO's End TB Strategy [2]. In 2023, RM, with a population of 3.1 million, reported 2300 new TB cases and 204 TB-related deaths (6.7/100000 people), reflecting a 33% reduction in TB mortality and a 26% reduction in TB incidence compared to 2015, still below the milestones of 75% and 50% reductions, respectively. According to national reports, the TB incidence rate in 2023 reached 76/100.000 population (range 64–88), reflecting a 2% increase from 2022 (74.5/100.000) and an 8.4% increase from 2021 (67.1/100.000). The incidence of TB relapses showed a fluctuating trend, with 14.8/100.000 population (range 11-19) in 2022 (457 cases), slightly increased from 14.7/100.000 population in 2021 (454 cases) and 13.9/100.000 population in 2020 (566 cases), then decreased compared with 18.8/100.000 population in 2019 (670 cases). The rate of destructive forms among new pulmonary cases increased from 37% in 2020 to 41% in 2023. Only 44% of estimated multidrug- or rifampicin-resistant TB (MDR/RR-TB) cases were treated in 2022, achieving a success rate of 68%, while in drug-susceptible TB, the rate remained high at 88% [5]. In this context, analyzing how risk factors affect the clinical course and paraclinical features across different periods of the COVID-19 pandemic and across various regions is essential to identify the main determinants of outcomes and to develop targeted interventions aimed at strengthening TB control in vulnerable populations [7]. The study aimed to conduct a comparative assessment of the main clinical and laboratory characteristics and their impact on case management and disease outcome before, during, and after the COVID-19 pandemic in the Republic of Moldova (MD) and Ukraine (UA).

2. MATERIAL AND METHODS

A prospective case-control study was conducted between 2018 and 2023, including two cohorts. The MD cohort comprised 1617 patients divided into three groups: 1st group (n=664) representing the pre-COVID-19 period (2018–2019), 2nd group (n=451) corresponding to the COVID-19 pandemic (2020–2021), and the 3rd group (n=502) representing the post-pandemic period (2022–2023). Similarly, the UA cohort was divided into three groups: 896 patients in the 1st group, 579 in the 2nd group, and 773 in the 3rd group.

3. RESULTS

The distribution of patients from MD cohort according to the pathway of accessing the healthcare system showed that the majority were detected by primary healthcare providers during the evaluation of TB-related symptomatic cases, which complained persistent cough with expectorations, associated with loss of weight and night sweets, with fluctuating trend from 451 (68%) in 1st G, to 282 (62%) in the 2nd G and increasing to 375 (74%) in the 3rd G. Similarly, in the UA cohort, the majority were detected by primary healthcare providers during the evaluation of TB-related symptomatic cases, in a similar proportion across all groups: 651 (73%) in the 1st G, 413 (71%) in the 2nd G, and 532 (69%) in the 3rd group, indicating the central role of the primary-care providers in the detection and clinical evaluation of TB cases. Detection through active screening by primary healthcare staff in the MD cohort showed an increasing trend over time, rising from 35 (5%) in the 1st G to 37 (8%) in the 2nd G, and 47 (9%) in the 3rd G. In the UA cohort, this pathway accounted for a higher initial proportion. Still, it demonstrated a decreasing trend, from 158 (18%) in the 1st G to 76 (13%) in the 2nd G and 100 (13%) in the 3rd G, indicating a gradual strengthening of active screening in MD and a relative decline in UA over the study period, with statistically higher rates in the UA cohort. Direct referrals to TB-specialized services (dispensaries), representing patients who went straight to TB-specialized facilities without contacting primary-care providers, in MD cohort had an important fluctuant rate from 127 (19%) in the 1st G decreasing to 36 (8%) in the 2nd G, and to 38 (7%) in the 3rd G, with a statically higher rate in the 1st G compared with other SGs ($\chi2$ =31; p=0). Medical specialists diagnosed TB during hospital stay in a higher proportion of patients in the MD cohort, with a significantly increased rate from 51 (8%) in the 1st G to 96 (21%) in the 2nd G, followed by a decrease to 42 (9%) in the 3rd G ($\chi2$ =52; p=0). In contrast, in the UA cohort, this pathway accounted for a lower proportion but demonstrated a gradual increase, from 55 (6%) in the 1st G to 62 (11%) in the 2nd G and 81 (10%) in the 3rd G, showing a significantly higher proportion in the MD 2nd G. Direct referrals to TB-specialized services (dispensaries) decreased from 127 (19%) in the 1st G, to 36 (8%) in the 2nd G and 38 (7%) in MD cohort, while in UA cohort showed a gradual increasing trend from 32 (4%) in the 1st G to 28 (5%) in the 2nd G, and 60 (8%) in the 3rd G, with a significantly higher rate in the Moldovan 1st G compared with Ukrainian 1st G (127 (19%) vs. 32 (4%), ($\chi2$ =80; p=0).

Patient distribution by TB localization showed that pulmonary TB was the most common diagnosis in both cohorts. Associated extrapulmonary TB, involving mediastinal lymph nodes, pleura, joints, or bones, occurred at low rates in the MD cohort with an increasing trend from 57 (8%) in the 1st G to 48 (11%) in the 2nd G, then reducing to 37 (7%) in the 3rd G. In comparison, in UA cohort it decreased from 47 (9%) in the 1st G to 29 (5%) in the 2nd G, and increased to 73 (9%) in the 3rd G. Generalized TB, including multisite or disseminated disease, was diagnosed in a slightly higher rates in MD cohort 21 (3%) in the 1st G, to 34 (7%) in the 2nd G then to 29 (6%) in the 3rd G compared with UA cohort: 13 (3%) in the 1st G, 18 (3%) in the 2nd G, and 20 (3%) in the 3rd G.

The distribution of TB cases by treatment history showed that new cases, patients who had never previously been treated for TB, constituted the largest category in both cohorts with increasing trend from 491 (74%) in the 1st G to 341 (76%) in the 2nd G and 422 (84%) in the 3rd G in MD cohort and decreasing trend in the UA cohort from 366 (74%) in the 1st G to 400 (69%) in the 2nd G, and 553 (71%) in the 3rd G. Relapse cases, patients who were successfully treated before and later developed TB again, were in a smaller in proportion and showed fluctuating trends in both cohorts: in UA from 61 (23%) in the 1st G to 105 (18%) in the 2nd G and 164 (21%) in the 3rd G and in lower rates in MD from 101 (15%) in the 1st G to 87 (19%) in the 2nd and 34 (7%) in the 3rd G, being significantly lower in 3rd Ukrainian group 34 (7%) compared with the 3rd Moldovan group 164 (21%); ($\chi2$ =130; p=0) Patients re-enrolled in treatment after treatment failure or after being lost to follow-up were relatively few and exhibited a marked decline over time in both cohorts: 69 (24%) in the 1st G, 74 (13%) in the 2nd G, and 56 (7%) in the 3rd G in UA cohort while in MD increased from 101 (15%) in the 1st G to 87 (19%) in the 2nd G and 34 (7%) in the 3rd G. Reduced proportions of re-enrolled cases in the 3rd SGs indicated improved treatment continuity and better retention in specialized healthcare in the post–COVID-19 period.

The tools used for TB diagnosis showed a distinct distribution and trend across the groups, with laboratory-diagnosed TB, based on Ziehl-Neelsen smear, conventional culture, and molecular-genetic tests being predominant with a sharply increasing trend in both cohorts: in MD from 252 (38%) in the 1st G to 201 (44%) in the 2nd G and 276 (55%) in the 3rd G. In comparison, in the UA cohort, it increased more evidently from 310 (35%) patients in the 1st G to 310 (53%) in the 2nd G and 582 (75%) in the 3rd G, which was statistically higher compared with the 3rd Moldovan group ($\chi2$ =11; p=0). Results reflected the expanding proportion of cases microbiologically confirmed in the post–COVID-19 period in line with WHO recommendations. The microbiological diagnostic results for acid-fast bacilli (AFB) and *Mycobacterium tuberculosis* (MTB) showed that microscopic AFB positivity increased in UA cohort from 192 (21%) in the 1st G to 292 (50%) in the 2nd G and further to 556 (72%) in the 3rd G while in MD cohort was a lower rates: 127 (19%) in the 1st G to 186 (41%) in the 2nd G and 201 (40%) in the 3rd G. Significant higher rate of positive AFB results were established in the 3rd Ukrainian compared with Moldovan group (556 (72%) vs 201 (40%); ($\chi2$ =34; p=0) which was the consequence of the specific case-management in the post–COVID-19 period. Conventional culture positivity for MTB followed a similar upward trend, rising from 210 (35%) in the 1st G to 322 (55%) in the 2nd G and 528 (68%) in the 3rd G in UA cohort, while in MD cohort was in lower rates: 176 (26%) in the 1st G to 222 (49%) in the 2nd G and 231 (46%) in the 3rd G. GeneXpert MTB positivity also increased progressively, from 310 (35%) in the 1st G to 321 (55%) in the 2nd G and 625 (81%) in the 3rd G in the UA cohort compared with 218 (33%) in the 1st G to 241 (53%) in the 2nd G and 267 (53%) in the 3rd G in MD cohort, being lower compared with UA 3rd G ($\chi2$ =128; p=0). So, laboratory-confirmed TB cases were significantly higher in the post–COVID-19 period in both countries, driven by WHO-recommended case management and the complex COVID-19-related constraints. GeneXpert results indicating rifampicin-sensitive TB rose from 142 (21%) in the 1st G to 189 (142%) in the 2nd G and 178 (35%) in the 3nd G in MD cohort, while in UA cohort rose from 210 (23%) in the 1st G to 243 (42%) in the 2nd G, but decreased to 102 (13%) in the 3rd G. In contrast, rifampicin-resistant cases remained relatively low and stable, with 100 (11%) in the 1st G, 56 (10%) in the 2nd G, and 71 (9%) in the 3rd G in UA cohort, compared with increasing trend in MD cohort: 76 (11%) in the 1st G, 52 (11%) in the 2nd G and 89 (18%) in the 3rd G. Confirmed sensible TB predominated, rising from 376 cases (42%) in the 1st G to 429 cases (74%) in the 2nd G and peaking at 672 cases (87%) in the 3rd G in UA cohort, while in MD cohort had a decreasing trend: 553 (82%) in the 1st G to 376 (83%) in the 2nd G and 349 (70%) in the 3rd G. Monoresistant TB was diagnosed in 29 (6%) cases in the 1st G, increasing slightly to 42 (7%) in 2nd G, then decreasing to 11 (1%) cases in the 3rd G in UA cohort while in MD was in a lower rates: 19 (3%) in 1st G, to 14 (3%) in the 2nd G and 38 (7%) in the 3rd G. Polyresistant TB remained low, with 9 (1%) cases in the 1st G, 12 cases (2%) in the 2nd G, and 18 cases (2%) in the 3rd G in UA cohort, while in MD was in 14 (2%) in 1st G, 9 (2%) in 2nd G and 24 (5%) in the 3rd G .Multidrug-resistant or rifampicin-resistant TB (MDR/RR-TB) was diagnosed in an increasing trend din MD cohort and was microbiologically confirmed in 76 (11%) cases in the 1st G and 52 (11%) cases in the 2nd group increasing to 89 (18%) in the 3rd G, while in UA cohort had a fluctuating trend from 84 (9%) cases in the 1st G, then rose to 96 (16%) cases in the 2nd G, and then decreased to 72 (9%) cases in the 3rd G, highlighting shifting patterns in drug resistance over the periods. The results showed divergent trends across both countries, with an increasing trend of MDR-TB in the Moldovan cohort, similarly identified in a wide national study [8]. The distribution of AFB positivity during the anti-TB treatment follow-up showed similar proportions across the three groups over time. At the end of the 2nd month of the anti-TB treatment, AFB positivity was moderately higher in UA cohort, being recorded in 52 (7%) cases in the 1st G, 42 (7%) in the 2nd G, and 52 (7%) in the 3rd G while in MD cohort was established in 23 (3%) in the 1st G, 12 (3%) in the 2nd G and 19 (4%) in the 3rd G. By the end of the 3rd month of the treatment, the proportions of the AFB-positive results decreased to 23 (4%) cases in the 1st G, 16 (3%) in the 2nd G, and 21 (3%) in the 3rd G in UA cohort and remained stable low in the MD cohort. The results demonstrated a stable trend in treatment effectiveness across both cohorts, as evaluated by microbiological status. The rate of cases diagnosed based on TB-related symptoms and chest imaging without microbiological confirmation decreased over time: 555 (62%) in the 1st G to 253 (44%) in the 2nd G, and 174 (22%) in the 3rd G in UA cohort while in MD the decrease was not so evident: 400 (60%) in the 1st H to 241 (53%) in the 2nd G and 216 (43%) in the 3rd G. The decreasing trend in the clinical radiological diagnosis in both countries reflected a shift toward more laboratory-based diagnosis, as recommended by the WHO, and in the context of the COVID-19 pandemic. In contrast, histologically diagnosed TB through tissue biopsy during bronchoscopic fibroscopy accounted for a small proportion in both cohorts, with slightly higher rates in MD: 12 (2%) in the 1st G, to 9 (2%) in the 2nd G and 10 (2%) in the 3rd G, as well in UA cohort: 31 (4%) in the 1st G, 16 (3%) in the 2nd G, and 17 (2%) in the 3rd G, indicating that histology played a minor role in TB diagnosis in both countries, compared with laboratory and clinical-radiological methods. Radiological evaluation showed a significantly higher proportion of patients meeting all severity criteria-extensive lung involvement (more than three segments), parenchymal destruction, and disseminated lesions - in the MD 2nd SGs (289 [64%]) vs. the UA cohort (52%). In the 1st SGs, the proportions were similar between cohorts: 201 (30%) in MD and 272 (30%) in UA. In the MD 3rd SG, the proportion was slightly higher than in UA: 356 (47%) vs 253 (51%). The treatment outcomes showed variation across both cohorts and according to the drug-resistance profile. In the MD cohort, the overall success rate was 517 (78%), with 420 (76%) for DS-TB and 54 (69%) for MDR-TB in the 1st G, 315 (70%) patients, comprising 269 (71%) with DS-TB and 32 (69%) with MDR-TB in the 2nd G cohort, 318 (76%), including 279 (80%) for DS-TB and 67 (74%) for MDR-TB in the 3rd G. The proportion of patients lost to follow-up rose slightly from 43 (6%) in the 1st G to 37 (8%) in the 2nd G and 38 (7%) in the 3rd G. the rate of patients who failed treatment and were switched to another regimen remained stable - 36 (5%) in the 1st G, 25 (6%) in the 2nd G and 32 (6%) in the 3rd G. There were 58 deaths (8%) in the 1st G, 51 (11%) in the 2nd G, and 37 (7%) in the 3rd G. Additionally, no outcome data were available for 58 (8%) patients in the 1st G, 51 (11%) in the 2nd G, and 37 (7%) in the 3rd G. In the UA cohort, treatment success increased over time, from 627 (70%) in the 1st G to 393 (68%) in the 2nd G, and reached 580 (76%) in the 3rd G. The proportion of patients lost to follow-up showed a fluctuating pattern, from 69 (5%) in the 1st G to 52 (9%) in the 2nd G, and then to 56 (7%) in the 3rd G. The treatment failure increased from 58 (6%) in the 1st G to 47 (8%) in the 2nd G, and declined to 39 (5%) in the 3rd G. Mortality remained relatively stable across groups, reported in 101 (11%), 78 (13%), and 92 (11%) of cases, respectively. No outcome data were available for 53 (6%) patients in the 1st G, 20 (4%) in the 2nd G, and 6 (1%) in the 3rd G. Lower treatment success rates in both countries during the COVID-19 period reflected resource shortages, while the subsequent improvement indicated enhanced effectiveness of TB care and rehabilitation, driven by gradual alignment with WHO standards in healthcare and TB treatment.

4. DISCUSSION

The majority of TB cases in both countries were identified by primary healthcare providers during the evaluation of symptomatic, TB-related presentations, underscoring their central role in case detection and clinical assessment, confirming the consistently high reliance on primary care in both countries, as stated in multiple studies [4-7]. Active screening conducted by primary healthcare staff showed divergent trends between the two cohorts, as in the MD cohort, the share remained low; in the UA cohort, the proportion initially higher, it declined, indicating a relative reduction in active-screening–driven detection, which is relevant for the actual epidemiological context [7]. Laboratory-diagnosed TB, based on Ziehl–Neelsen smear, conventional culture, and molecular genetic tests, showed a sharply increasing trend in both cohorts, in line with WHO recommendations [2]. The treatment outcomes varied across both cohorts and by drug resistance profile, with higher success rates observed in the post-COVID-19 period, reflecting improved effectiveness driven by the progressive alignment with WHO standards in healthcare and TB treatment. An important limitation of the study was its restricted geographical scope and time period, which limits the generalizability of the findings, particularly for the MDR-TB cohort at the national level, so the results may not fully reflect the broader epidemiological and programmatic context across the entire country.

5. CONCLUSIONS

Primary healthcare providers play a central role in detecting and clinically assessing the majority of TB cases in both countries, including those with clinical symptoms. Active screening by primary healthcare staff showed divergent trajectories: in the MD cohort, the contribution remained limited, while in the UA cohort, it was initially higher but declined over time. The marked increase in laboratory-diagnosed TB, based on Ziehl–Neelsen smear, conventional culture, and molecular genetic tests, reflected the progressive adoption of WHO-recommended diagnostic algorithms and improved laboratory capacity in both countries. Furthermore, treatment outcomes varied by cohort and drug-resistance profile, with higher success rates in the post-COVID-19 period. The COVID-19 pandemic impacted the case-management, determining a marked increase in laboratory-diagnosed TB, a higher rate of severe cases, and lower outcomes, particularly in drug-resistant TB. The results highlighted the importance of strengthening primary care–based case detection, expanding active screening, and maintaining robust laboratory diagnostics to sustain progress in TB control, while acknowledging the need for context-specific adaptations at the national level.

DECLARATIONS

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

Author Contributions: E. L. designed the study; E. L., L. T., and I. S. analyzed data; E. L. drafted the manuscript; all authors have reviewed and approved the final version of the manuscript.

Ethics Statement: This study was conducted in accordance with the Declaration of Helsinki and was approved by the State Pharmacy and Medicine University ethics committees on 13.11.2017. All participants provided informed consent, and data were anonymized to ensure confidentiality.

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

REFERENCES

1. Lipman, M, McQuaid, C.F., Abubakar, I., et al. (2021). The impact of COVID-19 on global tuberculosis control. Indian J Med Res, 153(4), 404-408. https://doi.org/10.4103/ijmr.IJMR_326_21
2. World Health Organization. Global Tuberculosis Report. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024
3. WHO. List of high TB burden countries. https://www.who.int/news/item/17-06-2021-who-releases-new-global-lists-of-high-burden-countries-for-tb-hiv-associated-tb-and-drug-resistant-tb
4. McQuaid, C.F., Vassall, A., Cohen, T., Fiekert, K., White, R.G. (2021). The impact of COVID-19 on TB: a review of the data. Int J Tuberc Lung Dis, 25(6), 436–446.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8171247).
5. Rapoarte SIME TB. http://simetb.ifp.md:8080/tbreps/
6. Compendium of TB/COVID-19 studies. Geneva: World Health Organization (2022). https://www.who.int/teams/global-tuberculosis-programme/covid-19/compendium).
7. Public Health Center of Ukraine. https://phc.org.ua/en/diseases-and-information/hivaids/monitoring-and-evaluation/surveillance/tb-statistics
8. Lan, Y, Crudu, V, Ciobanu, N., et al. (2024). Identifying local foci of tuberculosis transmission in Moldova using a spatial multinomial logistic regression model. EBioMedicine, 102, 105085. https://doi.org/10.1016/j.ebiom.2024.105085

EVALUATION OF THE TOLERANCE OF *SESAMUM INDICUM* L. SAMPLES TO WATER STRESS UNDER CONTROLLED EXPERIMENTAL CONDITIONS

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https://doi.org/10.52757/bsd26.11

ABSTRACT

Water stress is one of the major abiotic factors limiting the growth and productivity of agricultural crops, including *Sesamum indicum L.* The aim of this study was to evaluate the tolerance of sesame samples to water stress under controlled experimental conditions at early stages of development. The study included 15 samples obtained through X-ray induced mutagenesis from the genotypes *Zaltsadovski*, *Kadet* and *Adaptovanîi 2*, as well as the initial genotypes used as controls. Water stress was artificially simulated using a 15% PEG 6000 solution. Tolerance was assessed based on seed germination and seedling morphometric parameters. The results revealed significant differences among genotypes and irradiation treatments, with the most tolerant samples identified in the variants *Zaltsadovski* – 200 Gy, *Kadet* – 50 Gy and *Adaptovanîi 2* – 200 Gy. The results confirm the usefulness of induced mutagenesis for identifying sesame forms with increased tolerance to water deficit.

Keywords: *Sesamum indicum*, water stress, induced mutagenesis, PEG 6000

1. INTRODUCTION

Water stress is a major abiotic factor limiting crop growth, development, and productivity worldwide. Increasing drought frequency due to global climate change reduces agricultural yields and highlights the need to identify genotypes adapted to water-deficit conditions [4, 8]. In this context, investigating the physiological, biochemical, and molecular mechanisms underlying plant responses to water stress remains a key priority in modern agricultural research [1, 2, 3].

Sesamum indicum L. (sesame) is a valuable oilseed crop, appreciated for its high oil content and bioactive compounds of nutritional and medicinal importance. It is mainly cultivated in semi-arid and arid regions, where drought is the principal factor limiting production [5]. Although sesame is relatively drought-tolerant compared with many crops, severe water deficit can markedly reduce seed yield and quality [1]. For controlled evaluation of drought tolerance, water stress is often simulated using polyethylene glycol (PEG) solutions. This method reduces osmotic potential and provides uniform conditions for comparing genotypic responses during germination and early growth. Commonly assessed traits include germination percentage and rate, radicle and shoot length, vigor index, biomass, and relative water content [6, 7]. Tolerance to water stress is a complex polygenic trait strongly influenced by genotype × environment interactions. Recent studies emphasize the role of antioxidant defense mechanisms and the accumulation of osmolytes, such as proline and soluble sugars, in maintaining osmotic balance and protecting cellular structures under water-deficit conditions [1, 10]. Evaluation of drought-tolerance indices based on agromorphological and physiological traits also supports the identification of superior genotypes for breeding programs [3, 9].

In addition, induced mutagenesis and analysis of segregating generations help expand genetic variability and enable selection of stable lines with high adaptive potential [6]. Initial results on drought-tolerant *Sesamum indicum* mutants at early developmental stages confirm the effectiveness of this approach.

Overall, investigating water-stress tolerance in *Sesamum indicum* through modern artificial simulation methods and evaluation of morphophysiological traits is essential for developing drought-adapted varieties. Open-access scientific resources further strengthen the theoretical and applied basis needed to support breeding programs focused on maintaining yield stability under climate change conditions [3,11].

2. MATERIALS AND METHODS

To evaluate drought stress tolerance at the early developmental stage, 15 sesame (*Sesamum indicum* L.) samples obtained through X-ray–induced mutagenesis from the genotypes *Zaltsadovski*, *Kadet*, and *Adaptovanîi 2*, as well as their original genotypes used as controls, were investigated. The seeds were irradiated with doses of 50, 200, 400, and 500 Gy and cultivated in the M_1 generation. The plant material was obtained from the experimental field of the Institute of Genetics, Physiology and Plant Protection of MSU.

The assessment of drought stress tolerance was performed by artificial simulation of water deficit using a 15% polyethylene glycol (PEG 6000) solution, corresponding to an osmotic potential of 0.295 MPa. For each experimental variant, 50 seeds were placed in 90 mm Petri dishes on two layers of filter paper. Two treatments were applied: control (10 ml distilled water) and osmotic stress (10 ml of 15% PEG 6000 solution). The Petri dishes were incubated in darkness at 28°C for 5 days, and the experiment was repeated three times for each variant.

After the incubation period, the number of germinated seeds (G), root length (RL), and shoot length (SL) (mm) were recorded under both control and stress conditions [2].

Drought stress response was evaluated using a Water Stress Response Index (WSRI), which represents an integrative measure of the ability to maintain germination and growth under water deficit conditions. WSRI expresses, in percentage terms, the capacity of genotypes to sustain biological performance under stress and is calculated according to the following equation:

$$\mathrm{WSRI}(\%) = \frac{1}{3}\left(\frac{G_s}{G_m}\, x\, 100 + \frac{RL_s}{RL_m}\, x\, 100 + \frac{SL_s}{SL_m}\, x\, 100\right)$$

where:

G_s, RL_s, SL_s represent germination, root length, and shoot length under osmotic stress conditions;

G_m, RL_m, SL_m represent the corresponding values under control conditions.

As evaluation indicators, germination, root length, and shoot length were analyzed as parameters of growth and development at the early ontogenetic stage.

3. RESULTS AND DISCUSSION

Analysis of variation in the water stress response index (WSRI) of sesame samples obtained through X-ray-induced mutagenesis revealed significant differences among genotypes and irradiation doses. Lower WSRI values indicate greater tolerance to water stress, whereas higher values reflect increased sensitivity to water deficit and differences in phenotypic plasticity.

In the control treatment, seed germination of the studied *Sesamum indicum* samples ranged from 84 to 100%, confirming good suitability for testing. Under osmotic stress induced by PEG 6000, germination decreased to 20–76%.

In the case of samples derived from the *Zaltsadovski* genotype (Figure 1), a pronounced variability of index values is observed depending on the applied irradiation dose. The control variant shows an intermediate level of the index, reflecting the natural response of the genotype to osmotic stress conditions. The application of a 200 Gy dose resulted in the lowest index value, indicating a better capacity to adapt to water stress and, consequently, a higher tolerance of this sample during the early stages of development.

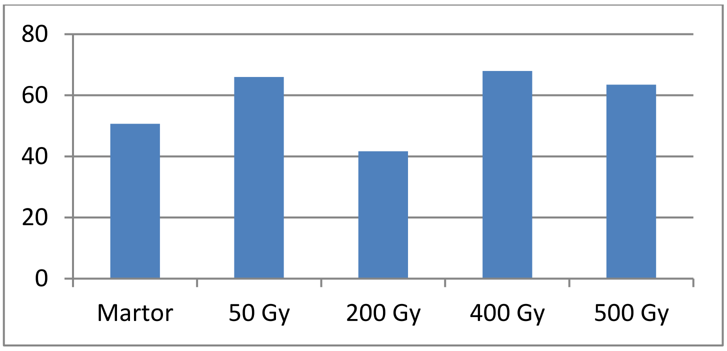

Figure 1. Variation of the water stress response index (%) in sesame samples derived from the Zaltsadovski genotype, depending on the X-ray irradiation dose, during the early stages of development

In contrast, at doses of 50 Gy, 400 Gy, and 500 Gy, higher index values are recorded, suggesting increased sensitivity to water stress, characteristic of genotypes with more pronounced phenotypic plasticity. This variability highlights that induced mutagenesis can generate both forms with enhanced adaptive potential and forms that are more sensitive to water-deficit conditions.

The obtained results are also confirmed by the analysis of seedling morphometric parameters. Thus, regarding root length, in the *Zaltsadovski* – 200 Gy variant, the smallest reduction under osmotic pressure is recorded, with a value of 11.05 mm, compared to the control variant, where root length reached 26.62 mm. A similar trend is observed for shoot length, where the 200 Gy variant shows a value of 12.45 mm, compared to 36.88 mm in the control, confirming a higher stability of growth processes under water stress conditions.

For the samples derived from the *Kadet* genotype (Figure 2), the dynamics of index values indicate a relatively stable response to water stress, but with clear differences among experimental variants. The lowest index value is recorded at the 50 Gy dose, indicating higher tolerance to osmotic stress compared to the control. At doses of 200 Gy, 400 Gy, and 500 Gy, higher index values are observed, suggesting relatively greater sensitivity to water deficit. At the same time, the gradual increase in index values at these doses may reflect more pronounced phenotypic plasticity, expressed through changes in germination rate and seedling growth under stress conditions.

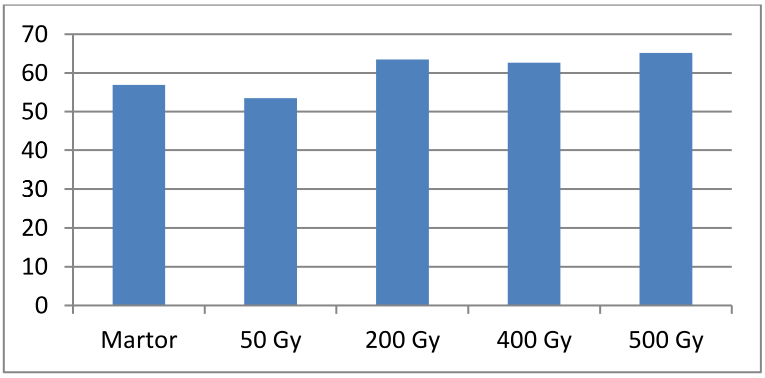

Figure 2. Variation of the water stress response index (%) in sesame samples derived from the Kadet genotype, depending on the X-ray irradiation dose, during the early stages of development

The analysis of growth parameters confirms these trends. Thus, in terms of root length, the smallest difference under osmotic pressure is recorded in the *Kadet* genotype – control, where root length is 11.60 mm, compared to the untreated control variant, where it reached 30.70 mm. A similar situation is observed for shoot length, where the recorded value is 11.75 mm, compared to 33.30 mm in the untreated control variant.

In the case of the *Adaptovanîi 2* genotype (Figure 3), the response of the samples to water stress shows a different pattern compared to the other analyzed genotypes. The 200 Gy dose results in the lowest index value, indicating increased tolerance to water-deficit conditions during the early stages of development. In contrast, the 50 Gy dose leads to the highest index value, suggesting pronounced sensitivity to water stress and reduced plasticity in terms of adaptation to osmotic stress conditions. The values recorded at 400 Gy and 500 Gy are intermediate, indicating a moderate response to stress and suggesting the presence of forms with a medium level of adaptability.

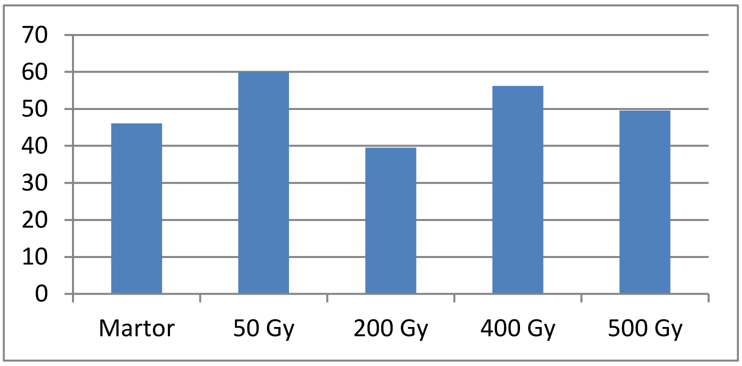

Figure 3. Variation of the water stress response index (%) in sesame samples derived from the Adaptovanîi 2 genotype, depending on the X-ray irradiation dose, during the early stages of development

The results are also supported by the analysis of morphometric parameters. Thus, in terms of root length, the *Adaptovanîi 2* – 200 Gy genotype shows the smallest difference under osmotic stress, with a value of 17.17 mm, compared to the control variant, where root length was 29.31 mm. A similar trend is observed for shoot length, where the 200 Gy variant records a value of 12.00 mm, compared to 36.93 mm in the control.

The comparative analysis of results for the three genotypes demonstrates that the response to water stress depends both on the genetic background of the initial genotype and on the intensity of the applied mutagenic factor. In general, intermediate irradiation doses favored the emergence of samples with lower values of the stress response index, indicating higher tolerance to water deficit. At the same time, higher index values reflect genotype sensitivity and differing levels of phenotypic plasticity, which may be explained by genetic modifications induced by ionizing radiation.

4. CONCLUSIONS

1. The testing of sesame samples obtained through X-ray-induced mutagenesis revealed significant variability in the response to water stress during the early stages of development, determined both by the genetic background of the genotypes and by the applied irradiation doses.
2. The artificial simulation of water stress using PEG 6000 (15%) resulted in a considerable reduction in seed germination (from 84–100% to 20–76%), confirming the effectiveness of the method for evaluating tolerance to water deficit.

3. The most tolerant samples were identified in the variants *Zaltsadovski* – 200 Gy, *Kadet* – 50 Gy, and *Adaptovanîi 2* – 200 Gy, which showed lower values of the stress response index and smaller differences in growth parameters under osmotic pressure conditions.
4. The obtained results confirm that X-ray-induced mutagenesis can generate sesame forms with enhanced adaptive potential to water stress, representing valuable material for breeding programs aimed at developing drought-tolerant varieties.

ACKNOWLEDGMENTS

The research was conducted within the framework of Subprogram 011102 ”Increasing and conservation genetic diversity, agricultural crop breeding in the context of climate change”, funded by the Ministry of Education and Research of the Republic of Moldova.

DECLARATION

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

Data Availability Statement: The datasets generated during the current study are available from the corresponding author upon reasonable request.

REFERENCES

1. Baghery, M. A., Kazemitabar, S. K., Dehestani, A., & Mehrabanjoubani, P. (2023). Sesame (*Sesamum indicum* L.) response to drought stress: Susceptible and tolerant genotypes exhibit different physiological, biochemical, and molecular response patterns. *Physiological and Molecular Biology of Plants, 29*(9), 1353–1369. https://doi.org/10.1007/s12298-023-01335-0
2. Donghua, L., Dossa, K., Zhang, Y., et al (2018). GWAS uncovers differential genetic bases for drought and salt tolerances in sesame at the germination stage. *Genes, 9*(2), 87. https://doi.org/10.3390/genes9020087
3. Dossa, K., Li, D., Wang, L., et al. (2017). The emerging oilseed crop sesame: Current status, challenges, and future prospects. *Frontiers in Plant Science, 8*, 1–17.. ISSN (online): 1664-462X.
4. Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. *Agronomy for Sustainable Development, 29*, 185–212. https://doi.org/10.1051/agro:2008021
5. Golestani, M., & Pakniyat, H. (2015). Evaluation of traits related to drought stress in sesame (*Sesamum indicum* L.) genotypes. *Journal of Asian Scientific Research, 5*(7), 388–396.
6. Kouighat, M., Hanine, H., El Fechtali, M., & Nabloussi, A. (2021). First report of sesame mutants tolerant to severe drought stress during germination and early seedling growth stages. *Plants, 10*(6), 1133. https://doi.org/10.3390/plants10061133
7. Mogilda, A. (2020). Toleranța genotipurilor de susan (*Sesamum indicum* L.) la stresul hidric în condițiile modelării lui artificiale. *Studia Universitatis Moldaviae (Seria Științe Reale și ale Naturii), 6*(136), 64–68.
8. Raza, A., Razzaq, A., Mehmood, S. S., et al. (2019). Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants, 8*(2), 34.. ISSN (online): 2223-7747.
9. Yemata, G., & Bekele, T. (2024). Evaluation of sesame (*Sesamum indicum* L.) varieties for drought tolerance using agromorphological traits and drought tolerance indices. *PeerJ, 12*, e17542. https://doi.org/10.7717/peerj.17542
10. You, J., Zhang, Y., Liu, A., et al. (2019). Transcriptomic and metabolomic profiling of drought tolerant and susceptible sesame genotypes in response to drought stress. *BMC Plant Biology, 19*, 267. https://doi.org/10.1186/s12870-019-1880-1
11. Food and Agriculture Organization of the United Nations. (2023). *Drought and agriculture*. https://www.fao.org/land-water/water/drought/droughtandag/en/.

CATALASE ACTIVITY OF FUNGAL STRAINS FROM THE GENERA *PENICILLIUM*, *ASPERGILLUS*, AND *TRICHODERMA*

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https://doi.org/10.52757/bsd26.12

ABSTRACT:

The catalase activity of fungi is particularly important, playing an essential role in protecting cells against oxidative stress by decomposing hydrogen peroxides. This enzyme contributes to maintaining cellular homeostasis and supports the adaptation of microbes to stressful environmental conditions. In addition, the antioxidant capacity of fungal strains has relevant applications in biotechnology, bioremediation and the pharmaceutical industry, offering prospects for the use of these microorganisms in industrial processes and fundamental research. In this study, a screening of 23 fungal strains from the genera *Penicillium*, *Trichoderma* and *Aspergillus* was performed to evaluate their catalase capacity. The results showed a variability of catalase activity between strains, with maximum values recorded in *Penicillium* spp. 18 and *Trichoderma* spp. 12 strains, highlighting differences in the capacity to neutralize oxidative stress between genera.

Keywords: catalase, antioxidant activity, fungi, biotechnology.

1. INTRODUCTION

Fungi represent an important biotechnological resource, being used as bioproducers of metabolites and enzymes with industrial and biomedical applications (Bils, 2016; Ayuningtyas, 2021). Among the enzymes of major interest, catalase occupies a central place due to its essential role in antioxidant protection and cellular detoxification processes.

Catalase is an antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen, preventing the accumulation of reactive oxygen species (ROS) and limiting oxidative stress. By regulating ROS levels, this enzyme contributes to maintaining cellular homeostasis and influences fundamental processes such as proliferation, apoptosis and metabolism. Alteration of catalase activity is associated with various metabolic and inflammatory conditions, and its therapeutic potential is being investigated in neurodegenerative, cardiovascular and inflammatory pathologies. At the same time, gene therapy strategies aimed at increasing catalase expression highlight its biological and applied importance (Rasheed, 2024; Gebicka, 2019).

From an industrial perspective, catalase is mainly obtained from mammalian liver and from the filamentous fungus *Aspergillus niger*, appreciated for its stability and high enzymatic yield. In parallel, microorganisms adapted to low temperatures (psychrophiles and psychrotrophs) represent an underexplored but promising source, due to their increased catalytic activity in the range of 0–20 °C and the reduced risk of microbiological contamination, characteristics relevant for industrial processes carried out at moderate or low temperatures (Gharaghani, 2022).

Catalase activity has also been demonstrated in species such as *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus nidulans*, *Aspergillus flavus* and *Aspergillus fumigatus*, where it contributes to survival under oxidative conditions and adaptation to the host environment.

However, for many other *Aspergillus* species, data on the regulation and expression level of catalase remain limited, which highlights the need for further research in this area (Aggez, 2022; Gharaghani, 2022).

The current interest in the valorization of fungal catalases is supported by their potential in sustainable industrial processes and in the development of enzymes adapted to extreme conditions. In particular, the exploration of psychrophiles and psychrotrophs for catalase biosynthesis is at an early stage, but offers relevant perspectives for expanding the biotechnological applications of these enzymes (El-Elimat, 2021; Gharaghani, 2022).

In conclusion, fungal catalases constitute an essential element of defense mechanisms against oxidative stress and represent an area of major interest from both a biological and biotechnological perspective; in this context, the aim of our research is to investigate and identify new fungal strains with increased catalase activity.

2. MATERIALS AND METHODS

Catalase activity was determined by the standard spectrophotometric method, based on the ability of hydrogen peroxide to react with ammonium molybdate, forming a stable-colored complex, quantifiable spectrophotometrically.

A quantity of 0.1 mL of metabolite solution and 2 mL of 0.03% H_2O_2 solution were introduced into the reaction mixture. After incubation for 10 minutes at room temperature, the reaction was stopped by adding 1 mL of 4% ammonium molybdate solution, which determines the formation of the colored complex. Catalase activity was expressed as a function of the variation of the optical density of the sample at a wavelength of 410 nm compared to the control (Komina, 2012; Titova, 2012).

Catalase activity was calculated using the formula:

Activity CAT (U/mg protein)= $(Abs_{control} - Abs_{sample}) \cdot V / v \cdot t \cdot \varepsilon \cdot C_{prot}$

where:

Abs_control – absorbance of the control sample

Abs_sample – absorbance of the analyzed sample

V – total reaction volume (mL)

v – sample volume (mL)

t – incubation time (min)

ε – extinction coefficient

C_prot – protein concentration in the sample (mg/mL)

Spectrophotometric determinations were performed, using cuvettes with an optical layer thickness of 1 cm, all samples were analyzed in triplicate to ensure reproducibility of the results, and the results were expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The study of the catalase activity of fungi is of particular importance, given the essential role of the catalase enzyme in cellular protection against oxidative stress, as well as the application potential of these microorganisms in fields such as biotechnology, bioremediation and the pharmaceutical industry (Poljovka, 2023; Dishliyska, 2023).

As part of the research, a screening of 23 fungal strains belonging to the genera *Penicillium*, *Trichoderma* and *Aspergillus* was carried out with the aim of evaluating catalase activity.

For the screening, 8 strains belonging to the genus *Penicillium*, 3 strains from the genus *Aspergillus*, 8 strains from the genus *Trichoderma* were selected. Their selection was based on the previously demonstrated biotechnological interest, since, in preliminary experiments, the analysed strains showed increased levels of catalase activity, determined by express methods of enzymatic evaluation.

Within the genus *Penicillium* spp., catalase activity showed considerable variations compared to other genera, with values ranging between 123.9 and 430.4mmol/min/mg protein. The lowest activity was recorded in strain *Penicillium* sp. 6 123.9861 mmol/min/mg protein, while strain *Penicillium* sp. 18 showed the highest enzymatic activity 430.42 mmol/min/mg protein. This variability suggests significant differences in the capacity to respond to oxidative stress, possibly correlated with adaptation to the specific conditions in the aquatic microhabitats of origin.

In the case of the genus *Aspergillus*, the catalase activity values were more homogeneous, ranging between 169 and 252 mmol/min/mg protein. The *Aspergillus* sp. 23 strain showed the highest activity 252.15 mmol/min/mg protein, closely followed by the *Aspergillus* sp. 22 strain 250.75 mmol/min/mg protein, while the *Aspergillus* sp.4 strain recorded a lower activity 169.8 mmol/min/mg protein. Compared to the *Penicillium* strains, the maximum level of catalase activity in the strains of the genus *Aspergillus* was lower, suggesting a relatively lower antioxidant capacity under the analyzed experimental conditions (Fig. 1).

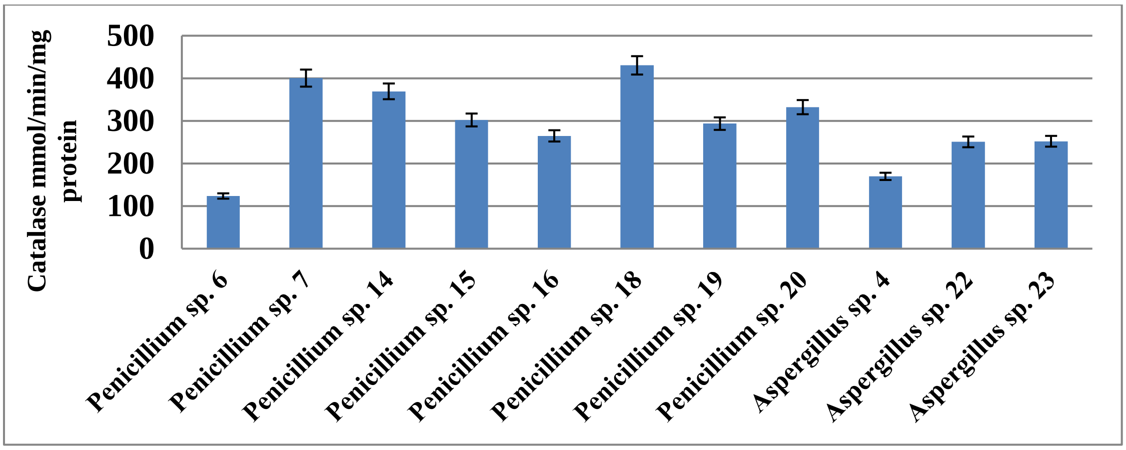

Figure 1. Catalase activity of *Penicillium* and *Aspergillus* fungal strains

Regarding strains belonging to the genus *Trichoderma*, catalase activity also revealed a pronounced metabolic variability, suggesting differences in the capacity to neutralize oxidative stress between isolates (Fig. 2).

The values recorded ranged from 118 to 342 mmol/min/mg protein. The lowest catalase activity was observed in *Trichoderma* sp. 9 strain 118.2 mmol/min/mg protein, closely followed by *Trichoderma* sp. 8 strain 122.84 mmol/min/mg protein. *Trichoderma* sp. 10, 11 and 13 strains showed relatively close values of 140–143 mmol/min/mg protein, indicating a moderate and relatively uniform level of enzymatic activity.

A higher level was revealed in the strains *Trichoderma* sp. 21 with values of 202 mmol/min/mg protein and the strain *Trichoderma* sp.17 228.8424 mmol/min/mg protein, which may reflect an increased capacity to adapt to conditions with higher oxidative potential. The strain *Trichoderma* sp. 12 is remarkable, which presented the highest catalase activity within this genus 342.2 mmol/min/mg protein, significantly exceeding the other isolates of the analyzedgenus *Trichoderma*.

Compared to other previously studied genera, the maximum level of catalase activity in *Trichoderma* sp. 12 is comparable to the high values recorded in some *Penicillium* strains. At the same time, most strains of the genus *Trichoderma* fall within an intermediate range of enzymatic activity, suggesting a balanced antioxidant profile. This distribution of catalase activity indicates a specific intra-genus variability, which may reflect differential adaptations to oxidative stress conditions and highlights the selective potential of certain strains for biotechnological applications.

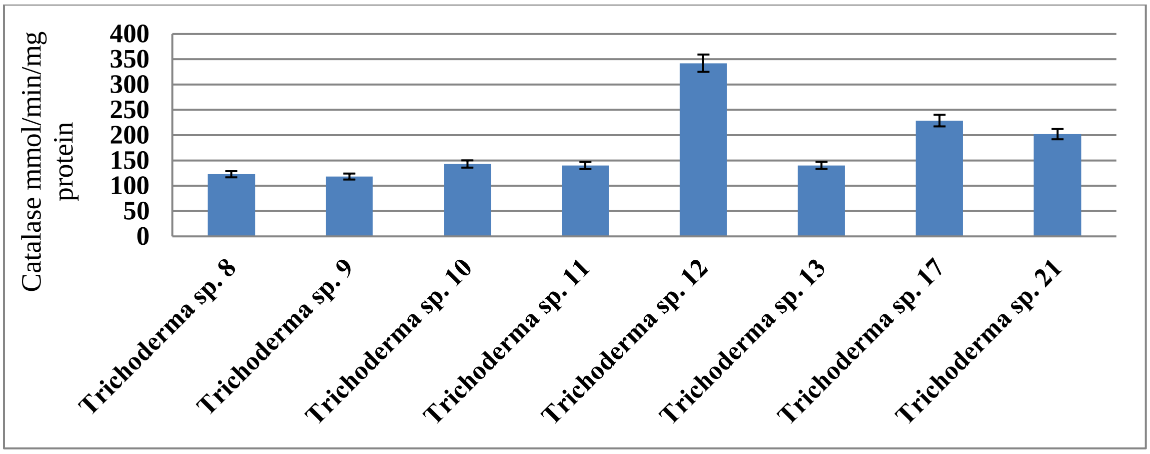

Figure 2. Catalaseactivity of micromycetes of thegenus*Trichoderma*

The data obtained in the study indicate that fungal strains from the genera *Penicillium*, *Aspergillus* and *Trichoderma* are significant producers of catalase, which is consistent with observations reported in the literature. In particular, strains *Penicillium* sp. 18 and *Trichoderma* sp. 12 exhibited high catalase activities (430.42 and 342.2 mmol/min/mg protein, respectively), highlighting a strong antioxidant profile and supporting the potential of these genera for biotechnological applications.

The results obtained are in accordance with those reported by Aggez C. (2022), who highlighted the maximum catalase production by *Aspergillus fumigatus* after 7 days of cultivation (213 U/mL). Regarding the genus *Trichoderma*, studies conducted by Zapata-Sarmiento D. (2025) demonstrated that the response to oxidative stress induces an increase in the activity of catalase and peroxidases, thus highlighting the role of these enzymes in the adaptation and survival of fungi under challenging environmental conditions.

Furthermore, Koleva Z. et al. (2024) highlighted the interest in extracellular catalase, due to its biotechnological applicability and ease of purification, indicating that *Penicillium* constitutes a valuable source of catalase for industrial and biotechnological processes. These findings support our observations on the high activity of *Penicillium* sp. 18 strains, compared to other analyzedstrains, and confirm their potential as efficient catalase producers.

4. CONCLUSIONS

As a result of the research, the strains *Penicillium* sp. 18 (430.42 mmol/min/mg protein) and *Trichoderma* sp. 12 (342.2 mmol/min/mg protein) were identified as producing catalase at significantly higher levels compared to the other analyzedstrains. These results highlight the metabolic variability between isolates and suggest a superior capacity to neutralize oxidative stress in these strains. The selection of these microorganisms for catalase production demonstrates their biotechnological potential, indicating the possibility of their use as efficient producers of antioxidant enzymes.

ACKNOWLEDGMENTS: This research was funded by Government of Republic of Moldova, Ministry of Educationand Research, Research Subprogram 020101 “InBioS – Innovative biotechnological solutions for agriculture, medicine andenvironment”. Institutional financing contract no. 4/FI of 22 February 2024.

DECLARATIONS

The authors declare that they have no conflict of interest.

Author contributions: All authors contributed equally to the development and writing of this article.

*Originality Statement:*The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

REFERENCES

1. Aggez, C. A., & Karakus, Y. Y. (2022). Production, purification and characterization of catalase from Aspergillus fumigatus. Journal of Microbiology & Biotechnology, 7. https://doi.org/10.23880/oajmb-16000246
2. Ayuningtyas, E., Sibero, M., Elisabet, B. R. H., Nadia, F., Evan, Murwani., Retno, Z., Dewi, S., Wijayanti, D., Sabdono, A., Pringgenies, D., Radjasa, O., & Zhang, Z. (2021). Screening of extracellular enzyme from phaeophyceae-associated fungi. IOP Conference Series: Earth and Environmental Science. https://doi.org/10.1088/1755-1315/750/1/012005
3. Bills, G. F., & Gloer, J. B. (2016). Biologically active secondary metabolites from the fungi. Microbiology Spectrum, 4(6). https://doi.org/10.1128/microbiolspec.FUNK-0023-2016
4. Dishliyska, V., Stoyancheva, G., Abrashev, R., Miteva-Staleva, J., Spasova, B., Angelova, M., &Krumova, E. (2023). Catalase from the Antarctic fungus *Aspergillus fumigatus* I-9: Biosynthesis and gene characterization. Indian Journal of Microbiology, 63(4), 541–548. https://doi.org/10.1007/s12088-023-01110-8
5. El-Elimat, T., Raja, H. A., Figueroa, M., Sharie, A. H., Bunch, R. L., & Oberlies, N. H. (2021). Freshwater fungi as a source of chemical diversity: A review. Journal of Natural Products, 84, 898–916. https://doi.org/10.1021/acs.jnatprod.0c01059
6. Gebicka, L., & Krych-Madej, J. (2019). The role of catalases in the prevention/promotion of oxidative stress. Journal of Inorganic Biochemistry, 197, 110699. https://doi.org/10.1016/j.jinorgbio.2019.110699
7. Gharaghani, M., Jafarian, H., Hatami, M., Shabanzadeh, M., & Zarei Mahmoudabadi, A. (2022). Evaluation of catalase activity of clinical and environmental isolates of *Aspergillus* species. Iranian Journal of Microbiology, 14(1), 133–137. https://doi.org/10.18502/ijm.v14i1.8815
8. Koleva, Z., Abrashev, R., Angelova, M., Stoyancheva, G., Spassova, et al. (2024). A novel extracellular catalase produced by the Antarctic filamentous fungus *Penicillium rubens* III11-2. Fermentation, 10, 58. https://doi.org/10.3390/fermentation10010058
9. Komina, A. V., Korostileva, K. A., Gyrylova, S. N., Belonogov, R. N., & Ruksha, T. G. (2012). Interaction between single nucleotide polymorphism in catalase gene and catalase activity under the conditions of oxidative stress. Physiological Research, 61, 655–658. https://doi.org/10.33549/physiolres.932333
10. Titova, N.M., Subbotina, T.N. (2012) Enzymology: Laboratory Workshop (in Russian). Krasnoyarsk: Siberian Federal University. 60 p. https://elib.sfu-kras.ru/handle/2311/63424
11. Poljovka, A., Musil, M., Bednář, D., Chovanová, K., Bauerová-Hlinková, V., Bellová, J., Kohútová, L., Baráth, P., &Zámocký, M. (2023). Comparison of fungal thermophilic and mesophilic catalase–peroxidases for their antioxidative properties. Antioxidants, 12(7), 1382. https://doi.org/10.3390/antiox12071382
12. Rasheed, Z. (2024). Therapeutic potentials of catalase: Mechanisms, applications, and future perspectives. International Journal of Health Sciences (Qassim), 18(2), 1–6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10915913/
13. Zapata-Sarmiento, D. H., Rodríguez-Hernández, A. A., Sepúlveda-Jiménez, G., & Rodríguez-Monroy, M. (2025). Tolerance and antioxidant response to heavy metals are differentially activated in *Trichoderma asperellum* and *Trichoderma longibrachiatum*. PeerJ, 13, e19016. https://doi.org/10.7717/peerj.19016

REMOTE SENSING-BASED ASSESSMENT OF LAND USE DYNAMICS AND ECOSYSTEM SERVICES IN ALBANIAN COASTAL LAGOONS: IMPLICATIONS FOR SUSTAINABLE TOURISM DEVELOPMENT

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https://doi.org/10.52757/bsd26.13

ABSTRACT

Coastal lagoons of Albania are multifunctional ecosystems that support biodiversity, regulate environmental processes, and sustain local economies through fisheries, agriculture, and tourism. In recent decades, urban expansion and tourism development have increased pressure on wetlands. The Kune-Vain wetlands, located between Shëngjin and Lezhë, are an important protected wetland on the northern Albanian coast and are increasingly affected by land transformation. This study investigates land use dynamics and vegetation changes in the Kune-Vain wetlands using remote sensing techniques and assesses their implications for ecosystem services and sustainable tourism development. The research covers the wetland ecosystem and adjacent coastal and urban areas influenced by the expansion of Shëngjin and Lezhë. Multi-temporal land use/land cover (LULC) datasets derived from Copernicus CORINE data were used to evaluate spatial changes. Vegetation dynamics were analyzed through the Normalized Difference Vegetation Index (NDVI) derived from satellite imagery representing historical conditions from the late 1980s–early 1990s and recent observations. GIS techniques were applied to identify patterns of urban growth, land transformation, and vegetation variability. Preliminary results indicate progressive land transformation around the wetlands, especially in areas affected by urbanization and tourism. Built-up areas expanded, surrounding landscapes were modified, and NDVI showed localized vegetation changes in sensitive wetland zones. Continuous spatial monitoring is essential for sustainable tourism planning and integrated coastal management.

Keywords: Kune-Vain Lagoon, LULC change, NDVI, ecosystem services, sustainable tourism, urban expansion

1. INTRODUCTION

Coastal wetlands are among the most productive and valuable ecosystems worldwide, providing essential ecological functions and ecosystem services such as habitat provision, water regulation, shoreline protection, nutrient retention, carbon storage, and support for fisheries and tourism-related activities [1, 2]. In the Mediterranean region, lagoon and wetland systems represent highly dynamic environments that are particularly sensitive to land-use change, hydrological alteration, and climate-related pressures [7]. Anthropogenic activities, including urban expansion, agricultural intensification, tourism development, and infrastructure growth, are widely recognized as major drivers of wetland transformation and habitat degradation in coastal regions [9]. In Albania, coastal lagoon ecosystems such as Kune–Vain, Karavasta, and Narta have been identified as areas of national and international ecological importance due to their biodiversity and ecological functions [3,4]. However, recent socio-economic development and land-use pressures have increased the vulnerability of these systems, particularly in areas influenced by settlement expansion and tourism-related activities. Remote sensing and

Geographic Information Systems (GIS) provide efficient tools for monitoring environmental changes in wetland ecosystems over extended time periods. Multi-temporal analysis of satellite imagery enables the identification of land-use and land-cover changes, while vegetation indices such as the Normalized Difference Vegetation Index (NDVI) are widely used to assess vegetation condition and ecosystem dynamics [5]. These techniques allow the detection of spatial and temporal changes that support environmental management and conservation planning.

Despite the recognized ecological importance of the Kune–Vain lagoon system, relatively limited studies have focused on long-term spatial analysis of vegetation dynamics and wetland transitions using multi-temporal satellite data. Therefore, the aim of this study is to assess vegetation dynamics and wetland extent changes in the Kune–Vain wetlands using remote sensing and GIS techniques. Specifically, the study evaluates: (i) long-term changes in NDVI-based vegetation density; (ii) vegetation dynamics between 1985 and 2024; and (iii) wetland extent changes between 2000 and 2018.

2. MATERIALS AND METHODS

2.1 Study Area

The Kune–Vain wetlands ecosystem is located along the northwestern coastal plain of Albania, near the mouth of the Drini River and in proximity to the city of Lezhë and the town of Shëngjin. The wetland complex consists of lagoons, marshes, agricultural lands, and transitional vegetation zones influenced by both marine and freshwater processes. The study area covers approximately 106.53 km², including wetland and surrounding land-use areas influencing wetland dynamics.

The ecosystem is recognized for its biodiversity and ecological importance but has experienced increasing pressure from land-use change, agricultural development, and urban expansion over recent decades [4,9] (Fig. 1).

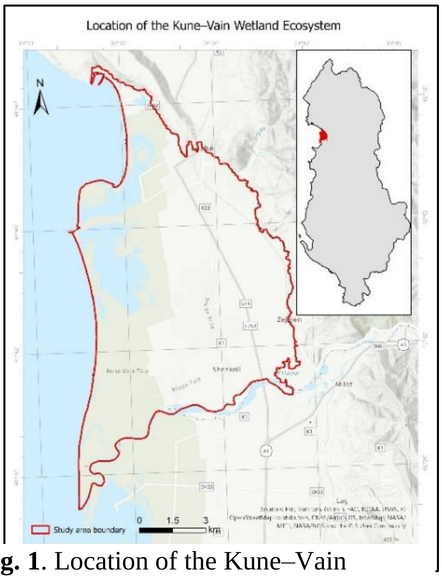

Fig. 1. Location of the Kune–Vain wetlands ecosystem

2.2 Satellite Data and Image Processing

Multi-temporal satellite imagery from the Landsat program was used to analyze vegetation dynamics in the Kune–Vain wetlands. Landsat 5 Thematic Mapper (TM) images were used for the years 1985 and 2004, while Landsat 9 Operational Land Imager (OLI) imagery was used for 2024. All images were selected from similar seasonal periods to minimize seasonal variability and ensure comparability between years. Preprocessing steps included radiometric and atmospheric corrections using standard surface reflectance products. The imagery was processed within a GIS environment, and all datasets were projected to a common coordinate system to ensure spatial consistency across temporal datasets.

2.3 NDVI Calculation and Change Detection

Vegetation dynamics were assessed using the Normalized Difference Vegetation Index (NDVI), which is widely applied in vegetation monitoring and wetland studies [6,8]. NDVI was calculated using the red and near-infrared spectral bands of Landsat imagery. The resulting NDVI values were classified into vegetation density categories to support spatial comparison of vegetation conditions across different time periods. To evaluate long-term vegetation dynamics, NDVI difference analysis was performed between 1985 and 2024, allowing identification of areas with vegetation increase, decrease, or stability. This approach enabled the detection of spatial patterns of vegetation change across the wetland ecosystem.

2.4 Land-Use Change and Wetland Transition Analysis

Land-use and wetland dynamics were analyzed using classified land-cover datasets representing wetland extent for the years 2000 and 2018. These datasets were used to evaluate wetland loss, conversion, and internal transformation processes. Spatial overlay analysis was applied to identify areas of wetland persistence, conversion to open water, and internal wetland transitions. Additionally, urban expansion patterns were assessed by comparing land-use classes between the selected years, allowing identification of areas where wetlands were affected by urban development. All spatial analyses were conducted using geographic information system (GIS) tools, enabling quantification of area changes and visualization of spatial transformation patterns.

3. RESULTS

The spatial distribution of NDVI-based vegetation density classes in the Kune–Vain wetlands for the years 1985, 2004, and 2024 is presented in Fig. 2, while the quantitative distribution of vegetation classes is summarized in Table 1.

In 1985, the study area was predominantly characterized by bare soil or very sparse vegetation, covering 75.29 km² (70.72%) of the total area. Areas classified as sparse to moderate vegetation occupied 24.64 km² (23.13%), while water bodies covered 6.53 km² (6.13%). Moderate to dense vegetation was nearly absent, accounting for only 0.01 km² (0.01%), indicating limited development of dense vegetation structures during this period.

By 2004, bare soil or very sparse vegetation decreased to 69.02 km² (64.83%), while sparse to moderate vegetation increased to 32.78 km² (30.79%). Water bodies declined to 4.67 km² (4.38%), indicating spatial redistribution of vegetation and surface water [5].

In 2024, bare soil or very sparse vegetation declined to 56.79 km² (53.33%), while sparse to moderate vegetation expanded to 40.38 km² (37.91%). Water coverage increased to 9.22 km² (8.66%), and moderate to dense vegetation slightly increased to 0.08 km² (0.07%), indicating localized vegetation densification [2]. Vegetation dynamics between 1985 and 2024 are illustrated in Fig. 3, and the corresponding spatial distribution of change categories is summarized in Table 2.

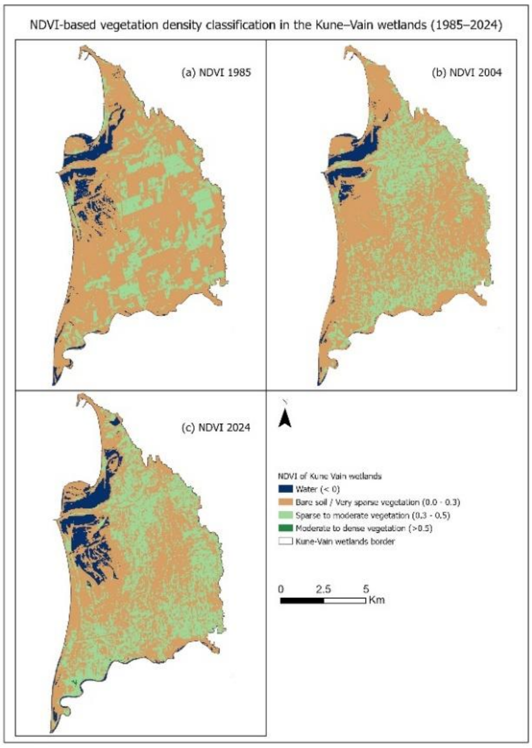

Fig. 2. Spatial distribution of NDVI-derived vegetation density classes in the Kune–Vain wetlands for (a) 1985, (b) 2004, and (c) 2024.

Table 1. Area (km²) and percentage (%) distribution of NDVI-based vegetation density classes in the Kune–Vain wetlands for the years 1985, 2004, and 2024.

NDVI Class	1985 Area (km²)	1985 (%)	2004 Area (km²)	2004 (%)	2024 Area (km²)	2024 (%)
Water (<0.0)	6.53	6.13	4.67	4.38	9.22	8.66
Bare soil /very sparse vegetation (0.0–0.3)	75.29	70.72	69.02	64.83	56.79	53.33
Sparse to moderate vegetation (0.3–0.5)	24.64	23.13	32.78	30.79	40.38	37.91
Moderate to dense vegetation (>0.5)	0.01	0.01	0.00	0.00	0.08	0.07

Table 2. NDVI change statistics

Change category	Area (km²)	Percentage (%)
Vegetation decrease (-1)	15.09	14.17
No change (0)	63.23	59.37
Vegetation increase (1)	28.21	26.45
Total	106.53	100

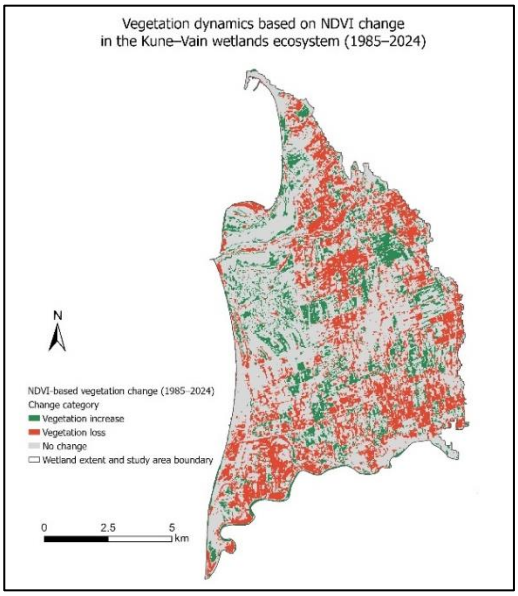

Fig. 3. Vegetation dynamics in the Kune–Vain wetlands ecosystem derived from NDVI change analysis between 1985 and 2024.

The results indicate that 63.23 km² (59.37%) of the study area remained stable during the analysis period, representing the dominant category of vegetation persistence. Areas experiencing vegetation increase accounted for 28.21 km² (26.45%), while vegetation decrease was observed across 15.09 km² (14.17%). Vegetation increase was primarily distributed in inland transitional zones and areas characterized by gradual vegetation recovery, whereas vegetation decrease was mainly concentrated along coastal margins and selected zones affected by land-use intensification. Such spatial patterns of vegetation stability combined with localized increase and decrease are commonly observed in coastal wetland ecosystems undergoing both natural and anthropogenic pressures [1,7]. Wetland extent changes between 2000 and 2018 are presented in Fig. 4, showing spatial patterns of wetland persistence and localized wetland loss.

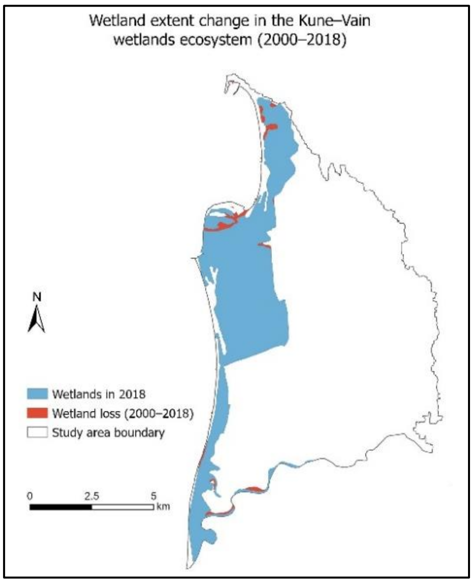

Fig. 4. Wetland extent change in the Kune–Vain wetlands ecosystem between 2000 and 2018.

The spatial analysis indicates that large portions of the wetland system remained stable during the observation period, while localized wetland loss occurred along lagoon margins and coastal sectors. These zones of wetland reduction were mainly concentrated along dynamic shoreline environments and transitional wetland boundaries, reflecting areas sensitive to hydrological variability and land-use pressures. Such localized wetland losses are consistent with patterns reported in Mediterranean coastal systems influenced by shoreline processes, water-level fluctuations, and human-induced landscape modification [9].

4. DISCUSSION

The results indicate clear spatial changes in vegetation distribution and wetland extent within the Kune–Vain wetland system. The reduction of sparsely vegetated surfaces and expansion of moderate vegetation zones suggest gradual vegetation development influenced by natural succession and land-use adjustments [2]. Localized vegetation decrease along coastal margins and wetland loss near lagoon boundaries highlight areas sensitive to shoreline dynamics and hydrological variability, consistent with observations from Mediterranean wetland environments [7].

The applied GIS and remote sensing approach proved effective for detecting vegetation and wetland dynamics; however, medium-resolution imagery may limit detection of small-scale features. Future studies may benefit from higher-resolution datasets and additional environmental indicators.

5. CONCLUSIONS

This study assessed vegetation dynamics and wetland extent changes in the Kune–Vain wetland ecosystem using multi-temporal satellite imagery and GIS-based analysis. The results indicate a gradual reduction in sparsely vegetated areas and localized wetland loss, while large portions of the system remained stable over time. NDVI-based change detection proved effective in identifying spatial vegetation trends and wetland dynamics. The applied methodology provides a practical framework for supporting wetland monitoring and environmental management in coastal ecosystems.

DECLARATIONS

Conflict of Interest Statement: The authors declare no conflict of interest.

Author Contributions: S.P. conceptualized the study, processed the data, prepared all maps and figures, drafted the manuscript, and finalized the paper. A.H. contributed to literature review and interpretation. V.P. contributed to methodology and revision. All authors approved the final manuscript.

Data Availability Statement: The data used in this study are available from the corresponding author upon reasonable request.

*Ethics Statement***:** Not applicable.

*Originality Statement***:** The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

REFERENCES

1. Costanza, R., Anderson, S. J., Sutton, P., Mulder, K., Mulder, O., Kubiszewski, I., Wang, X., Liu, X., Pérez-Maqueo, O., Martinez, M. L., Jarvis, D., & Dee, G. (2021). The global value of coastal wetlands for storm protection. Global Environmental Change, 70, 102328. https://doi.org/10.1016/j.gloenvcha.2021.102328
2. Finlayson, C. M., Gardner, R. C., & Davidson, N. C. (2020). The Ramsar Convention and its role in sustainable wetland management. Marine and Freshwater Research, 71, 1–5.
3. Miho, A., & Shumka, S. (2007). The lagoons of Albania: Ecological features and conservation status. Mediterranean Marine Science, 8, 5–20.
4. Miho, A., Vasjari, M., Wallja, L., Kashta, L., & Qirjo, M. (2019). Climate change adaptation interventions of the Kune–Vaini lagoon system – ecological approach. In Third International Conference “Biotechnology in Agriculture”. Agricultural University of Tirana.
5. Muro, J., Varea, A., Strauch, A., Guelmami, A., Fitoka, E., Thonfeld, F., Diekkrüger, B., & Waske, B. (2020). Multitemporal optical and radar metrics for wetland mapping at national level in Albania. Heliyon, 6, e04496. https://doi.org/10.1016/j.heliyon.2020.e04496
6. Pettorelli, N., Vik, J. O., Mysterud, A., Gaillard, J.-M., Tucker, C. J., & Stenseth, N. C. (2005). Using the satellite-derived NDVI to assess ecological responses to environmental change. Trends in Ecology & Evolution, 20(9), 503–510. https://doi.org/10.1016/j.tree.2005.05.011
7. Ramsar Convention Secretariat. (2021). Global Wetland Outlook: Special Edition 2021. Ramsar Convention Secretariat.
8. Tucker, C. J. (1979). Red and photographic infrared linear combinations for monitoring vegetation. Remote Sensing of Environment, 8(2), 127–150.
9. UNEP & Ministry of Environment (MoE). (2010). Land use change in Kune-Vain and Patok. United Nations Environment Programme & Ministry of Environment, Albania.

VIABILITY OF FUNGAL AND BACTERIAL STRAINS AFTER LYOPHILIZATION IN AN OPTIMAL PROTECTIVE MEDIUM

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https://doi.org/10.52757/bsd26.14

ABSTRACT:

In recent decades, microorganisms have increasingly influenced all human activity, which requires special attention to studies of estimating the biodiversity of microorganisms and their application in various biotechnological processes. Storage, maintaining the viability and long-term stability of microorganisms of scientific and practical interest are the main tasks of all collections in the world. Lyophilization is one of the main methods of preserving microorganisms and long-term storage. Maintaining the viability of lyophilized microorganisms depends on various factors: taxonomic peculiarities of the strains, the usedprotective medium, the revitalization medium, etc.

In this study, the viability of fungal and bacterial strains after lyophilization on optimized protective media containing skimmed milk supplemented with carbohydrates and ascorbic acid as an antioxidant was evaluated. The results obtained demonstrated a high viability of 92.7 - 98%, of fungal strains from the genus *Penicillium* and *Trichoderma* and of 75.5 - 91.2% of bacteria from the genera *Bacillus*, *Pseudomonas*, *Micrococcus*.

Keywords: fungi, bacteria, conservation, lyophilization, viability.

1. INTRODUCTION

For long-term preservation of most bacteria, yeasts and fungi, lyophilization is used as a safe method of preservation with conservation of the initial productive properties and a long storage period. Lyophilization is based on a biological principle – anabiosis and on two physical processes: freezing and sublimation. Freezing allows to keep intact the physiological state from the initial moment, as a result of stopping any enzymatic activity. The efficiency of the method of preserving microorganisms in a lyophilized state is determined by several factors, such as: the culture subjected to lyophilization, the phases of cell growth and concentration, the composition of the protective medium, the rehydration of lyophilized cells and the packaging and storage conditions. The use of inappropriate protective media in the lyophilization process leads to significant changes in microorganisms after lyophilization: of morpho-cultural and biosynthetic properties, in the cell membrane, the secondary protein structure, DNA damage and even their death (Sîrbu T., 2005; Грачева И.В., 2016; Guowei Shu 2017; Xiao-min Li, 2024).

Cryopreservation and lyophilization are currently considered the safest methods for storing active cells, which would allow for greater shelf-life stability at ambient temperatures and facilitate easier transportation and storage (Morgan, 2006).

During freeze-drying, drying (primary and secondary), also the revitalization step, the risk of potential cell damage arises influenced by various physicochemical factors, including the operational parameters during freezing, sublimation, thawing and the type of cryoprotectants and used reconstitution medium. These factors play a crucial role in determining both the survival and metabolic activity of microorganisms during freezing and lyophilization processes.

To prevent these harmful processes, a wide range of protective excipients have emerged, which can be classified, depending on their chemical affiliation, into sugars, macromolecules, polyols, antioxidants and chelating agents (Егинчибаева, А.Д., 2015; Ute Rockingera, 2021; Wang, J., 2025).

Among the soluble substances in protective media, sugars and protein products are used. Carbohydrates are usually used in combination with colloids, less often as simple protective media. Various concentrations from 1 to 10% or more are used. However, the effect of the carbohydrate suspension added to the protective medium varies depending on the culture subjected to lyophilization (ОхапкинаВ.Ю., 2009). Very often, skimmed milk is used as a protective in combination with carbohydrates (XymenaPołomska, 2012; Peiren J., 2015; Valdez-Tenezaca, A., 2025).

The objectives of this study consisted in evaluating the viability of filamentous fungal strains and bacterial strains after lyophilization in the optimal protective medium.

2. MATERIALS AND METHODS

The objects of study were 10 strains of filamentous fungi and 14 strains of bacteria, isolated from aquatic reservoirs in Chisinau municipality, Republic of Moldova. The fungal strains were represented by the genera *Penicillium* (4 strains) and *Trichoderma* (6 strains), and the bacterial strains by the genera: *Bacillus* (9 strains); *Peribacillus* (1 strain), *Pseudomonas* (2 strains) and *Micrococcus* (2 strains).

As a protective medium for lyophilization of fungi, the medium with the composition: skimmed milk with 5% glucose, 5% sucrose and 0.01% ascorbic acid was used, and for lyophilization of bacteria, the medium containing: skimmed milk with 5% glucose; 5% glycerin; 0.01% ascorbic acid. The titer of the spore suspensions before lyophilization and the viability of the strains after lyophilization were determined.

The dried samples after lyophilization were rehydrated with 1 ml of sterile distilled water for 2 hours at a temperature of 28-30°C, after appropriate dilutions they were plated on nutrient agar (bacteria) and acidified agar (fungi). The plates with fungi were incubated at 28-30°C for 96 hours, and those with bacteria at 36°C for 24-48 hours. After cultivation, the colony forming units (CFU/ml) were counted. The results were expressed as a percentage of viable cells compared to the initial number of cells in the suspensions before lyophilization.

The number of viable cells was expressed as $lg10$ colony forming units (CFU) in 1.0 ml of suspension. Viability was calculated according to the formula $BSR = (lgBL/lgAL) \times 100$, where BSR is the viability of the strain in %, $lgBL$ – the logarithm of the CFU number before lyophilization and $lgAL$ – the logarithm of the CFU number after lyophilization or storage (Muñoz-Rojas J. et al., 2006).

3. RESULTS AND DISCUSSION

Preservation of microorganisms by lyophilization still appears to be a science based on empirical tests rather than on tested facts and theories. The literature describes different methodologies for different species and even different strains of the same species, often being strain-specific and dependent on the induction of lyophilization tolerance in microbial cells (Morgan 2006; Guowei Shu, 2017).

The results obtained after lyophilization of the fungal strains in the usedprotective medium (skimmed milk with 5% glucose, 5% sucrose and 0.01% ascorbic acid), demonstrated a very high viability in all studiedstrains (Fig. 1). The viability of fungal strains of the genus *Penicillium* after lyophilization varied within the limits of 92.7 - 98%, compared to the initial titer of the suspension subjected to lyophilization. The highest viability of 98% was recorded in the strain *Penicillium* sp. 8. In strains of the genus *Trichoderma*, a high viability was also recorded after lyophilization, which constituted 94 - 97.5% compared to the initial titer. We can consider that the high viability of fungal strains after lyophilization is due to the optimal protective medium as well as the tolerance of the strains to the lyophilization process (freezing and sublimation), but also to rehydration.

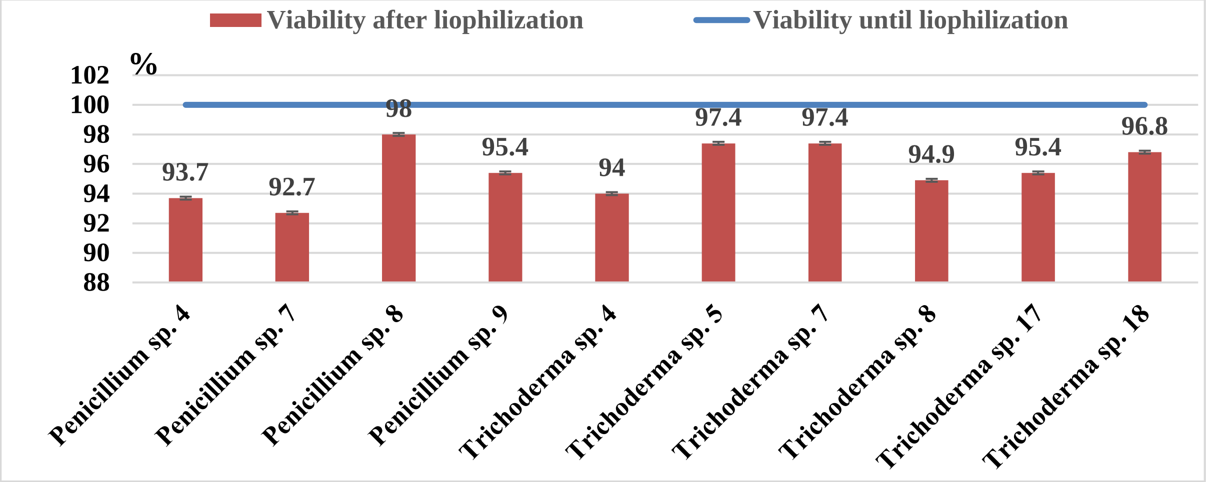

Figure 1. Viability of filamentous fungal strains after lyophilization

The bacterial strains studied reacted differently to the lyophilization process regardless of genus (Fig. 2; Fig. 3), thus demonstrating the different tolerance of the cultures to the freezing and vacuum sublimation process.

Out of the 14 strains lyophilized in the protective medium: skimmed milk with 5% glucose, 5% glycerin and 0.01% ascorbic acid, the viability of 6 strains varied within the limits of 89.1 - 91.2% (Tab. 2). Thus, demonstrating a high viability of bacterial cultures from the genera *Bacillus*, *Pseudomonas* and *Micrococcus*.

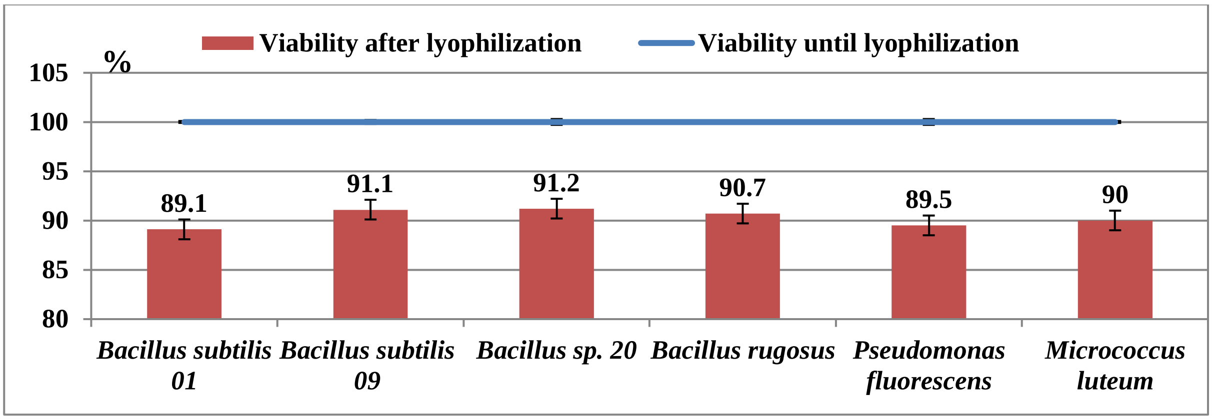

Figure 2. Viability of bacterial strains after lyophilization

In 8 strains of bacteria (Fig. 3), which belong to the same genera (*Bacillus*, *Pseudomonas*, *Micrococcus*) viability was lower after lyophilization, their value varying within the limits of 75.5 - 83.9%. These strains tolerate the lyophilization process less.

Bacteria of the genus *Bacillus* recorded a different viability. Viability in 3 strains out of 5 exceeded 80%, and in 2 strains the recorded viability value was 77.8% and 79.3%.

The viability value of strains of the genera *Pseudomonas* and *Micrococcus* was lower than 78% and 78.4% compared to 89.5% and 90%, respectively compared to the strains in Tab. 2.

The lowest viability after lyophilization of 75.5%, compared to the initial titer, was recorded in the strain *Peribacillus simplex*.

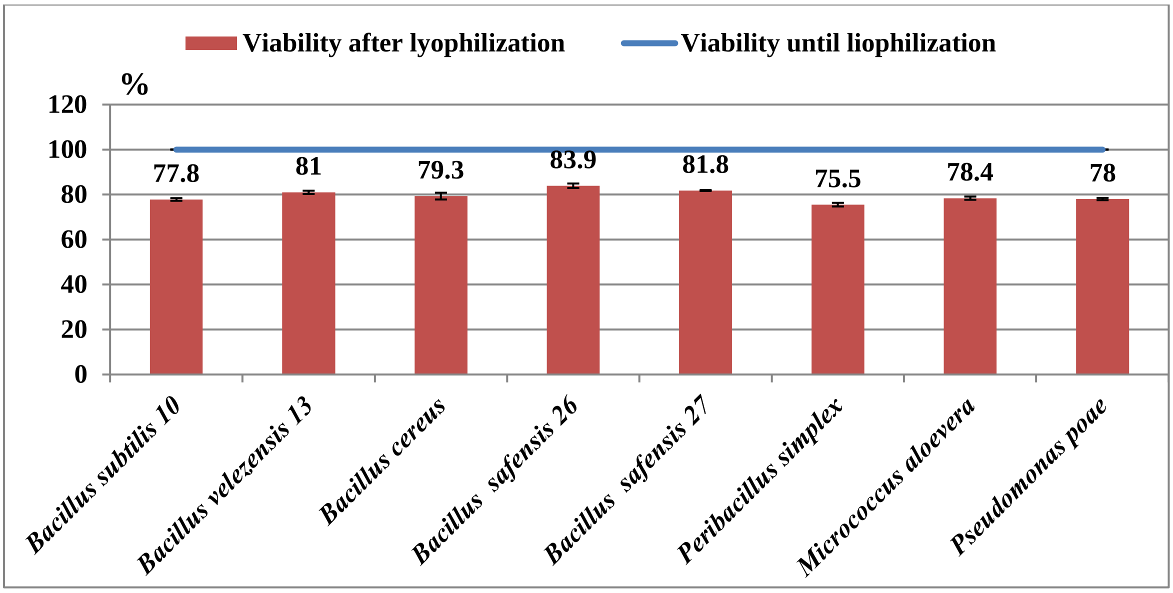

Figure 3. Viability of some bacterial strains after the lyophilization process

The results obtained are consistent with data from the specialized literature, which mentions that microorganisms suffer varying degrees of damage during dehydration and the stabilizing effects of excipients (increased interaction of membrane lipids, accompanied by leakage of intracellular components, replacement of water with small polar substances, etc.) which induces loss of cellular viability and stability (Егинчибаева, А.Д.,2015;Li, X.-M., 2024).

4. CONCLUSIONS

The viability of lyophilized microbial cultures varies not only between different genera but also among strains within the same genus.

The results of this study demonstrated that fungal cultures are more tolerant to the lyophilization process than bacterial ones. The viability of filamentous fungal strains ranged between 92.7–98%, while bacterial viability ranged between 75.5–91.2%, relative to the initial titer. These viability levels indicate that the applied preservation conditions are suitable for maintaining microbial cultures over long-term storage.

DECLARATIONS

Conflict of interest: The authors declare that they have no conflict of interest.

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

Data Availability Statement: The datasets generated during the current study are available from the corresponding author upon reasonable request.

ACKNOWLEDGMENTS: This research was funded by the project 25.80012.7007.32SE NARD (Innovative protective media for the preservation of aquatic microorganisms)

REFERENCES

1. Guowei Shu, Bowen Zhang, Yixin Hui, He Chen, Hongchang Wan.(2017) Optimization of cryoprotectants for *Streptococcus thermophilus* during freeze-drying using Box-Behnken experimental design of response surface methodology. Emirates Journal of FoodandAgriculture. 29(4): 256-263 doi: 10.9755/ejfa.2016-07-960(https://www.sciencedirect.com/science/article/pii/S0167701206000583)

2. Li, X.-M.; Che, L.-H.; Wu, Y.; Li, C.; Xu, B.C. (2024). An effective strategy for improving the freeze-drying survival rate of Lactobacillus curvatus and its potential protective mechanism. Food Biosci,. 58, 103794
3. Morgan C.A., N. Herman, P.A. White, G. Vesey. (2006) Preservation of micro-organismsbydrying; A review, Journal of MicrobiologicalMethods, Volume 66, Issue 2, Pages 183-193, ISSN 0167-7012, https://doi.org/10.1016/j.mimet.2006.02.017.
4. Muñoz-Rojas J. et al. (2006).Involvement of Cyclopropane Fatty Acids in the Response of Pseudomonas putida KT2440 to Freeze-Drying. Applied Environmental Microbiology, vol. 72, № 1, p. 472-477.
5. Peiren, J., Buyse, J., DE Vos, P. *et al.* (2015). Improving survival and storage stability of bacteria recalcitrant to freeze-drying: a coordinated study by European culture collections. *Appl Microbiol Biotechnol*99, 3559–3571. https://doi.org/10.1007/s00253-015-6476-6
6. Rockingera Ute, Funkb Martin, Wintera Gerhard. (2021). Current Approaches of Preservation of Cells During (freeze-) Drying. Journal of Pharmaceutical Sciences 110, p. 2873−2893.
7. Sîrbu T., Codreanu S., Rudic V. (2005). Optimizarea procesului de păstrare a tulpinilor de micromicete în colecție. Buletinul AȘM, științele vieții, 2(297), p. 136-142.
8. Valdez-Tenezaca, A.; Gutiérrez, M.E.; Guerra, M.; Castro, J.F.; Covarrubias, S.A.; Díaz, G.A. (2025). Methods to Stimulate Sporulation and Freeze-Drying Strategies for the Conservation of *Diplodia mutila*, *Diplodia seriata*, *Lasiodiplodia theobromae*, and *Neofusicoccum arbuti* Isolated from Apple Trees with Canker and Dieback Symptoms. *J. Fungi.11*, 640. https://doi.org/10.3390/jof11090640
9. Wang, J.; Wu, P.; Dhital, S.; Yu, A.; Chen. X.D. (2025). Impact of Freezing and Freeze Drying on *Lactobacillus rhamnosus* GG Survival: Mechanisms of Cell Damage and the Role of Pre-Freezing Conditions and Cryoprotectants. *Foods*, *14*, 1817. https://doi.org/10.3390/foods14101817
10. Xiao-Min LI, Lie-Hua Che, Ying Wu, Cong Li, Bao-Cai Xu. (2024). An effective strategy for improving the freeze-drying survival rate of Lactobacillus curvatus and its potential protective mechanism, Food Bioscience,Volume 58, 103794, ISSN 2212-4292, https://doi.org/10.1016/j.fbio.2024.103794.(https://www.sciencedirect.com/science/article/pii/S2212429224002244)
11. Xymenapołomska, Maria, Wojtatowicz, Barbara Zarowska, Marekszołtysik, Józefachrzanowska. (2012). Freeze-drying preservation of yeast adjunct cultures for cheese production. Pol. J. FoodNutr. Sci., Vol. 62, No. 3, pp. 143-150 DOI: 10.2478/v10222-011-0045
12. Грачева И.В., Осин А.В. Механизмы повреждений бактерий при лиофилизации и протективное действие защитных сред. (2016).Проблемы особо опасных инфекций, 3:5–12. DOI: 10.21055/0370-1069-2016-3-5-12
13. Егинчибаева А.Д., Бекенова Н.Е., Ануарбекова С.С. (2015). Хранение железоокисляющих и сероокисляющих микроорганизмов методом криоконсервации и лиофилизации. // Вестник науки Казахского агротехнического университета им. С. Сейфуллина (междисциплинарный).,№2 (85), с. 12- 18.
14. Охапкина В.Ю. (2009). Методы поддержания микробных культур. Часть 2. Лиофилизация. Теоретическая и прикладная экология, .№4, с. 21-32.

A COMPARATIVE STUDY OF THE GROWTH AND STRUCTURAL ORGANIZATION OF *GANODERMA LUCIDUM* AND *GANODERMA RESINACEUM* MYCELIA FOR BIOCOMPOSITE DEVELOPMENT

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https://doi.org/10.52757/bsd26.15

Background: The building sector is a major contributor to greenhouse gas emissions owing to the widespread use of petroleum-derived materials and the high energy demand associated with heating and air conditioning. In this context, improving thermal insulation represents an important lever for reducing building-related environmental impacts. Mycelium-based composites are therefore increasingly investigated as sustainable alternatives to conventional insulation materials. As their performance strongly depends on fungal colonization and network architecture, understanding mycelial growth and structural organization is a necessary preliminary step for strain selection and biocomposite development.

Aim of the study: The aim of this study is to characterize and compare the growth behavior and structural organization of *Ganoderma lucidum* and *Ganoderma resinaceum* mycelia under different temperature conditions, as a preliminary step toward strain selection for biocomposite development.

Materials and methods: To address this objective, the mycelial growth dynamics of the two species were analyzed on a nutrient-rich solid medium under controlled laboratory conditions. The effect of temperature was assessed through radial growth rate, network densification, and hyphal organization. Mycelial development was monitored over time and complemented by imaging analyses. Particular attention was given to the temporal evolution of the mycelium, which grows radially and exhibits clear spatial stratification. Sampling at four positions along a radial axis was performed to evaluate spatial variations associated with mycelial development.

Results: The results showed distinct temperature optima for the two species. *G. lucidum* exhibited maximal radial growth (11 mm/day) and increased network densification at 25 °C after 10 days, whereas *G. resinaceum* reached optimal growth at 30 °C within 6 days (21 mm/day). Under the tested conditions, *G. lucidum* consistently formed denser mycelial networks, while *G. resinaceum* exhibited a more structured and organized mycelial network.

Conclusions: Overall, these findings highlight species-specific thermal preferences and contrasting strategies of mycelial organization, providing valuable insights for strain selection in the development of high-performance mycelium-based composites.

Keywords: *Ganoderma lucidum, Ganoderma resinaceum,* growth dynamics, hyphal organization, mycelium-based composites.

Acknowledgments: Communaute Urbaine du Grand Reims, Departement de la Marne, Region Grand Est and European Union (FEDER Champagne-Ardenne 2014-2020, FEDER Grand Est 2021-2027) are acknowledged for their financial support to the Chair of Biotechnology of CentraleSupelec and the Centre Europeen de Biotechnologie et de Bioeconomie (CEBB).

SURVEY OF '*CANDIDATUS* PHYTOPLASMA SOLANI' PRESENCE IN SOME AGRICULTURAL CROPS

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https://doi.org/10.52757/bsd26.16

Background: '*Candidatus* Phytoplasma solani' is a distinct species of the class Mollicutes (16SrXII group), characterized by phloem-limited localization and strong host dependence. It is widely distributed, mainly in Europe, and affects over 100 plant species, including economically important crops for Moldova. The name reflects its significant impact on plants of the *Solanaceae* family, causing stolbur disease. It also infects grapevine, pepper, maize, lavender, and other crops. Climate change has favored the spread of the pathogen and its insect vectors into new regions and host plants. Therefore, monitoring and controlling its dissemination in economically important crops is essential. Accurate diagnosis can be achieved by molecular methods, with PCR-based techniques offering a reliable and cost-effective approach.

The aim of the study was to determine the presence or absence of infection in some agricultural crops: tomato, maize, potato, pepper, eggplant, wheat, plum and apple growing in experimental fields of Institute of Genetics, Physiology and Plant Protection, MSU.

Materials and methods: Various DNA extraction methods were used depending on plant culture: boiling in alkaline solution, DNAzol, SDS, CTAB. Nested PCR analysis was carried out using specific to '*Ca*. P. solani' primers designed on the base of chaperonine or 16S rRNA gene sequences. Amplification results were recorded using gel agarose electrophoresis (1.5% agarose, 0.5 x TBE buffer) stained with ethidium bromide and visualized in UV.

Results: A comprehensive study of tomato conducted during 2016–2025 showed the continuous presence of the pathogen, with infection rates reaching up to 72% depending on annual conditions, confirming the high susceptibility of tomato to '*Ca. P. solani*'. In potato, 14.6% of plants were infected, while sweet pepper showed a much lower incidence of 4%. Overall, the results indicate the predominant distribution of the pathogen within the *Solanaceae* family, further supported by its detection in the ruderal species *Solanum nigrum*. The phytoplasma was not detected only in eggplant among the analyzed Solanaceae species. In contrast, molecular diagnostics gave negative results in crops from other families, including maize and wheat (cereals), as well as apple and plum (fruit crops). The pathogen was not detected in orchard trees during either the dormant or fruit-bearing stages.

Conclusions: Thus, obtained results demonstrate an ecological and epidemiological specificity of '*Ca*. P. solani' for certain host species, characterized by a high incidence in Solanaceae, particularly tomato. Additionally, the study confirms the potential role of ruderal species within the same family as natural reservoirs of the pathogen. These data contribute to a deeper understanding of the spread of the phytoplasma providing a base for effective monitoring and phytosanitary management strategies.

Keywords: '*Candidatus* Phytoplasma solani', stolbur spread, molecular diagnosis, host plants.

Acknowledgements: This study was supported by the research project 011101 “Genetic and biotechnological approaches of management of agroecosystems under climate change conditions” funded by the Ministry of Education and Research.

EFFECT OF PRE-FREEZING REGIMES AND REACTIVATION CONDITIONS ON THE VIABILITY OF LYOPHILIZED *SACCHAROMYCES CEREVISIAE* STRAINS

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https://doi.org/10.52757/bsd26.17

Background: Long-term preservation of *Saccharomyces cerevisiae* by lyophilization is highly important for scientific research and for the biotechnological and food industries. Maintaining cell viability and genetic stability is essential for reproducible results and consistent product quality. Lyophilization includes rapid freezing of microbial cultures followed by water removal through sublimation under vacuum. Therefore, pre-freezing temperature is a key factor affecting post-lyophilization survival, as it influences ice crystal formation, membrane integrity, and intracellular stability. Higher pre-freezing temperatures cause slower freezing and larger ice crystals, leading to irreversible cell damage and reduced viability. Lower temperatures ensure faster freezing, smaller and more uniform ice crystals, less mechanical injury, and higher survival rates.

The aim of this study was to evaluate the influence of pre-freezing temperature and reactivation time on the survival capacity of *Saccharomyces cerevisiae* yeasts after 13 years of storage in a lyophilized state.

Materials and methods: The study included 11 strains of *Saccharomyces cerevisiae* from the National Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Technical University of Moldova, lyophilized in 2013 using skimmed milk as a protective agent. Prior freezing was performed at two temperature regimes: −50°C and −80°C. Cultures were reactivated with 1 mL of distilled water for 4 and 16 hours, and viability was assessed by colony-forming unit (CFU) counts, followed by logarithmic transformation of the data.

Results: The results demonstrated that rapid freezing at −80°C led to higher cell viability, ranging from 38.2% to 67.8%, depending on the strain analyzed. The use of a higher pre-freezing temperature (−50°C) was associated with a slightly reduced survival rate, with values between 33.0% and 67.1%. Additionally, standard reactivation (4 hours) resulted in low titers of 2.1–3.7 $\log_{10}$ CFU·mL^{-1}. Under these conditions, optimization of reactivation parameters became necessary; extending the reactivation time to 16 hours led to a significant increase in yeast titers, reaching up to 6.8 $\log_{10}$ CFU·mL^{-1}.

Conclusions: This study demonstrates significant long-term survival of lyophilized yeast strains after 13 years of storage. Selecting an optimal pre-freezing temperature, such as −80°C, minimizes the negative effects associated with ice crystal formation, osmotic stress, and molecular instability, thereby enhancing the survival capacity of *Saccharomyces cerevisiae* strains. Furthermore, extended reactivation time significantly improves viability parameters, highlighting the importance of post-lyophilization recovery conditions.

Keywords: yeasts, viability, freezing

Acknowledgements:This research was carried out within project 020101 InBioS – “Innovative biotechnological solutions for agriculture, medicine and environment,” funded by the Ministry of Education and Research of the Republic of Moldova.

BIOCONTROL OF PHYTOPATHOGENIC FUNGI BY AQUATIC ACTINOBACTERIA

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https://doi.org/10.52757/bsd26.18

Background: Among microbial control agents, actinobacteria are of particular interest due to their ability to produce antimicrobial compounds and inhibit numerous phytopathogens. Effective strains have been reported in the genera *Actinomadura, Actinoplanes, Arthrobacter, Micromonospora, Rhodococcus, and Streptomyces*. Their metabolic versatility allows them to compete in root exudates and plant litter, colonize the rhizosphere and plant tissues, and form stable plant associations [2]. Aquatic actinobacteria also contribute to water quality improvement, pathogen suppression, and the health of aquatic organisms [1].

Aim of the study: This study aimed to screen actinobacteria strains isolated from the Lake La Izvor system for their ability to inhibit the growth of phytopathogenic fungi.

Materials and methods: Thirteen actinobacterial strains were cultivated for 14 days on Gause medium. Antifungal activity was evaluated by the disc-diffusion method against *Alternaria alternata, Botrytis cinerea*, *Fusarium solani*, and *Fusarium oxysporum* on malt-agar medium [3].

Results: The tested strains showed variable antifungal activity, with inhibition zones ranging from 8.0 to 22.0 mm. The most active against *A. alternata*, were *Actinoplanes* sp. 26, *Actinomadura* sp. 37, and *Actinoplanes* sp. 43, producing zones of 20.33–21.33 mm. Only five strains inhibited *B. cinerea*, with the highest activity shown by *Actinomadura* sp. 37 (22.0 mm). Nine strains suppressed *F. oxysporum* (8.0–17.67 mm), with maximum activity recorded for *Actinoplanes* sp. 42. Ten strains were active against *F. solani* (8.33–19.67 mm).

Conclusions: Aquatic actinobacteria belonging to the genera *Actinomadura*, *Actinoplanes*, and *Streptomyces* demonstrated significant potential for suppressing phytopathogenic fungi.

was to perform the antimicrobial screening of actinobacteria strains isolated from the lake system La izvor, for their ability to suppress the growth and development of phytopathogenic fungi.

Keywords: actinobacteria, antifungal activity, aquatic environment, phytopathogenic fungi.

Acknowledgments: This research was found by the support of Government of the Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 “InBioS - Innovative biotechnological solutions for agriculture, medicine and environment”.

References:

1. Balagurunathan, R., Radhakrishnan, M., Shanmugasundaram, T., Gopikrishnan, V., Jerrine, J. (2020). Protocols in actinobacterial research. 1. New York: Springer. Evaluation of Actinobacteria for aquaculture applications; pp. 181–188.
2. Meliani, H., Makhloufi, A., Cherif, A., Mahjoubi, M., Makhloufi, K. (2022). Biocontrol of toxinogenic *Aspergillus flavus* and *Fusarium oxysporum* f. sp. *albedinis* by two rare Saharan actinomycetes strains and LC-ESI/MS-MS profiling of their antimicrobial products. *Saudi Journal of Biological Sciences*, 29(6), 103288.
3. Rizk, M., Abdel-Rahman, T., Metwally, H. (2007). Screening of antagonistic activity in different *Streptomyces* species against some pathogenic microorganisms. *Journal of Biological Sciences*, 7(8):1418–1423.

MICROBIOLOGICAL ASSESSMENT OF BACTERIAL CONTAMINATION IN BOAR SEMEN FROM PIETRAIN AND DUROC BREEDS

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https://doi.org/10.52757/bsd26.19

Background: Boar semen used for artificial insemination must meet strict microbiological quality standards because bacterial contamination can negatively affect sperm viability, fertility, and biosafety in pig breeding systems.

The aim of this study was to evaluate the presence of selected bacterial contaminants in semen collected from breeding boars.

Materials and methods: Semen samples were collected from two boars belonging to different breeds (Pietrain No. 88730 and Duroc No. 39631) and microbiologically analyzed using selective culture media. Bacteriological examinations were performed by inoculating semen dilutions on Bile-Esculin Azide Agar for detection of *Enterococcus* spp., Endo Agar for identification of *Escherichia coli*, and Salmonella–Shigella agar for isolation of *Salmonella* spp.

Results: The microbiological analysis demonstrated that *Escherichia coli*, *Enterococcus* spp., and *Salmonella* spp. were detected only up to the third dilution of semen samples, with no bacterial growth observed in higher dilutions. Quantitative assessment indicated that the concentration of *Escherichia coli* reached 1.8×10^2 CFU/ml in Duroc semen and 4.0×10^3 CFU/ml in Pietrain semen, while *Enterococcus* spp. were present at levels of 3.3×10^2 CFU/ml and 5.6×10^2 CFU/ml, respectively. In addition, *Salmonella* spp. were identified at concentrations of 1.5×10^2 CFU/ml and 2.2×10^2 CFU/ml, respectively.

The occurrence of microorganisms only in the initial dilution suggests superficial contamination of semen, most likely originating from the preputial microflora or environmental sources during semen collection. In contrast, the persistence of *Escherichia* spp. in higher dilutions, together with their relatively high concentration, may indicate a higher initial bacterial load or an increased capacity of these bacteria to survive in the seminal environment.

Conclusions: These findings highlight the importance of strict hygienic conditions during semen collection and handling in order to minimize bacterial contamination and to ensure the microbiological safety and reproductive quality of boar semen used for artificial insemination.

Keywords: boar semen, *Enterococcus* spp., *Escherichia coli*, *Salmonella* spp.

EFFECT OF PHYTOSUBTIL ON PHOTOSYNTHETIC PIGMENTS AND PRODUCTIVITY OF WHEAT UNDER FIELD CONDITIONS

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https://doi.org/10.52757/bsd26.20

Background: Plant growth-promoting bacteria of the genus *Bacillus* are considered promising tools for improving crop productivity under field conditions. However, the effectiveness of microbial biopreparations depends not only on the strain's properties, but also on the dose and application method, which may differentially affect plant growth, grain filling, and final yield components.

Aim of the study: This study aimed to evaluate the effect of the biopreparation Phytosubtil, based on a *Bacillus velezensis* strain, on the physiological traits and yield components of soft wheat (*Triticum aestivum* L.) cv. Zymoyarka under field conditions.

Materials and methods: A small-plot field experiment was conducted on sod-podzolic soil in Kyiv, Ukraine, during the 2024 growing season. Four treatments were compared: water-treated control; pre-sowing seed treatment with Phytosubtil at 2.5×10^7 CFU/mL; pre-sowing seed treatment with undiluted Phytosubtil at 2.5×10^9 CFU/mL, and foliar application of Phytosubtil at 2.5×10^7 CFU/mL. The pigment content in wheat leaves was assessed at different phenological stages using the BBCH scale, and yield structure was determined at full maturity.

Results: The response to Phytosubtil strongly depended on the treatment regime. The most consistent positive effects were obtained with the 2.5×10^7 CFU/mL treatment, particularly when applied foliarly, which improved chlorophyll-related characteristics and assimilation activity. Among the yield components, the strongest effects were observed for grain number and grain weight per spike. Foliar application resulted in the highest grain weight per spike (0.99 g) and thousand-kernel weight (30.27 g), compared with 0.94 g and 26.13 g in the control. Thus, under field conditions, Phytosubtil was most effective when applied as a foliar treatment at 2.5×10^7 CFU/mL, whereas increasing the dose for seed treatment did not provide additional benefits.

Conclusions: The effectiveness of Phytosubtil in soft wheat cultivation was determined by both application method and concentration. Foliar treatment at 2.5×10^7 CFU/mL proved to be the most efficient regime, enhancing physiological performance and improving key yield components. These findings indicate that optimized foliar application of Phytosubtil may represent a practical strategy for increasing wheat productivity under field conditions and highlight the importance of optimizing the application strategy when using *Bacillus*-based biopreparations in wheat cultivation.

Keywords: *Triticum aestivum* L., Phytosubtil, foliar application, pre-sowing seed treatment.

EFFECT OF Β-GLUCAN ADDITION ON MICROBIOLOGICAL QUALITY AND STABILITY OF VEGAN SAUCE

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https://doi.org/10.52757/bsd26.21

Background: The growing demand for plant-based foods has stimulated the development of vegan sauces with improved stability and safety characteristics. This trend is driven by increasing consumer awareness of health, sustainability, and the environmental impact of food production. Nevertheless, the formulation of vegan sauces remains a technological challenge because the exclusion of egg-based ingredients may reduce emulsion stability and affect product quality. In this context, β-glucans have gained attention as promising functional ingredients due to their thickening, water-binding, and stabilizing properties. Their incorporation into vegan sauces may contribute not only to improved structural stability but also to enhanced microbiological quality and overall product acceptability.

Aim of the study: The study aimed to evaluate the effect of β-glucan concentrations as fat-replacing and stabilizing agents of vegan sauces.

Materials and methods: Three experimental samples containing different β-glucan levels were prepared and compared with a control sample without β-glucans. The physicochemical properties of the developed sauces were evaluated by determining dry matter content, moisture, pH, total acidity, ash content, viscosity, and organoleptic characteristics. In addition, microbiological analysis and emulsion stability assessment were carried out to evaluate the safety and shelf-life potential of the developed formulations.

Results: The results showed that increasing β-glucan concentration led to an increase in dry matter content and viscosity, while moisture content slightly decreased. The pH values remained within acceptable limits for microbiological safety, and all samples demonstrated satisfactory microbiological quality without exceeding permissible limits. Emulsion stability improved with increasing β-glucan concentration, indicating the structural role of β-glucans in stabilizing the dispersed system.

Overall, the findings confirm that β-glucans can effectively contribute to improving the stability and microbiological safety of vegan sauces. Their incorporation positively influenced the structural properties of the emulsified system and supported the maintenance of acceptable quality parameters. In addition, the microbiological results indicated that the developed formulations remained within satisfactory safety limits, which is essential for the production of plant-based sauces.

Conclusions: These results demonstrate that β-glucans can serve not only as texture-modifying agents, but also as valuable functional ingredients in vegan food formulations. Therefore, the obtained data support the use of β-glucans for the development of stable, safe, and nutritionally enhanced plant-based sauces.

Keywords: β-glucans; vegan sauce; emulsion stability; microbiological quality

Acknowledgments: The research was supported by Institutional Project, subprogram 020405 "Optimizing food processing technologies in the context of the circular bioeconomy and climate change", Bio-OpTehPAS, being implemented at the Technical University of Moldova.

EMULSION STABILITY OF Β-GLUCANS ISOLATED FROM WINE LEES IN OIL-BASED SYSTEMS

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https://doi.org/10.52757/bsd26.22

Background: β-Glucans are natural polysaccharides widely distributed in various biological sources, including cereals, fungi, yeast, bacteria, and algae, each source providing β-glucans with distinct structural and functional characteristics. Their physiological properties depend strongly on origin, molecular structure, molecular weight, degree of branching, and solubility, which influence their biological activity and functional performance. Cereal β-glucans are typically characterized by linear β-(1→3)(1→4) linkages associated with cholesterol-lowering and glycemic control effects, whereas yeast and fungal β-glucans mainly contain β-(1→3)(1→6) linkages responsible for immunomodulatory activity. Additionally, extraction methods and processing conditions significantly affect the molecular conformation, hydration behavior, viscosity, gel-forming capacity, and interactions with lipid phases, determining their suitability for applications in food, pharmaceutical, and cosmetic systems.

The aim of this study was to evaluate the ability of β-glucans isolated from wine lees to stabilize oil-in-water emulsions prepared with two lipid phases: avocado oil and the bio-based ester diisopropyl adipate (DIPA).

Materials and methods: Aqueous suspensions were prepared and subsequently homogenized with the oil phases by ultrasonic treatment to obtain the emulsified systems. The prepared emulsions were stored for 14 days, and their stability was assessed at 0, 7, and 14 days through macroscopic and microscopic observations.

Results: The results showed that all emulsions exhibited phase separation during storage, leading to the formation of two distinct layers. The lower phase was more transparent and contained a lower concentration of suspended material, whereas the upper phase was enriched in oil, β-glucans, and microstructural aggregates of different sizes. Differences in the stability profile were observed depending on the oil type and on the characteristics of the β-glucans used. Microscopic evaluation indicated changes in droplet distribution and particle aggregation over time, confirming the progressive destabilization of the systems during storage. Despite the observed separation phenomena, the studied β-glucans demonstrated their capacity to participate in emulsion formation and to influence the structural organization of the dispersed systems.

Conclusions: These findings support the potential valorization of β-glucans derived from wine lees obtained from indigenous wine varieties remaining after wine production, highlighting the significant availability of such by-product in the Republic of Moldova and their potential use as sustainable functional ingredients for emulsion-based applications in the food, cosmetic, and pharmaceutical sectors.

Keywords: wine yeast by-products, emulsifying properties, natural emulsifiers

Acknowledgments: The research was supported by the Project 25.80013.5107.03RE “Sustainable valorization of residual yeasts from winemaking: exploring multifunctional bioingredients for innovative applications”, implemented at the Technical University of Moldova.

ANTIOXIDANT BIOMARKERS IN ASSESSING MAIZE GERMINATION AND SEEDLING GROWTH FOLLOWING EXPOSURE TO NEGATIVE TEMPERATURE STRESS

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https://doi.org/10.52757/bsd26.23

Background: In the current climate change environment, many breeding programs in Northern Europe are focused on developing cold-resistant maize genotypes. In this context, it is important to study and understand the morphophysiological and biochemical traits of maize that facilitate adaptation to such conditions, as the resulting information can be used for further improvement of breeding material. Therefore, five hybrids were evaluated for their response to negative temperature stress (NTS) at −4°C for 16 h before germination, followed by growth at 26°C for 7 days.

The aim of the study was to determine the role of antioxidant system components in ensuring the defensive potential of maize seedlings with different levels of resistance to a specific dose of negative temperature during the early stages of ontogenesis.

Materials and methods: Seeds of different maize hybrids used in this study were provided by the Public Institution "National Center for Seed Research and Production," Paşcani, Republic of Moldova. Before germination, maize seeds were exposed to a specific dose of subzero temperature (−4°C) for 16 h. Germination, seedling growth and development were carried out under controlled conditions with regulated air temperature. Morphological and physiological parameters, as well as antioxidant system components, including peroxidase and catalase activities, total polyphenol and flavonoid contents, ascorbic acid, and malondialdehyde (MDA), were determined in the seedlings.

Results: Data were obtained on the role of antioxidant system components in providing the protective potential of maize seedlings with varying levels of resistance to NTS. The results revealed a significant ($P<0.05$) decrease in growth parameters such as seminal root length, plant height (cm), and fresh seedling biomass (g) caused by NTS. However, a significant ($P<0.05$) increase in lipid peroxidation was observed. Changes were also recorded in the activities of antioxidant enzymes, including catalase, benzidine peroxidase, and ascorbate peroxidase, as well as non-enzymatic antioxidant compounds, such as total phenolic compounds, flavonoids, and ascorbic acid.

Conclusions: The study showed that pre-treatment of maize seeds with negative temperature (−4°C) for 16 h significantly affected germination, growth, and antioxidant metabolism in the seedlings. Antioxidant enzymes and compounds played a crucial role in protecting plants against oxidative damage. However, the hybrids responded differently depending on their genetic background.

Keywords: maize seeds, negative temperature stress, antioxidant biomarkers.

Acknowledgments: The research was carried out within the subprogram 011101 „Genetic and biotechnological approaches to the agroecosystems management under climate change", financed by the Ministry of Education and Research of the Republic of Moldova.

EFFECTS OF SILVER NANOPARTICLES ON BIOMASS, PROTEIN, AND CARBOHYDRATE ACCUMULATION IN *HAEMATOCOCCUS LACUSTRIS* DURING THE GREEN GROWTH PHASE

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https://doi.org/10.52757/bsd26.24

Aim of the study: Evaluation of the effects of citrate-stabilized AgNPs (10 nm and 20 nm), applied at different concentrations, on biomass accumulation, protein content, and carbohydrate content during the green growth phase of *Haematococcus lacustris* strain CNMN-AV-05.

Materials and methods: Microalgal cultures were exposed to AgNPs at concentrations ranging from 0.01 to 5 mg/L, applied from the onset of the green growth phase. At the end of this stage, the following parameters were determined: biomass (g/L), protein content (% of dry biomass), and carbohydrate content (% of dry biomass). Statistical significance of differences compared to the control was assessed using Student's t-test ($p < 0.05$).

Results: Exposure to AgNPs induced concentration- and size-dependent effects on all aanalyzed parameters. Low concentrations of 10 nm AgNPs (0.01-0.1 mg/L) stimulated biomass accumulation by 14.12-16.23%, while concentrations of 0.5-1 mg/L resulted in more pronounced increases (25.94-27.95%). 20 nm AgNPs produced biomass increases of 14.40% and 27.47% within the stimulatory concentration ranges. In contrast, the highest concentration (5 mg/L) exerted an inhibitory effect, reducing biomass by 29.39-32.75% compared to the control. Protein content increased at all tested concentrations, regardless of nanoparticle size. For 10 nm AgNPs, increases ranged from 8.54% to 15.94%, while 20 nm AgNPs induced higher increases of 13.98-24.88%, with maximum values recorded at 0.1-1 mg/L. Carbohydrate content showed the greatest change among the biochemical parameters. For 10 nm AgNPs, concentrations of 0.05-1 mg/L led to increases of 28.13-64.74%, while extreme concentrations (0.01 and 5 mg/L) resulted in more moderate increases (16.10% and 16.60%, respectively). 20 nm AgNPs induced significant carbohydrate accumulation across the 0.05-5 mg/L range, with the greatest increases observed at 0.5 and 1 mg/L (62.03% and 68.13%, respectively). Overall, a hormetic response was observed, characterized by stimulation at low concentrations and inhibition at high concentrations. Effects were stronger for 20 nm AgNPs, particularly for proteins and carbohydrates.

Conclusions: This study demonstrates that controlled application of AgNPs can enhance productivity and stimulate biomolecular biosynthesis in *Haematococcus lacustris* biotechnology, with effects that depend on both concentration and nanoparticle size.

Keywords: AgNPs, *Haematococcus lacustris*, biomass, proteins, carbohydrates, hormesis.

Acknowledgments: This work was supported by research subprogram 020101 funded by the Ministry of Education and Research of the Republic of Moldova.

MICROALGAE-BASED BIOSTIMULANTS FOR ENHANCING TOMATO RESILIENCE UNDER CZECH CLIMATIC CONDITIONS

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https://doi.org/10.52757/bsd26.25

Background: Sulfated polysaccharides (SPS) are biopolymers located on cell surfaces and in the extracellular matrix of microalgae and cyanobacteria. They are known for antioxidant, antiviral, and immunomodulatory properties. Due to their high sulphation and polyanionic nature, SPS interact with plant cells as natural elicitors and biostimulants. Their application can enhance seed germination, stimulate root and shoot growth, improve nutrient uptake, strengthen defense responses, and increase tolerance to abiotic stress. These effects are relevant for tomato seedlings in the Czech Republic, which are vulnerable to low spring temperatures and to summer heat and drought stress.

Aim of the study: The main objective of this study is to establish baseline methodologies and assess seed treatments with SPS biostimulants derived from *Arthrospira platensis* (spirulina), developed at IMB UTM, for enhancing tomato resilience to temperature extremes and water deficit, in relation to cumulative Growing Degree Days (GDD).

Materials and methods: For this purpose, SPS were extracted from spirulina biomass using distilled water at a ratio of 1:45 (w/v) at 90 °C for 2 h, followed by centrifugation to remove residual biomass. The resulting supernatant was subsequently treated with cetyltrimethylammonium bromide to precipitate the polysaccharide fraction, purified, and re-dissolved in distilled water. Tomato seeds were treated with obtained SPS before sowing.

Results: Under controlled greenhouse conditions, treated seeds exhibited faster, more synchronized emergence, with a reduction in mean germination time of approximately 2–3 days and a 25–35% increase in seedling vigor index compared to the untreated control. These responses were associated with enhanced root elongation and shoot growth. The most pronounced effects of the biostimulant application were observed at the seedling stage, where treated plants showed more vigorous and structurally balanced development, including a better-developed root system and improved physiological readiness for transplanting. Although the risk of late-spring frosts constrained transplanting dates, seedlings were successfully transplanted into open-field conditions in the Elbe Lowland region of the Czech Republic. Following transplanting, treated plants demonstrated faster field establishment, characterized by more rapid root anchorage and an earlier resumption of growth. Moreover, biostimulant-treated seedlings exhibited increased tolerance to early-season environmental variability, particularly spring temperature fluctuations, resulting in reduced transplanting stress and improved early field performance.

Conclusions: These findings highlight the relevance of SPS biostimulants for improving tomato development and adaptation to extreme temperature and water deficit.

Keywords: spirulina, sulphated polysaccharides, tomato, seed treatment, heat stress, drought

Acknowledgments: Supported by the ReBrain project “Nanobiotechnology and Modelling for Climate-Smart Agriculture in the Republic of Moldova,” initiated by ANCD.

NANOBIOTECHNOLOGY OF PHOTOSYNTHETIC MICROORGANISMS FOR SUSTAINABLE DEVELOPMENT: CONTROLLED OXIDATIVE STRESS AND METABOLIC RESPONSES

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https://doi.org/10.52757/bsd26.26

Background: Interactions between engineered nanoparticles (NPs) and photosynthetic microorganisms represent a rapidly developing field at the interface of nanotechnology and microbial biotechnology. Metal and metal oxide nanoparticles can act as both stressors and modulators of cellular metabolism, inducing oxidative stress responses that may lead to either growth inhibition or adaptive metabolic redirection.

Aim of the study: The study aims to elucidate the physiological and biochemical responses of selected photosynthetic microorganisms (cyanobacteria and microalgae) to exposure to engineered nanoparticles, with a focus on oxidative stress modulation and its impact on biomass productivity and metabolite accumulation.

Materials and methods: Model strains of cyanobacteria and microalgae (e.g., *Arthrospira platensis, Nostoc linckia, Haematococcus lacustris*) were cultivated under controlled laboratory conditions and exposed to metal and metal oxide nanoparticles of defined sizes and concentrations. Growth parameters, pigment content (chlorophylls, carotenoids), and oxidative stress markers were monitored. Spectrophotometric and biochemical assays were used to assess cellular responses, and comparative analyses between treatments and controls were performed.

Results: Exposure to engineered NPs induced dose- and size-dependent responses across all tested strains. At higher concentrations, NPs inhibited growth, increased oxidative stress, degraded pigments, and impaired photosynthetic activity. In contrast, sublethal concentrations triggered hormetic effects, reflected in moderate stimulation of growth and metabolism. Photosynthetic microorganisms activated antioxidant defense mechanisms, leading to increased levels of protective pigments and improved redox balance. In *Haematococcus lacustris*, controlled nanoparticle exposure promoted a shift toward carotenoid biosynthesis, resulting in enhanced astaxanthin accumulation under moderate stress conditions. Cyanobacteria such as *Arthrospira platensis* and *Nostoc linckia* exhibited adaptive responses mediated by extracellular polymeric substances (EPS), which reduced nanoparticle bioavailability and toxicity. Nanoparticle size also influenced biological effects, with smaller particles inducing stronger responses. Overall, the results highlight the dual role of nanoparticles as both stressors and modulators of metabolism, with controlled oxidative stress enhancing the production of valuable metabolites.

Conclusions: The results demonstrate that controlled nanoparticle-induced stress can be exploited as a biotechnological tool to modulate metabolic pathways in photosynthetic microorganisms. Understanding the balance between toxicity and hormesis is essential for developing sustainable biotechnological applications, including high-value metabolite production.

Keywords: nanoparticles, microalgae, cyanobacteria, oxidative stress, hormesis, metabolites.

Acknowledgments: This work was supported by research subprogram 020101 funded by the Ministry of Education and Research of the Republic of Moldova.

PROTEIN-ENRICHED *PORPHYRIDIUM PURPUREUM* BIOMASS VIA NITROGEN AND LIGHT MODULATION FOR BIOTECHNOLOGICAL APPLICATIONS

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https://doi.org/10.52757/bsd26.27

Background: *Porphyridium purpureum* is a red microalga with significant biotechnological potential, whose biomass composition can be modulated under controlled culture conditions. Increased nitrogen availability and optimized light intensity stimulate protein accumulation, reflecting adaptive metabolic responses to environmental variations. This metabolic plasticity enables the redirection of biosynthetic pathways toward target compounds. Moreover, the resulting biomass can serve as a biological matrix for generating reducing biomolecules applicable in the functionalization of silver nanoparticles and the development of stable, biocompatible nanobiomaterials.

Aim of the study: To identify optimal nutritional and illumination conditions for maximizing protein accumulation in *Porphyridium purpureum* and to validate the obtained biomass as a source of targeted proteins for subsequent use in the biofunctionalization of silver nanoparticles.

Materials and methods: *Porphyridium purpureum* was cultivated in three nutrient media under controlled temperature, pH, and continuous illumination to determine the optimal growth conditions. Nitrogen source optimization was performed using a single-factor experimental design followed by the Box-Wilson method. The illumination regime was adjusted stepwise according to the growth phases (adaptation and exponential). Biomass productivity and protein accumulation were assessed by protein quantification, MDA content evaluation, and antioxidant activity assays (DPPH and FRAP) using specific spectrophotometric methods.

Results: Optimization of cultivation conditions—by increasing nitrogen from 5.5 to 6.5 g/L and applying phased illumination (56 μmol photons·m^{-2}·s^{-1} for 6 days, then 72 μmol photons·m^{-2}·s^{-1} during the exponential phase)—significantly enhanced protein content compared to standard conditions, while maintaining low oxidative stress (reduced MDA). The optimized biomass reached ~45% protein versus ~33% under conventional conditions.

Proteins were extracted via alkaline solubilization, followed by centrifugation and pH adjustment, yielding a soluble fraction of ~16 g/100 g extract. The extract showed antioxidant and reducing activity (DPPH inhibition ~20%; FRAP ~27 mg ascorbic acid equivalents/g extract), confirming its suitability as a biological matrix.

Conclusions: Optimization of nutritional and illumination conditions significantly enhances protein accumulation in *Porphyridium purpureum* without inducing substantial oxidative stress. The resulting protein extract exhibits antioxidant and reducing capacity, supporting its applicability as a functional biological matrix for the biofunctionalization of silver nanoparticles.

Keywords: *Porphyridium purpureum*, targeted proteins, optimization, biofunctionalization, silver nanoparticles.

Acknowledgments: This research was conducted within the framework of the bilateral ROMD complex project – 25.80013.8007.12ROMD (NARD) and PN-IV-PCB-RO-MD-2024-0254 (UEFISCDI).

INTERACTION MODELS OF ZnO MULTIPOD NANOPARTICLES WITH CYANOBACTERIUM *ARTHROSPIRA PLATENSIS* AND RED MICROALGA *PORPHYRIDIUM PURPUREUM*

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https://doi.org/10.52757/bsd26.28

Background: Zinc oxide-based nanomaterials exhibit distinct physicochemical properties that influence their interactions with biological systems. In microalgae and cyanobacteria, these interactions exert effects that are strongly dependent on morphology, concentration, and the timing of application relative to the culture's physiological state.

Aim of the study: To investigate interaction models of ZnO multipod nanoparticles with cyanobacterium *Arthrospira platensis* and red microalga *Porphyridium purpureum*, focusing on metabolic response and oxidative stress.

Material and methods: *Arthrospira platensis* and *Porphyridium purpureum* cultures were grown under controlled conditions and exposed to ZnO multipod nanoparticles at concentrations ranging from 0.1 to 30 mg/L. Nanoparticles were applied at different growth stages (lag, exponential, and pre-stationary/tardo-exponential phases). Biomass, biochemical composition (proteins, carbohydrates, lipids, photosynthetic pigments, and phycobiliproteins), and oxidative stress marker (MDA) were assessed. Statistical analysis was performed using Student's t-test ($p < 0.05$).

Results: The interaction of ZnO multipod nanoparticles with microalgal systems follows a general dose-dependent model, differentiated according to the cultures' physiological state. In *Arthrospira platensis*, the response is phase-dependent. It follows a sequence of patterns: an initial adaptation model characterized by minor changes at low doses, followed by a compensatory model in the exponential phase, defined by the accumulation of carbohydrates and lipids and a reduction in phycobiliproteins, and finally an advanced oxidative stress model marked by decreased protein content and increased lipid peroxidation markers. In *Porphyridium purpureum*, the interaction is predominantly dose-dependent and less influenced by growth phase, characterized by an early and persistent oxidative stress model, with lipid reduction, pigment variations, and a continuous increase in malondialdehyde levels. At low doses, a moderate metabolic adjustment model is observed, whereas at high concentrations, phycobiliproteins undergo progressive degradation.

Conclusion. The data obtained indicate that the interaction between ZnO multipod nanoparticles and microalgae and cyanobacteria follows a sequence of functional patterns: initial adaptation → compensatory response → advanced/persistent oxidative stress, characterized by correlated changes in biomolecules and oxidative stress markers. These results support the controlled use of nanomaterials to modulate microalgal metabolism, provided that the applied concentrations are strictly optimized.

Keywords: ZnO nanoparticles, multipod morphology, microalgae, *Arthrospira platensis*, *Porphyridium purpureum*, oxidative stress, metabolic response.

Acknowledgments: This work was supported by research project 25.80012.7007.26 SE funded by the National Agency for Research and Development, Republic of Moldova.

AGRI-FOOD WASTES AS AN EFFICIENT SUBSTRATE FOR THE PIGMENTED YEAST CULTUVATION

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https://doi.org/10.52757/bsd26.29

Background: Agri-food wastes represent an increasingly acute global problem, with approximately one billion tons generated worldwide each year. In addition to the high costs associated with its management, it also creates serious environmental problems. At the same time, agri-food wastes are a sustainable source of various substances with biological activity and nutritional value, which can be used in animal feed or as a substrate for the production of value-added products. In this context, the utilization of agri-food wastes in various biotechnological processes, as a nutrient substrate for the cultivation of microorganisms and the synthesis of biologically active substances, is a promising research direction. Yeasts are considered excellent producers of biologically active compounds because they are characterized by a high rate of biomass accumulation, the ability to metabolize a wide range of carbon sources, resistance to contamination by foreign microflora, and the capacity to grow on relatively inexpensive nutrient media, including by-products from the agricultural and food industries. This significantly reduces the production costs of biologically active substances.
The aim of this research was to evaluate the influence of liquid nutrient media based on carrot and/or celery wastes on the synthesis of exometabolites in the culture liquid of the *R. gracilis* CNMN-YS-03 and *R. mucilaginosa* CNMN-YS-10 pigmented yeast strains.
Materials and methods: Spectrophotometric methods were used to determine the content of proteins, carbohydrates, and total antioxidant activity (TAA) in the culture liquid.
Results: It was established that the culture liquid obtained after cultivating the *R. gracilis* CNMN-YS-03 strain on YPD nutrient medium contained up to 52.22±1.72% d.w. proteins, 3.93±0.09% d.w. carbohydrates, and exhibited a TAA of 75.11±1.29% inhibition. For the *R. mucilaginosa* CNMN-YS-10 strain, these values were 60.93±4.99% d.w. proteins, 4.10±0.16% d.w. carbohydrates, and the TAA of 83.64±5.50% inhibition. The use of nutrient media based on aqueous extracts of carrot peels, celery peels, and the 1:1 mixture of carrot and celery peels as the sole nutrient source significantly increased ($p \leq 0.05$), depending on the used strain and medium, the protein content by up to 39.1%, the carbohydrate content by 2.9-4.2 times, and the TAA in the culture liquid by up to 106.6%, compared to the YPD control medium.
Conclusions: Thus, agri-food wastes represent a sustainable and efficient substrate for the cultivation of pigmented yeasts. Nutrient media based on carrot and celery wastes stimulate the synthesis of exometabolites. The culture liquid obtained after cultivating *Rhodotorula* yeasts on these media exhibits high antioxidant activity and is a natural source rich in proteins and carbohydrates, paving the way for its multiple applications in various fields.

Keywords: yeasts, carrot peels, celery peels, culture liquid

Acknowledgments: The research was carried out within project 020101 InBioS - “Innovative biotechnological solutions for agriculture, medicine and environment” funded by Ministry of Education and Research of the Republic of Moldova.

INITIATION OF *IN VITRO* CULTURES OF *HEUCHERA* × *HYBRIDA*

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https://doi.org/10.52757/bsd26.30

Background: *In vitro* micropropagation is a branch of plant biotechnology that encompasses methods for propagating plants through cultures of cells, tissues and organs, offering efficiency far superior to conventional propagation techniques.

Aim of the study: The present study aimed to initiate and multiply *in vitro* the species ***Heuchera* × *hybrida*** to produce high-quality, genetically uniform and contamination-free planting material within a relatively short time.

Materials and methods: The experiments were conducted in the Embryology and Biotechnology Laboratory at the “Alexandru Ciubotaru” National Botanical Garden (Institute) of MSU. The cultivar ‘Regina’ was selected for inoculation, being appreciated for its ornamental qualities and suitability to the pedoclimatic conditions of the Republic of Moldova. This cultivar is fast-growing and combines attractive foliage with abundant flowering.

For *in vitro* culture initiation, plant material was collected from donor plants in early May, during active vegetative growth. Three types of explants were tested: apical meristems with leaf primordia, petiole segments and petiole segments with leaf primordia. Explants were excised and inoculated under strict aseptic conditions to ensure culture sterility and to initiate *in vitro* regeneration. The biological material was disinfected using a 0.1% diacid solution for 9 minutes.

Results: Multiple formulations of Murashige & Skoog (MS) agar medium, supplemented with the cytokinin 6-benzylaminopurine (BAP) at various concentrations (BAP 0.1–0.5 mg/L), were evaluated for culture initiation. The highest frequency of viable explants was achieved using petiole fragments with leaf primordia. No contamination was observed with this inoculation protocol. In contrast, explants consisting of apical meristems with leaf primordia exhibited a high contamination rate of 30-40%. Although the inoculation of petiole fragments did not show contamination, it failed to produce microclones, unlike the protocol using petiole fragments with leaf primordia.

Conclusions: Overall, the results suggest that the use of petiole fragments with leaf primordia, 1.0-1.5 cm in size, proved to be an efficient and proliferative material for the *in vitro* multiplication of this species.

Keywords: inoculation, explant, nutrient medium.

Acknowledgments: This research was conducted under Subprogram 010101 “Ex situ and in situ research and conservation of plant diversity in the Republic of Moldova” (2024-2027).

A NEW APPROACH FOR SELECTING BIOCONTROL BACTERIAL AGENTS AGAINST FUSARIUM

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https://doi.org/10.52757/bsd26.31

Background: Fusariosis is a widespread infection by fungi of the genus *Fusarium*, affecting a wide range of crops and causing significant economic losses in global agriculture. Biological control using antagonistic microorganisms represents an effective and environmentally friendly strategy for managing fusariosis. In particular, bacteria are known for their ability to secrete fungicidal compounds while promoting root growth, to compete for iron, and to induce systemic resistance in plants. Conventionally, microorganisms with antifungal activity are identified through *in vitro* screening on agar media. However, this approach does not account for the influence of the host plant on microbial activity at the initial screening stage. Since environmental conditions can significantly affect microbial behavior, this may lead to the selection of strains that are active only in the absence of plants or the rejection of those that exhibit activity only in their presence.

The aim of this study was to develop a method for evaluating antimicrobial activity in the presence of agricultural plants and to compare the results with those from conventional screening.

Materials and methods: Wheat seeds (cv. Moldova 16), a highly phytopathogenic strain (*Fusarium solani* CNMN-FF-07), and four bacterial strains from the National Collection of Non-pathogenic Microorganisms (*Bacillus cereus* var. *fluorescens* CNMN-BB-07, *Bacillus velezensis* CNMN-BB-31, *Pseudomonas fluorescens* CNMN-PsB-01, and CNMN-PsB-02) were used as experimental materials. The bacterial strains exhibited antimicrobial activity against the phytopathogen, as determined by the agar block method, with inhibition zones of up to 23 mm.

Results: As a result, a novel methodology was developed for screening microorganisms capable of protecting plants against *Fusarium* infections. The method involves the germination and growth of bacterized seeds in test tubes containing semi-solid Knop agar medium. The treated seeds were placed on the surface of the medium, after which different concentrations of the phytopathogen were applied dropwise onto the seeds. The test tubes were subsequently sealed with air-permeable cotton plugs and incubated at room temperature for up to 14 days.

Conclusions: Of all tested bacteria, only *Bacillus cereus* var. *fluorescens* CNMN-BB-07 retained its antifungal activity in the presence of the host plant. These results highlight the importance of screening potential biocontrol agents under conditions that include the target plant, thereby ensuring the selection of strains effective in real plant–microbe–pathogen interactions.

Keywords: fusariosis, biocontrol, antimicrobial activity, plant protection

Acknowledgement: the research was funded within the project 020101 “InBioS - Innovative biotechnological solutions for agriculture, medicine and environment”.

BIOPROTECTIVE NON-*SACCHAROMYCES* YEASTS AS AN ALTERNATIVE TO SULFUR DIOXIDE IN WINEMAKING

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https://doi.org/10.52757/bsd26.32

Background: Sulfur dioxide has served as the main antimicrobial and antioxidant agent in winemaking for more than two millennia. However, the increasing consumer demand for “low-sulfite” wines requires alternative strategies for the microbiological stabilization of wines. The use of yeasts with bioprotective activity presents an alternative for winemakers, with the aim of limiting the development of spoilage microorganisms and preserving the sensory quality of the products. Non-*Saccharomyces* yeasts (*Metschnikowia pulcherrima* and *Torulaspora delbrueckii*) suppress spoilage organisms through bioprotection, without compromising fermentation.

Aim of the study: The objectives of the study were to quantify the results of reducing sulfite content in wines, present chemical and microbiological safety profiles, and demonstrate the practical applicability of bioprotective yeasts in Moldovan winemaking conditions.

Materials and methods: Two dry still white wines were produced during the 2025 vintage in the Codru wine region (Glera and Feteasca regală). For bioprotection, the commercial preparation Zymaflore Égide TDMP (Laffort Groupe, Bordeaux, France) was used, consisting of a certified mixture of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*, intended for environmental colonization, with no fermentation activity detected. All analytical measurements of wines were performed according to the methods approved by the Technical Regulation "Analysis methods in the field of wine production”.

Results: The results demonstrated a 32-50% reduction in sulfites compared to traditional methods, while maintaining volatile acidity at only 40% of the EU threshold. The levels of heavy metals and sodium remained well below the regulatory limits, confirming the wine’s compliance with market requirements and the potential for premium positioning of Moldovan wines with low sulfite content. The antimicrobial activity of *Metschnikowia pulcherrima* yeasts is mediated by iron sequestration, limiting the growth of iron-dependent spoilage microorganisms. Oxygen consumption by bioprotective yeasts further contributes to the early establishment of quasi-anaerobic conditions, restricting aerobic spoilage metabolism before active alcoholic fermentation. Sequential inoculation of *Saccharomyces cerevisiae*, 24-48 hours after the addition of bioprotective yeasts, proved to be essential for the success of the fermentation.

Conclusions: Bioprotective yeasts application provides a scientifically validated pathway for Moldavian producers to meet the growing global demand for "low-sulfite" and natural wines. This strategy aligns environmental sustainability with high-quality enological standards, ensuring competitive positioning in premium international markets.

Keywords: wines, bioprotection, sulfur dioxide, spoilage microorganisms, sensory quality.

Acknowledgments: The research was funded under the institutional project, subprogram 020405 "Optimization of food processing technologies in the context of circular bioeconomy and climate change", Bio-OpTehPAS.

SIMULATING WATER DEFICIT IN CULTIVATED FLAX (*LINUM USITATISSIMUM L.*)

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https://doi.org/10.52757/bsd26.33

Background: The reintroduction into cultivation of resilient, native, or historically cultivated species such as flax (*Linum usitatissimum* L.) represents an opportunity for sustainable agriculture, as these species demonstrate a higher capacity to adapt to water scarcity and unfavorable soil conditions, compared to agricultural systems based on intensive monocultures.

Aim of the study: To evaluate the germination behavior of local flax forms (*Linum usitatissimum* L.) under water stress induced by PEG 6000, in order to identify genotypes with enhanced drought tolerance.

Materials and methods: Two local flax (*Linum usitatissimum* L.) accessions from the Gene Bank's *ex situ* collection were used as study material: *DRS-09* (Sofia vlg., Drochia dist.) and *CSG-22* (Grigorievca vlg., Causeni dist.). To investigate plant responses to water stress, a polyethylene glycol 6000 (PEG 6000) solution was applied at five concentrations (0% – control, 10, 15, 20 and 25%) as a standard method for simulating drought conditions under controlled laboratory environments. The samples were exposed to a temperature of 20±1° for 6 days.

Results: The results showed a clear variation in germination capacity and seedling growth depending on the intensity of osmotic stress induced by PEG 6000. Under non-stress conditions (0% PEG), the genotypes exhibited high germination rates, 90% for *DRS-09* and 88% for *CSG-22*. At 10% PEG, a slight stimulation of germination was observed, with both genotypes reaching values of up to 94%. As the concentration increased to 15% and 20% PEG, germination remained relatively stable, ranging between 88-92% and 86-88%, respectively, while at 25% PEG germination was completely inhibited (0%). In the absence of osmotic stress, the *DRS-09* genotype exhibited superior vigor, with an average seedling length of 15.67±0.60 cm compared to 9.96±0.57 cm for *CSG-22*. PEG application resulted in a progressive reduction in growth, which became more pronounced with increasing stress intensity. At 10% PEG, seedling length was 11.40±0.65 cm (*DRS-09*) and 9.02±0.53 cm (*CSG-22*), followed by marked decreases at 15% PEG (6.82±0.40 cm and 4.26±0.26 cm). At 20% PEG, growth was severely inhibited, reaching values of 1.75±0.07 cm and 1.67±0.09 cm and at 25% PEG, complete suppression of germination and growth was observed. Overall, the results indicate increased sensitivity to severe stress and differential tolerance among genotypes under moderate stress conditions.

Conclusions: The *DRS-09* genotype showed higher overall tolerance to PEG-induced water deficit and represents the most promising material for further study. A PEG concentration of up to 20% is recommended for future screening of flax genotypes, as it effectively differentiates stress responses.

Keywords: *Linum usitatissimum* L., local forms, water deficit, PEG 6000.

Acknowledgments: The research was conducted within the framework of Subprogram 011102 "Increasing and conservation genetic diversity, agricultural crop breeding in the context of climate change", financed by the Ministry of Education and Research of the Republic of Moldova.

CHARACTERIZATION OF MICROORGANISMS ASSOCIATED WITH POTATO TUBER ROTS

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https://doi.org/10.52757/bsd26.34

Background: Potato tuber rots are one of the major causes of post-harvest losses worldwide and are commonly associated with both fungal and bacterial pathogens. Dry rot is primarily caused by several species of the genus *Fusarium*, whereas soft rot is mainly associated with *Pectobacterium* and *Dickeya* genera. Infected tubers can contribute to the persistence and spread of these pathogens in storage facilities and agricultural systems. A comprehensive understanding of the diversity of microorganisms associated with potato tuber rot is essential for the development of effective disease management strategies.

The aim of the present study was to isolate and identify microorganisms associated with dry rot and soft rot of potato tubers from the active collection of local potato germplasm maintained at the National Gene Bank of the Institute of Genetics, Physiology and Plant Protection.

Materials and methods: Tubers from ten potato genotypes exhibiting symptoms of different types of rot were analyzed. A total of eighteen microbial isolates were obtained in pure culture from infected potato tissues displaying symptoms of dry rot and soft rot. Fresh fungal mycelium or bacterial biomass grown on culture media was collected after five days of incubation and subjected to DNA extraction using a modified CTAB protocol. The concentration and quality of the extracted DNA were assessed by spectrophotometric analysis and agarose gel electrophoresis. Polymerase chain reaction (PCR) was employed for the molecular identification of the isolates. Fungal isolates were amplified using universal ITS primers targeting the internal transcribed spacer (ITS) region of ribosomal DNA, whereas bacterial isolates were analyzed using primers specific to the 16S rRNA gene. PCR reactions were carried out in a total volume of 25 μL containing 66 mM Tris-HCl (pH 8.4), 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1% Tween 20, 7% glycerol, 100 μg mL^{-1} bovine serum albumin, 0.2 mM of each dNTP, 1 U Taq DNA polymerase, 5 pM of each primer, 50 ng of genomic DNA. Amplification was performed under the following conditions: initial denaturation at 95 °C for 4 min, followed by 35–40 cycles of denaturation at 95 °C for 30 s, primer annealing at 55–60 °C for 30 s (depending on the primer set), and extension at 72 °C for 45–60 s, with a final extension at 72 °C for 10 min.

Results: The results showed that twelve isolates produced positive amplification with ITS primers, indicating their fungal origin, whereas six isolates yielded positive amplification with 16S rRNA primers, suggesting a bacterial identity. Subsequent PCR analysis of the ITS-positive isolates using β-tubulin gene primers confirmed the presence of *Fusarium* spp.

Conclusions: These findings contribute to a better understanding of the microbial complex associated with potato tuber rot and provide a basis for further molecular identification and characterization of the pathogens involved.

Keywords: potato germplasm, dry rot, soft rot, PCR.

Acknowledgments: This study was supported by the research project 25.80012.7007.31SE funded by the National Agency for Research and Development, Government of Republic of Moldova.

THE EFFECT OF UV RADIATION ON THE CONIDIA OF SOME NATIVE FUNGAL STRAINS FORMULATED WITH VEGETABLE OILS.

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https://doi.org/10.52757/bsd26.35

Background: To protect crops, farmers are increasingly resorting to chemical control of insects. However, chemicals not only destroy harmful insects but also have significant environmental consequences, such as inducing resistance or harmful effects on non-target organisms. Additionally, their ability to penetrate and accumulate in plant organs can drastically reduce crop quality. All these concerns have led to the search for natural remedies, such as entomopathogenic fungi, recognized as promising biological control agents due to their proven efficacy against various insect pests. They are naturally found in the soil, are widespread across different climatic regions, and have the capacity to produce epizootics. In addition, biopesticides based on native strains of entomopathogenic fungi present several advantages over the use of chemicals. These strains are better adapted to environmental conditions, and local production would be beneficial for farmers, as they can always have access to these products. However, their use as biological control agents is hampered by physical factors, with solar radiation being one of the most destructive ones. This problem can be solved by adding protective substances to the biopreparation formula, such as optical brighteners, like Tinopal, sunscreens, and natural additives, such as vegetable oils, teas, or humic acids.

The present study aimed to investigate the effect of UVB radiation on the viability of conidia of two native micromycete strains with bioinsecticidal potential, formulated with vegetable oils as protective additives, under laboratory conditions.

Materials and Methods: The study included two strains of native entomopathogenic fungi, identified as *B. bassiana* and *I. fumosorosea*, and ten vegetable oils. The compatibility of the investigated strains with the selected vegetable oils was studied. Subsequently, the conidia emulsified in the selected oils were exposed to UVB irradiation ($\lambda = 312$ nm) for 15, 30, 45, 60, and 90 min.

Results: The compatibility of the fungal strains formulated with different vegetable oils was confirmed, the germination rate varying significantly between the two strains, with ranges from 7.0% to 82% for *I. fumosorosea* and from 0% to 43% for *B. bassiana*, compared to the control sample, in which the germination percentage was 100% for both strains. The protective effect of oils such as sunflower oil, soybean oil, almond oil, sea buckthorn oil, and pumpkin oil was found, confirming the survival of conidia exposed to UVB for up to 90 min., with a germination rate of 10% in almond oil for the *B. bassiana* strain and 9% in pumpkin oil for the *I. fumosorosea* strain. **Conclusions:** Vegetable oils are compatible with the conidia of the tested fungal strains and can partially reduce the damage caused by UVB radiation. Further research will be conducted to confirm the preservation of the bioinsecticidal properties of the tested fungal strains in emulsion form and to evaluate the protective effect under both protected and open field conditions.

Keywords: entomopathogenic micromycetes, UVB radiation, additives

Acknowledgments: This study was supported by the project 20.80009.7007.12, funded by the National Agency for Research and Development and subprogram 010701 ZOOAQUATERRA, (Moldova State University, Institute of Zoology).

THE YEAST *WICKERHAMOMYCES ANOMALUS* AS A PROMISING BIOCONTROL AGENT AGAINST *ASPERGILLUS* PLANT PATHOGENS

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https://doi.org/10.52757/bsd26.36

Background: The sustainability of modern agricultural systems is critically dependent on the availability of safe and effective plant protection pathways. Synthetic pesticides, despite their efficacy, are associated with a range of negative consequences including environmental persistence, disruption of beneficial soil communities, the progressive development of resistant pathogen strains. In recent years the contamination of crops and post-harvest plant products by *A. fumigatus* has been increasingly reported. Also, *Aspergillus flavus* is an opportunistic phytopathogen that affects numerous crops, including *Zea mays*. The integration of biological control strategies into modern crop production provides an alternative to the application of synthetic chemical fungicides.

The aim of the present study was to identify the antifungal activity of *Wickerhamomyces* yeast against *Aspergillus* spp. strains.

Materials and methods: For the study as the objects of study were used yeast and fungal strains from the National Collection of Nonpathogenic Microorganisms (NCNM) of the IMB of the Technical University of Moldova. The antifungal activity was investigated using cup plate agar diffusion method.

Results: Following investigation, the antagonistic potential of *W. anomalus* CNMN-YS-07 biomass and liquid culture was clearly evidenced by its capacity to suppress mycelial development in both *A. fumigatus* and *A. flavus*. The yeast biomass exhibited superior inhibitory performance, with the greatest suppression recorded against *A. fumigatus* (inhibition zone 34.33 ± 0.57 mm), followed by *A. flavus* (20.66 ± 0.57 mm). Importantly, antifungal activity, also detected in the liquid culture filtrate, has demonstrated similar antagonistic effect, expressed by the inhibition zone of 25.66 ± 0.50 mm and 18.66 ± 0.57 mm against *A. fumigatus* and *A. flavus*, respectively.

Conclusions: These observations suggest the contribution of both biomass and culture liqiud to the overall antifungal mechanism of this strain. The findings indicate that *Wickerhamomyces anomalus* CNMN-YS-07 possesses high potential as a biofungicide for the management of *Aspergillus* spp. in agricultural systems offering a basis for its further evaluation under laboratory and field conditions.

Keywords: yeast, antifungal activity, biocontrol agent, *Aspergillus* spp.

Acknowledgments: The results were obtained within the Project 25.80012.5107.16SE - DEFEND – Development of ecological and natural fungicides based on yeast- funded by the National Agency for Research and Development (NARD).

EVALUATION OF YEAST STRAINS FOR BIOSTIMULANT POTENTIAL APPLICATIONS

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https://doi.org/10.52757/bsd26.37

Background: The intensification of global agricultural production in response to a rapidly growing population has led to extensive reliance on synthetic fertilizers and pesticides, which, despite their short-term agronomic efficacy, might caused significant long-term deterioration of soil health, disruption of native microbial communities, and accumulation of environmental pollutants. Microbial biostimulants, such as yeasts, serve as promising alternatives to chemical pesticides through mechanisms including competition, production of bioactive compounds, and induction of plant growth. According to recent data, yeasts are increasingly recognized as promising biostimulants due to their ability to enhance plant growth, improve nutrient uptake, and stimulate plant defense responses.

The aim of the present study was to select yeast strains belonging to the genera *Wickerhamomyces* (*Hansenula)*, *Saccharomyces*, and *Lipomyces*, and to evaluate the biochemical composition of their biomass as a potential source of bioactive compounds and derived preparations with practical applications in sustainable agriculture.

Materials and methods: The screening of yeast strains was carried out using spectrophotometric methods, based on productivity indices, protein and carbohydrate content, total antioxidant activity, and the activity of the enzyme superoxide dismutase.

Results: As a result of the study, the yeast strain *Wickerhamomyces anomalus* CNMN-YS-07, demonstrating superior biomass accumulation (5.45 g/L) and high protein (48.22% dry weight) and carbohydrate (19.89% dry weight) content compared with the other investigated strains, was selected for further research. The enzymatic and antioxidant profiling of the yeast biomass revealed strong antioxidant activity in *W. anomalus* CNMN-YS-07 (62.36% ABTS radical scavenging), while superoxide dismutase activity reached 225.5 U/mg protein, exceeding that of strains from other genera.

Conclusions: The results obtained has demonstrated that the yeast strain *Wickerhamomyces anomalus* CNMN-YS-07 represents an promising biotechnological object with significant biocontrol potential. I

Keywords: yeast, screening, biostimulant, antioxidant activity

Acknowledgments: The research was supported by 020101 InBioS – “Innovative biotechnological solutions for agriculture, medicine and environment,” funded by the Ministry of Education and Research of the Republic of Moldova.

FEATURES OF WATER RELATIONS IN FRUIT TREES DEPENDING ON GROWING CONDITIONS AND GENOTYPE

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https://doi.org/10.52757/bsd26.38

Background: Water stress is one of the most damaging abiotic stressors affecting agricultural crops. The physiological study of plant responses during periods of declining soil and air moisture made it possible not only to determine the influence of dehydration on tree condition, but also to assess their drought resistance.

The aim of this study was to evaluate the influence of growing conditions and genotype on water exchange and drought tolerance of plum and apple trees throughout the annual vegetation cycle.

Materials and methods: Two fruit tree species were included in the study: pome fruits represented by the apple varieties *Granny Smith* and *Golden Spur*, and stone fruits represented by the plum varieties *Udlinionnaya* and *Stanley*. The research was conducted under laboratory and field conditions and included the assessment of physiological indicators related to the water regime, namely total water content, relative leaf turgidity, water deficit, water-holding capacity, and leaf transpiration rate.

Results: During the dry period of the growing season (July–August), more pronounced changes occurred in the water regime than during the more humid months (May–June). Studies showed that the quantitative losses of total water content in the leaves of both apple varieties during the growing season were greater than those in plum varieties. The decrease in water content led to reduced transpiration intensity and an increased ability of the leaves to retain water. Maximum transpiration was observed in spring, followed by a continuous decline until autumn. The transpiration intensity of apple leaves, which had a larger foliar surface and a thinner cuticle, exceeded that of plum leaves. In trees grown under conditions of insufficient humidity, transpiration was less intense, whereas the water-holding capacity of the leaves was more pronounced. On hot and dry summer days, strong transpiration combined with reduced water uptake caused water deficiency in the leaves, which correlated with the degree of plant water supply. Lower water deficiency was recorded in the leaves of plum trees grown under both optimal and insufficient humidity conditions. Drought-resistant fruit trees were characterized by higher tissue water content and lower water deficit, resulting in relatively high turgor. The relative leaf turgidity of trees grown under optimal conditions showed higher values than that of trees grown under insufficient humidity, especially during July–August. Plum trees exhibited greater leaf turgidity than apple trees.

The study demonstrated that plum varieties, during the growing season, were characterized by higher tissue water content, greater transpiration intensity, lower water deficit, higher water-holding capacity, and a smaller decline in leaf turgidity compared with apple varieties. Growth of both apple and plum trees under insufficient humidity reduced the efficiency of physiological processes compared with trees grown under optimal humidity, as reflected in the studied indicators.

Conclusions: Plum varieties are characterized by moderate drought resistance, whereas apple trees exhibit low drought resistance in both species. As a result of the study, we concluded that plum trees are characterized by moderate drought resistance, whereas apple trees exhibit low drought resistance.

Keywords: fruit crops, water regime, optimal humidity, drought resistance, water deficiency.

OPTIMIZATION OF MASS REARING TECHNOLOGY OF THE ENTOMOPHAGOUS *BRACON HEBETOR* Say. UNDER CONTROLLED LABORATORY CONDITIONS

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https://doi.org/10.52757/bsd26.39

Background: The entomophagous *Bracon hebetor* Say (Hymenoptera: Braconidae) is a larval ectoparasitoid used as a biological control agent against lepidopteran pests of the Pyralidae family. Due to its high reproductive capacity and short ontogenetic development cycle, *B. hebetor* can be successfully multiplied under controlled laboratory conditions. Its mass rearing requires a viable susceptible host, such as *Plodia interpunctella*, which supports completion of the parasitoid life cycle. The larvae of *P. interpunctella* also allow evaluation of the biological and reproductive parameters of the entomophagous species.

The aim of the present study was to optimize the multiplication technology of the entomophagous *Bracon hebetor* Say under controlled laboratory conditions, by estimating the influence of the host *Plodia interpunctella* on the biological and reproductive parameters of the parasitoid.

Materials and methods: Larvae of the species *Plodia interpunctella* were reared on a nutrient medium of cornmeal, dried apple, glycerin and yeast. Controlled laboratory conditions of 27 ± 1°C and 65 ± 5% humidity ensured uniform development and reduced physiological variability. 24-hour-old adults of the entomophagous insect *Bracon hebetor* were used for infestation. 25 pairs were introduced into 1 L boxes with 25 host larvae, respecting the optimal parasitism density. The containers were covered with gauze and maintained in a 12:12 photoperiod environment. Monitoring was performed daily for parasitism. The experiment included three independent replicates, each with 10 experimental units. After 10–12 days, emerging imagoes were evaluated, determining the parasitism rate, development duration, and sex ratio (♀: ♂).

Results: It was demonstrated that *Plodia interpunctella* larvae of III–V instars are optimal for the complete development of the entomophagous *Bracon hebetor*. The high parasitism rate indicates strong acceptability and trophic compatibility between parasitoid and host. Females of *B. hebetor* showed high reproductive potential, achieving on average up to 7 successive infestations during their life cycle. Total fecundity reached about 128 eggs per female, of which 122 larvae hatched (≈95.3%) and 115 reached the pupal stage (≈89.8%), reflecting high biological efficiency and offspring viability. Sex ratio analysis showed a predominance of males, with approximately 60.7% males and 39.3% females. The development cycle from egg to adult remained within species-specific limits and was strongly influenced by temperature (27 ± 1°C) and humidity (65 ± 5%). Maintaining these conditions ensured synchronized development and reduced mortality in immature stages, favoring the production of viable new generations.

Conclusion: The species *Plodia interpunctella* represents a suitable and efficient host for the multiplication of the entomophagous *Bracon hebetor* under controlled laboratory conditions, ensuring complete development and major viability of the offspring.

Acknowledgments: The research was conducted within the framework of Subprogram 011103 Development of ecologically harmless means of reducing the impact of harmful organisms on agricultural crops against the background of climate change, funded by the Ministry of Education and Research of the Republic of Moldova.

NON-*SACCHAROMYCES* YEASTS IN FERMENTED BEVERAGES

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https://doi.org/10.52757/bsd26.40

Fermented beverages are drinks obtained through microbial fermentation and include both nonalcoholic products and widely consumed alcoholic beverages such as cider, beer, and wine. Their production is traditionally based on *Saccharomyces cerevisiae strains*, valued for their strong fermentative capacity and technological traits that ensure stable, uniform, and high-quality products. For many years, non-*Saccharomyces* yeasts were considered undesirable contaminants in alcoholic beverage manufacture. However, this perception has changed in recent years, as studies of spontaneous wine fermentation have shown their important contribution to the sensory quality of the final product. Although many non-*Saccharomyces* yeasts have lower fermentative power and ethanol productivity than *S. cerevisiae*, they possess valuable properties absent or less pronounced in this species. These include the production of elevated levels of aromatic compounds, such as esters and fatty acids, as well as the ability to inhibit spoilage microorganisms [1].

Selected and inoculated strain of *S. cerevisiae* can suppress any non-*Saccharomyces* species and dominate the fermentation process, but several studies have shown that non-*Saccharomyces* yeasts can persist during fermentations inoculated with pure cultures of *S. cerevisiae*. These yeasts predominate at the initial stage of the spontaneous fermentation until certain concentration of ethanol is reached up to 5% v/v, then being inhibited due to the effect of the ethanol and the decreasing of dissolved oxygen. The fermentative activity of non-*Saccharomyces* is manifested in the presence of small amounts of oxygen which leads to an increase in cell biomass and the decrease in ethanol yield, a strategy that can be used to reduce the ethanol content of wines produced in coculture with *S. cerevisiae* for low-alcohol beverages obtaining [3]. It is important to note an increasing production of low-alcohol products such as wine and beer in the past decades, which shows that this technology has become more promising. Among the most studied non-*Saccharomyces* yeasts that reached special importance for researchers include *Kloeckera*, *Hanseniaspora*, *Brettanomyces*, *Pichia*, *Lanchacea*, *Kluyveromyces*, *Torulaspora delbrueckii*, *Metschnikowia pulcherrima* [2,3].

Keywords: low-alcohol, fermentation, beverages

Acknowledgments: This study was supported by the research project 25.80012.5107.12TC „Elaboration of the grapes and berries low-alcohol beverages technology”, funded by National Agency for Research and Development.

References:

1. Desiderio Estela Escalante, W. (2019). Perspectives and uses of non-saccharomyces yeasts in fermented beverages. In Frontiers and New Trends in the Science of Fermented Food and Beverages. IntechOpen. https://doi.org/10.5772/intechopen.81868
2. Klimczak, K., Cioch, M., Ciosek, A., & Poreda, A. (2024). Application of non-Saccharomyces yeast for the production of low-alcohol beer. Foods. 13, 3214. https://doi.org/10.3390/foods13203214
3. Klimczak, K., Cioch, M., & Poreda, A. (2024). Application of non-Saccharomyces yeast for low-alcohol beer production. Applied Sciences. 14(15), 6755. https://doi.org/10.3390/app14156755

IMPACT OF A GREEN-SYNTHESIZED NANOBIOCOMPLEX ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF JUVENILE WHEAT PLANTS

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https://doi.org/10.52757/bsd26.41

Background: Nanotechnology is increasingly applied in agriculture due to the unique properties of nanoparticles and nanomaterials, while green synthesis has emerged as an environmentally friendly approach for obtaining plant-active nanoformulations. However, further research is needed to better understand the physicochemical organization of these systems and their biological effects in plants.

Aim of the study: To synthesize a multicomponent nanobiocomplex (NBC) containing Ag, Cu, Mn, Zn, Fe, Mg, Mo, Se, and I, using *Cucumis sativus* and *Aloe vera* extracts as reducing and stabilizing agents, and to evaluate its physicochemical properties and physiological effects on juvenile wheat plants.

Materials and Methods: The NBC was produced by green synthesis using plant extracts of *Cucumis sativus* and *Aloe vera*. Physicochemical characterization was performed by UV–Vis spectroscopy, transmission electron microscopy (TEM), and morphometric analysis. Physiological effects were assessed on wheat through seed priming and foliar application using biochemical, biophysical, biometric, morphometric, and statistical methods. Parameters analyzed included growth indices, electrolyte leakage, pigment content, antioxidant enzyme activity, and chlorophyll fluorescence.

Results: The NBC supernatant showed a broad UV band at ~270–300 nm, indicating that its optical properties were mainly determined by plant-derived chromophores and metal–organic interactions. TEM and morphometric analysis revealed a hierarchical nanostructure composed of two primary-particle fractions and a heterogeneous aggregate fraction: ultrasmall primary particles of 1.88–5.64 nm (3.70 ± 1.12 nm, median 3.59 nm; n = 21), dominant primary particles of 6.06–13.13 nm (10.06 ± 2.23 nm, median 10.42 nm; n = 41), aggregates mainly ranging from 20.05 to 81.82 nm (39.33 ± 16.03 nm, median 33.47 nm; n = 115) and larger aggregates ranged from 84.38 to 125.12 nm (107.64 ± 12.83 nm, median 107.77 nm; n = 8).

In wheat, NBC produced positive physiological effects. Seed priming with 0.75–1.25% NBC stimulated early seedling growth, increasing seedling length and fresh biomass. Treatment with 1.25% NBC improved the VI_1 index, while 2.0% NBC increased the integrated viability index VI_2. The strongest reduction in electrolyte leakage was observed at 1.5% NBC, indicating membrane-protective effects. Foliar application of 0.75% NBC increased chlorophyll a, carotenoids, and total chlorophyll content at the 5–6 leaf stage. NBC also induced dose-dependent increases in POD and CAT activities and favorable chlorophyll fluorescence responses without marked photoinhibition.

Conclusions: The green-synthesized NBC based on Cucumis sativus and Aloe vera extracts is a plant-active nanobiocomplex composed of ultrasmall and small particles assembled mainly into medium-sized aggregates. Its application improved growth and physiological performance of juvenile wheat plants through coordinated effects on early development, membrane stability, pigment accumulation, antioxidant activity, and photosynthetic function.

Keywords: *Triticum aestivum* L., *Apium graveolens* L., green synthesis, seed priming, nanoparticles.

CYANOBACTERIAL EXOPOLYSACCHARIDES: FROM PRODUCTION TO APPLICATIONS IN FOREST SEEDLING SUPPORT

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https://doi.org/10.52757/bsd26.42

Background: Enhancing Forest seedling resilience and improving soil quality are critical challenges under current climate change conditions. Cyanobacterial exopolysaccharides (EPS) represent a promising biotechnological solution due to their capacity to improve water retention, stabilize soil structure, and modulate rhizosphere interactions, thereby supporting plant adaptation to abiotic stress.

Material and Methods: The cyanobacterial strain *Nostoc linckia* CNMN-CB-03 was cultivated on a range of nutrient media to identify optimal conditions for EPS production. Key physicochemical parameters, including temperature, light intensity, and pH, were systematically optimized. EPS were isolated from the culture medium by ethanol precipitation, then dried to constant weight and formulated into a stable powder via spray drying. The resulting product was applied in a nursery experiment on oak seedlings using localized rhizosphere treatment (0.1 g/L), with untreated plants serving as controls.

Results: Comparative evaluation of nutrient media identified MN-1 and BG-11 supplemented with $NaHCO_3$ as the most efficient systems for EPS biosynthesis, with MN-1 providing consistently higher yields under optimized conditions. Implementation of a stage-specific cultivation regime - 30°C and 60 μmol photons m^{-2} s^{-1} during the initial growth phase, followed by reduced temperature (25°C) and light intensity (35 μmol photons m^{-2} s^{-1}), significantly enhanced EPS production, reaching up to 2.68±0.09 g/L after a 16-day cultivation cycle. The developed protocol proved robust and reproducible, integrating biomass cultivation, EPS recovery, and downstream processing into a coherent technological workflow. Spray drying enabled the production of a homogeneous, stable, and easily applicable powder suitable for field use. Nursery trials demonstrated that EPS application did not negatively affect seedling physiological status and contributed to maintaining root system integrity. Moreover, treated seedlings showed indications of improved rhizosphere conditions, likely related to enhanced soil moisture retention and structural stability. These effects are expected to translate into improved establishment rates and root regeneration capacity during the subsequent growing season.

Conclusions: *Nostoc linckia*-derived EPS demonstrate strong potential as a sustainable biostimulant for forestry applications, supporting root protection and improving rhizosphere functionality. The developed technology is experimentally validated, scalable, and relevant for practical implementation in nursery and reforestation systems.

Keywords: cyanobacteria, *Nostoc linckia*, exopolysaccharides; biostimulants, forest seedlings; soil stabilization; stress tolerance.

Acknowledgments: This work was supported by research project 25.80015.7007.06VI funded by the National Agency for Research and Development, Republic of Moldova.

IN VITRO SENSITIVITY OF MICROFUNGAL STRAINS TO GLYPHOSATE

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https://doi.org/10.52757/bsd26.43

Background: Glyphosate (N-(phosphonomethyl)glycine, GP) is one of the most widely used herbicides globally. It inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme of the shikimate pathway present in plants, algae, bacteria, fungi, and apicomplexan parasites. Disruption of this pathway blocks the biosynthesis of aromatic amino acids and essential secondary metabolites, which is lethal to target plants and may affect non-target microorganisms. Fungi are of particular interest in this context: sensitive species may experience growth inhibition, whereas tolerant strains can survive and utilize glyphosate as a nutrient source.

The aim of this study was to assess the *in vitro* sensitivity of 15 microfungal strains to glyphosate (50 and 100 mg/L) using an agar block assay.

Materials and methods: The strains, representing *Ascomycota*, *Basidiomycota*, and *Mucoromycota*, were isolated from plastic-contaminated soil and the external surfaces of saproxylic beetles and their larvae from the "Plaiul Fagului" Nature Reserve (Moldova). Fungal growth on control and GP-supplemented Czapek agar was evaluated by measuring colony diameters at days 3 and 7; sensitivity was quantified using the inhibitory activity index (IA, %).

Results: GP induced a wide range of responses, from growth stimulation (-8.00%) to strong inhibition (71.33%). *Peniophora cinerea* showed the highest sensitivity, with inhibition increasing from 40.0% (50 mg/L, 3 days) to 71.33% (100 mg/L, 7 days). *Alternaria tenuissima* demonstrated a consistent increase in inhibition from 29.08% to 42.61% under intensified exposure. *Penicillium citreonigrum* exhibited a hormetic-like response, with initial growth stimulation (-8.00%, 50 mg/L, 3 days) followed by inhibition at higher concentration and longer exposure (13.33%, 100 mg/L, 7 days). *Trichoderma* sp. 1 showed no inhibition under any tested conditions, whereas *T. harzianum* exhibited minor and variable responses (12.78% to 0.56%). *Fusarium* sp. 1 showed a decrease in inhibition over time at 100 mg/L (from 13.95% to 0%), suggesting potential adaptive responses. In several strains, inhibition increased after 7 days, indicating a time-dependent effect, and was generally higher at 100 mg/L.

Conclusions: Overall, the results reveal pronounced inter-strain variability in glyphosate susceptibility, with implications for microbial ecology and the selection of fungal strains for bioremediation of herbicide-contaminated environments. The observed hormetic and adaptive-like responses warrant further investigation.

Keywords: micromycetes, inhibitory activity, organophosphorus pesticide

Acknowledgments: This research work was funded by Government of Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 ”InBioS - Innovative biotechnological solutions for agriculture, medicine and environment”.

COMPARATIVE ANALYSES OF CHEMICAL CONSTITUENTS FROM *Juniperus sabina* AND *Juniperus communis* FRUITS

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https://doi.org/10.52757/bsd26.44

Background: *Juniperus sabina* L. and *Juniperus communis* L. *J.* are valuable plants due to their hardiness and usefulness, serving both ecological (landscape maintenance) and practical purposes (ornamental use, essential oil production, biological activity of their secondary metabolites). The fruits of these plants vary significantly in weight, chemical composition, and antioxidant activity depending on the growing location and year of harvest.

The aim of this study was to compare some morphological properties, chemical constituents and antioxidant activity of the fruits of two juniper species: *J. sabina* L. and *J. communis* L. growing in the Republic of Moldova and the Republic of Armenia.

Materials and methods: The juniper fruits were collected from Chisinau region of the Republic of Moldova and from three regions of Armenia: Yerevan, Kotayk and Tavush. The total phenolic content of juniper fruits was determined in gallic acid equivalent (GAE) using the Folin-Ciocalteu procedure. Antioxidant activity of fruit extracts was evaluated by DPPH method, and expressed in Trolox equivalent antioxidant capacity (TEAC).

Results: The weight of 100 *Juniperus sabina* fruits was 1.3–1.5 times greater than that of *Juniperus communis* fruits grown in the Republic of Moldova. The weight of 100 *Juniperus communis* fruits from Kotayk was 3.6–3.9 times greater than that of fruits grown in Tavush and 2.7–2.9 times greater than those from Yerevan.

The total phenolic content of juniper fruits was strongly influenced by species, ripening stage (first or second year), and extraction procedure. In hydroalcoholic extracts, total phenolic content ranged widely from 7.7 to 56.2 mg GAE/g dry residue. Comparative analysis showed that fruits from the Yerevan floristic region had a 1.5-fold higher total phenolic content than fruits from the Tavush and Kotayk regions.

The antioxidant activity of fruit extracts ranged from 15.32 to 103.12 mg TEAC/g dry residue. The fruit extract demonstrated the high antioxidant activity that was directly correlated (Pearson correlation 0.8020) with the total phenolic content. However, no correlation was found between the total phenolic content, antioxidant activity, and the weight of 100 fruits.

Conclusions: The study demonstrates that both species and geographic origin significantly influence the morphological traits and bioactive composition of juniper fruits.

Keywords: juniper berry, weight, total phenolic content, antioxidant activity

Acknowledgments. This joint research was initiated with the support of the National Agency for Research and Development of the Republic of Moldova and the Higher Education and Science Committee, Ministry of Education, Science, Culture and Sports of the Republic of Armenia through the bilateral project 26.80013.5107.02ARM and 26NARD-1A003 between the Republic of Moldova and the Republic of Armenia.

IMPACT OF BIOREGULATOR ON CORN RESILIENCE TO NON-OPTIMAL TEMPERATURES

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https://doi.org/10.52757/bsd26.45

Background: A plant's ability to maintain productivity under extreme temperatures and drought depends on its genotype and stress response. Therefore, current research focuses on identifying corn lines and hybrids with high germination energy and vigor in cold soils, as well as stable growth under drought and temperature fluctuations. Their performance can be improved through an integrated approach combining genetic potential with pre-sowing seed treatment using natural growth regulators.

The aim of this study was to evaluate the effect of the bioregulator JS, extracted from *Juniperus sabina* L. plant, on the early stages of seed development of the mid-season corn hybrid Porumbeni 396 MRf (FAO 390) under temperature stress.

Materials and methods: The corn hybrid Porumbeni 396 MRf (FAO 390) has a horny endosperm, ensuring specific adaptability. Its high grain density slows moisture absorption, causing low germination energy in cold soil ("difficult start"). At the same time, it shows higher heat tolerance at early growth stages than hybrids with flint and dent endosperm.

Seeds were exposed to non-optimal temperatures in two tests: a cold test, with germination for 7 days at +10°C followed by 4 days under optimal conditions; and a heat test, with seeds kept in a water bath at +50°C for 30 min, then germinated for 7 days under optimal conditions, according to international rules.

Results: At low temperature (+10°C), pre-treatment of seeds with the bioregulator JS stimulated metabolism and showed a strong protective effect. Root and seedling vigor increased by 17.5% compared with the untreated control. Energy metabolism was also optimized: the share of biomass mobilized for root and seedling growth rose from 49.58% (control) to 53.98% (treated), while respiration costs decreased by 8.7%. As a result, metabolic efficiency increased by 19.3%, indicating that endosperm resources were used mainly for morphogenesis rather than compensation of temperature stress.

Under heat stress (+50°C), the effect of JS shifted from growth stimulation to protection and adaptation. Despite maintaining high root and seedling vigor, 28.4% above the control, a marked restructuring of bioenergetics was observed: metabolic efficiency decreased by 31.5%, while respiration increased by 18.4%. This suggests activation of energy-demanding repair mechanisms and heat shock protein synthesis. Resources were redirected from biomass accumulation to viability maintenance and protection against overheating. Thus, JS was most effective at low temperature, where it optimized endosperm resource use. Under heat stress, it stimulated defense systems, temporarily reducing metabolic efficiency in favor of survival.

Conclusions: Pre-treatment of the Porumbeni 396 MRf corn hybrid seeds with the JS bioregulator demonstrates high effectiveness in mitigating temperature stress, although the adaptation mechanisms are fundamentally different, depending on the influence of sub-optimal and super-optimal temperatures.

Keywords: bioregulator JS, corn seed, vigor, metabolic efficiency, non-optimal temperature

Acknowledgments: This study was supported by the Ministry of Education and Research of the Republic of Moldova within the Subprogram 011101 "Genetic and biotechnological approaches to agro ecosystem management under climate change conditions" for 2024-2027.

EVALUATION OF CHANGES IN STARCH MOBILIZATION DURING CORN GERMINATION UNDER THE INFLUENCE OF NON-OPTIMAL TEMPERATURES

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https://doi.org/10.52757/bsd26.46

Background: Starch, a complex carbohydrate, is a reserve substance and one of the primary sources for corn seed germination. Breaking down into simple sugars (glucose) during the early growth stage of corn seeds, the starch supports both root and seedling biomass accumulation, participating in the synthesis of cells structural components and providing energy for plant respiration and growth. Thus, the proportion of starch mobilized from the corn seeds determines their metabolic efficiency, and changes in starch mobilization indicate modification to the physiological processes of corn germination and growth in response to external abiotic factors.
The aim of this study was to investigate the effect of two abiotic factors - temperature and treatment with the natural bioregulator JS, dry extract obtained from *Juniperus sabina* and diluted to a concentration of 0.0001%, on starch mobilization during corn seed germination and its relationship with plant biomass and metabolic efficiency.
Materials and methods: Corn seeds were germinated in six variants: two under optimal conditions - control seeds and seeds pretreated with JS; two under cold test - control and pretreated seeds with JS, which were germinated at 10°C for 7 days, followed by 4 days under optimal conditions; and two under heat test - the seeds exposed at 50°C for 30 minutes, then germinated under optimal conditions - control and seeds pretreated with JS. Starch content in intact and post-germinated seeds was determined using the Ewers-Grossfeld polarimetric method. Seeds of the maternal inbred line of the Porumbeni 455 MRf were used as study object.
Results: The grains of the maternal line contain 63.35±1.79% starch under optimal germination conditions for control seeds, 51.8% of the initial starch content was mobilized from endosperm and used for root and seedlings growth, as well as for energetic support of physiological processes. At non-optimal temperatures, the starch utilization for germination of control seeds changed significantly: at low temperatures, its consumption decreased by 9.5%, while at high temperatures it increased by 45.3% compared to the control seeds germinated under optimal conditions. The metabolic efficiency of these seeds changed similarly. This fact confirms that the studied hybrid is more resistant to high temperatures than to low ones. Pretreatment of corn seeds with the JS solution increased the consumption of starch used for seed germination under non-optimal conditions by 2.4-11.5%, which indicates the synergistic effect of two abiotic factors. A direct correlation between the amount of mobilized starch and plant biomass (0.6069), as well as between mobilized starch and metabolic efficiency (0.6192) was established. Similar results were obtained when studying changes in metabolic efficiency after treating corn seeds of the P427 hybrid with JS solutions of varying concentrations (0.1...0.0001%).
Conclusions: Thus, an assessment of changes in plant biomass, metabolic efficiency and mobilization of reserve substances, especially starch, showed that the maternal inbred line of the Porumbeni 455MRf corn hybrid has significant resistance to elevated temperature.
Keywords: corn seed, germination, starch, bioregulator JS, non-optimal temperature
Acknowledgments: This study was supported by the Ministry of Education and Research of the Republic of Moldova within the Subprogram 011101 “Genetic and biotechnological approaches to agro ecosystem management under climate change conditions” for 2024-2027.

EFFECT OF ALTERNATIVE NUTRIENT MEDIA ON BIOMASS AND ANTIOXIDANT STATUS OF *SACCHAROMYCES CEREVISIAE* STRAINS

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https://doi.org/10.52757/bsd26.47

Background: *Saccharomyces cerevisiae* baker's yeasts represent a promising platform for bakery biotechnology, particularly when cultivated using nutrient media derived from wastes such as fruit and vegetable peels. These low-cost substrates can effectively support yeast growth and biomass production. Such approaches align with circular economy principles by converting waste materials into valuable microbial products with potential antioxidant properties. This highlights the relevance of baker's yeasts not only in baking, but also in sustainable biotechnology and functional food research.

The aim of this research was to evaluate the growth performance and bioprotective potential of *S. cerevisiae* yeasts isolated in pure culture from different artisanal bakery sourdoughs cultivated on natural nutrient media, obtained from agro-industrial wastes of carrot (CE), red beet (BE) and barley (BaE), as the single source of nutrition.

Materials and methods: Four *S. cerevisiae* strains were used in the study: *S. cerevisiae* 7P, isolated from artisanal sourdough prepared from rye flour (Ukraine); *S. cerevisiae* 10P, isolated from artisanal sourdough prepared from homemade rye flour (Ukraine); *S. cerevisiae* 9P, isolated from the commercial dried sourdough "O-Tentic-Durum" (Romania); and *S. cerevisiae* 11P, isolated from the commercial dried sourdough "4199 Spring Pane" (MILBO S.P.A., Italy). The studied strains cultivated on liquid YPD nutrient medium served as the control. Total antioxidant activity of biomass samples was determined using the spectrophotometric ABTS•+ radical cation method.

Results: It was established that the use of nutrient media based on CE, BE and BaE increases the biomass productivity of yeasts compared to the control medium. Thus, the biomass production of strains *S. cerevisiae* 7P, 9P, 10P and 11P on YPD medium constituted 4.73±0.23, 3.67±0.19, 3.72±0.31 and 4.28±0.15 g L^{-1} d.w., and on CE, BE and BaE media it was significantly ($P \leq 0.05$) higher by 52.34-89.25%, 66.12-134.04% and 95.35-119.89% depending on the strain. The use of the BE nutrient medium led to a significant increase ($P \leq 0.05$) of 154.06-202.38% in the total antioxidant activity of yeast biomass, whereas CE and BaE media exhibited less pronounced differences compared to the control medium.

Conclusions: Overall, nutrient media based on carrot, beet, and barley wastes are effective nutrient sources for the cultivation of baker's yeast and the accumulation of biomass with antioxidant potential.

Keywords: *Saccharomyces cerevisiae* yeasts, wastes, biomass, antioxidant activity

Acknowledgments: The research was carried out within project 020101 InBioS - "Innovative biotechnological solutions for agriculture, medicine and environment" funded by Ministry of Education and Research of the Republic of Moldova.

FORMATION OF GENERATIVE ORGANS OF APPLE TREE UNDER WATER DEFICIENCY CONDITIONS

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https://doi.org/10.52757/bsd26.48

Background: In apple trees, floral bud initiation, differentiation and formation of generative organs occur in the summer-autumn period preceding flowering. Among the factors that can change the regularity of these processes, water regime is a crucial aspect.

The aim of this study was to identify the characteristics of the formation of apple tree generative organs depending on the variety and to evaluate the effect of water deficiency on floral morphogenesis and the quality of flower buds.

Materials and methods: Floral buds of apple trees of the Granny Smith and Golden Spur varieties were examined in dynamics, from the moment of initiation (June of the previous year) until the moment of flowering, 50 buds in each sample from control trees (irrigation, around 90% soil water content) and trees growing at a moisture level of 30% of the control.

Results: The formation of flower buds in the apple tree begins in June after the end of the first wave of shoot growth. The beginning of formation and the rate of differentiation largely depend on climatic factors and the characteristics of the variety. The generative organogenesis is the most important element of tree productivity. In the summer, the differentiation of fruit buds depended on the degree of water stress, which often led to a stop of this process. Trees affected by drought in the moment of bud initiation were able to recover the differentiation process after optimization of the water regime, showing physiological plasticity expressed in the normalization of the formation of flower buds. During the drought that lasted throughout July, the trees' ability to recover was not observed, which disrupted the process of flower bud differentiation. It was found that trees exposed to summer water deficits had bigger buds. This may be due to increased levels of abscisic acid, which stops plant growth and stimulates earlier bud formation in drought conditions as a response to water stress. In addition to slowing down development and changes in bud size, water deficiency leads to the formation of abnormal flowers. Water stress in the autumn negatively affected bud size. During this period, water deficit can cause strong competition between the buds, which complete the organogenesis of the flower in early autumn. As a result, morphological abnormalities and signs of browning and tissue necrosis were observed in the flower buds. Almost all the examined flower buds collected from control trees had morphologically well-developed generative organs, and only 3% showed abnormalities. In contrast, the number of abnormal flower buds in trees growing in drought conditions varied from 10% to 12%. The most frequent abnormalities were associated with necrosis at the ovary or pistil level. Flowering, as usual, occurred in the 2-3 decade of April without differences between the variants. However, there is a difference in the varieties: Granny Smith begins to bloom in the second decade of April, while Golden Spur - in the third. Apple trees exposed to water deficit showed a reduced number of flowers compared to the control.

Conclusions: This study highlights the importance of water availability in the summer-autumn period to provide better conditions for the formation of normal floral buds to avoid crop decline or loss in the coming year. With the dry and hot autumn-winter seasons increasing in recent years, optimal water supply is becoming essential.

Keywords: floral buds, differentiation, apple trees, drought, water deficit.

THE BIOACTIVE POTENTIAL OF NATIVE AROMATIC HERBS USED IN FOOD

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https://doi.org/10.52757/bsd26.49

Background: In the Republic of Moldova, there is a long-standing tradition of cultivating and using aromatic herbs in culinary practices. Beyond enhancing the flavor of meat and vegetable dishes, these herbs contribute to digestive health and support detoxification processes. Moreover, many aromatic plants are recognized as valuable sources of bioactive compounds with significant antioxidant potential, which are associated with various health-promoting effects.

The aim of this study was to evaluate the content and potential of biologically active compounds in selected native aromatic herbs.

Materials and methods: The study was carried out on a representative selection of widely consumed herbs: mint, dill, parsley, lovage, red basil, green basil, celery, rosemary, thyme, and lettuce. Active acidity and the content of simple sugars were determined in the juice extracted from fresh plant material. Total chlorophyll content, including chlorophyll a and chlorophyll b fractions, was determined spectrophotometrically using a UV-Vis-1800 spectrophotometer (Japan). Vitamin A content, expressed as β-carotene, was quantified following extraction with hexane and measurement of absorbance at 450 nm.

Results: The determination of dry matter content revealed considerable variation among the analyzed samples, ranging from 19.2% in thyme to a significantly lower value of approximately 7.8% in lettuce leaves. The active acidity (pH) of the herb extracts showed a narrow variation, with values between 6.85 in celery and 7.02 in parsley, indicating a slightly acidic to neutral character. The content of simple sugars varied from 2.55% in celery to 6.43% in mint, suggesting differences in metabolic composition among species. High chlorophyll content was observed particularly in mint (388 mg%), dill (370 mg%), and green basil (247 mg%), indicating their strong photosynthetic capacity and potential antioxidant activity. The assessment of vitamin A content, expressed as β-carotene, also demonstrated notable variability. Lovage exhibited the highest concentration (9.3 mg/100 g), followed by lettuce (7.56 mg/100 g) and dill (5.68 mg/100 g), whereas thyme recorded the lowest value (3.30 mg/100 g). These differences highlight the nutritional diversity of the studied herbs.

Conclusions: The investigated native aromatic herbs represent valuable sources of bioactive compounds, particularly chlorophylls and provitamin A (β-carotene), with significant nutritional and functional potential. The variability observed among species underscores the importance of their informed selection and combination in the diet to maximize health benefits. Furthermore, these findings support the potential application of aromatic herbs as functional ingredients in the development of health-oriented food products.

Keywords: bioactive compounds, aromatic herbs, chlorophyll, vitamin A, nutritional value

Acknowledgments: This research was supported by the Institutional Project, subprogram 020405 “Optimizing food processing technologies in the context of the circular bioeconomy and climate change” (Bio-OpTehPAS), implemented at the Technical University of Moldova.

STUDY OF ANTIMICROBIAL ACTIVITY OF FUNGAL STRAINS FROM THE GENERA *PENICILLIUM* AND *TRICHODERMA* AGAINST CERTAIN PHYTOPATHOGENS

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https://doi.org/10.52757/bsd26.50

Background: Fungi represent an essential source of bioactive compounds used in combating antimicrobial resistance, due to their ability to produce antibiotics and secondary metabolites with strong activity against pathogenic agents. In the face of growing drug resistance, the exploration of fungi from diverse environments, including extreme ones, is crucial for the discovery of new effective antimicrobial substances. In agriculture, fungi play an important role both in plant protection through the production of antifungal and antibacterial compounds and in improving soil fertility and stimulating crop growth.

The aim of the study was to evaluate the antimicrobial activity of fungal strains against phytopathogens in order to support sustainable plant protection strategies and reduce the use of chemical pesticides.

Material and methods: The study material consisted of 20 fungal strains belonging to the *Penicillium* and *Trichoderma* genera, previously isolated from Chisinau water reservoirs. To determine their antimicrobial properties, these strains were tested against eight plant pathogens: *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani*, *Fusarium oxysporum*, *Agrobacterium tumefaciens*, *Corynebacterium michiganensis*, *Erwinia carotovora*, and *Xanthomonas campestris*.

Results: The selected fungal strains exhibited pronounced antagonistic activity against the tested phytopathogens. The largest inhibition zones were recorded for *Penicillium* sp. strains 1, 2, 5, and 6, with values of up to 24–32 mm against *Alternaria alternata* and 18–30 mm against *Botrytis cinerea*, while *Trichoderma* strains showed inhibition zones of up to 35 mm against the phytopathogenic fungi *Alternaria alternata* and *Botrytis cinerea*. In the case of phytopathogenic bacteria, the activity was lower; however, some *Trichoderma* strains (such as *Trichoderma* sp. 7, 9, and 14) demonstrated inhibition zones of 15–22 mm against *Corynebacterium michiganensis* and *Erwinia carotovora*, while *Penicillium* strains showed inhibition of up to 20 mm against *Xanthomonas campestris*, confirming the potential of these microorganisms for use in biocontrol and plant protection.

Conclusions: Fungal strains from the genera *Penicillium* and *Trichoderma* demonstrated the ability to produce bioactive metabolites with significant antifungal and antibacterial effects. In particular, *Trichoderma* sp. 3 and *Trichoderma* sp. 14 exhibited the highest antimicrobial activity, with inhibition zones of 20–34 mm against phytopathogenic fungi and 15–20 mm against phytopathogenic bacteria. These results indicate the potential of these microorganisms to inhibit pathogen development and to be used as biological control agents in agriculture and biotechnology, as an eco-friendly alternative to chemical treatments.

Keywords: Fungi, antimicrobial activity, pathogens, biotechnology.

Acknowledgments: This research was funded by the project 25.80012.7007.32SE NARD (Development of optimal protective media for the lyophilization of aquatic bacteria and fungi).

ALTERATION OF THE COUPLING BETWEEN PHOTOSYSTEM II ELECTRON TRANSPORT AND CO_2 ASSIMILATION IN *TRITICUM AESTIVUM* L. UNDER WATER DEFICIT

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https://doi.org/10.52757/bsd26.51

Background: Water deficit is one of the major factors limiting the photosynthetic productivity of plants. Under drought conditions, the decline in CO_2 assimilation may result not only from stomatal limitations, but also from impaired coordination between photochemical reactions and carboxylation processes.

The aim of the present study was to assess changes in the relationship between electron transport in photosystem II (ETR) and net CO_2 assimilation (*A*) across different genotypes of *Triticum aestivum* L. under contrasting water supply conditions.

Materials and Methods: Plants of the wheat cultivars Moldova 5 and Meleag were grown in 10-L vegetation pots under two soil moisture regimes: 70% of full water capacity (control) and 35% of full water capacity (water deficit). The functional state of photosystem II was assessed using a PAM-2100 chlorophyll fluorometer by determining the effective quantum yield and electron transport rate (ETR). Gas exchange was assessed based on the rate of net CO_2 assimilation using a PTM-48A portable photosynthesis system. Plants were grown at a photosynthetically active radiation (PAR) level of 100-120 μmol m^{-2} s^{-1} under a 16 h light/8 h dark photoperiod. Soil moisture was monitored using ZTS-3000-TR sensors calibrated for the substrate used and the pot volume.

Results: Under optimal water supply (70% FWC), both cultivars maintained a positive relationship between ETR and CO_2 assimilation, indicating coordinated functioning of photochemical reactions and carbon fixation processes. In cv. Moldova 5, ETR ranged from 20 to 27 μmol $e^-m^{-2}s^{-1}$, while *A* varied from 3.2 to 3.8 μmol CO_2 $m^{-2}s^{-1}$; in cv. Meleag, the corresponding values were 21-27 and 3.3-3.8 μmol m^{-2} s^{-1}, respectively.

Under water deficit conditions (35% FWC), both cultivars exhibited a sharp decline in CO_2 assimilation, whereas the decrease in ETR was less pronounced. In cv. Moldova 5, *A* decreased to 0.8-1.1 μmol CO_2 m^{-2} s^{-1}, while ETR declined by 9-12 μmol e^- m^{-2} s^{-1}; in cv. Meleag, *A* ranged from 0.9 to 1.4 μmol CO_2 m^{-2} s^{-1}, where as ETR decreased by 11-13 μmol e- m^{-2} s^{-1}. This was accompanied by an increase in the ETR/*A* ratio, reflecting the apparent electron requirement for CO_2 fixation: in cv. Moldova 5, it increased from 6.2-7.1 in the control to 10.9-11.2 under water deficit, and in cv. Meleag from 6.3-7.1 to 9.2-12.2.

Conclusions: Thus, water deficit weakens the functional coupling between electron transport in photosystem II and CO_2 assimilation. The increase in the ETR/*A* ratio indicates that, under limited water availability, carbon assimilation is more strongly constrained than the photochemical generation of electron flow. This points to a disruption in the coordination between photochemical reactions and carbon fixation and likely increases the importance of photoprotective mechanisms in maintaining the stability of the photosynthetic apparatus. These results highlight the value of combining chlorophyll fluorescence and gas exchange analyses for assessing the physiological response of wheat to water deficit.

Keywords: PSII, ETR, real assimilation CO_2

Acknowledgements: The research was carried out within the sub-programme 011101 "Genetic and biotechnological approaches to management of agroecosystems in the conditions of climate change", funded by the Ministry of Education and Research.

LIPOSOMAL STABILIZATION OF SEA BUCKTHORN CAROTENOIDS FOR FUNCTIONAL FOOD APPLICATIONS

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https://doi.org/10.52757/bsd26.52

Background: Carotenoids from sea buckthorn *(Hippophae rhamnoides* L.) are valuable lipophilic bioactive compounds known for their strong antioxidant activity and health-promoting properties. However, their application in food systems is often limited by low stability and high susceptibility to oxidative degradation. Liposomal encapsulation represents an effective strategy to protect carotenoids, improve their stability, and enhance their functionality in functional food applications.

The aim of this study was to develop and characterize liposomal formulations encapsulating carotenoids extracted from sea buckthorn (*Hippophae rhamnoides* L.) in order to assess their potential application as functional food ingredients.

Materials and Methods: Liposomal formulations containing sea buckthorn (*Hippophae rhamnoides* L.) carotenoids were prepared in an aqueous medium using an adapted Mozafari method. The obtained systems were characterized by determining encapsulation efficiency and carotenoid retention rate. Antioxidant activity was evaluated using the DPPH radical scavenging assay. Storage stability of the liposomal formulations was assessed over a period of 4 weeks by monitoring the retention of bioactive compounds.

Results: The obtained liposomal formulations showed high encapsulation efficiency (92.0 ± 3.0%) and retention rate (89.2 ± 3.5%), demonstrating effective incorporation of carotenoids into liposomal structures. Encapsulated carotenoids exhibited strong antioxidant activity, with 96.0 ± 1.5% DPPH inhibition. Stability studies indicated that the liposomal systems preserved a significant amount of carotenoids after 4 weeks of storage, confirming their protective effect against oxidative and storage degradation.

Conclusions: These findings confirm that liposomal encapsulation is an efficient delivery system for improving the stability of sea buckthorn carotenoids. The developed formulations show strong potential for application in functional foods, contributing to enhanced bioactive compound retention and improved nutritional value.

Keywords: liposomes; carotenoids; functional foods; bioactive stability

Acknowledgement: The research was supported by the State Project for Young Researchers 25.80012.5107.10TC “Stabilization of Plant-derived Bioactive Compounds by Liposomal Encapsulation”, running within Technical University of Moldova

LIPOSOMAL STABILIZATION OF GRAPE POMACE POLYPHENOLS FOR FUNCTIONAL FOOD MATRICES

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https://doi.org/10.52757/bsd26.53

Background: Grape pomace represents a rich and sustainable source of polyphenols with strong antioxidant and bioactive properties. However, their incorporation into food matrices is often limited by low stability, sensitivity to environmental factors, and reduced functionality during storage. Liposomal encapsulation offers a promising approach to protect polyphenols, enhance their stability, and improve their applicability in functional food matrices.

Aim of the study: To develop and characterize liposomal formulations loaded with grape pomace polyphenols, and to evaluate their encapsulation efficiency, antioxidant activity, and stability during storage for potential application in functional food matrices.

Materials and methods: In this study, liposomal formulations loaded with grape pomace polyphenols were developed using an adapted Mozafari method in an aqueous medium and characterized in terms of particle size, encapsulation efficiency, retention rate, antioxidant activity (DPPH assay), and storage stability over 4 weeks.

Results: The liposomal formulations exhibited nanoscale particle sizes, reaching up to 168.58 ± 2.48 nm, confirming their suitability for incorporation into food systems. High encapsulation efficiency (>80%) and retention rates were achieved, indicating effective incorporation of polyphenols within liposomal structures. The antioxidant activity of encapsulated polyphenols remained significant during storage, demonstrating the protective effect of the phospholipid bilayer against oxidative degradation. After 4 weeks, liposomal systems preserved a considerable proportion of bioactive compounds compared to non-encapsulated extracts.

Conclusions: These findings highlight the potential of liposomal encapsulation as an efficient delivery system for stabilizing grape pomace polyphenols and enhancing their functionality in food matrices. The developed formulations support the valorization of agro-industrial by-products and their application in functional foods with improved bioactive compound retention and nutritional value.

Keywords: liposomal encapsulation; grape pomace polyphenols; functional foods.

Acknowledgments: The research was supported by Institutional Project, subprogram 020405 “Optimizing food processing technologies in the context of the circular bioeconomy and climate change”, Bio-OpTehPAS, being implemented at the Technical University of Moldova.

ECOLOGICAL VULNERABILITY OF THE VILUNI LAGOON SYSTEM IN ALBANIA USING COPERNICUS SENTINEL-2 IMAGERY

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https://doi.org/10.52757/bsd26.54

Background: The Viluni Lagoon, a critical coastal ecotone in northern Albania, is shaped by hydrological interactions between the Shkodra Lake system, the Buna River, and the Adriatic Sea. This dynamic interface between freshwater and marine environments supports diverse migratory bird populations and specialized species adapted to transitional conditions, making it highly sensitive to environmental change. Anthropogenic pressures, including tourism, sand extraction, and altered river discharge, disrupt the lagoon's natural balance and contribute to habitat degradation. At the same time, climate-driven processes such as coastal erosion, floodplain shifts, and intensified storm surges increasingly influence shoreline dynamics, water levels, and salinity patterns.

The aim of this study was to asses the ecological vulnerability of the Viluni Lagoon system (Albania), based on Copernicus Sentinel-2 Imagery .

Materials and methods: To define the study area, a Digital Elevation Model (DEM) was used to delineate the lagoon's catchment, limited to elevations below 10 m above sea level and within a 10 km buffer. A multi-temporal analysis was conducted using Sentinel-2 (MSI) imagery from 2017 to 2025, with one representative June image selected annually to ensure phenological consistency. Shorelines were digitized manually from True Color Composites (TCCs), with supervised classification used to improve boundary accuracy. Biophysical conditions were assessed using spectral indices (NDVI, NDMI, NDWI, and MNDWI). Binary rasters were converted to vector format to quantify total lagoon area and the proportion of open water. A rule-based classification identified five vegetation and habitat classes.

Results indicate that fluctuations in lagoon extent and water coverage reflect interactions between sea-level rise and sediment inputs from the Buna River. The observed expansion of the lagoon, peaking in 2021, suggests habitat loss in salt marshes and wet meadows, likely replaced by shallow water and mudflats. The western shoreline emerges as a critical zone of ecological vulnerability and a potential tipping point for habitat loss, underscoring conservation priorities.

Conclusion: The results demonstrate that the Viluni Lagoon system is highly vulnerable to the combined effects of sea-level rise and river sediment dynamics, leading to shoreline instability and progressive habitat transformation, particularly along the western sector, which should be considered a priority area for conservation and management.

Keywords: vulnerability; coastal wetlands; Sentinel-2; Copernicus; Adriatic lagoon; Albania

Acknowledgments: This research was supported by the Student Grant Competition (SGS) of the Czech University of Life Sciences Prague, project no. 2025/21/IGA2025, entitled "Sustainable Use of Agricultural Landscape".

PREDICTING TOMATO YIELD IN CENTRAL EUROPE THROUGH INTEGRATION OF FIELD EXPERIMENTS, DSSAT-CROPGRO-TOMATO SIMULATIONS AND MACHINE LEARNING

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https://doi.org/10.52757/bsd26.55

Background: Tomato (*Solanum lycopersicum* L.) is a major vegetable crop worldwide, valued for its nutritional quality, rich in antioxidants such as lycopene, and its economic significance.
The aim of this study was to test three high-yielding tomato varieties (drought-tolerant Cocktail Crush F1, heat-sensitive Nagina F1, and the stable line Momini Salzi) at the Ostra site (2023–2025) to develop models for climate adaptation, disease risk management, and production efficiency.
Materials and methods: The Ostra site features an automated weather station operated by the Department of Agro ecology and Crop Production (ČZU), which provides continuous, canopy-level monitoring of daily meteorological conditions directly within the crop environment. We integrated field observations with the Cropping System Model CSM-CROPGRO-Tomato, part of the Decision Support System for Agrotechnology Transfer (DSSAT) software, and calibrated the model using Generalized Likelihood Uncertainty Estimation with 50,000 evaluations per cultivar, then validated against independent 2025 data.
Results: The calibrated model generated synthetic datasets that captured cultivar-specific physiological responses and were used to train 10 machine learning algorithms. Long Short-Term Memory networks achieved the highest prediction accuracy ($R^2 = 0.94$, RMSE = 0.31 t ha^{-1}), providing reliable yield forecasts 30 days before the initial harvest. The drought-tolerant hybrid Cocktail Crush F1 demonstrated superior stability, maintaining 85% of yield potential under severe stress compared to 76–78% for other cultivars. Under optimized management (double-row planting, late-May transplanting), yield gains reached 2.8 t ha^{-1} under favourable scenarios and 1.6 t ha^{-1} under high-emission scenarios. Thirty-day forecasts enable early-market entry with 35–45% price premiums and reduce last-minute labour costs by 20–25%.
Conclusions: The key innovation is a hybrid framework that leverages the CSM-CROPGRO-Tomato within DSSAT–a sophisticated Fortran-based system–as a knowledge engine to generate physiologically robust synthetic data. These datasets train accessible machine learning algorithms for practical 30-day yield forecasting. The study presents a hybrid framework combining DSSAT-based modelling with machine learning to deliver practical, accurate farm-level yield predictions.

Keywords: DSSAT-CROPGRO-Tomato; Long Short-Term Memory; machine learning; ClimRisk; Central Europe

Acknowledgments: This work was supported by the PERUN project (Prediction, Evaluation and Research for Understanding National Sensitivity and Impacts of Drought and Climate Change for Czechia), co-financed with state support from the Technology Agency of the Czech Republic under the Environment for Life Programme.

BIOREMEDIATION OF SOIL HEAVILY CONTAMINATED WITH ORGANOCHLORINE PESTICIDES: DDTs AND HCHs

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https://doi.org/10.52757/bsd26.56

Background: Dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) are organochlorine pesticides (OCPs), or chlorinated hydrocarbons used extensively from the 1940s till the 1970s in agriculture. Although their production and agricultural use was banned under the Stockholm Convention on Persistent Organic Pollutants (DDT in 2001 and HCH in 2009), their traces can still be found in water and soil. Due to their environmental persistence, toxicity, and pronounced bioaccumulation and biomagnification potential, these OCPs are of great concern. The reported half-lives of DDT in soil range from 4 to 35 years, while those of HCH vary between 2 and 10 years depending on the isomer and soil properties. Even decades ago, remain locations with highest concentrations of these contaminants.

The aim of this study was to evaluate the effectiveness of bioremediation strategies for soil heavily contaminated with organochlorine pesticides (DDT, HCH, and their metabolites).

Materials and methods: A bench scale experiment was carried out, the treatment of contaminated soil included 8 cycles of alternating anaerobic and aerobic conditions. Bioremediation was enhanced through the addition of a locally sourced amendment consisting of 40% iron powder (0.3–0.5 mm), 50% wood shavings, and 10% composted chicken manure.

Results: After eighth consecutive bioremediation cycles, the total concentration of OCPs decreased from initial 600-700 mg/kg dry sol to 90-145 mg/kg, depending on the amount of amendment. The total concentration of DDTs (∑DDTs) in soil, compared to the experimental variant without additives, decreased twofold, while the concentration of DDT declined by 5.5 times. This reduction was attributable both to a decrease in p,p'-DDT and to the near-complete mineralization of o,p'-DDT. The DDD/DDE ratio reached 4.5, indicating that the parent DDT degradation accompanied by the accumulation of metabolites formed under anaerobic conditions.

The application of the amendment also resulted in a 4.6-fold reduction in total HCHs concentrations compared to variant without amendments: γ-HCH isomer decreased by 5.6-fold, while α-HCH declined by 28-fold, accompanied by the formation β-HCH.

The degradation of OCPs was accompanied by a pronounced stimulation of soil microbiota, including both bacteria and microscopic fungi, whose abundance increased tenfold.

Conclusions: The alternating anaerobic–aerobic bioremediation strategy, enhanced with an iron–organic amendment, proved highly effective for the degradation of organochlorine pesticides in contaminated soil. The process favored reductive transformation pathways and stimulated soil microbial activity.

Keywords: organochlorine pesticides, bioremediation, soil microbiota

Acknowledgments: This research work was funded by Government of Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 ”InBioS - Innovative biotechnological solutions for agriculture, medicine and environment”.

INTENSIFICATION OF VIOLACEIN PRODUCTION FROM AGRO-INDUSTRIAL BY-PRODUCTS USING SOLID-STATE FERMENTATION BY *JANTHINOBACTERIUM LIVIDUM*

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https://doi.org/10.52757/bsd26.57

Background: The accumulation of agro-industrial residues is a major environmental and economic challenge, encouraging sustainable bioprocesses for their conversion into high-value products. Solid-state fermentation (SSF) is a promising strategy due to low water use, reduced energy demand, and high substrate compatibility. In this context, violacein, a bisindole-derived purple pigment produced by several bacterial species, has gained interest because of its broad biological activities, including antimicrobial, antitumor, and immunomodulatory effects. However, its industrial application remains limited by low production yields under conventional submerged fermentation conditions.

Aim of the study: This study aimed to evaluate the potential of diverse agro-industrial by-products as low-cost substrates for violacein production by *Janthinobacterium lividum* under SSF conditions, and to optimize key process parameters to maximize production efficiency.

Materials and methods: A range of lignocellulosic and protein-rich residues, including hemp seed cake, grape marc, coffee grounds, brewers' spent grains, wheat bran, date pits, sunflower seed cake, and rapeseed cake, were screened as fermentation substrates. Their biochemical composition was determined by standard methods: Kjeldahl for protein, Soxhlet for lipids, AOAC for moisture and ash, and Folin-Ciocalteu for total phenolics. SSF experiments were performed under controlled conditions (70% initial moisture, 25 °C, 24 h inoculum age, 7 days fermentation). Parameters affecting violacein biosynthesis (moisture, inoculum volume, temperature) were optimized using a Box-Behnken design and response surface methodology to assess factor interactions and identify optimal conditions.

Results: Substrate characterization showed marked compositional variability: hemp seed cake had the highest protein and lipid contents, while date pits had the highest total phenolic content (14.63 g GAE/100 g). Among the tested substrates, hemp seed cake provided the best conditions for bacterial growth and violacein biosynthesis. Optimization identified 25 °C, 4 mL inoculum, and 80% moisture as optimal parameters, yielding 0.99 mg/g dry matter after 7 days of fermentation. This was a significant improvement over non-optimized conditions, confirming the efficiency of the applied strategy.

Conclusions: This study demonstrates that agro-industrial by-products, especially hemp seed cake, are efficient and sustainable substrates for violacein production by SSF. The findings support integrated waste valorization processes and the transition toward a circular bioeconomy through coupling residue management with high-value bioactive compound production

Keywords: solid-state fermentation, violacein, *Janthinobacterium lividum*, agro-industrial residues, process optimization.

Acknowledgments: Communauté Urbaine du Grand Reims, Département de la Marne, Region Grand Est and European Union (FEDER Champagne-Ardenne 2014-2020, FEDER Grand Est 2021-2027) are acknowledged for their financial support to the Chair of Biotechnology of Centrale Supélec and CEBB.

ZooBioR-INDUCED CHANGES IN EGG PRODUCTION AND MORPHOMETRIC PARAMETERS OF QUAILS

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https://doi.org/10.52757/bsd26.58

Background: Process-related stress negatively affects animal health, welfare, productivity, and the quality of animal-derived products, contributing to disease emergence and increased risks to both animal and human health. This has led to growing interest in sustainable strategies to mitigate stress, particularly the use of natural alternatives to antibiotic growth promoters. The use of antibiotics in poultry feed is associated with the development of antibiotic-resistant bacteria and the accumulation of residues in eggs and meat.

Aim of the study: Evaluation of the effects of ZooBioR, a natural product derived from *Arthrospira platensis*, on egg production and morphometric characteristics in quails.

Materials and methods: ZooBioR contain amino acids and oligopeptides (4.5-10%), proteoglycans including sulphated polysaccharides (15-20%), proteins (45-50%), phospholipids (0.2-0.25%), zinc (2.5-5 mg), and selenium (25-50 µg). The study was conducted under poultry farm conditions using 200 quails divided into two groups: control (n=100) and experimental (n=100). The experimental group received a basal diet supplemented with 0.15 mg ZooBioR/kg feed, while the control group received only the basal diet. Birds had ad libitum access to feed and water for 71 days. Egg production was recorded daily, and eggs were collected and analyzed separately for each group.

Results: ZooBioR was well tolerated and contributed to improved overall bird condition. The supplementation resulted in approximately a 7% increase in egg production and a 4.5% increase in average egg weight compared with the control group. Consequently, total egg mass production was about 11% higher in the experimental group. Feed conversion efficiency (kg feed per 10 eggs and per 1 kg egg mass) was also improved. Egg quality analysis showed higher albumen and yolk weights and heights, as well as more favorable proportions in the experimental group. Additionally, lower pH values of both albumen and yolk were recorded at the end of the study and during storage (evaluated at ~2-week intervals), indicating improved egg freshness and quality.

Conclusions: ZooBioR demonstrated clear efficacy as a functional feed additive, significantly improving productive performance, egg quality, and feed efficiency in quails. These findings support its potential as a sustainable alternative to antibiotic growth promoters in poultry production systems.

Keywords: *Arthrospira platensis*, ZooBioR, quails, egg production, egg quality, morphometric traits, feed efficiency.

Acknowledgments: This work was supported by research subprogram 020101 funded by the Ministry of Education and Research of the Republic of Moldova.

ZooBioR EFECTS ON MUSCLE PROTEIN METABOLISM AND BODY WEIGHT DYNAMICS

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https://doi.org/10.52757/bsd26.59

Background: Rapid global population growth is placing increasing pressure on animal food production systems. In the Republic of Moldova, poultry farming represents a key agricultural sector; however, birds raised under intensive conditions are frequently exposed to technological stress, which can negatively affect health, metabolic balance, and productivity. In this context, natural biologically active compounds are increasingly investigated as sustainable alternatives to support physiological functions and improve performance.

Aim of the study: Assessment of the effects of ZooBioR, a natural product derived from the cyanobacterium *Arthrospira platensis*, on muscle protein metabolism and body weight dynamics in quails.

Materials and methods: The experiment was conducted under poultry farm conditions on 200 adult laying quails (Texas universal breed), randomly assigned to control (n = 100) and experimental (n = 100) groups. The experimental group received a basal diet supplemented with ZooBioR (15 mg/kg feed), while the control group received only the standard diet. Blood, muscle, and liver samples were collected from 5 birds per group at days 0, 35, and 71. Biochemical parameters (total proteins, albumin, AST, ALT, CK-NAK, and G-6-PDH) were measured spectrophotometrically, with $p < 0.05$ considered statistically significant.

Results: No adverse effects were observed during the experimental period. Total muscle protein content increased initially in both groups (control: +8.1%, $p < 0.05$; experimental: +13.6%, $p < 0.001$), then declined toward the end of the trial, reflecting physiological adaptation to growth and production stages. Albumin levels decreased slightly over time in both groups. AST and ALT activities decreased throughout the experiment, with more pronounced reductions in the experimental group at the final stage (AST: -27.7%; ALT: -28.6%), indicating improved liver function. CK-NAK activity decreased initially in both groups, followed by a moderate increase in the experimental group (+17.5%) at the end of the trial. G-6-PDH activity remained consistently higher in the experimental group ($p < 0.05$), suggesting enhanced cellular antioxidant defense and increased resistance to oxidative stress.

Conclusions: The study demonstrates that ZooBioR supplementation improves metabolic efficiency, optimizes protein turnover, and enhances oxidative balance and adaptation to intensive rearing conditions.

Keywords: *Arthrospira platensis*, quail, ZooBioR, protein metabolism, muscle tissue, body weight.

Acknowledgments: This work was supported by research subprogram 020101 funded by the Ministry of Education and Research of the Republic of Moldova.

INCORPORATING PROBIOTICS IN FERMENTED LOW ALCOHOLIC BEVERAGES: BENEFITS, CHALLENGES AND TECHNOLOGICAL PERSPECTIVES

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https://doi.org/10.52757/bsd26.60

Background: Incorporating probiotics into food is associated with multiple health benefits, including boosting the immune system, preventing gastrointestinal diseases, reducing cholesterol, and balancing the gut microbiota. They can also increase the nutritional value of food by improving nutrient absorption. Although the consumption of alcoholic beverages should not be encouraged at the population level, functional fermented beverages with minimal alcohol content can become vehicles for the delivery of probiotics, although the challenges related to their survival in the alcoholic environment remain significant.

Aim of the study: The present research focuses on the production and stabilization of probiotic beverages based on local and traditional materials (wheat bran, sour borscht) with the addition of red grape pomace extracts and sea buckthorn.

Materials and methods: Microscopic method was used for microbial identification and analysis of the morphology of microorganisms. Indigenous (autochthonous) microorganisms and those added during the production of probiotic drinks were inoculated on Sabureaud, IGC, MRS, BRODTH and other nutrient media by the "exhausted plate" method.

Results: The fermentation capacity and quality indices of beverages obtained on the basis of endogenous and exogenous microorganisms were tested. The identified microorganisms are yeasts, lactic acid bacteria, acetic bacteria, micrococci, streptococci. In the fermentation processes, they are activated in mutual symbiotic relationships, which is demonstrated by the formation of films on the surface of the beverages and associations on the bottom of the vessel, as well as dissemination on solid nutrient media. To monitor the fermentation process of apple beverages, the growth rate of the number of NTG-producing microorganisms was determined. The latent phase of adaptation is relatively short, the accelerated phase is fast (3-4 days). The fermentation process of beverages can be completed in 6-10 days. The technological scheme of production was developed, the physicochemical and sensory properties of low-alcohol beverages of the "Cider" type were analyzed, obtained on the basis of apple pulp with the addition of extracts from red grape pomace and sea buckthorn.

Conclusions: The manufactured low-alcohol probiotic beverages have high sensory and tonic properties, ensured by the natural chemical composition of organic acids, vitamins, enzymes. Native and added microorganisms, yeasts, lactic acid bacteria transform fermented solutions into acid-sugar beverages with an original taste.

Keywords: Beverages, probiotics, prebiotics, stabilization, clay-based materials

Acknowledgments: The research was funded undertheinstitutionalproject, subprogram 020405 "Optimization of foodprocessingtechnologies in the context of circular bioeconomyand climate change", Bio-OpTehPAS,

INHIBITORY EFFECT OF BIOACTIVE PREPARATIONS FROM MICROALGAE AND CYANOBACTERIA ON LDL OXIDATION

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https://doi.org/10.52757/bsd26.61

Background: Extracts from microalgae and cyanobacteria represent important sources of bioactive compounds with antioxidant potential. Their efficacy depends on their chemical nature and behavior in different environments. For assessing activity in lipid systems, the low-density lipoprotein (LDL) oxidation inhibition assay is an appropriate functional model for comparing the capacity to prevent lipid peroxidation.
Aim: To comparatively evaluate the inhibitory effect on LDL oxidation of selected bioactive preparations of cyanobacterial and microalgal origin.
Materials and methods: Phycobiliproteins from *Porphyridium cruentum* and *Arthrospira platensis* (aqueous extracts), as well as astaxanthin from *Haematococcus pluvialis* (ethanolic extract from aplanospores), were analyzed. The inhibitory effect on LDL oxidation was assessed using a spectrophotometric assay, with results expressed as percentage inhibition at defined concentrations of the tested preparations.
Results: All three preparations exhibited inhibitory activity against LDL oxidation, with variations depending on the chemical nature of the bioactive compounds and their concentration. Astaxanthin from *Haematococcus pluvialis* showed the strongest inhibitory effect, reaching 67.3% at 5 µg/mL, indicating high antioxidant potency at very low concentrations. This effect may be attributed to the specific chemical structure of astaxanthin, which enables activity in lipid systems by localizing within hydrophobic membrane regions and at the lipid-water interface, the primary site of LDL lipid peroxidation initiation. Phycobiliproteins from *Arthrospira platensis* showed an inhibitory effect of 27.9% at 3.99 µg/mL, suggesting high specific activity at low concentrations. Phycobiliproteins from *Porphyridium cruentum* induced 43.4% inhibition at 136.6 µg/mL. These differences in potency may be related to the distinct compositions of phycobilin chromophores (phycocyanobilin vs. phycoerythrobilin), which influence radical-scavenging efficiency and electron-transfer processes. Phycocyanin exhibits high activity at low concentrations through chain-breaking antioxidant mechanisms in the early stages of oxidation. In contrast, phycoerythrin may exhibit higher activity at elevated concentrations due to structural differences that affect interactions at the lipid-water interface of LDL.
Conclusions: The study demonstrates that algal and cyanobacterial preparations inhibit LDL oxidation to varying degrees, depending on compound type and concentration. Astaxanthin from *Haematococcus pluvialis* was the most potent inhibitor at µg/mL levels, while phycobiliproteins from *Arthrospira platensis* and *Porphyridium cruentum* also exhibited relevant antioxidant activity. The LDL oxidation assay proved to be a suitable model for evaluating antioxidant potential in lipid systems.

Keywords: LDL oxidation, inhibitory effect, phycobiliproteins, astaxanthin, *Arthrospira platensis*, *Porphyridium cruentum*, *Haematococcus pluvialis*.

Acknowledgments: This work was supported by research subprogram 020101 funded by the Ministry of Education and Research of the Republic of Moldova.

EFFECTS OF TiO_2, Cu, AND CuO NANOPARTICLES ON BIOMASS ACCUMULATION AND BIOCHEMICAL COMPOSITION IN THE MICROALGA *PORPHYRIDIUM PURPUREUM*

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https://doi.org/10.52757/bsd26.62

Background: Metal and metal oxide nanoparticles can modulate microalgal metabolism. However, their effects strongly depend on chemical nature, applied concentration, and species specificity. *Porphyridium purpureum* is a red microalga of high biotechnological interest due to its rich content of phycobiliproteins, exopolysaccharides, and fatty acids.

Aim of the study: To evaluate the effects of TiO_2, Cu, and CuO nanoparticles on biomass accumulation and the biochemical profile of *Porphyridium purpureum*.

Materials and methods: TiO_2 (20 nm), CuNPs (25 nm), and CuO (50 nm) nanoparticles (Sigma-Aldrich, Merck KGaA, Germany; size confirmed by TEM) were applied to *Porphyridium purpureum* cultures at 0.1-30 mg/L. At the end of cultivation, biomass, proteins, carbohydrates, lipids, phycobiliproteins, photosynthetic pigments (chlorophyll a, b, carotenoids), and malondialdehyde (MDA) were determined.

Results: TiO_2 nanoparticles induced moderate effects on biomass, causing reductions of 6.8-17% compared to the control at all tested concentrations (0.1-30 mg/L). Protein content generally remained at control levels or showed slight increases (up to +8.6% at 0.1 mg/L), except at 30 mg/L, where a decrease of approximately 2% was observed. Carbohydrates remained stable at low concentrations and increased significantly at 30 mg/L (+31.3%). Lipids decreased moderately (11.6-9.4%), while phycobiliproteins fluctuated around control values. MDA levels did not show significant changes, indicating low oxidative stress induced by TiO_2. Cu nanoparticles exerted the most pronounced effects, causing consistent reductions in biomass across all tested concentrations (1.097-1.223 g/L). Protein content remained stable or slightly increased at low concentrations, whereas phycobiliproteins decreased dramatically at 10 mg/L (- 49.9%), indicating high sensitivity of accessory pigments to copper ions. MDA levels increased significantly at 10 mg/L (+72%), confirming the onset of intense oxidative stress. CuO nanoparticles induced milder effects than metallic Cu. Biomass ranged from 1.177 to 1.374 g/L, with a tendency to increase at higher concentrations (30 mg/L: 1.374 g/L). Protein content ranged from 85.7% to 108.9% of the control, with no clear concentration-dependent trend. Carbohydrates decreased at all tested concentrations (10.1-12.6% vs. 16.04% in the control), while lipids increased progressively, reaching 14.4% at 30 mg/L (compared to 13% in the control). MDA levels showed a significant, concentration-dependent increase, from +13% at 0.1 mg/L to +155% at 20 mg/L, indicating the strongest cumulative oxidative stress among all tested nanoparticles.

Conclusions: The obtained data demonstrate that the chemical nature of nanoparticles determines the response pattern of the red microalga *Porphyridium purpureum*. Cu nanoparticles exhibit acute toxicity, CuO induces a dominant pro-oxidative effect, while TiO_2 shows the highest level of biological compatibility.

Keywords: *Porphyridium purpureum*; nanoparticles; TiO_2, Cu, CuO; oxidative stress; biochemical composition.

Acknowledgments: This work was supported by research subprogram 020101 funded by the Ministry of Education and Research of the Republic of Moldova.

PHENOTYPING CHARACTERIZATION OF THE LANDRACES (LD) FROM THE NATIONAL TOMATO, EGGPLANT, PEPPER COLLECTIONS OF ARMENIA

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https://doi.org/10.52757/bsd26.63

Background: Farmers in our republic have a number of ancient forms belonging to the *Solanaceae* family (*Salanaceae* Juss), which are not only important for use in breeding, but they are also highly resistant and rich in phyto-insecticidal compounds, medicinal plants, and have ethnobotanical and ethnopharmaceutical significance. The collection and preservation of these local forms has enriched the amount of genetic resources and their use in the breeding process has become important. Since the territories of a number of regions of the republic are geographically distant from the vegetable growing regions of the Ararat Plain, the local ancient forms (LD) of the main vegetable crops of the *Solanaceae* family - tomato, eggplant, sweet and hot pepper - that are present in these regions and that have been formed by local farmers have been collected and are currently being studied to characterize their phenotype.

Aim of the study: Research work was carried out to improve the phenotypic characteristics of the local ancient (LD) forms of Armenian eggplant, pepper, tomato stored at the Scientific Center and make them available through the EURISCO database, ensuring access to information for breeders, thus strengthening the information system of plant genetic resources. This approach has increased the valorization of Armenian genetic resources, promoting its use for breeding purposes, facilitating the use of spermatozoa for sustainable use.

Materials and methods: The characterization of landraces of *Solanaceae* vegetable crops from the National Solanaceae Vegetable Crop Collection of Armenia was carried out using plant descriptors (IPGRI, AVRDC and CATIE, 1995, Descriptors for Capsicum (*Capsicum* spp.), IPGRI, AVRDC and CATIE, 1990, Descriptors for Eggplant (*Solanum* L), IPGRI, AVRDC and CATIE, 1995, Descriptors for Tomato (*Lycopersicon* spp.)) and compiled into a phenotypic record of the main collection.

Results: Landraces in the National Solanaceae Vegetable Crop Collection have been comprehensively studied in morphological characteristics and phenotypes have been assessed. A core collection of around 42 samples (10 tomato, 13 eggplant, 7 sweet pepper, 12 hot pepper) was created, which is as diverse and representative as possible. Selection criteria were based on the main characteristics of the LR fruits (e.g. fruit size, shape, color) and quality. A common protocol for descriptors and basic phenotypic data was created, and the seed plasm was characterized according to the guidelines. The Armenian local variety information catalog was improved, creating a comprehensive collection, providing phenotypic information and deeply assessing genetic diversity.

Conclusions: A powerful collection with phenotypic diversity was created, which will be used for research and breeding purposes. All this will improve the value of local high-quality products in the food chain, with a concrete and positive impact on end users and the breeding of new, more adapted crops to feed future generations

Keywords: tomato, eggplant, sweet pepper, hot pepper, landraces

Acknowledgements and Funding: The research was supported by the Higher Education and Science Committee of MESCS (Ministry of Education, Science, Culture and Sports) RA (Research project № 25RG-4B011).

GENETICALLY PREDICTED EFFECTS OF DHA AND EPA ON COGNITIVE PERFORMANCE: EVIDENCE FROM TWO-SAMPLE MENDELIAN RANDOMIZATION

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https://doi.org/10.52757/bsd26.64

Background: Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are biochemically distinct long-chain omega-3 polyunsaturated fatty acids with different brain distributions and metabolic roles. Despite their biological specificity, most intervention trials administer combined supplements, precluding fatty acid-specific causal conclusions. Whether individual circulating DHA and EPA levels exert independent effects on cognitive function remains unresolved.

Aim of the study: To estimate and formally compare the genetically predicted causal effects of circulating DHA and EPA on reaction time and general cognitive performance using a two-sample Mendelian randomization (MR) framework, with explicit assessment of FADS-cluster pleiotropy.

Materials and methods: Genome-wide significant SNPs were selected as instrumental variables for circulating DHA (N = 115,082; 45 independent instruments after LD clumping) and EPA (N = 7,367; 1 instrument), both from European-ancestry GWAS. Outcome GWAS included reaction time (N = 443,396; UK Biobank) and general cognitive performance (N = 257,828; Savage et al. 2018). LD clumping was performed locally using PLINK v1.90 and the 1000 Genomes Phase 3 European reference panel ($r^2 < 0.001$). Primary analysis used the inverse-variance weighted (IVW) method; sensitivity analyses included MR-Egger, weighted median, weighted mode, and MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) with outlier correction.

Results: Neither DHA nor EPA showed causal effects on reaction time across all analytical methods; null results were confirmed by MR-PRESSO outlier correction (distortion test $p = 0.464$). For cognitive performance, apparent negative DHA effects detected by pleiotropy-robust methods (weighted median: $\beta = -0.049$, $p = 5.2 \times 10^{-6}$; MR-Egger: $\beta = -0.064$, $p = 0.011$) did not survive MR-PRESSO outlier correction: removal of four fatty acid desaturase (FADS)-cluster instruments reversed the DHA estimate to non-significance ($\beta = +0.008$, $p = 0.555$; distortion test $p = 0.002$), indicating material violation of the exclusion restriction assumption. The single-instrument EPA estimate ($\beta = -0.395$, $p = 5.4 \times 10^{-6}$) cannot be assessed for pleiotropy and remains hypothesis-generating.

Conclusions: Genome-wide instrument selection for circulating DHA and EPA at the FADS locus does not support fatty acid-specific causal inference on cognitive outcomes. Apparent effects reflect FADS-cluster pleiotropy rather than direct neurobiological action of individual omega-3 fatty acids. Future MR studies on omega-3 cognition require larger EPA GWAS datasets with multiple independent instruments outside the FADS region.

Keywords: Mendelian randomization, omega-3 fatty acids, cognitive performance, FADS gene cluster, pleiotropy, causal inference

Acknowledgments: This research was funded by the National Agency for Research and Development (NARD), grant no. 25.00208.7007.05/PD "Nutritional Resilience of the National Army Personnel: Integrating Smart Systems for Food Management and Deficiency Prevention", Technical University of Moldova.

ANTAGONISTIC EFFECTS OF FUNGAL AND ACTINOMYCETE STRAINS ON SELECTED PATHOGENS

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https://doi.org/10.52757/bsd26.65

Background: Filamentous fungi and actinobacteria are valuable biotechnological resources that play a key role in the pharmaceutical industry as sources of antibiotics effective against pathogenic bacteria and fungi. Identifying microbial strains active against opportunistic pathogens, including *Candida albicans*, *Escherichia coli*, and *Salmonella*, is essential for developing new therapeutic strategies.

The aim of this study was to select new strains with antimicrobial potential against some opportunistic pathogens and pathogens.

Materials and methods: A screening of 24 *Penicillium* and *Trichoderma* strains of fungi and 7 strains of *Streptomyces* actinobacteria was performed. The strains of fungi and actinobacteria were tested against the opportunistic pathogenic fungi *Aspergillus fumigatus*, *Fusarium solani*, *Fusarium oxysporum*, the pathogen *Candida albicans* and the pathogenic bacteria *Escherichia coli*, *Salmonella* and *Enterococcus* spp. The tests were performed by the disk-diffusion method adopted by Egorov [1].

Results: The study results showed that, out of 24 fungal strains evaluated for activity against the opportunistic pathogens *A. fumigatus*, *F. oxysporum*, and *F. solani*, as well as the pathogen *C. albicans*, 2 strains were highlighted: *Penicillium* sp. 12 and *Penicillium* sp. 14. The inhibition zones against the opportunistic pathogens varied within the range of 22–32 mm, whereas against *C. albicans*, the inhibition zones were insignificant, measuring 14–15 mm. Against the pathogenic bacteria *E. coli*, *Salmonella*, and *Enterococcus* spp. (*E. faecium* and *E. faecalis*), none of the studied fungal strains showed antagonistic activity.

Among the 7 actinobacterial strains studied for their antifungal properties against the opportunistic fungi *F. solani*, *F. oxysporum*, and *A. fumigatus*, 2 strains were identified—*Streptomyces gougerotii* CNMN-Ac-14 and *Streptomyces fradiae* CNMN-Ac-11—with inhibition zones of 23–28 mm. The strain *S. fradiae* CNMN-Ac-11 also showed antagonism against *C. albicans*, with insignificant inhibition zones of 14–15 mm. None of the actinobacterial strains tested against the pathogens *E. coli*, *Salmonella*, and *Enterococcus* spp. (*E. faecium* and *E. faecalis*) showed antagonistic activity.

Conclusions: According to the obtained results, we can conclude that the strains: *Penicillium* sp. 12, *Pencillium* sp. 14, *S. gougerotii* CNMN-Ac-14 and *S. fradiae* CNMN-Ac-11 are promising for combating opportunistic pathogenic fungi such as *A. fumigatus*, *F. solani*, *F. oxysporum*, but cannot combat pathogenic bacteria.

Keywords: fungi, actinobacteria, antagonism, pathogens, inhibition zones.

Acknowledgments: This research was funded by Government of Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 “InBioS – Innovative biotechnological solutions for agriculture, medicine and environment”.

References:

1. Egorov, N. S. (2004). *Fundamentals of the study of antibiotics*. Moscow: Nauka Publishing House. 528 p. ISBN 5-02-033595-92

ACTION OF Fe_2O_3 AND Fe_2ZnO_4 NANOPARTICLES ON STRAINS OF ACTINOBACTERIA AND FILAMENTOUS FUNGI

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https://doi.org/10.52757/bsd26.66

Background: Nanoparticles can exert both beneficial and adverse effects on microorganisms, including DNA modification, accelerated aging, and cell death. They may also act as biostimulants, enhancing the biosynthetic properties of microorganisms of industrial, medical, and agricultural importance. Therefore, studying the effects of nanoparticles on the biosynthesis of biologically active substances is both relevant and necessary.

The aim of this study was to evaluate the effects of Fe_2O_3 and Fe_2ZnO_4 nanoparticles on the biosynthesis of biologically active substances in strains of actinobacteria and filamentous fungi.

Materials and methods: The study subjects included two actinobacterial strains (*Streptomyces gougerotii* CNMN-Ac-14 and *Streptomyces fradiae* CNMN-Ac-11) and three filamentous fungi (*Penicillium* sp. 7, *Penicillium* sp. 12, and *Penicillium* sp. 14). The main parameters assessed were anti-inflammatory and antioxidant activities, along with protein content and catalase activity. Fe_2O_3 and Fe_2ZnO_4 nanoparticles, at concentrations of 1, 5, and 10 mg/L, were tested as stimulators of bioactive compound biosynthesis in the studied strains. The following opportunistic microorganisms were used as test strains: *Aspergillus fumigatus*, *Fusarium solani*, *Fusarium oxysporum*, and the pathogenic yeast *Candida albicans*.

The results indicate that the addition of Fe_2O_3 and Fe_2ZnO_4 nanoparticles to the nutrient medium during subculturing of actinobacteria and filamentous fungi reduced antifungal and antioxidant activities in all studied strains. In the actinobacterial strains *Streptomyces gougerotii* CNMN-Ac-14 and *Streptomyces fradiae* CNMN-Ac-11, an increase in catalase activity was observed in variants containing Fe_2ZnO_4 nanoparticles at concentrations of 1–5 mg/L. The highest protein content in the *Streptomyces fradiae* CNMN-Ac-11 strain was recorded in the variant treated with 1 mg/L Fe_2O_3 nanoparticles, while in the *Streptomyces gougerotii* CNMN-Ac-14 strain, it was observed in the variant containing 10 mg/L Fe_2ZnO_4 nanoparticles. The fungal strain *Penicillium* sp. 7 showed stimulation of catalase activity compared to the control sample in the variant treated with 10 mg/L Fe_2ZnO_4 nanoparticles. In the *Penicillium* sp. 14 strain, enhanced catalase activity was also observed at 10 mg/L Fe_2ZnO_4 nanoparticles, along with an increase in protein content in variants with 5–10 mg/L Fe_2O_3. Regarding the *Penicillium* sp. 12 strain, the tested nanoparticles exerted a stimulatory effect on protein biosynthesis at 1 mg/L for both Fe_2O_3 and Fe_2ZnO_4.

Conclusions: According to the results of this study, it can be concluded that nanoparticles used in the subculturing of microorganisms (actinobacteria and filamentous fungi) can either stimulate or suppress their biosynthetic processes, depending on the strain, nanoparticle composition, and concentration.

Key words: fungi, actinobacteria, nanoparticles, antifungal activity, catalase.

Acknowledgments: This research was funded by Government of Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 “InBioS – Innovative biotechnological solutions for agriculture, medicine and environment”.

A STUDY OF *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* IN SUSTAINABLE BIOTECHNOLOGIES FOR APPLE AND WALNUT CROP PROTECTION

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https://doi.org/10.52757/bsd26.67

Background: Apple and walnut orchards are often affected by lepidopteran pests that reduce yield, impair fruit and nut quality, and decrease economic efficiency. Intensive use of synthetic insecticides has raised concerns regarding environmental contamination, pesticide residues, effects on non-target organisms, and resistant pest populations. These issues highlight the need for sustainable and biologically based alternatives. This study evaluated the relevance of *Bacillus thuringiensis* var. *kurstaki* as a microbial bioinsecticide for apple and walnut protection, focusing on insecticidal potential, environmental compatibility, and its role in integrated pest management.

Materials and methods: The study was based on a review and comparative analysis of recent literature regarding the biological properties, insecticidal activity, ecological safety, and practical application of *Bacillus thuringiensis* var. *kurstaki* in orchards. Particular attention was given to its use against lepidopteran pests in apple and walnut crops and its compatibility with sustainable protection strategies. Field observations on treatments against *Cydia pomonella* L. in apple were also included.

Results: The analysis showed that *Bacillus thuringiensis* var. *kurstaki* is an effective and selective agent against lepidopteran larvae. Its activity is due to Cry proteins which, after ingestion, damage the larval midgut and cause mortality. In apple orchards, treatments against codling moth reduced fruit damage from 21.3% in the untreated control to 4.3% at 10.0 L/ha, corresponding to 79.8% efficacy. In walnut orchards, application against *Cydia pomonella* L. achieved 82.8% biological efficiency. These findings highlight the potential of *Bacillus thuringiensis* var. *kurstaki* as an important component of environmentally friendly protection programs for both apple and walnut crops. Compared with conventional insecticides, Bt-based products have a lower ecological impact, pose a reduced risk to non-target organisms, and show greater compatibility with integrated pest management strategies. Their field effectiveness depends on application timing, pest developmental stage, and environmental conditions.

Conclusions: *Bacillus thuringiensis* var. *kurstaki* is a valuable component of sustainable apple and walnut protection strategies. Its use reduces dependence on synthetic insecticides, improves ecological safety, and supports long-term orchard sustainability. Bt-based bioinsecticides should be considered important tools in modern environmentally responsible biotechnologies for perennial fruit crop protection.

Keywords: *Bacillus thuringiensis* var. *kurstaki*, apple crop protection, walnut crop protection, bioinsecticide, sustainable biotechnology, integrated pest management.

Acknowledgements: Research was carried out within the subprogram 011103: “Development of environmentally friendly means of reducing the impact of harmful organisms on agricultural crops against the background of climate change” and the bilateral Project Moldo-Turc 25.80013.5107.01TR: “Eco-farming for innovative protection of new walnut varieties: harnessing methodologies and technologies”, funded by the National Agency for Research and Development and the Ministry of Education and Research.

IN VITRO PROPAGATION OF PLANTS AT THE “ALEXANDRU CIUBOTARU” NAȚIONAL BOTANICAL GARDEN (INSTITUTE)

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https://doi.org/10.52757/bsd26.68

Biotechnological investigations at the National Botanical Garden (Institute) "Alexandru Ciubotaru" were formally initiated in 1985 with the establishment of the Biotechnological Complex, under the coordination of acad. Al. Ciubotaru. The initial phase of development was primarily oriented toward the systematic acquisition, mobilization, and introduction of novel species and taxa into the institutional collections and exhibitions. This period was distinguished by extensive planting programs, employing seedlings obtained from diverse regions of the former USSR as well as from international sources.

In this context, the prevalence of plant diseases particularly viral infections affecting ornamental crops was identified as a critical challenge, necessitating the development of effective diagnostic and control strategies for both viral and fungal pathogens. In response, a specialized research group dedicated to plant protection and microclonal propagation was established. The investigations were conducted within the Embryology and Biotechnology Laboratory and were directed toward elucidating callusogenic, androgenic, and gynogenic processes, as well as toward the production of pathogen-free biological material suitable for breeding programs. Furthermore, the research agenda encompassed the introduction of novel species to the RM, including *Stevia rebaudiana*, *Actinidia chinensis*, *Rosmarinus officinalis*, and *Withania somnifera.*

In parallel with the advancement of scientific investigations, the research infrastructure underwent significant modernization through the acquisition of contemporary laboratory equipment. Applied projects with socio-economic impact were also implemented, notably the design and establishment of urban green spaces utilizing ornamental plant material produced via *in vitro* culture. Through targeted technology transfer initiatives, micropropagation protocols for fruit shrub varieties of the *Rubus* genus were successfully developed and disseminated.

Concurrently, the application of *in vitro* culture techniques has proven particularly effective in the *ex situ* conservation of rare taxa from spontaneous flora, offering viable solutions to mitigate the adverse impacts of anthropogenic pressures. Current investigations are directed toward the refinement of micropropagation technologies and the systematic expansion of the plant genetic pool. The novelty of this research lies in the incorporation of previously unstudied species into culture systems and in the advancement of efficient biotechnological propagation methodologies, which collectively contribute to both biodiversity conservation and the enhancement of breeding resources.

Keywords: Micropropagation, explants, nutrient medium.

Acknowledgements: The research was conducted within the framework of the State Program for Research and Innovation (2020-2023), entitled “Introduction and development of multiplication and cultivation technologies through conventional techniques and in vitro cultures of new woody plant species” (Project No. 20.80009.7007.19) and continued under Subprogram 010101, entitled “Research and ex situ and in situ conservation of plant diversity in the RM.”

MICROORGANISMS FOR MUSTARD GROWTH PROMOTION

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https://doi.org/10.52757/bsd26.69

Background: Medicinal plants are natural sources of bioactive compounds whose therapeutic properties have been recognized for millennia. Among these, mustard stands out due to its wide range of biological properties, being used as an expectorant, analgesic, antibacterial, antioxidant, digestive stimulant, and diuretic. Mustard cultivation is influenced by several risk factors, including soil quality, susceptibility to diseases common to other crops, pest pressure, and occasionally reduced seed germination capacity.

The aim of this study was to evaluate the potential of three *Pseudomonas* bacterial strains as plant growth-promoting agents for mustard, with the objective of improving seed germination and supporting plant development under cultivation constraints.

Materials and methods: mustard seeds were treated with suspensions of *Pseudomonas fluorescens* CNMN-PsB-01, CNMN-PsB-02, and CNMN-PsB-12, cultivated in King B liquid medium.

Results: The treatment of mustard seeds led to increases in seed germination, seedling length, and dry weight by 11.6–15.0%, 16.0–18.7%, and 20.0–22.9%, respectively, compared with the control. In addition, total chlorophyll content—particularly chlorophyll *b*—increased significantly. In the most effective treatments (*Pseudomonas fluorescens* CNMN-PsB-02 and *Pseudomonas fluorescens* CNMN-PsB-12), chlorophyll *b* content was 52.6–71.8% higher than in the control.

An increase in chlorophyll content in mustard leaves has important physiological and agronomic implications, as this pigment plays a central role in photosynthesis. Elevated chlorophyll levels, especially chlorophyll *b*, enhance the efficiency of light energy capture. Consequently, an intensified photosynthesis leads to greater production of assimilates necessary for plant growth and development, which likely explains the observed stimulation of mustard growth. In mustard, enhanced photosynthetic activity may contribute to faster leaf development and improved formation of reproductive organs. Plants with a well-developed photosynthetic apparatus can sustain active metabolic processes for longer periods, providing a competitive advantage. Furthermore, increased chlorophyll content may reflect improved mineral nutrition, particularly with respect to nitrogen and magnesium—key elements in the chlorophyll molecule. Thus, the stimulation of chlorophyll biosynthesis may serve as indirect evidence of enhanced nutrient uptake and utilization.

Conclusion: The tested *Pseudomonas* spp. strains demonstrate significant potential as growth promoters for mustard. Their application improves seed germination, plant growth, and chlorophyll content, thereby contributing to increased productivity and resilience under variable environmental conditions.

Keywords: microorganisms, plant growth promoters, mustard

Acknowledgments: The research was carried out within project 020101 InBioS – “Innovative biotechnological solutions for agriculture, medicine and environment” funded by Ministry of Education and Research of the Republic of Moldova.

INITIATION OF MICROPROPAGATION OF THE FERN – *OSMUNDA REGALIS L.*

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https://doi.org/10.52757/bsd26.70

Background: *In vitro* micropropagation is a modern and efficient technique for plant multiplication under aseptic laboratory conditions, enabling the production of large numbers of virus-free and pathogen-free microplants. This method is independent of climatic conditions and is carried out in controlled growth chambers with regulated airflow, optimal temperature and humidity, and appropriate lighting. Biotechnological approaches are widely applied for the conservation and propagation of rare and endangered species, including ferns. The growing demand for ornamental ferns highlights the need for propagation techniques that allow rapid production of large numbers of plants.

The present study aimed to initiate and multiply *in vitro* the species *Osmunda regalis* L. within the Laboratory of Embryology and Biotechnology of “Al. Ciubotaru” National Botanical Garden (Institute).

Materials and methods: *O. regalis* L. is a perennial, deciduous fern with large, striking leaves (fronds), which grow about 150-200 cm, and are light green in summer and turn yellow-brown in autumn. The species is frost-tolerant (down to −25°C), thrives in moist, acidic soils, prefers shaded or semi-shaded sites and develops from a short, erect, massive rhizome, forming a small, trunk-like structure in mature plants. Its fronds are dimorphic: *sterile* outer fronds and *fertile* central fronds, the latter producing reddish-brown sporangia arranged in dense panicles at maturity. For *in vitro* initiation, the inoculum consisted of rhizome fragments, frond primordia and apices, incompletely differentiated sporangium fragments and immature spores, collected in early April from juvenile sporophytes. The plant material was sterilized in three stages using various sterilizing agents, while spores were packaged in filter-paper envelopes. Both sterilized plant material and spores were aseptically transferred onto nutrient media, the composition of which was tailored to specific experimental goals: spore germination, gametophyte differentiation, sporophyte development, or rhizogenesis induction. The initiated cultures were maintained in a growth chamber under 3000 lux illumination for 16 hours per day at 23 - 24°C.

Results: Following spore germination, gametophyte development begins with the formation of the prothallus, a filamentous, irregular structure differentiated into a basal chlorophyll-poor region bearing numerous trichorhizoids and unicellular brown trichomes, and several distal lobes with well-developed meristematic tissue on which antheridia and archegonia are formed. When rhizome fragments, primordia, and frond apices were used as inoculum, no positive results were observed after 30 days of incubation. However, when sporangium fragments and solitary spores were used, abundant spore germination occurred after six weeks, followed by prolific development of multicellular gametophytes.

Conclusion: The success of cultures initiated from spores depends on spore size and viability, sterilization method, culture medium, pH, temperature, light and humidity.

Keywords: *in vitro*, inocula, ferns, sporangia, spores, gametophyte.

Acknowledgments: This research was conducted under Subprogram 010101 “Ex situ and in situ research and conservation of plant diversity in the Republic of Moldova” (2024-2027).

BIOTECHNOLOGICAL ASPECTS OF THE USE OF PHEROMONE TRAPS FOR MONITORING POPULATIONS OF *HALYOMORPHA HALYS* ON ORNAMENTAL PLANTS

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https://doi.org/10.52757/bsd26.71

Background: The rapid expansion of invasive species in urban ecosystems represents a major challenge for plant protection and the maintenance of ecological balance. The invasive species *Halyomorpha halys* (Stal, 1855) is a highly polyphagous pest with a strong capacity for adaptation and dispersal, frequently utilizing ornamental plants as food sources and shelters in urban environments. In this context, biotechnological methods based on the use of aggregation pheromones are becoming essential for the effective monitoring of this pest. Pheromone traps enable early detection and assessment of population dynamics, contributing to the development of integrated pest management strategies.

The aim of this study was to evaluate the efficiency of pheromone traps in monitoring *Halyomorpha halys* populations on ornamental plants in the municipality of Chișinău.

Materials and methods: The research was conducted during the 2025 growing season in the urban environment of Chișinău, on various ornamental plant species. Monitoring of *Halyomorpha halys* populations was carried out using pheromone traps. The traps were installed in the plant canopy at a height of 1–1.5 m, on the territory of the Institute of Genetics, Physiology and Plant Protection and the Botanical Garden. Data were recorded weekly by counting the number of captured individuals.

Results and discussion: The results obtained from pheromone trap monitoring revealed a well-defined seasonal dynamic of *Halyomorpha halys* populations in the urban ecosystems of Chisinau, characterized by fluctuations in density depending on the vegetation period and host plant species. The onset of captures was recorded in the last decade of may, marking the emergence of overwintered adults, followed by a progressive increase in the number of captured individuals.

A pronounced population peak was recorded in July, with average values of 35–45 individuals per trap per week in areas dominated by *Ailanthus altissima* and *Syringa spp.*, confirming their role as key elements in the trophic structure and aggregation behavior of the pest. In the case of *Hibiscus spp.*, the distribution of captures showed a bimodal pattern, with two distinct peaks in June and August. For *Tilia spp.* and *Rosa majalis*, capture levels were moderate (18–25 individuals per trap per week).

Conclusions: Seasonal monitoring of *Halyomorpha halys* populations using pheromone traps highlights the importance of continuous surveillance throughout the entire vegetation period. The use of aggregation pheromones allowed for precise identification of host plant preferences and seasonal fluctuations in population density. Ornamental plants, particularly lilac (*Syringa spp.*) and tree of heaven (*Ailanthus altissima*), demonstrated an essential role in maintaining and reproducing pest populations in urban environments. The obtained results confirm the efficiency of pheromone traps as a biotechnological tool and emphasize the need for their integration into phytosanitary monitoring systems in urbanized areas.

Keywords: *Halyomorpha halys*, invasive species, pheromone traps.

Acknowledgments: Research was carried out within the subprogram 011103 „Development of ecologically harmless means of reducing the impact of harmful organisms of agricultural crops against the background of climate change”, financed by the Ministry of Education and Research of the Republic of Moldova.

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF SEEDS OF DIFFERENT *TRITICUM AESTIVUM* L. GENOTYPES TO OSMOTIC STRESS

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https://doi.org/10.52757/bsd26.72

Background: Drought is one of the main abiotic factors limiting wheat productivity, especially during the early stages of development, when seed uptake and germination are directly dependent on water availability. Simulating water deficit under controlled conditions allows us to identify genotypic differences in plant tolerance to osmotic stress during the seedling stage.

The aim of this study was to evaluate the physiological response of seeds of different *Triticum aestivum* L. genotypes to osmotic stress induced by varying sucrose concentrations in order to identify genotypes characterized by increased tolerance to water deficit during the early stages of development.

Materials and methods: Seeds of six wheat genotypes were used as experimental material. Drought conditions were simulated using aqueous sucrose solutions with concentrations of 3%, 5%, 10%, and 16%, while distilled water served as a control. Seeds were germinated in a laboratory incubator at a temperature of 20–22°C in a Friocell thermostat. Germination parameters were recorded on days 3, 5, 7, and 10. The percentage of germinated seeds, root length, and shoot length were assessed.

Results: Increasing sucrose concentration generally resulted in a significant reduction in germination and seedling growth in all studied genotypes, although the magnitude of the response varied significantly between them. The greatest differentiation between genotypes in both germination and growth traits was observed with treatment with a 16% sucrose solution. Root length proved to be a more sensitive indicator of osmotic stress than other growth parameters. In sensitive varieties, this trait decreased 3-10 times depending on the sucrose concentration.

An analysis of antioxidant compounds showed that osmotic stress was accompanied by a decrease in catalase activity in all studied wheat genotypes. The content of total phenolic compounds also changed under osmotic stress. Several genotypes showed an accumulation of phenolic compounds, which may indicate a nonspecific defense response. In more sensitive genotypes, biochemical changes were less pronounced or unstable.

These differences reflect genotype-specific physiological and biochemical adaptation mechanisms, including the ability to maintain water balance through osmotic regulation and provide effective antioxidant protection during the early stages of development.

Conclusions: Drought simulation using sucrose solutions is an effective tool for early selection of wheat genotypes for tolerance to water stress, and a comprehensive assessment of germination traits, seedling growth, and antioxidant system status can serve as an informative criterion for selecting drought-tolerant varieties in breeding programs.

Keywords: wheat, osmotic stress, seed germination, sucrose-induced drought simulation, catalase activity, phenolic compounds.

Acknowledgments: The research was carried out within the sub-programme 01110 ”Genetic and biotechnological approaches to management of agroecosystems in the conditions of climate change”, funded by the Ministry of Education and Research.

INVESTIGATION OF NON-*SACCHAROMYCES* YEAST BIODIVERSITY AND THEIR PERSISTENCE ACROSS VINTAGES AND WINEMAKING STAGES IN MOLDOVAN VINEYARDS

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https://doi.org/10.52757/bsd26.73

Background: Historically, non-*Saccharomyces* yeasts were considered spoilage organisms in winemaking. Recent research, however, recognizes their ability to enhance wine complexity, improve aromatic profiles, and provide bioprotection by suppressing undesirable microbes. **Aim of the study**: This study aims to investigate the diversity and persistence of non-*Saccharomyces* species in several Moldovan vineyards, assessing their presence across three vintages and two vinification stages to better understand their role in terroir-driven wines. **Material and methods:** Four sets of wine samples were collected at two key stages - initial must and mature wine - from three grape varieties: Feteasca Neagră, Feteasca Albă, and Nistreana, grown in four Moldovan viticultural areas: Purcari, Strășeni, Mileștii Mici, and Chișinău. Vinification was performed under controlled conditions at the micro-winery of the Technical University of Moldova using traditional protocols. Must samples were taken before commercial yeast inoculation, and mature wine samples just before bottling.

DNA was extracted using the SDS-PVP method with bead-beating, followed by real-time PCR (qPCR) with SYBR Green and custom primers targeting 16 species: *Torulaspora delbrueckii*, *Candida stellata*, *Hanseniaspora occidentalis*, *Hanseniaspora vineae*, *Hanseniaspora uvarum*, *Lachancea kluyveri*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Meyerozyma guilliermondii*, *Pichia farinosa*, *Pichia fermentans*, *Pichia membranifaciens*, *Pichia occidentalis*, *Pichia terricola*, *Wickerhamomyces anomalus*, and *Zygoascus meyerae*.

Results: Screening showed that 5 of the 16 target species appeared in at least one sample: *H. uvarum*, *M. pulcherrima*, *M. guilliermondii*, *P. occidentalis*, and *P. terricola*. *H. uvarum* was the most prevalent yeast, detected in 100% (12/12) of must samples and 83.3% (10/12) of finished wines across all vintages.

Conclusions: These results highlight the native microbial ecology of Moldovan vineyards, showing that some non-*Saccharomyces* yeasts survive fermentation and persist in the final wine. Their stability suggests potential use as bio-tools to enhance the typicity and quality of regional wines and support the unique sensory identity of Moldovan viticulture.

Keywords: non-*Saccharomyces* yeasts, micro-winery, primers, SYBR Green, real-time PCR

Acknowledgments: This work was funded within Institutional Project, subprogram 020405 “Optimizing food processing technologies in the context of the circular bioeconomy and climate change”, Bio-OpTehPAS, being implemented at the Technical University of Moldova; Subprogram 011101 “Genetic and biotechnological approaches to agroecosystem management under conditions of climate change”, funded by The Ministry of Education and Research of the Republic of Moldova.

APPLICATION OF $MgFe_2O_4$/PVP NANOCOMPOSITE FOR LDPE FILMS TREATMENT

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https://doi.org/10.52757/bsd26.74

Background: Magnesium ferrite nanoparticles ($MgFe_2O_4$ NPs) exhibit intrinsic peroxidase-like activity and have been effectively used as nanozymes – enzyme mimics that catalyze biochemical reactions similarly to natural enzymes. These nanomaterials have been used in wastewater treatment as adsorbents to remove heavy metal ions and as Fenton-like catalysts for break down contaminants. Due to their biocompatibility and low toxicity these nanoferrites find their application in medicine; their semiconducting and superparamagnetic properties are used to set up bioelectronic sensor devices for environmental monitoring.

The aim of this study was to synthesize and to evaluate magnesium ferrite nanocomposites application in the treatment and functional modification of LDPE films.

Materials and methods: Magnesium ferrite nanocomposite was synthesized by chemical reduction procedures. Low-molecular weight poly-N-vinylpyrrolidone (PVP) was used as a stabilizer. The resulting nanocomposite has been characterized by scanning electron microscopy (SEM), X-ray powder diffraction (XRD), and FT-IR spectroscopy.

Results: The obtained $MgFe_2O_4$/PVP nanoparticles are spherical, 5–10 nm in size, and tend to aggregate into 80–120 nm structures due to high surface energy. EDX analysis showed an Mg:Fe ratio of 1:2.5. The nanocomposites exhibited peroxidase-like activity in the 0.01–0.16 μM range. They were applied to LDPE films, which were then incubated in soil from the Țintareni landfill contaminated with plastic waste. Microbial consortia were obtained from the enrichment cultures cultivated on two MSM media, with counts ranging from 12.20×10^6 CFU/ml (MSM 4, pH 6.5) to 27.93×10^6 CFU/ml (MSM 2, pH 5.5). All consortia had mixed composition. A change in the acidity of the culture liquid was observed during the growth of microorganisms on both media: a decrease in pH to 3.9-4.0, which is associated with the predominant development of fungi in the consortia. The micromycetes isolated from the consortia belonged to different genera: *Trichoderma*, *Penicillium*, *Aspergillus*. The isolated bacteria were part of the genus *Pseudomonas* and *Streptomyces*.

Conclusions: Modifying the surface structure of LDPE films by treating polyethylene with $MgFe_2O_4$/PVP nanocomposites can influence the formation of microbial consortia in soil, the titer and composition of microorganisms depend on the initial acidity of medium, and the concentration of the nanocomposite.

Keywords: ferrite nanoparticles, LDPE, plastic waste, microbial consortia

Acknowledgments: This research work was funded by Government of Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 "InBioS - Innovative biotechnological solutions for agriculture, medicine and environment".

IDENTIFICATION OF QUALITY PRODUCTIVE COMMON WHEAT VARIETIES IN UNFAVORABLE ENVIRONMENTAL CONDITIONS

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Background: The reduction in crop yields under unfavorable climatic conditions in recent years has led to enormous losses, both in terms of food supply for the population and livestock, and for the national economy. Unfavorable conditions negatively affect the productivity and quality of seeds, especially during dry summers, particularly at the stages of wheat flowering and maturation. The country is forced to import high-quality wheat from other countries, as the wheat varieties cultivated domestically, both in previous years and at present, do not ensure the maintenance of quality under these conditions. These factors constitute the main causes of economic losses recorded in years with unfavorable climatic conditions. The cause of this situation lies in the irrational use of wheat varieties cultivated in the country in relation to these extreme climatic conditions. From the above, it follows that there is an urgent need to identify, within the country, varieties that can ensure productivity and grain quality at an optimal level, meeting domestic requirements.

Aim of the study: Identification of productive and quality varieties under unfavorable conditions.

Materials and methods: In this study, seeds of six hexaploid wheat varieties (Kuialnic, Meleag, Moldova 5, Moldova 66, Moldova 614, and Bijuteria Zâmbrenilor) were used, cultivated on the experimental field of the Institute of Genetics, Physiology, and Plant Protection of the ASM during 2024–2025. At full maturity, the seeds of these plants were harvested and analyzed to determine productivity and quality, by evaluating the yield, gluten content, and gluten quality. The data obtained were statistically processed using the ANOVA software package, calculating mean values and standard deviations, as well as significant differences among the varieties.

Key results: The analysis of dry gluten content in different common winter wheat samples revealed differences among the varieties. The highest dry gluten content was recorded in the Moldova 66 sample, approximately 14%, indicating superior quality gluten with high potential for premium bakery products. The Moldova 5 and Kuialnic samples also showed high values, ranging between 12.5% and 12.7%, suggesting gluten with good dough-forming and baking properties.Moderate values, between 11.0% and 12.1%, were observed in the Meleag, Moldova 614, and Bijuteria Zâmbrenilor samples, indicating a gluten content suitable for standard bakery products. The results reflect genetic differences among the varieties and allow the identification of those with the greatest potential for use in the baking industry. Regarding the harvest, no significant differences were observed among the samples.

Conclusions.The analysis of wet and dry gluten content in different common winter wheat samples revealed significant differences among the varieties. The Moldova 66 and Moldova 5 samples stood out with the highest gluten values, indicating high potential for premium bakery products. Samples with moderate values, such as Kuialnic, Moldova 614, Meleag, and Bijuteria Zâmbrenilor, exhibited gluten suitable for standard bakery products. The results confirm the influence of variety genetics on gluten quality and allow the selection of samples with the greatest potential for the baking industry.

Keywords: *Triticum aestivum* L., dry gluten content, harvest.

Acknowledgment. This research was supported by Subprogram 011101: Genetic and Biotechnological Approaches to the Management of Agroecosystems under Climate Change Conditions, funded by the Ministry of Education and Research.