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AQUATIC BASINS — NATURAL SOURCE OF FUNGI WITH ANTIFUNGAL POTENTIAL

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Abstract.*The study of aquatic fungi is an area of major interest in microbiology, due to their essential ecological role and valuable biotechnological potential. In this context, the purpose of the paper was to test 71 strains of aquatic micromycetes isolated from the water basins of Chisinau municipality, in order to evaluate their antifungal activity against phytopathogens of agricultural importance. The results showed that over 80% of the analysed strains showed high antifungal activity, the most effective being against Fusarium solani (93%) and Botrytis cinerea (85.9%). The Trichoderma genus was shown to have remarkable antifungal potential, with inhibition areas ranging from 16 to 40 mm. Following the tests, two strains - T. atrobruneum CNMN FD 25 and T. longibrachiatum CNMN FD 27 - were selected to obtain biopreparations, which demonstrated increased efficiency in inhibiting the development of phytopathogenic fungi.*

Keywords: aquatic fungi, biotechnology, durable agriculture, antifungal activity, biopesticides.

INTRODUCTION

The aquatic microbiota encompasses a wide range of microorganisms belonging to diverse taxonomic groups. Among them, filamentous fungi play a pivotal role in aquatic ecosystems, contributing significantly to nutrient cycling and the decomposition of organic matter, as well as to the formation of subsurface mycelial networks. Numerous fungal genera—such as *Aspergillus*, *Penicillium*, *Talaromyces*, and *Trichoderma*—are commonly found in aquatic environments and are represented by a variety of species.

These fungi have garnered considerable attention due to their ecological significance, as well as their capacity to produce energy and serve as raw materials in biotechnological applications, including the production of bioactive compounds of high relevance. In particular, filamentous fungi from the genus *Trichoderma* have been extensively employed in sustainable agriculture, where they contribute to soil improvement and are incorporated into the formulation of biopesticides and biofertilizers [1–5].

The genus *Trichoderma* is cosmopolitan, occurring ubiquitously in soil, water, and air. These fungi are well recognized for their ability to enhance plant growth and suppress phytopathogens through mechanisms of biological control. Notable species include: *T. harzianum*, which promotes plant growth by colonizing root systems and facilitating nutrient uptake, while also acting as an effective biocontrol agent through competition and the secretion of hydrolytic enzymes; *T. asperellum*, which provides plant protection via the production of antimicrobial compounds and the induction of systemic resistance; *T. viride* and *T. virens*, noted for their synergistic interactions in commercial formulations of biofertilizers and biopesticides; *T. longibrachiatum* and *T. saturnisporum*, which enhance plant tolerance to abiotic stressors such as salinity and drought [6–11].

These fungi also contribute to improved soil fertility and the suppression of soilborne pathogens. All higher plants form symbiotic associations with micromycetes that establish mycorrhizal networks on root surfaces. In agricultural systems, technological interventions are employed to create conditions that favor plant development, modulate soil microbial communities, and aid in the management of infectious soil diseases [12–14].

In light of these findings, the investigation of aquatic fungal strains and their antifungal potential constitutes a research area of high importance, given their critical ecological functions and the growing interest in their exploitation for biotechnological innovations, sustainable agricultural practices, and the development of bioactive molecules with broad-spectrum applications.

MATERIALS AND METHODS

A total of 71 micellar fungal strains isolated from aquatic basins located in the municipality of Chișinău—specifically "La Izvor", "Valea Morilor", and "Valea Trandafirilor"—were used as objects of study. These isolates belong to the genera *Trichoderma*, *Talaromyces*, and *Penicillium*. The fungal strains were evaluated for their antifungal activity against five phytopathogenic fungi.

Antifungal activity was assessed using the agar block diffusion method, as described by Egorov (2004). The following phytopathogenic test strains were employed: *Alternaria alternata* CNMN FF 09, *Aspergillus niger* CNMN FD 01, *Botrytis cinerea* CNMN FF 10, *Fusarium oxysporum* CNMN FF 06, and *F. solani* CNMN FF 07.

The selected fungal strains were cultivated on malt agar medium in Petri dishes for 96 hours at 28–30°C. Agar blocks (8 mm in diameter) were excised using a sterile cork borer from areas of dense fungal growth. These blocks were then transferred into wells prepared in Petri dishes containing solidified nutrient medium inoculated with freshly subcultured test organisms. The Petri dishes were kept at low temperature for 24 hours prior to incubation to allow for the diffusion of

bioactive compounds.

The diameter of the inhibition zones was measured after incubation at 28–30°C for 96 hours for fungal pathogens and at 37°C for 24 hours for bacterial strains, respectively.

Among the analyzed strains, *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 exhibited high antifungal potential. These strains were subsequently cultivated under submerged conditions to obtain the cultural filtrate. The composition of the liquid medium (g/L) was as follows: glucose – 30.0; $NaNO_3$ – 1.0; KH_2PO_4 – 1.0; $MgSO_4 \cdot 7H_2O$ – 1.0; $CaCO_3$ – 1.0; pH – 6.0, with distilled water up to 1 liter. The medium was supplemented with 10 mg/L yeast extract for *T. atrobruneum* CNMN FD 25 and 20 mg/L corn extract for *T. longibrachiatum* CNMN FD 27.

The strains were cultivated in 1 L Erlenmeyer flasks containing 200 mL of inoculated liquid medium (1×10^7 spores/mL), at 28–30°C, under continuous agitation (160 rpm), for 6 days (*T. atrobruneum*) and 5 days (*T. longibrachiatum*). The obtained culture supernatant was subsequently tested for antifungal activity using the agar block diffusion technique.

Experimental results were subjected to statistical analysis. Statistical indicators were calculated using Microsoft Office Excel 2021.

RESULTS AND DISCUSSIONS

Testing the antifungal properties of 71 strains against the studied phytopathogens showed that over 80% of them exhibit high antifungal activity. Thus, out of the total number of 71 tested strains, 57.75% showed antagonism against A. niger, 84.5% against A. alternata, 85.9% against B. cinerea, 93% against Fusarium solani, and 81.7% against F. oxysporum (Figure 1).

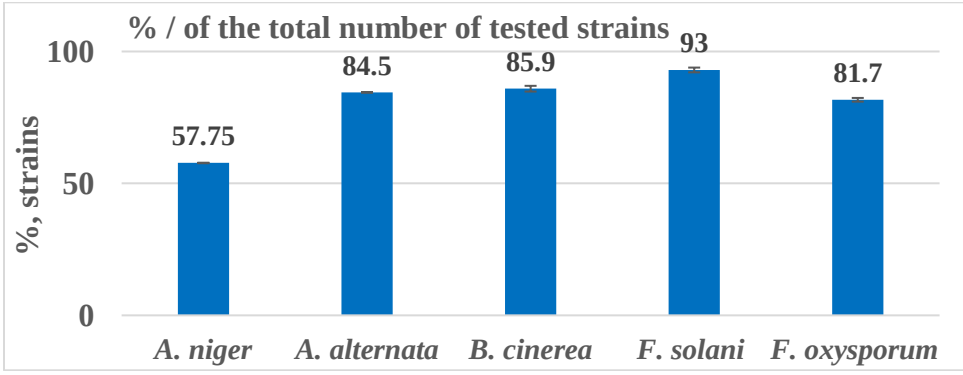


Figure 1. Stems (% of the total number of 71) that have shown antagonism towards some phytopathogens.

It was demonstrated that certain strains of aquatic micromycetes possess

significant inhibitory activity against the tested pathogens, with the largest inhibition zones observed in the case of *Trichoderma* strains. The diameter of the inhibition zones ranged from 16 to 40 mm (Figure 2).

Among all tested genera of micromycetes, the genus *Trichoderma* proved to have remarkable antifungal and antibacterial potential, which is why two strains were selected to be tested on wheat cultures. *Trichoderma* is a fungal genus that includes species widely used in the biocontrol of plant diseases, due to their ability to inhibit the growth of other phytopathogenic fungi through physical and chemical mechanisms, such as enzyme production.

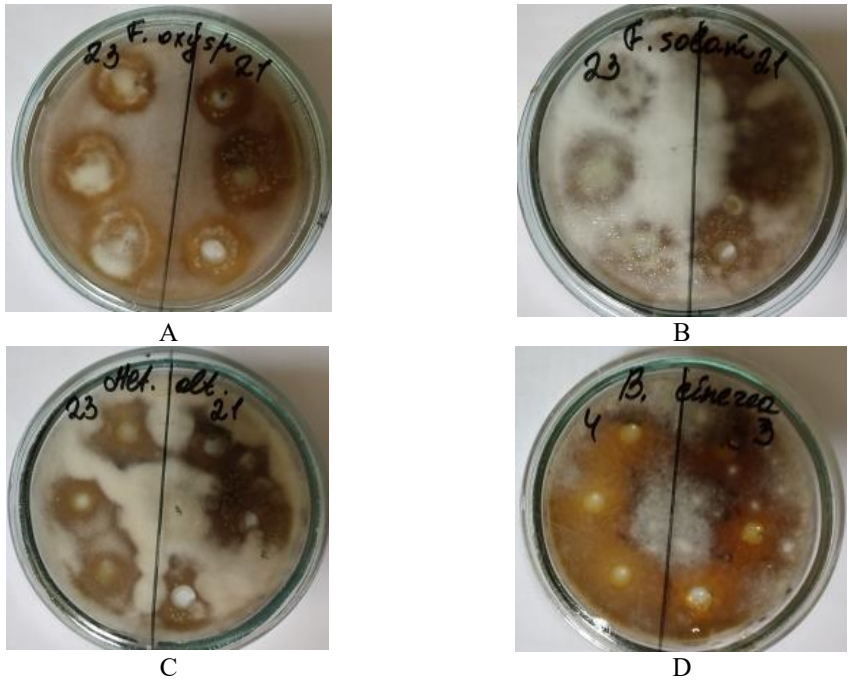


Figure 2. Areas of inhibition of some strains of fungi of the genus *Trichoderma* against phytoppathogens:

a) Fusarium oxysporum; Fusarium solani; Alternaria alternata; Botrytis cinerea.

Of all the genera of micromycetes tested, the genus *Trichoderma* was found to have remarkable antifungal potential, which is why 2 strains were selected, which were grown submersed in the liquid medium and obtained biopreparations. The biopreparations obtained have been tested against phytopathogens and the results obtained are shown in Figure 3.

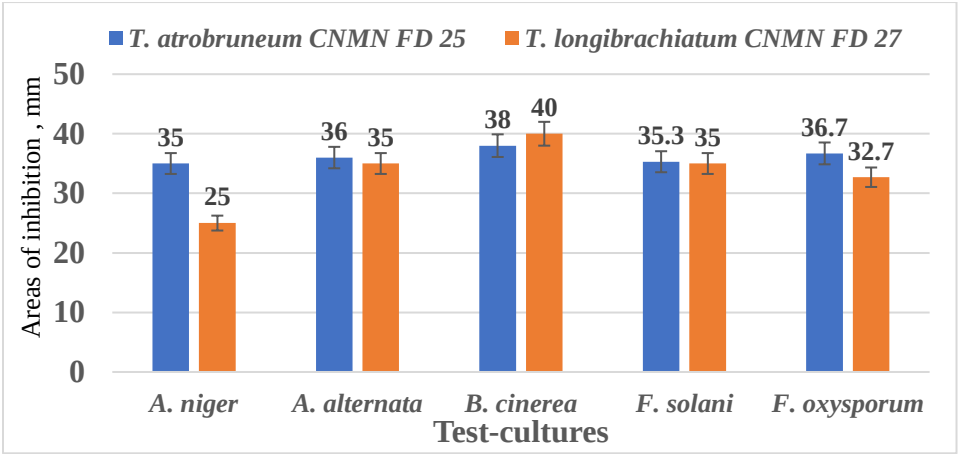


Figure 3. Diameter of phytopathogenic inhibition zones under the action of biopreparations obtained from *Trichoderma* strains

The biopreparation obtained from the strain *T. atrobruneum* CNMN FD 25 exhibited high sensitivity against phytopathogenic fungi, with inhibition zone diameters ranging from 35.3 mm (*F. solani*) to 38 mm (*B. cinerea*). The inhibition zones of phytopathogenic fungi under the action of the biopreparation derived from the strain *T. longibrachiatum* CNMN FD 27 varied from 25 mm (*A. niger*) to 40 mm (*B. cinerea*).

CONCLUSIONS

Aquatic basins can serve as a source of fungi with antifungal potential applicable in the agricultural field. Over 80% of the studied strains exhibited antifungal activity against five phytopathogenic fungi. Fungi of the genus *Trichoderma* demonstrated the highest antifungal potential, with inhibition zones ranging from 16 to 40 mm. Biopreparations obtained from the strains *T. atrobruneum* CNMN FD 25 and *T. longibrachiatum* CNMN FD 27 showed high antifungal activity against phytopathogens and could be used as potential producers of antifungal substances in agriculture.

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