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**MORPHO-CULTURAL AND BIOSYNTHETIC PROPERTIES OF MICROMYTES IN
AQUATIC BASINS OF CHISINAU MUNICIPALITY**

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CONCEPTUAL FRAMEWORK OF THE RESEARCH

Relevance and importance of the topic addressed. Aquatic microfungi are eukaryotic microorganisms that have been spread across Earth for millions of years and are closely related to both animals and plants. They belong to a diverse group morphologically, phylogenetically, and ecologically, depending on aquatic ecosystems and submerged substrates. The first research on freshwater microfungi dates back to the 19th century, and their initial identification was based on morphology and biochemical tests. Although progress has been made in understanding them, knowledge about filamentous freshwater fungi remains limited, and their ecology is a neglected field [5; 6; 9; 12; 22].

Filamentous fungi are the main microorganisms in aquatic ecosystems with the capacity to decompose organic matter, a unique characteristic of which is their quality as active enzyme producers. Extracellular enzymes or non-specific exoenzymes allow aquatic fungi to use as substrate diverse structural organic compounds, which correspond to different classes of pollutants. Therefore, these fungi could represent a new resource used in biotechnology [7; 8; 17; 23].

Although there is a great diversity of aquatic microfungi, only a few species have been studied and used for the production of industrial enzymes. Filamentous fungi species such as *Aspergillus*, *Penicillium*, and *Trichoderma* are capable of producing highly efficient enzymes, which are used in industries such as food, textiles, and agriculture due to their protein stability and ability to function under various conditions. Fungal enzymes are also compatible with safety requirements and regulations for sensitive industries, and the trend toward ecological and sustainable usage continues to grow, which is very important given the increasing trend for ecological and sustainable economic development [13; 14; 16; 18; 21; 23].

In addition to the benefits of using aquatic fungi in the enzymatic industry, they are of particular interest in biotechnology, being used as biocontrol agents. Aquatic fungi promote plant growth, provide safer and more effective solutions, contributing to reducing the use of chemical pesticides, thus protecting the environment and promoting more ecological agriculture. These microorganisms have antimicrobial effects, stimulate the systemic immunity of plants, and contribute to improving agricultural production [4; 10; 11; 15; 19; 26].

Based on the above, we can emphasize the importance of studying the diversity of filamentous aquatic fungi and their biotechnological properties, which are essential for identifying valuable enzymes and bioactive compounds with multiple industrial applications. These studies can facilitate the development of more efficient and ecological industrial processes.

Purpose of the thesis: Isolation and study of the morpho-cultural and biosynthetic properties of microfungi from the aquatic basins in Chişinău municipality.

Research objectives:

1. Isolation of micromycetes in pure culture from the aquatic basins of Chişinău municipality;
2. Study of the morpho-cultural peculiarities of the isolated micromycetes and their identification up to the genus level;
3. Identification of the enzymatic potential of the selected micromycetes;
4. Evaluation of the antimicrobial and phytostimulatory potential of some isolated aquatic micromycetes.

Research hypothesis: The aquatic basins in Chişinău municipality can be considered a valuable source of micromycetes strains capable of synthesizing bioactive substances with enzymatic, antimicrobial, and phytostimulatory properties. It is assumed that these strains have high biotechnological potential. The identification, characterization, and selection of these microorganisms could contribute to the development of new, efficient, and ecological bioproducts with applications in agriculture, the pharmaceutical industry, and environmental protection.

Synthesis of the research methodology and justification of the chosen research methods:

For the study, 476 strains of micromycetes isolated from the „La Izvor”, „Valea Trandafirilor” and „Valea Morilor” lakes in Chişinău municipality were used. Based on the proposed aim and objectives to demonstrate the advanced hypothesis, both classical and modern methods were applied:

Microbiological methods: Inoculation on agar media (malt 6 B, Czapek, Sabouraud agar without additives, Raistrick) of micromycetes from collected samples, isolation, and selection in pure culture (malt agar medium), study of the morpho-cultural peculiarities (visual and microscopic) of the isolated micromycetes;

Biochemical methods: Enzymatic activity of aquatic micromycetes (determination of catalase activity, amylase activity, lipase activity, cellulase activity, antimicrobial activity determination (antifungal and antibacterial properties));

Standardized methods: Obtaining and testing biopreparations on seeds of agricultural plants, which allow the identification of the phytostimulatory properties of fungal strains.

Statistical data analysis methods: Interpretation of results was performed after each stage in accordance with the results obtained from the conducted research and the method used. Statistical analysis of data was prepared by using MS Office Excel 2010.

Scientific novelty and originality: For the first time, a study was conducted on the diversity of microfungi in the „La Izvor”, „Valea Morilor”, and „Valea Trandafirilor” lakes in Chişinău municipality. The morpho-cultural characteristics of the isolated micromycetes were analyzed, and they were classified at the genus level. The predominant groups were identified, and the frequency of their occurrence was evaluated based on their location in the aquatic basins. The enzymatic biosynthesis capacity of the isolated micromycetes (amylase, catalase, lipase, and cellulase) was determined, and high-potential strains were selected and included in the National Collection of Non-Pathogenic Microorganisms as new resources of biotechnological and scientific interest. The antimicrobial potential of the micromycetes isolated from the lakes of Chişinău municipality was evaluated, particularly representatives from the *Trichoderma*, *Penicillium*, and *Talaromyces* genera, against phytopathogens. The phytostimulatory action of bioproducts based on *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 strains on wheat seeds was demonstrated.

Result: The result, which contributes to solving an important scientific problem, consists of evaluating the diversity of microfungi from the „La Izvor”, „Valea Morilor”, and „Valea Trandafirilor” aquatic basins in Chişinău, identifying the dominant genera, and estimating their enzymatic and antimicrobial potential. This led to the selection and storage of 14 strains with valuable biosynthetic properties, two of which belong to the *Trichoderma* genus and have positive effects on wheat seed germination. This allowed the expansion of the National Collection of Non-Pathogenic Microorganisms with new strains of interest for microbial biotechnology.

Theoretical significance: Were obtained next outcomes: i) accumulated data on the diversity and density of micromycetes from 3 aquatic basins of Chisinau municipality; ii) identified the dominant genera and evaluated their enzymatic and antimicrobial potential; iii) selected and deposited in the Collection of Non-Pathogenic Microorganisms 14 strains of micromycetes of biotechnological interest from the aquatic environment, 2 of which with phytostimulatory properties, which contributes to the development of new scientific resources for future research in microbiology, biotechnology, and other fields.

Applicative value: The strains of aquatic micromycetes from the *Trichoderma* and *Penicillium* genera can be used for the production of enzymes (amylase, catalase, lipase, and cellulase), while bioproducts with phytostimulatory properties can be developed based on *Trichoderma atrobruneum* CNMN FD 25 and *Trichoderma longibrachiatum* CNMN FD 27 strains.

Implementation of scientific results: The microfungi strains with enzymatic, antimicrobial, and phytostimulatory potential have enriched the National Collection of Non-

Pathogenic Microorganisms with promising producers. The bioproducts obtained from the *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 strains were tested in laboratory conditions on wheat seeds, demonstrating their phytostimulatory action on wheat seedlings.

Thesis content: The thesis consists of an Introduction, 4 chapters, General Conclusions and Recommendations, Bibliography with 342 references, 9 annexes, 134 pages of basic text, 23 figures, and 21 tables. The results obtained have been published in 35 scientific papers and 2 invention patents.

1. „MICROMYCETES - DISTRIBUTION AND USE IN BIOTECHNOLOGY”

Chapter 1 presents a critical analysis of the specialized literature regarding the importance of aquatic micromycetes and their fundamental role in aquatic environments, emphasizing their significance in various ecological and biotechnological processes. The main genera of aquatic filamentous fungi are described.

Additionally, this chapter highlights the importance of aquatic fungi in biotechnology, a continuously expanding field that greatly benefits from the biological diversity provided by these microorganisms. Aquatic micromycetes have the unique ability to produce highly stable and efficient enzymes that are much more resistant to extreme environmental conditions, such as high salinity and water pressure.

The study focuses on the strong antibacterial functions of aquatic filamentous fungi, which give them significant potential in plant protection, helping to combat diseases and pests, thereby supporting sustainable and ecological agriculture. These antibacterial capacities can be exploited for the development of biofertilizers and biocides, providing ecological and safer solutions for the environment and human health.

The chapter also emphasizes recent research on the secondary metabolites of aquatic filamentous fungi, bringing important genera such as *Trichoderma*, *Penicillium*, *Aspergillus*, *Fusarium*, and *Cladosporium* to the forefront. This research is essential for discovering new bioactive substances with biotechnological potential.

Aquatic micromycetes represent a vast and promising research field with significant applications in biotechnology, ecology, and pharmacology. These organisms not only help maintain the health of aquatic ecosystems but also offer innovative solutions for the development of more sustainable and eco-friendly industrial technologies.

In Chapter 2, **„OBJECT OF THE STUDY AND APPLIED METHODS IN RESEARCH”**, the main object of study is described, represented by filamentous fungi isolated from the aquatic basins „La Izvor”, „Valea Morilor” and „Valea Trandafirilor” in Chişinău municipality.

To isolate and analyze the aquatic micromycetes, classical microbiological methods were applied, which included sample collection from water, sediments (silt), and biofilms from different areas of the lakes. The samples were then transferred to the laboratory, where the microorganisms from each sample were isolated [1; 24; 25; 26].

An important aspect of the research was the use of specific culture media for isolation of micromycetes from the aquatic basins. These media were elaborated to favor the growth of filamentous fungi and facilitate their isolation from various types of the taken samples, thus ensuring a wide diversity of cultures.

In addition to the microbiological analysis, the study was expanded to include the evaluation of the enzymatic activities of the aquatic microfungi using rapid methods. The enzymatic activity of micromycetes has been investigated for such important enzymes as: catalase, amylase, lipase and cellulase, particularly essential for the decomposition of organic matter, with an important role in the bioremediation of aquatic environments.

Screening methods were used to assess the antimicrobial activity of the aquatic filamentous fungi, identifying strains with significant antimicrobial potential [2; 3; 19; 23]. Additionally, for two strains from the *Trichoderma* genus, their phytostimulatory potential was evaluated against wheat seeds.

Thus, this chapter details not only the microbiological techniques but also the biochemical approaches used to better understanding of the diversity and biotechnological properties of aquatic micromycetes.

Chapter 3. „ISOLATION, IDENTIFICATION AND ANALYSIS OF THE MORPHOLOGICAL AND CULTURAL PECULIARITIES OF AQUATIC MICROMYCETES”

This chapter includes the results of the research on the isolation and identification of aquatic micromycetes from the lakes „La Izvor”, „Valea Trandafirilor” and „Valea Morilor”.

3.1. - 3.2. Isolation and identification by genus of micromycetes in basins of Chisinau municipality

Within this compartment, the research carried out consisted of sampling water, biofilm and

silt sediment, which were then inoculated in agar media to promote the development and allow the isolation of micromycetes. After 5-7 days of incubation, the density of UFC (Colony-forming unit) was calculated. The results showed that the average density of UFC/ml varied from one lake to another, the highest density being in lake „La Izvor” in all samples regardless of the location taken – water, biofilm or silt sediments. The highest density in all lakes was found in sediments, which ranged from 4×10^4 in lake „Valea Morilor” to 2×10^6 UFC/g in lake „La Izvor”.

Were examined and isolated in pure culture on the malt agar medium strains that differed according to morpho-cultural peculiarities. From lake „La Izvor” were isolated 247 strains, lake „Valea Morilor” – 124 strains, and from lake „Valea Trandafirilor” – 105 strains.

The morpho-cultural peculiarities of isolated strains and genus affiliation have been studied, highlighting their wide diversity, thus demonstrating the ecological and functional variability of these organisms in aquatic environments. Each of the lakes from which the samples were taken had specific characteristics, depending on the location (water, biofilm, sediment), being identified certain predominant genera of micromycetes. The results obtained are presented in table 3.1.

Table 3.1. Number of fungal isolates extracted from aquatic basins

Gender of strains	„La Izvor”		„Valea Morilor”		„Valea Trandafirilor”	
	Nr. strains	frequency, %	Nr. strains	frequency, %	Nr. strains	frequency, %
<i>Penicillium spp.</i>	40	16,19	27	21,78	22	20,96
<i>Aspergillus spp.</i>	41	16,6	29	23,40	28	26,68
<i>Trichoderma spp.</i>	17	6,88	10	8,06	12	11,43
<i>Alternaria spp.</i>	23	9,31	10	8,06	5	4,76
<i>Fusarium spp.</i>	12	4,86	6	4,84	7	6,67
<i>Mucor spp.</i>	9	3,64	5	4,03	4	3,81
<i>Rhizopus spp.</i>	9	3,64	3	2,42	2	1,90
<i>Acremonium spp.</i>	9	3,65	2	1,62	2	1,90
<i>Talatomyces spp.</i>	5	2,02	3	2,42	2	1,90
<i>Botrytis spp.</i>	8	3,24	2	1,62	2	1,90
<i>Phoma spp.</i>	4	1,62	1	0,8	1	0,95
<i>Chaetomium spp.</i>	5	2,02	1	0,8	2	1,90
<i>Ulocladium spp.</i>	3	1,21	1	0,8	0	0
<i>Monilia spp.</i>	6	2,43	2	1,62	1	1,90
<i>Arthrimum spp.</i>	4	1,62	0	0	0	0
<i>Ambrosiella spp.</i>	6	2,43	0	0	0	0
<i>Trichocladium spp.</i>	3	1,21	0	0	0	0
<i>Cladosporium spp.</i>	6	2,43	2	1,62	1	0,95
Nedetrminate	37	15,0	20	16,13	14	13,34
Total	247	100 %	124	100 %	105	100 %

According to the study carried out, the isolates from the lake „La Izvor” are representatives of 18 genera which constitute 85% of the total number of isolates, of which the strains of the genus *Penicillium* constitute 16%, *Aspergillus* – 17%; *Alternaria* – 9%, *Trichoderma* – 7%, *Fusarium* – 5%. Other genera were recorded with a frequency of less than 4%.

Of 105 isolates from the „Valea Trandafirilor” lake, 14 genera were identified, which is 87% of the total number of isolates. Most strains belong to the genus *Penicillium*, the frequency of which constitutes 21%, strains of the genus *Aspergillus* – 26%, and the genus *Trichoderma* 11%. A significant number was represented by the genera: *Fusarium*, *Alternaria* and *Mucor* (4-6%), the other genera recorded a lower frequency.

Of 124 strains, isolated from the lake „Valea Morilor”, were identified 15 genera, which constitute 84% of the total number of isolates. The frequency of occurrence of the fungi *Aspergillus* spp. is 23%, of the *Penicillium* – 22%, followed by genera *Trichoderma* spp. and *Alternaria* spp. with a frequency of 8%. Other genera were in a number less than 4%.

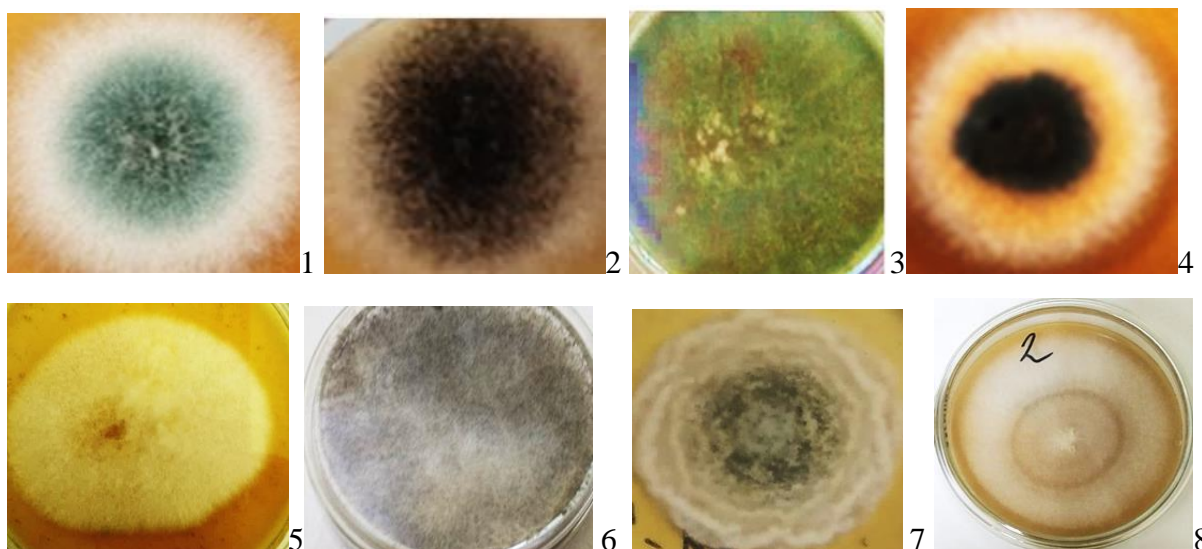


Figure 3.1. Colonies of fungal strains of the genera: 1. *Penicillium*, 2. *Aspergillus*, 3. *Trichoderma*, 4. *Talaromyces*, 5. *Fusarium*, 6. *Mucor*, 7. *Trichocladium*, 8. *Chaetomium*

It was found that in the water basins of Chisinau municipality conveys a wide range of micromycetes, predominantly representatives of the genera *Aspergillus*, *Penicillium*, *Trichoderma* followed by the genera *Fusarium*, *Mucor*, *Alternaria* (figure 3.1) [20].

3.3. Identification of fungal strains by molecular biology techniques

Fourteen strains of micromycetes, with significant enzymatic and antimicrobial potential were identified at species level by molecular biology techniques. The identification was carried out at the Institute of Biology in Bucharest, Romania in collaboration with Ruginescu R. and

Enache M., being used kits for the identification of fungi. The results obtained are presented in table 3.2.

As a result of research, it was found that fungi belong to the genera *Talaromyces* (7 strains), *Trichoderma* (5 strains) and *Aspergillus* (2 strains) [22].

Table 3.2. Identification of fungal strains by molecular biology techniques

Strain	Base pairs (pb)	Similarity
B 1	461	<i>Talaromyces tumuli</i> NRRL 62151
B 5	528	<i>Trichoderma atrobrunneum</i> CBS 548.92
B 8	477	<i>Talaromyces adpressus</i> CBS 140620
A 1	549	<i>Trichoderma atrobrunneum</i> CBS 548.92
A 3	474	<i>Talaromyces purpureogenus</i> CBS 286.36
A4	513	<i>Talaromyces purpureogenus</i> CBS 286.36
A5	513	<i>Talaromyces purpureogenus</i> CBS 286.36
A 13	575	<i>Trichoderma longibrachiatum</i>
N 3	513	<i>Talaromyces purpureogenus</i> CBS 286.36
N 7	509	<i>Talaromyces purpureogenus</i> CBS 286.36
N8	520	<i>Aspergillus fumigatus</i> ATCC 1022
A 23	520	<i>Aspergillus fumigatus</i> ATCC 1022
N 9	537	<i>Trichoderma simmonsii</i> CBS 130431
N 14	555	<i>Trichoderma longibrachiatum</i>

Note: A – strains isolated from water; B – strains isolated from biofilms and N – strains isolated from sediments

After identification, for each strain the passport was developed and stored in the CNMN as potential producers of bioactive substances. The strains were assigned a registration number, with subsequent inclusion in the Collection of Non-Pathogenic Microorganisms Catalogue, which can be accessed on the website cnmn.imb.md.

4. BIOSYNTHETIC PROPERTIES OF MICROMYCETS ISOLATED FROM AQUATIC LAKE OF CHISINAU MUNICIPALITY

The enzymatic, antifungal and antibacterial properties of some strains of micromycetes isolated from lakes „La Izor”, „Valea Trandafirilor” and „Valea Morilor” were evaluated.

Of the 14 strains identified up to the species level, 2 strains *Trichoderma atrobruneum* and *T. longibrachiatum* were selected, which demonstrated high enzymatic and antimicrobial activity based on which biopreparations were obtained, and then tested on wheat seeds.

4.1 Study of the enzymatic activity of micromycetes isolated from the lakes of Chisinau

The enzymatic capacities of the selected strains were tested for producing activity for some essential enzymes: amylase, catalase, cellulase and lipase. These enzymes are involved in breaking down carbohydrates, lipids and other organic substances, and studying them helps to understand the role of these filamentous fungi in ecological processes and identify opportunities for various biotechnological applications/processes.

From the set of fungi isolated from Lake „La Izvor”, 93 strains were tested on the enzymatic capacities: 35 strains isolated from water, 28 strains – from biofilm and 30 strains – from sediments (silt). The results are shown in figure 4.1.

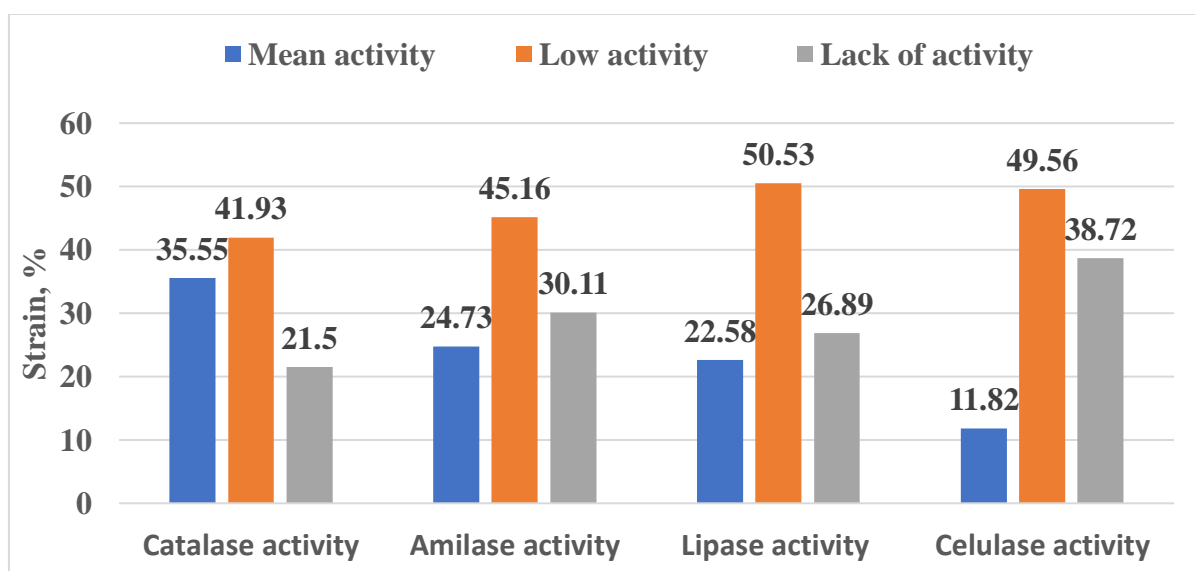


Figure 4.1. Rate of micromycete strains in „La Izvor” lake, with specific enzymatic activity

In figure 4.1, the results of enzyme activity testing are presented. According to the data, 36.55% of the tested strains showed mean intensity catalase activity, 41.93% – low intensity catalase activity, and 21.5% did not show activity. Also, a significant percentage of strains showed amylase activity, with 24.73% having mean intensity amylase activity and 45.16% – low activity. Lipase activity was more diverse, with 22.58% of strains having mean intensity activity and 50.53% at low level. Cellulase activity was recorded in 11.82% of strains as mean and 49.46% as low.

In terms of enzymatic capacities, most strains of the genera *Trichoderma*, *Talaromyces* and *Penicillium* have demonstrated medium or low enzyme activity for the enzymes studied. Most micromycetes with medium activity were isolated from water.

Forty-three strains from „Valea Morilor” lake, were tested on enzymatic activity: 19 strains isolated from water, 17 strains – from sediments and 7 strains – from biofilm, the data being presented in figure 4.2.

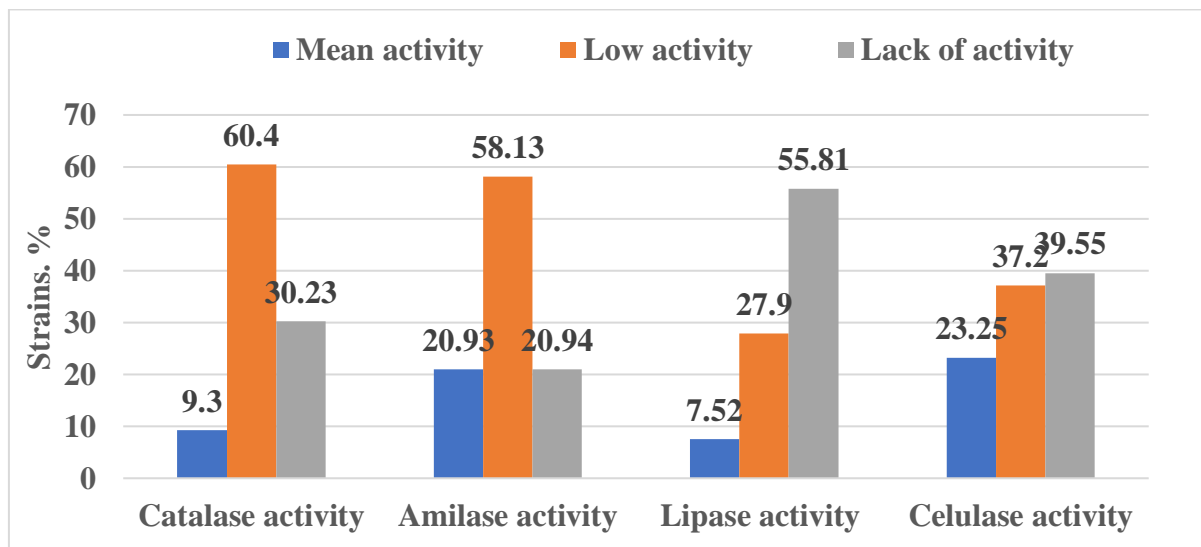


Figure 4.2. Rate of micromycetes strains in „Valea Morilor” lake, with specific enzymatic activity

According to the data obtained, a considerable variability in the enzymatic activity of the analyzed strains is noted. Thus, of the studied strains, 9.3% showed mean catalytic activity, 60.4% - decreased activity, and 30.23% were inactive.

Amylase activity was different from catalase activity, so 20.93% of strains showed average activity, and a similar percentage: 20.93% – low intensity amylase activity, while 58.13% showed no activity.

The lipase activity was lower: 7.52% of the strains showed mean intensity lipase activity, 27.9% – low intensity, and 55.81% did not show lipase activity.

In terms of **cellulase activity**, 23.25% of the strains had mean cellulase activity, 37.2% had low intensity activity and 39.53% had no activity.

The most active isolates from „Valea Morilor” lake, depending on the enzymatic activity, were represented genera *Trichoderma* and *Aspergillus*, most of which are isolated from water.

From „Valea Trandafirilor” lake, 28 strains were tested: 9 strains isolated from water, 14 strains from sediments and 5 strains from biofilm. In comparison with the „La Izvor” lake and the

„Valea Morilor” lake, the enzymatic activity of the strains from „Valea trandafirilor” lake was lower. The data obtained are presented in Figure 4.3.

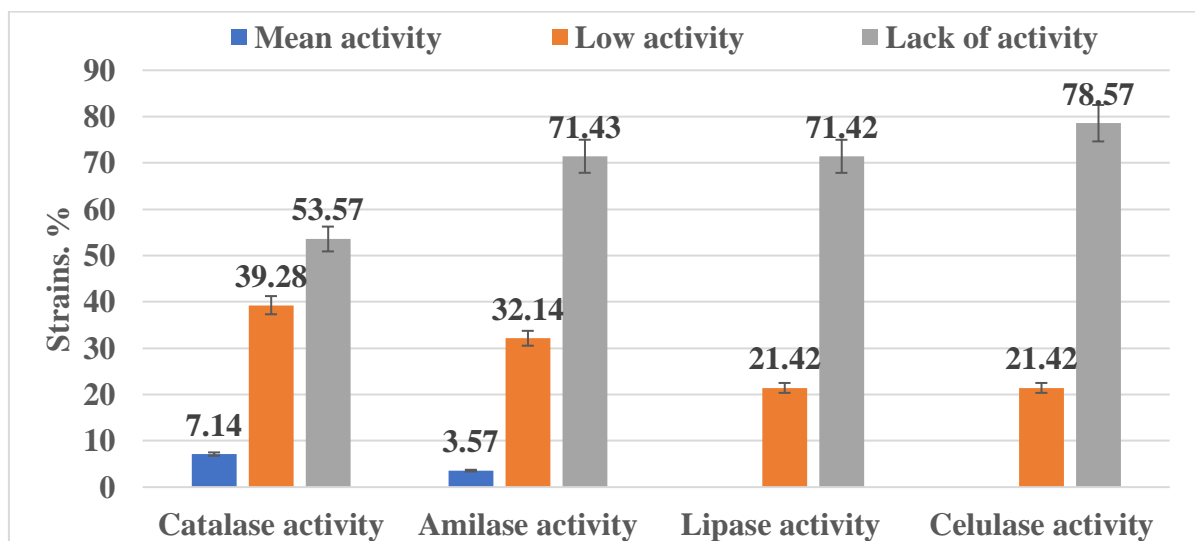


Figure 4.3. Rate of micromycete strains in „Valea trandafirilor» lake, with specific enzymatic activity

Following the analysis of the data obtained, an insignificant variability was found in the enzymatic activity of the studied strains. Thus, 7.14% of them had mean-intensity of catalase activity, 39.28% decreased activity, and 53.57% did not show activity. Similarly, the strains showed amylase activity: 3.57% had mean activity and 32.14% – low. Lipase activity was poorly expressed, with only 21.42% of strains having low activity, while 71.42% had no activity. Cellulase activity was also decreased to 21.42% of strains and 78.57% had no activity.

The results obtained in this compartment have demonstrated that most of the aquatic micromycetes studied possess significant activity of one or more enzymes, which facilitate the decomposition of vegetable matter and organic substances from lakes into simple carbohydrates, thus contributing to the purification of water in aquatic ecosystems. The most enzymatically active strains were presented by fungi belonging to the genera *Penicillium*, *Talaromyces*, *Aspergillus*, *Trichoderma* and *Fusarium*. Of the total number of 164 strains tested, over 12.2% had an average activity for the 4 enzymes in the study.

Given this wide range of enzymes produced, aquatic micromycetes have a remarkable potential of biotechnological interest in obtaining bioactive substances for industries of various fields – food (food additives), medical (medicines), pharmaceutical, cosmetic, agricultural, etc.

4.2 Antimicrobial properties of micromycetes isolated from aquatic basins

In addition to the enzymatic properties of isolates from lakes „La Izvor”, „Valea Trandafirilor” and „Valea Morilor”, antimicrobial properties against phytopathogens were also studied. For this purpose, 71 aquatic strains of genera *Talaromyces*, *Penicillium*, *Trichoderma* were tested. As pathogens served 5 species of fungi – *A. niger*, *A. alternata*, *B. cinerea*, *F. solani*, *F. oxysporum* and 5 bacteria – *B. subtilis*, *X. campestris*, *C. michiganensis*, *A. tumefaciens*, *E. caratovora*. From „La Izvor” lake were tested 33 strains (14 isolates from water, 3 – biofilm, 16 – sediments); from „Valea Trandafirilor» lake – 20 strains (10 – water isolates, 6 – sediments and 4 – biofilm); from lake „Valea Morilo” – 18 strains (8 – water isolates, 6 – sediments and 4 – biofilm).

A pronounced antifungal activity was recorded in a considerable number of tested strains, with the results being illustrated in figure 4.4.

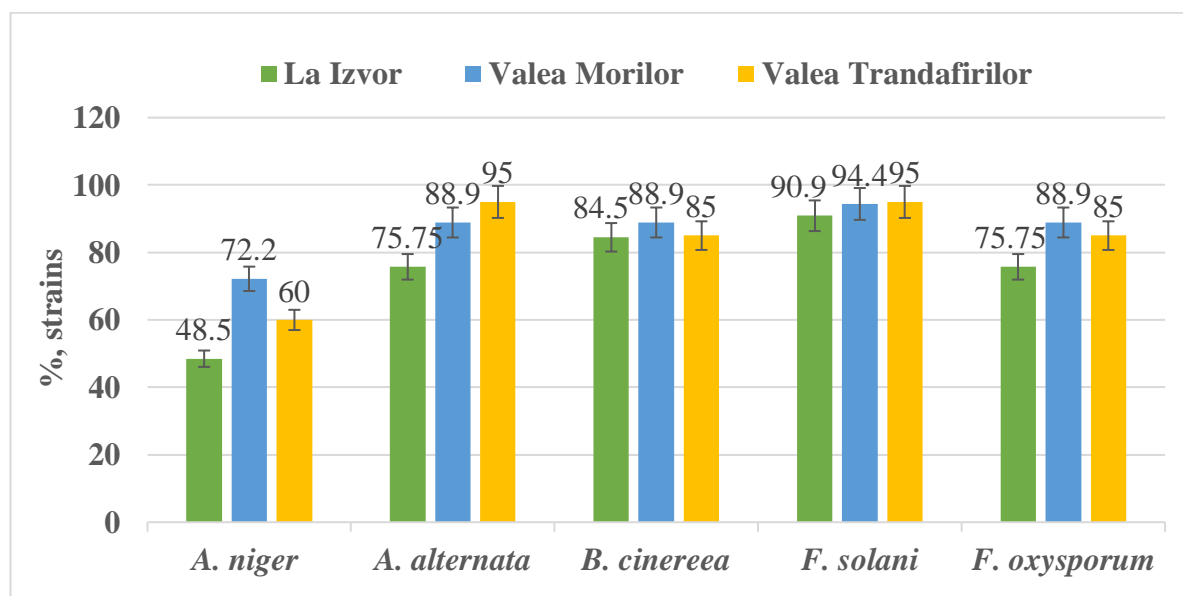


Figure 4.4. Rate of isolates of micromycetes from „Valea Morilor”, „Valea Trandafirilor” and „La Izvor” lakes, possessing antifungal activity

Thus of 33 isolates from lake „La Izvor”, 48.5% showed antifungal activity against *A. niger*, 75.75% – for *A. alternata*, 84.85% – *B. cinerea*, 90.9% – *F. solani* and 75.75% – *F. oxysporum*. The diameter of the inhibition zones of phytopathogens varied within 16-40 mm. Based on antifungal capabilities against the tested phytopathogens, more active manifested representatives of the genus *Trichoderma*, in comparison with representatives of the genus *Penicillium* and *Talaromyces*, regardless of the place of isolation.

Of the 20 strains, isolated from the „Valea Trandafirilor», showed antifungal activity against the phytopathogen *A. niger* 60%, *A. alternata* and *F. solani* - 95%, in comparison with *B.*

cinerea and *F. oxysporum* – 85%. Phytopathogenic inhibition zones ranged from 16 mm to 29 mm. Comparing the antifungal activity of the tested strains from „Valea Trandafirilor” lake depending on the place of isolation, it was found that the most active were isolated strains from the water, represented by the genus *Trichoderma*.

Of 18 strains, isolated from the „Valea Morilor” lake, 72.2% showed activity against phytopathogen *A. niger*, 88.9% – for: *A. alternata*, *B. cinerea* and *F. oxysporum*; and for *F. solani* – 94.4%. The diameter of the inhibition zones of phytopathogens varied within 14-40 mm.

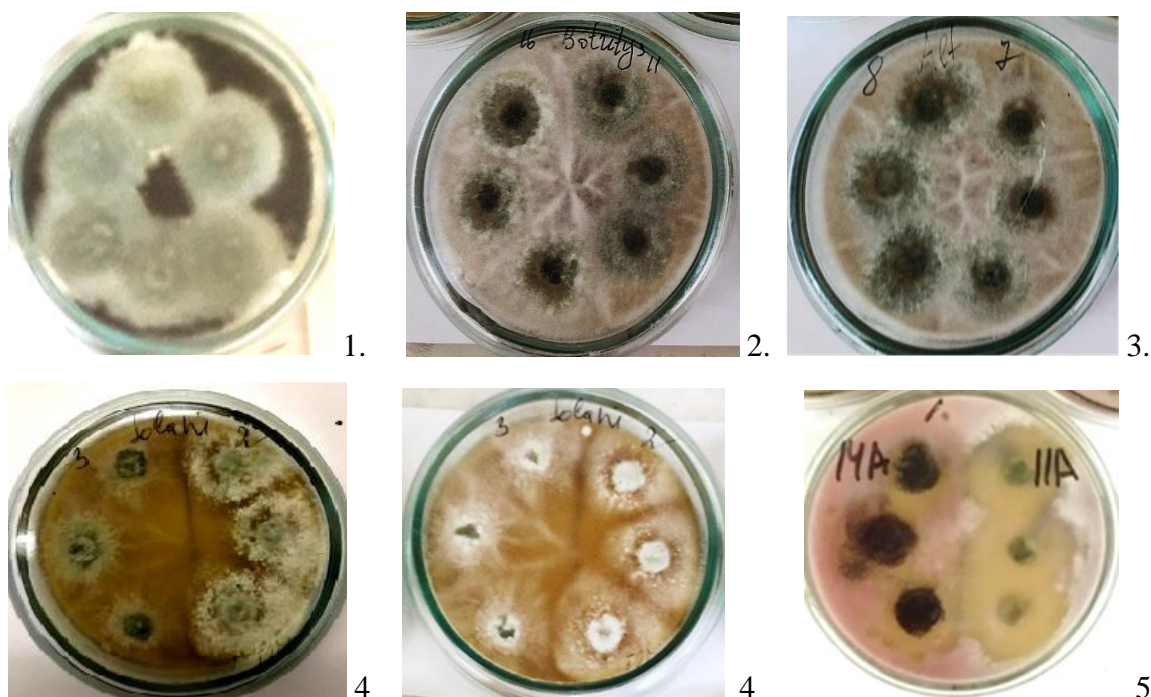


Figure 4.5. Inhibition zones of phytopathogenic fungi under the influence of metabolites of micromycetes isolated from Chisinau municipality lakes: 1. *A. niger*, 2. *B. cinerea*, 3. *A. alternata*, 4. *F. solani*, 5. *F. oxysporum*

By obtained results we can find that the isolated micromycetes from the „La Izvor”, „Valea Trandafirilor” and „Valea Morilor” lakes, show increased antifungal activity against the tested phytopathogens, with inhibition zones ranging from 15 mm to 40 mm, more active proving the strains of the genus *Trichoderma* (figure 4.5).

Research on phytopathogenic bacteria has demonstrated different antibacterial activity than antifungal (figure 4.6)

The results show that aquatic micromycete strains isolated from the «Valea Trandafirilor” lake possess high antibacterial properties against the tested strains, except for the phytopathogen *E. carotovora*, for which only 10 out of the 20 tested strains exhibited activity, representing 50%. Against the phytopathogens *B. subtilis*, *C. michiganensis*, and *A. tumefaciens*, 80% of the tested

strains exhibited activity, while 85% showed activity against *X. campestris*, with inhibition zones ranging from 15-36 mm.

Out of the total of 18 strains isolated from the „Valea Morilor” lake, 61.1% showed antibacterial activity against *B. subtilis*, 88.9% against *X. campestris* and *A. tumefaciens*, 83.3% against *C. michiganensis*, and 66.7% against *E. carotovora*. The inhibition zones for the phytopathogens ranged from 15-32 mm.

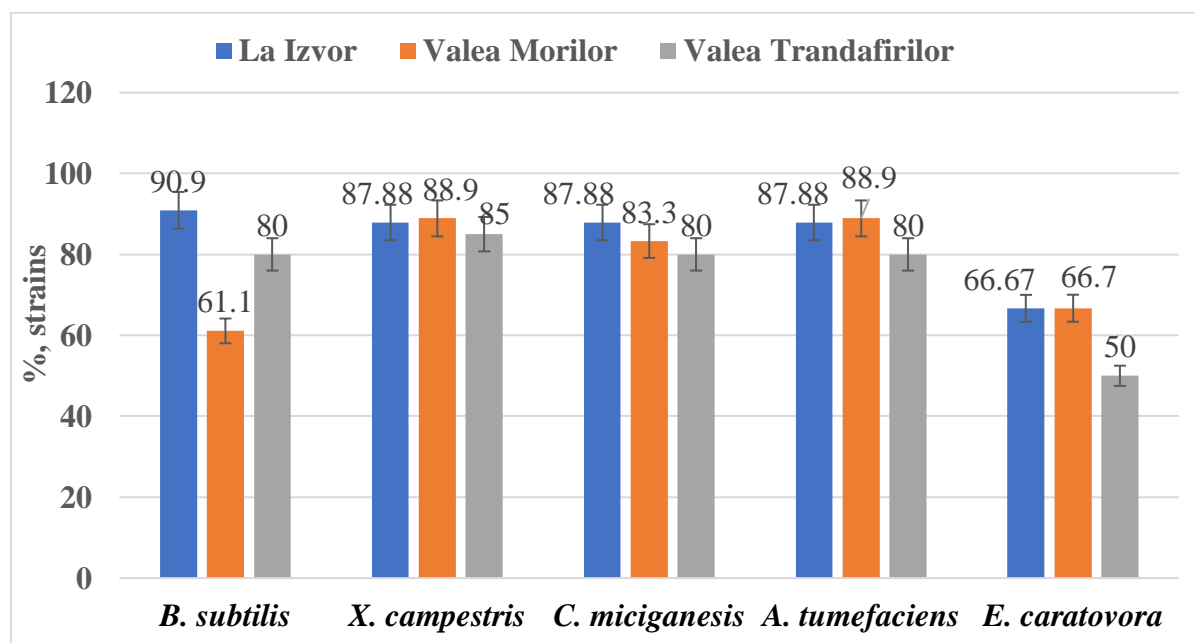


Figure 4.6. Rate of isolates of micromycetes from „Valea Morilor”, „Valea Trandafirilor” and „La Izvor” lakes, possessing antibacterial activity

The strains isolated from the sediments of the studied lakes demonstrated higher antimicrobial activity in comparison to those isolated from water and biofilm. Representatives of the genus *Trichoderma* were more active in comparison with representatives of the genera *Penicillium* and *Talaromyces* (figure 4.7).

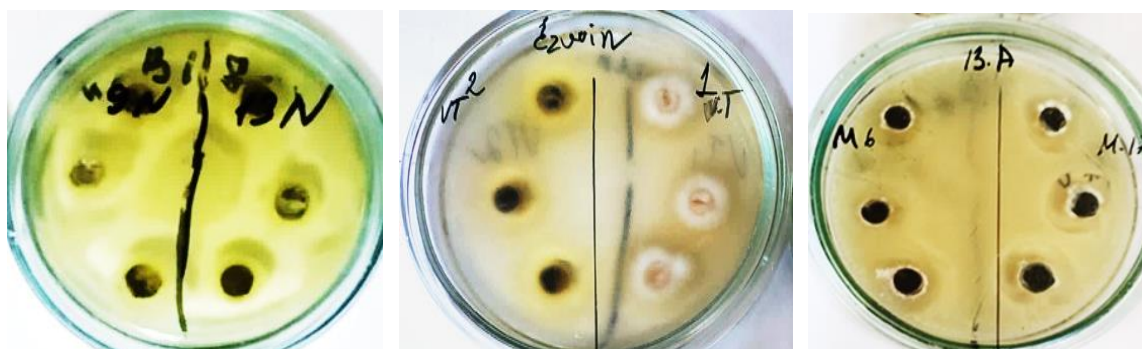


Figure 4.7. Inhibition zones of bacteria under the influence of metabolites of micromycetes isolated from Chisinau municipality lakes: a) *A. tumefaciens*; b) *B. subtilis*; c) *E. carotovora*

The results obtained in this section demonstrate that the majority of the micromycete strains isolated from the „La Izvor”, „Valea Morilor”, and „Valea Trandafirilor” lakes possess high antifungal and antibacterial properties and can be used in biotechnology as sources of bioactive substances.

4.3. Evaluation of the phytostimulatory potential of some aquatic micromycetes

To identify the phytostimulatory capacity, from the wide range of aquatic micromycete strains studied, two strains belonging to the genus *Trichoderma*, isolated from the „La Izvor” lake, were selected, which possess significant enzymatic and antimicrobial properties against the tested phytopathogens: *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27, based on which biopreparations were obtained.

The biopreparations, obtained through submerged cultivation, were tested to identify their interaction characteristics with certain phytopathogens. The results obtained in this study are presented in figure 4.8.

The biopreparation obtained from the *T. atrobruneum* CNMN FD 25 strain exhibited high activity against phytopathogenic fungi, with inhibition zone diameters ranging from 35-38 mm, and against phytopathogenic bacteria with inhibition zones ranging from 23-32.3 mm. The inhibition zones for phytopathogenic fungi under the action of the biopreparation obtained from the *T. longibrachiatum* CNMN FD 27 strain ranged from 25-40 mm, while for phytopathogenic bacteria, the inhibition zones ranged from 20.2-25 mm.

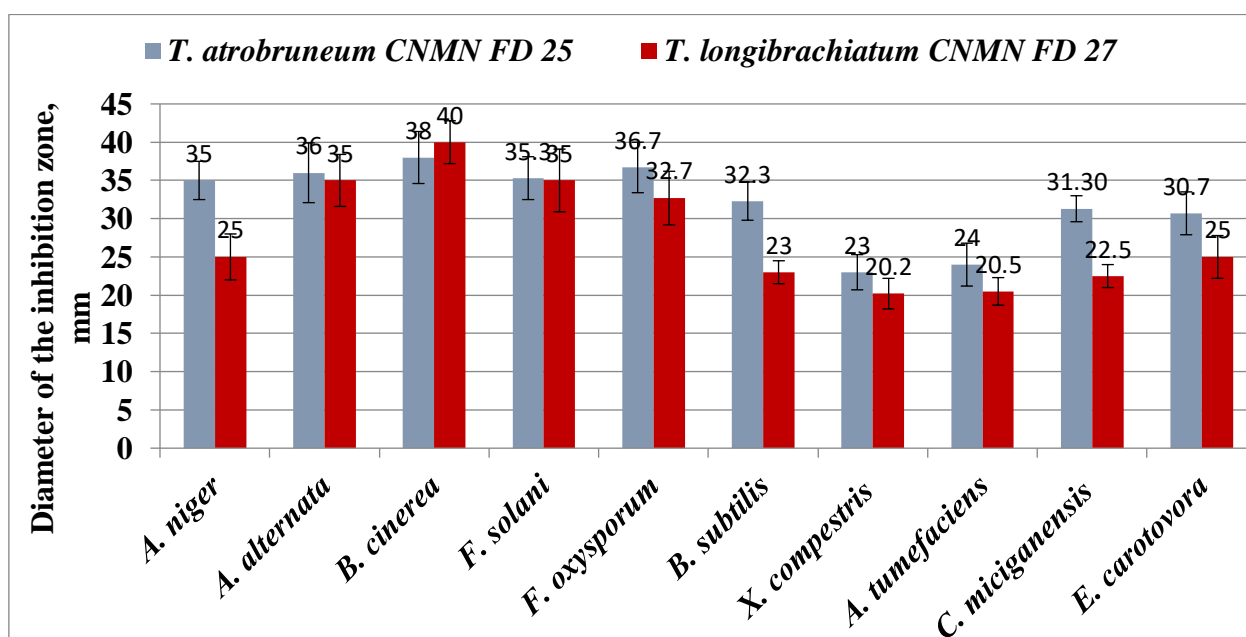


Figure 4.8. Antimicrobial activity of *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 in relation to phytopathogens

The phytostimulatory efficiency of the biopreparations was tested on wheat seeds (Moldova 16 variety), kindly provided by Dr. G. Lupașcu from the Institute of Genetics, Physiology, and Plant Protection, SUM.

Wheat grains were treated with microbial preparations in concentrations of 1%, 0.5%, 0.33% and 0.25% for 1 hour, then sown in soil in 4 variations and cultivated at a temperature of 18-20°C under controlled conditions, with daylight. In the control variant, the seeds were treated with tap water. After 14 days of cultivation, several biometric indices of the wheat seedlings were evaluated: germination, average seedling length, dry weight of the seedlings and roots. The results obtained from the testing of the biopreparation based on the *T. atrobruneum* CNMN FD 25 strain are presented in figure 4.9.

The data presented in figure 4.9 demonstrate that treating wheat seeds with the *T. atrobruneum* CNMN FD 24 biopreparation, regardless of the applied concentration, positively impacts the seeds, stimulating both germination and seedling growth. The most relevant results were recorded in the variant where the seeds were treated with a 0.5% exometabolite solution, where germination increased by 6.11%, and seedling length increased by 12.61%, in comparison with the control variant.

The indices for the dry weight of the seedlings and dry weight of the roots also showed positive results. The results for the dry weight of the seedlings varied within $\pm 4\%$ in comparison with the control variant. The highest results of 106.77% in comparison with the control variant, were obtained in the variant with exometabolite solution of 0.25%. In contrast, the variant where the exometabolite solution of 0.5% was applied, the index had only 92.42%.

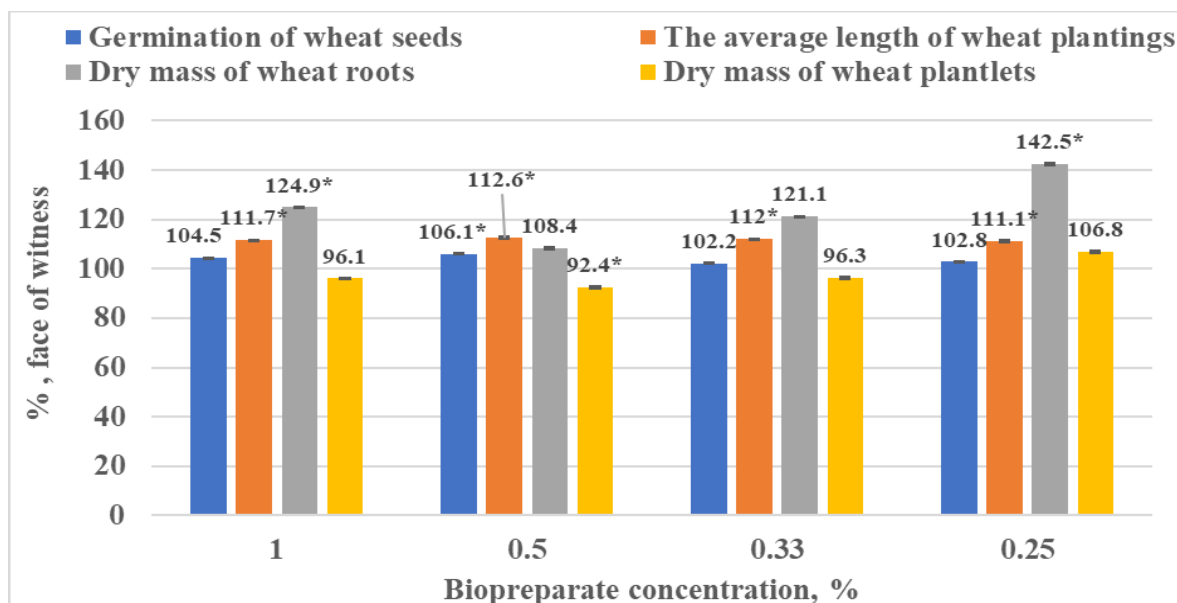


Figure 4.9. Action of the biopreparate obtained based on the *T. atrobruneum* CNMN FD 24 strain on wheat seeds, dry mass of seedlings and roots, * $p \leq 0,05$

Thus, it can be concluded that this biopreparation had a more beneficial effect on the root system and less on the overall growth of the wheat seedlings.

Treating wheat seeds with the biopreparation obtained from the *T. longibrachiatum* CNMN FD 27 strain also demonstrated stimulation of some morphological and biological traits of the wheat seedlings (figure 4.10.).

Treating wheat seeds with the biopreparation obtained from the *T. longibrachiatum* CNMN FD 27 strain did not stimulate the germination capacity. Only in the variant where the seeds were treated with a 0.33% exometabolite solution did germination reach the level of the control group, while in the other variants, this index showed a slight, insignificant decrease.

Evaluating the biopreparation's effect on seedling growth, we can note a slight stimulation, with seedling length varying between 106.4% (for the 1% concentration variant) and 108.3% (for the 0.33% variant) in comparison with the control.

A stimulatory effect was observed for the indices of dry weight of the roots and dry weight of the seedlings. The maximum advantage for the dry weight of the roots index, 123.71% in comparison with the control, was recorded in the variant where the 0.5% biopreparation concentration was used. The dry weight index of the seedlings showed lower values. In the variant where the seeds were treated with a 0.5% exometabolite solution, this index was 108.64% in comparison with the control.

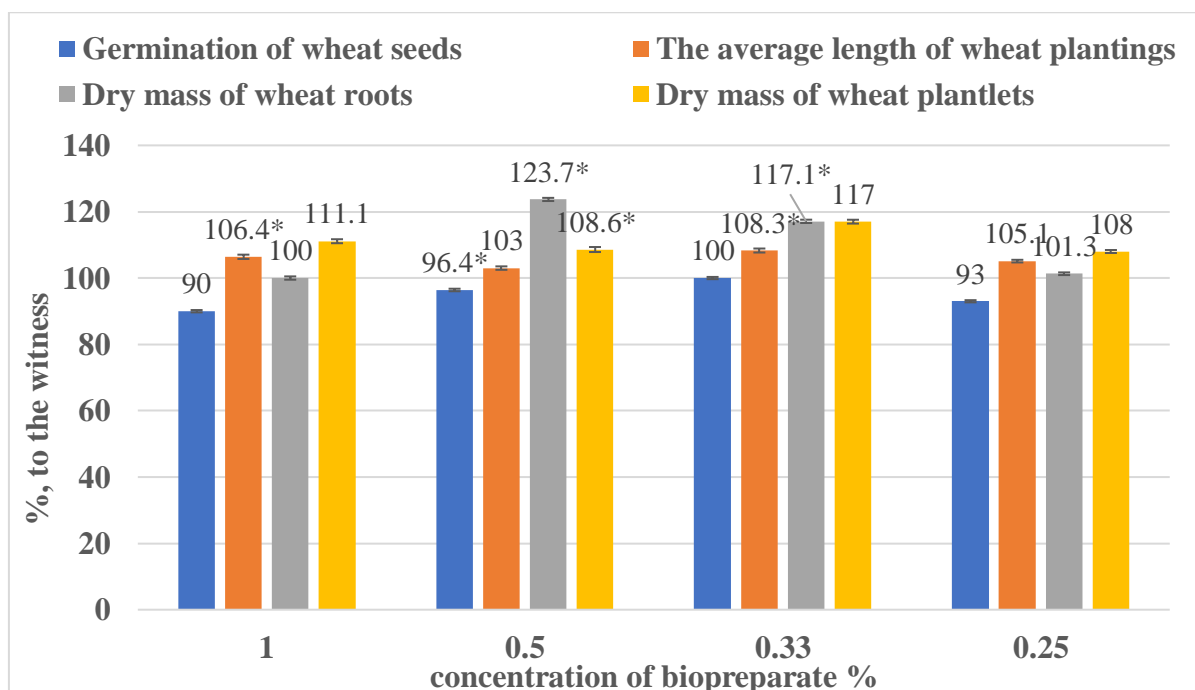


Figure 4.10. Biopreparate action obtained from *T. longibrachiatum* CNMN FD 24 strain on wheat seed, plant and root dry mass, *p≤0,05

As with the biopreparation obtained from *T. atrobruneum* CNMN FD 25, treating wheat seeds with the biopreparation from *T. longibrachiatum* CNMN FD 27 had a more beneficial effect on the root system and less on the overall growth of the wheat seedlings, which was demonstrated by the significant stimulation of the dry weight of the roots index in comparison with the control.

Based on the results obtained, we can conclude that the strains *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 can be considered promising for use in agriculture as phytostimulators for crop plants.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

General conclusions

1. From the «La Izvor», «Valea Morilor», and «Valea Trandafirilor» lakes in Chişinău, 476 micromycete strains were isolated from water, sediment, and biofilm samples. Eighteen genera were identified, representing 85% of the total isolates, among which the predominant genera were *Aspergillus*, *Penicillium*, and *Trichoderma*, with frequencies of 17–26%, 16.2–21.8%, and 6.88–11.43%, respectively.
2. From the total number of micromycetes isolated from the aquatic environment, 14 strains with high biosynthetic potential were selected, identified through morphocultural and molecular biology techniques. These were documented by creating passports and deposited in the National Collection of Non-Pathogenic Microorganisms (CNMN) as objects of biotechnological interest.
3. The evaluation of enzymatic activities (amylase, catalase, cellulase, and lipase) of 164 strains showed that 12.2% of them exhibited activity for all four enzymes. Pronounced activity was recorded for strains isolated from the sediments and water of the «La Izvor» lake.
4. Testing the antimicrobial properties of 71 strains against phytopathogens showed that over 80% exhibited high activity, with inhibition zones up to 40 mm for fungi and up to 35 mm for phytopathogenic bacteria. The most active strains were isolated from the sediments and water of the «La Izvor» lake.
5. The phytostimulatory capacity of two biopreparations, developed from the *Trichoderma atrobruneum* CNMN FD and *Trichoderma longibrachiatum* CNMN FD 27 strains, was tested on wheat seeds. Applying biopreparations at concentrations of 0.5% and 0.33% resulted in an increase of the germination rate by 6%, seedling length by 12.6%, seedling dry weight by 8.6%, and root dry weight by 23.7%.
6. **The research hypothesis** underlying this work was confirmed, demonstrating that the aquatic basins in Chisinau represent a valuable source of micromycete strains with high potential for the synthesis of bioactive substances, with applicability in biotechnology.

Practical recommendations

It is recommended: the use of aquatic micromycete strains with high enzymatic and antimicrobial potential for biotechnology to obtain bioactive substances.

It is proposed to use biopreparations obtained from the fungal strains *Trichoderma atrobruneum* CNMN FD 25 and *Trichoderma longibrachiatum* CNMN FD 27 as phytostimulators for grass plants.

Personal contribution: The results obtained in the development of the doctoral thesis, the analysis, generalizations and conclusions belong to the author.

In the patents MD 4896. "*Trichoderma atrobrunneum* fungal strain with antimicrobial properties" and "Nutrient medium for cultivation of the fungal strain *Trichoderma longibrachiatum* CNMN-FD-27", Entry No. 2552 of 06.06.2024, the author is entitled to a share according to the list of authors.

Implementation of Scientific Results: Micromycete strains with enzymatic, antimicrobial, and phytostimulatory potential have been added to the National Collection of Non-Pathogenic Microorganisms as promising producers, and biopreparations based on *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 strains have been tested in laboratory conditions on wheat seeds, demonstrating their phytostimulatory action on wheat seedlings.

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2.1. In journals from the Web of Science and SCOPUS databases

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2. SÎRBU, T.; **MOLDOVAN, C.** Studiul activității enzimatice a fungilor acvatice. In: *Instruire prin cercetare pentru o societate prosperă*. Biologie. 2022. Ediția 9, Vol.1, pp. 136-139. doi.org/10.52757/imb21.088 ISBN 78-9975-76-390-5.

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5. **MOLDOVAN, C.** Study of the enzymatic properties of fungi in the "La izvor" aquatic ecosystem. În: *„Microbial Biotechnology”*, international scientific conference. 5th International Scientific Conference on Microbial Biotechnology, Chisinau, Moldova, October 12-13, 2022. 71. ISBN 978-9975-3555-7-5
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4. Patents and other intellectual property (OPI)

4.2. Issued by the State Agency on Intellectual Property

1. SÎRBU Tamara, **MOLDOVAN Cristina**, ȚURCAN Olga. Tulpină de fungi *Trichoderma atrobrunneum* cu proprietăți antimicrobiene. Brevet de invenție 4896. MD - BOPI 7/2024, p. 23.
2. SÎRBU Tamara, **MOLDOVAN Cristina**, ȚURCAN Olga. Bîrsa Maxim. Mediul nutritiv de cultivare a tulpinii fungice *Trichoderma longibrachiatum* CNMN-FD-27. Hotărârea de acordare a brevetului de invenție Nr. 10574 din 2025. 02. 18

ADNOTARE

MOLDOVAN Cristina: „Proprietățile morfo-culturale și biosintetice ale micromicetelor din bazinele acvatice ale municipiului Chișinău”, teză de doctor în științe biologice, Chișinău, 2025. **Structura tezei:** introducere, 4 capitole, concluzii generale și recomandări, bibliografie cu 342 titluri, 9 anexe, 134 pagini text de bază, 23 figuri, 21 tabele. Rezultatele obținute sunt publicate în 35 lucrări științifice, 2 brevete de invenție.

Cuvinte-cheie: micromicete, izolare, particularitățile morfo-culturale, zone de inhibiție, medii nutritive, activitate enzimatică, activitate antimicrobiană, proprietăți fitostimulatoare.

Scopul lucrării: izolarea și studierea proprietăților morfo-culturale și biosintetice ale micromicetelor din bazinele acvatice ale municipiului Chișinău.

Obiectivele cercetării: Izolarea în cultură pură a micromicetelor din bazinele acvatice ale municipiului Chișinău; Studiul particularităților morfo-culturale ale micromicetelor izolate și identificarea lor la nivel de gen; Evaluarea potențialului enzimatic al micromicetelor identificate; Evaluarea potențialului antimicrobian și fitostimulator al micromicetelor din mediul acvatic.

Noutatea și originalitatea științifică. Pentru prima dată a fost realizat un studiu privind diversitatea micromicetelor din lacurile „La Izvor”, „Valea Morilor” și „Valea Trandafirilor” din municipiul Chișinău. S-au analizat particularitățile morfo-culturale ale micromicetelor izolate, acestea fiind clasificate la nivel de gen. Genurile predominante au fost identificate și s-a evaluat frecvența de apariție în funcție de localizarea în bazinele acvatice. A fost determinată capacitatea de biosinteză enzimatică a micromicetelor izolate (amilaza, catalaza, lipaza și celulaza), fiind selectate tulpinile cu potențial înalt, care au fost incluse în CNMN ca noi resurse de interes biotehnologic și științific. A fost evaluat potențialul antimicrobian al micromicetelor izolate din lacurile municipiului Chișinău, în special aparținând genurilor *Trichoderma*, *Penicillium* și *Talaromyces*, împotriva fitopatogenilor. S-a demonstrat acțiunea fitostimulatoare a biopreparatelor pe bază de tulpinile *T. atrobruneum* CNMN FD 25 și *T. longibrachiatum* CNMN FD 27 asupra semințelor de grâu.

Rezultatul obținut, care contribuie la soluționarea unei probleme științifice importante, constă în fundamentarea științifică a diversității micromicetelor din bazinele acvatice „La Izvor”, „Valea Morilor” și „Valea Trandafirilor” din municipiul Chișinău, identificarea genurilor dominante și evaluarea potențialului lor enzimatic și antimicrobian, ceea ce a condus la selectarea și depozitarea a 14 tulpini cu proprietăți biosintetice valoroase, dintre care două tulpini din genul *Trichoderma* cu efecte pozitive asupra germinării semințelor de grâu, fapt ce a permis extinderea Colecției Naționale de Microorganisme *Nepatogene* (CNMN) cu noi tulpini de interes pentru domeniul biotehnologiilor microbiene.

Semnificația teoretică: Au fost acumulate date privind diversitatea și densitatea micromicetelor din 3 bazine acvatice ale municipiului Chișinău. Au fost identificate genurile dominante și evaluat potențialul lor enzimatic și antimicrobian. Au fost selectate și depuse în Colecția Națională de Microorganisme *Nepatogene* 14 tulpini de micromicete de interes biotehnologic din mediul acvatic, dintre care 2 cu proprietăți fitostimulatoare, ceea ce contribuie la dezvoltarea de noi resurse științifice pentru cercetări viitoare în microbiologie, biotehnologie, și alte domenii.

Valoarea aplicativă: Tulpinile de micromicetele din mediul acvatic din genurile *Trichoderma* și *Penicillium* pot fi utilizate pentru obținerea enzimelor (amilaza, catalaza, lipaza și celulaza), iar pe baza tulpinilor *Trichoderma atrobruneum* CNMN FD și *Trichoderma longibrachiatum* CNMN FD 27 pot fi dezvoltate biopreparate cu proprietăți fitostimulatoare.

Implementarea rezultatelor științifice: Tulpinile de micromicete cu potențial enzimatic, antimicrobian și fitostimulator au completat Colecția Națională de Microorganisme *Nepatogene* cu producători perspectivi, iar biopreparatele obținute în baza tulpinilor *T. atrobruneum* CNMN FD 25 și *T. longibrachiatum* CNMN FD 27 au fost testate în condiții de laborator pe semințele de grâu, demonstrând acțiunea lor fitostimulatoare asupra plantulelor de grâu.

АННОТАЦИЯ

МОЛДОВАН Кристина: „Морфокультуральные и биосинтетические свойства микромицетов из водоемов муниципия Кишинев”, диссертация на соискание учёной степени доктора биологических наук, Кишинев, 2025.

Структура диссертации: введение, 4 главы, общие выводы и рекомендации, цитируемые источники в библиографии – 342, 9 приложений, 134 страниц основного текста, 23 рисунка, 21 таблиц. Полученные результаты опубликованы в 35 научных работах, два патента.

Ключевые слова: микромицеты, изоляция, морфо-культуральные свойства, экзометаболические, зоны ингибции роста, питательные среды, ферментативная активность, антимикробная активность, фитостимулирующие свойства.

Цель работы: выделение и изучение морфо-культуральных и биосинтетических свойств микромицетов из водоемов муниципия Кишинев.

Задачи исследования: Выделение чистых культур микромицетов из водоемов муниципия Кишинев; Изучение морфо-культуральных особенностей выделенных микромицетов и их идентификация на уровне рода; Оценка ферментативного потенциала идентифицированных микромицетов; Оценка антимикробного и фитостимулирующего потенциала микромицетов из водной экосистемы.

Научная новизна и оригинальность. Впервые проведено исследование разнообразия микромицетов из озер «Ла Извор», «Валя Морилор» и «Валя Трандафирилор» муниципия Кишинев. Проанализированы морфо-культурные характеристики выделенных микромицетов и проведена их классификация на уровне рода. Выявлены преобладающие роды и оценена частота встречаемости в зависимости от местонахождения в водоёмах. Определена ферментная активность выделенных микромицетов (амилаза, каталаза, липаза и целлюлаза), отобраны штаммы с высоким потенциалом, которые включены в Национальную Коллекцию Непатогенных Микроорганизмов (НКНМ) в качестве новых ресурсов, представляющих биотехнологический и научный интерес. Оценен антимикробный потенциал выделенных микромицетов из озер мун. Кишинев, принадлежащих к родам *Trichoderma*, *Penicillium* и *Talaromyces*, по отношению к фитопатогенам. Показана фитостимулирующая активность биопрепаратов на основе штаммов *T. atrovirens* CNMN FD 25 и *T. longibrachiatum* CNMN FD 27 на семенах пшеницы.

Полученный результат, способствующий решению важной научной проблемы, заключается в научном обосновании разнообразия микромицетов водоемов «Ла Извор», «Валя Морилор» и «Валя Трандафирилор» муниципия Кишинев, выявлении доминирующих родов и оценке их ферментативного и антимикробного потенциала, что привело к отбору и хранению 14 штаммов с ценными биосинтетическими свойствами, из которых два штамма из рода *Trichoderma* с положительным влиянием на прорастание семян пшеницы, что позволило пополнить НКНМ новыми штаммами, представляющими интерес в области микробных биотехнологий.

Теоретическая значимость: Полученные данные о разнообразии микромицетов из 3 водоёмов муниципия Кишинев. Определены доминирующие роды и оценен их ферментативный и антимикробный потенциал. Отобраны и депонированы в НКНМ 14 штаммов микромицетов, представляющих биотехнологический интерес, 2 из которых обладают фитостимулирующими свойствами, что способствует разработке новых научных исследований в области микробиологии, биотехнологии и других областей.

Практическая значимость: Штаммы водных микромицетов родов *Trichoderma* и *Penicillium* могут быть использованы для получения ферментов (амилазы, каталазы, липазы, целлюлазы), а на основе штаммов *Trichoderma atrovirens* CNMN FD 25 и *Trichoderma longibrachiatum* CNMN FD 27 могут быть созданы биопрепараты с фитостимулирующими свойствами.

Внедрение научных результатов: Штаммы микромицетов с ферментативным, антимикробным и фитостимулирующим потенциалом дополнили НКНМ перспективными продуцентами, а биопрепараты, полученные на основе штаммов *T. atrovirens* CNMN FD 25 и *T. longibrachiatum* CNMN FD 27, испытаны в лабораторных условиях на семенах пшеницы, показав их фитостимулирующее действие на проростки пшеницы.

ANNOTATION

MOLDOVAN Cristina: „Morpho-cultural and biosynthetic properties of micromycetes from the water basins of Chisinau municipality”, PhD thesis in biological sciences, Chişinău, 2025.

Thesis structure: introduction, 4 chapters, general conclusions and recommendations, bibliography with 342 titles, 9 annexes, 134 pages of basic text, 23 figures, 21 tables. The results obtained are published in 35 scientific papers, tow patent.

Key-words: micromycetes, isolation, morpho-cultural characteristics, exometabolites, zones of inhibition, nutrient media, enzymatic activity, antimicrobial activity, phytostimulation properties.

Purpose of the work: isolation and study of the morpho-cultural and biosynthetic properties of micromycetes from the water ponds of Chisinau municipality.

Research objectives: Isolation in pure culture of micromycetes from the water basins of Chisinau municipality; Study of the morpho-cultural characteristics of the isolated micromycetes and their identification at the genus level; Evaluation of the enzymatic potential of the identified micromycetes; Evaluation of the antimicrobial and phytostimulatory potential of micromycetes from the aquatic ecosystem.

Scientific novelty and originality. For the first time, a study was conducted on the diversity of micromycetes from the lakes «La Izvor», «Valea Morilor» and «Valea Trandafirilor» in the Chisinau municipality. The morpho-cultural characteristics of the isolated micromycetes were analyzed and classified at the genus level. The predominant genera were identified and the frequency of occurrence was evaluated depending on the location in the water basins. The enzymatic biosynthesis capacity of the isolated micromycetes (amylase, catalase, lipase, and cellulase) was determined, and strains with high potential were selected, which were included in the National Collection of Nonpathogenic Microorganisms (NCNM) as new resources of biotechnological and scientific interest. The antimicrobial potential of the isolated micromycetes from the lakes of the Chisinau municipality, especially belonging to the genera *Trichoderma*, *Penicillium*, and *Talaromyces*, against phytopathogens was evaluated. The phytostimulation activity of biopreparations based on the strains *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 on wheat seeds was demonstrated.

The result obtained, which contributes to solving an important scientific problem, consists of the scientific substantiation of the diversity of micromycetes from the «La Izvor», «Valea Morilor» and «Valea Trandafirilor» water basins in Chisinau, the identification of the dominant genera and the evaluation of their enzymatic and antimicrobial potential, which led to the selection and storage of 14 strains with valuable biosynthetic properties, of which two strains from the *Trichoderma* genus with positive effects on wheat seed germination, which allowed the replenishing of the NCNM with new strains of interest for the field of microbial biotechnologies.

Theoretical significance: Data on the diversity and density of micromycetes from 3 water basins of Chisinau municipality were accumulated. The dominant genera were identified and their enzymatic and antimicrobial potential was evaluated. Fourteen strains of micromycetes of biotechnological interest from the aquatic ecosystem were selected and deposited in the NCNM, 2 of which possess phytostimulation properties, which contributes to the development of new scientific resources for future research in microbiology, biotechnology, and other fields.

Practical significance: Strains of aquatic micromycetes of the genera *Trichoderma* and *Penicillium* can be used to obtain enzymes (amylase, catalase, lipase, and cellulase), and based on the strains *Trichoderma atrobruneum* CNMN FD 25 and *Trichoderma longibrachiatum* CNMN FD 27, biopreparations with phytostimulation properties can be developed.

Implementation of scientific results: Micromycete strains with enzymatic, antimicrobial and phytostimulation potential have replenish the National Collection of Nonpathogenic Microorganisms with promising producers, and biopreparations obtained based on the strains *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 were tested under laboratory conditions on wheat seeds, demonstrating their phytostimulatory action on wheat seedlings.

MOLDOVAN CRISTINA

**MORPHO-CULTURAL AND BIOSYNTHETIC PROPERTIES OF MICROMYTES IN
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