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AQUATIC MICROMYCETS WITH PHYTOSTIMULATORY POTENTIAL FOR GRAMINEAE

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Abstract: Fungal strains of the genus *Trichoderma* are widely used in agriculture as biological disease control agents, due to their ability to suppress pathogens and stimulate plant growth. Identification of new strains with valuable biosynthetic properties is a topic of major interest for the scientific community, having direct implications in the development of sustainable agricultural biotechnologies. In this work was tested strain *Trichoderma atrobruneum* CNMN FD 25, applied on wheat and triticale grains. The results showed a significant incentive effect on the development of the root system, and to a lesser extent - on the aerial growth of plantlets. Treatment of wheat seeds with biopreparations in concentrations of 0.5 % and 0.33 % led to an increase in the germination rate by 6 %, the length of the plantlets by 12.6 %, the mass of the plantlets by 8.6 %, and the root mass by 23.7 %. In the case of triticale, the same concentrations resulted in an increase in the germination rate by 16 %, in the length of the plantlets by 15 %, in the mass of the plantlets by 7 % and in the root mass by 32 %.
These results confirm the biostimulant potential of the *T. atrobruneum* CNMN FD 25 strain, in particular through its influence on the development of the root system.

Keywords: *biopreparation, biotechnology phytostimulants, sustainable agriculture, Trichoderma*

INTRODUCTION

Excessive use of chemical fertilizers and pesticides leads to soil degradation and could lead to a food crisis in the future. Population growth and the need for ever-increasing amounts of food require researchers to look for new innovative solutions and technologies. These technologies are a promising alternative to alleviate biotic and abiotic stress, providing resistance to various diseases, also improving the quality of nutrients and agricultural crops, as a result stimulating the quantity and quality of harvest in agricultural plants [1 – 3].

According to Bano *et al*. [4], phytostimulants are natural substances that, applied to plants, seeds, or growth substrates, can modify the physiological processes of plants, improving their growth, development and response to stress, without including nutrients or pesticides. Their use in sustainable agriculture helps protect the environment by reducing chemical inputs while preserving crop yields and quality" [4].

As an alternative to agricultural chemicals, microorganisms are widely used, including plant growth-promoting fungi, which are beneficial microorganisms that improve the defense mechanisms against diseases, fight the invasion of phytopathogens and stimulate plant growth. The most well-known beneficial fungi belong to the genera *Gliocladium*, *Penicillium*, *Phoma*, *Rhizoctonia* and *Trichoderma*, which have shown positive effects on the growth of crops such as corn, wheat, sunflower, tobacco, soybean, eggplant, tomato, cucumber, carrot and cotton [5 – 10].

Fungal phytostimulants are micromycetes, which benefit plants through various mechanisms, helping to improve their growth and tolerance to stress. They are classified into two main categories: mycorrhizal micromycetes and plant growth-promoting micromycetes [4, 11, 12].

*Trichoderm*a-based products are used as phytostimulators to accelerate germination and rooting, fructification ability and reduce the period of maturation, resistance to diseases and adverse factors. The most important stimulating factor in almost all stages of plant growth and development is the synthesis of enzymes, phytohormones and phyto-regulators [13 – 17].

Thus, micro-mycoses of the genus *Trichoderma*, due to its antimicrobial properties to pathogenic, plant-growing stimulating microorganisms, participating in soil fertilization, are more reliable compared to other microorganisms for use in agriculture, causing the increase of both biological and economic yield of crops by reducing the use of harmful chemicals and the development of organic agriculture.

Biopreparations obtained from strains of micromycetes of the genus *Trichoderma* are known for their phytostimulatory properties, which is why they are widely used in agriculture as a growth stimulator of agricultural plants [4, 18 – 20].

Some of the most important species of this genus [1, 7, 5, 21 – 25] are:

- *T. harzianum*: promotes plant growth by colonizing roots and improving nutrient absorption. It is also an effective biological control agent, inhibiting the growth of pathogens through competition and production of hydrolytic enzymes.
- *T. asperellum*: is used for biocontrol, protects plants through the production of antimicrobial compounds and inducing systemic resistance;
- *T. viride* and *T. virens*: are recognized for their synergistic effects in commercial blends of bio-fertilizers and biopesticides; *T. longibrachiatum* and *T. saturnisporum* –

contribute to improving plant tolerance to abiotic stresses, and such as salinity and drought.
Thus, due to its broad biofertilization, biocontrol and biostimulation potential, *Trichoderma* seems to be the best option for use in green technologies.
In the National Collection of Nonpathogenic Microorganisms of Moldova (CNMN), a number of fungal strains of the genus *Trichoderma* are stored and preserved, isolated from soil, water, air, etc., with high antimicrobial potential against a wide spectrum of phytopathogens [26].
In this study, the phytostimulatory potential of a fungal strain of the genus *Trichoderma* on crop plants was evaluated. The studied strain was isolated from the "La Izvor" lake, Chisinau municipality and exhibits antagonism against a wide spectrum of phytopathogens. The tests were performed on gramineae (wheat, triticale).

MATERIALS AND METHODS

Object of the study

To identify phytostimulatory capacity, from the wide range of previously studied aquatic micromycete strains isolated from the "La Izvor" lake, Chisinau municipality, the *Trichoderma atrobruneum* CNMN FD 25 strain was selected, which demonstrated significant antimicrobial properties against a wide range of phytopathogens [27].
The *T. atrobruneum* CNMN FD 25 strain was cultivated submerged in the medium with the composition: ($g \cdot L^{-1}$): glucose – 30.0; $NaNO_3$ – 1.0; KH_2PO_4 – 1.0; $MgSO_4x7H_2O$ – 1.0; $CaCO_3$ – 1.0; *p*H - 6.0, distilled water up to 1 L and supplemented with 10 $mg \cdot L^{-1}$ yeast extract (for *T. atrobruneum* CNMN FD 25) at a temperature of 28 - 30 °C, with continuous stirring (160 rpm), for 6 days. After cultivation, the metabolite solution together with the biomass were homogenized (dry mass is 13 $g \cdot L^{-1}$), then used as a microbial biopreparation, which was subsequently applied to wheat and triticale grains.
Wheat grains, Moldova 16 variety and triticale, Costel variety, harvest of 2023 were tested. The mentioned varieties exhibit tolerance to drought, wintering and the main plant diseases, and are recommended for all cultivation areas of the Republic of Moldova.
The obtained biopreparation was tested on wheat grains, Moldova 16 variety and triticale, Costel variety, harvest year 2023. The gramineae varieties were created at the Institute of Genetics, Physiology and Plant Protection, State University of Moldova and offered to the Institute of Microbiology and Biotechnology of Technical University of Moldova. These gramineae varieties exhibit tolerance to drought, wintering and the main plant diseases and are recommended for all cultivation areas of the Republic of Moldova.
Wheat and triticale grains were treated with the biopreparation obtained based on the *T. atrobruneum* CNMN FD 25 strain for 1 hour. Subsequently, the seeds were sown in pots with soil (chernozem) and cultivated at a temperature of 18-20 °C, with daylight. Four variants (100 grains each) with wheat and 4 variants with triticale were mounted, in which the grains were treated with the studied biopreparation in concentrations of: 1 %; 0.5 %, 0.33 % and 0.25 %. In the control sample, the grains were treated with tap water. After 14 days of cultivation, the following indices were evaluated: seed germination, average seedling length, seedling dry mass and dry mass of wheat and triticale roots.

Statistical analysis

Data are displayed as mean ±SEM. The statistical significance was evaluated using a one-way ANOVA with post hoc Tukey test. A $p \leq 0.05$ value was considered statistically significant. Data were analyzed using PAST 3.26 statistical software [28].

RESULTS AND DISCUSSION

The phytostimulating efficiency of the biopreparation obtained based on the fungal strain *Trichoderma atrobruneum* CNMN FD 25 was tested on wheat grains, Moldova variety, and triticale, Costel variety.
The results obtained from testing the biopreparate obtained from the *T. atrobruneum* CNMN FD 25 strain on wheat seeds are shown in Figure 1.

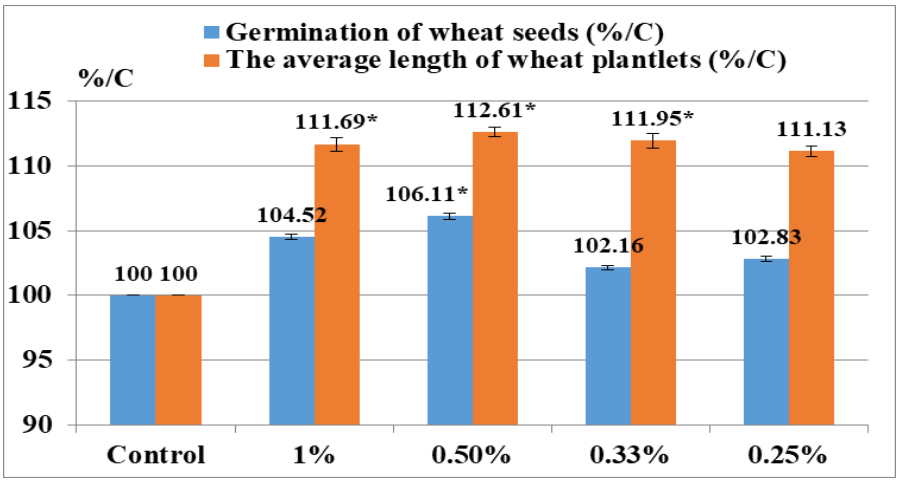

Figure 1. *Action of the biopreparation obtained based on the T. atrobruneum CNMN FD 25 strain on wheat grains, *p ≤ 0.05*

The data presented in Figure 1 demonstrate that the treatment of wheat seed *with T. atrobruneum* biopreparation, regardless of the applied concentration, acts positively on wheat seed, stimulating both germination and plant growth. The most relevant results were recorded in the variant in which the grains were treated with the 0.5 % metabolite solution, thus seed germination increased by 6.11 % and wheat plant length by 12.61 % compared to the control variant.
On the indices of dry mass of seedlings and dry mass of roots, the tested biopreparation also demonstrated positive results (Figure 2).
The results obtained on the dry mass of wheat seedlings varied within ± 4 % compared to the control variant. The most significant results of 106.77 %, compared to the control variant, were obtained in the variant in which the concentration of the biopreparation was applied at 0.25 %, and in the variant in which the concentration of the biopreparation was 0.5 %, this index constituted only 92.42 % (Figure 2).
The tested biopreparation had a more beneficial effect on the dry mass of the roots, stimulating this index significantly and constituting 124.9 % in the variant in which the seeds were treated with 1 % biopreparation, and in the variant in which a biopreparation

with a concentration of 0.25 % was used, this index constituted 142.53 %, compared to the control variant.

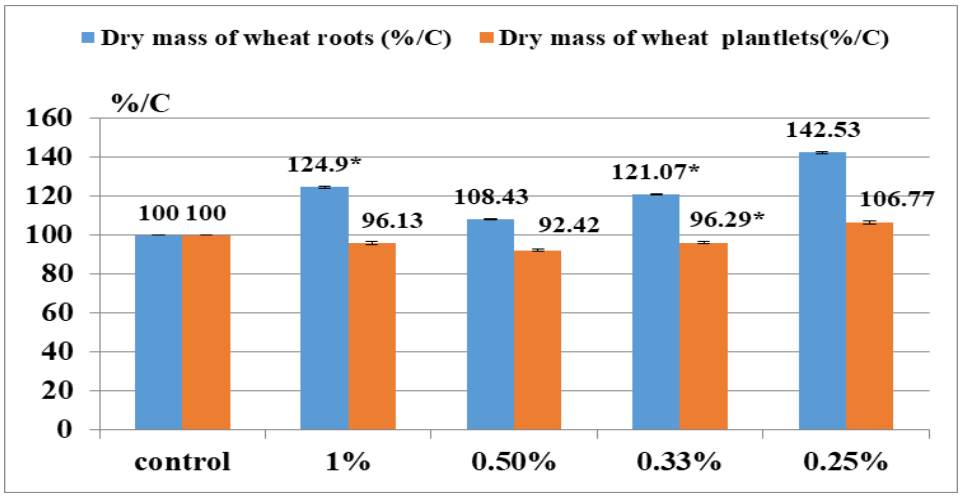

Figure 2. *Action of the biopreparation T. atrobruneum CNMN FD 25 on the dry mass of wheat seedlings and roots, *p ≤ 0.05*

On the dry mass of the roots, the tested biopreparate acted more beneficially, stimulating this significant index and constituting 124.9 %, in the variant in which the seeds were treated with a biopreparate of 1 %, and 142.53 %, in the variant in which the biopreparate was used with the concentration of 0.25 %, compared to the control variant.

Thus, we find that this biopreparation acted more beneficially on the root system and less on the growth of wheat plantlets.

In various studies it has been shown that *Trichoderma* strains have a beneficial effect on the growth of wheat plantlets through several mechanisms. They produce phytohormones such as indole-3-acetic acid (IAA), harzianic acid and harzionalide, which stimulate the development of the root system and the growth of vegetative mass. In addition, *Trichoderma* solubilizes phosphates and microelements (Fe, Zn), making them more accessible to plants. Some strains can colonize the roots as endophytes, boosting the immunity of the plant and protecting it from pathogens. These effects lead to faster growth and greater tolerance to abiotic and biotic stresses. Therefore, *Trichoderma* is considered an effective and environmentally friendly biostimulant for wheat crop [8, 15, 16, 23, 29, 30].

The studied biopreparation, tested on triticale grains, also demonstrated significant stimulation of germination and seedling growth (Figure 3).

In all the mounted variants, an increase in the germination index of triticale grains was recorded, which varied within the limits of 112.48 - 116.19 %, compared to the control variant. The maximum germination value was recorded in the variant in which the triticale grains were treated with biopreparation in a concentration of 0.33 %, constituting 116.19 %, and with increasing concentration of the biopreparation, germination gradually decreases. On the growth of triticale seedlings, the effect was weaker, the increase in this index varying within the limits of 107.4 - 115.65 %. The highest result was obtained in the variant in which the concentration of the biopreparation constituted 0.5 % (Figure 3).

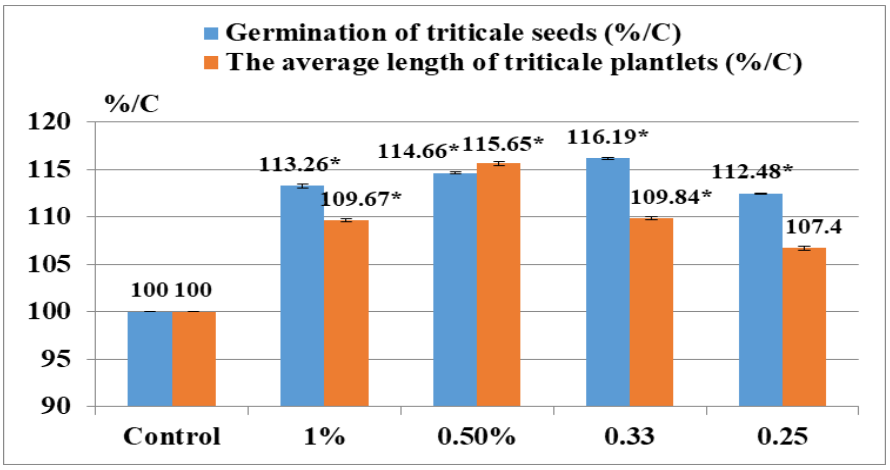

Figure 3. *Action of the biopreparation obtained based on the T. atrobruneum CNMN FD 25 strain on triticale grains, *p ≤ 0.05*

As a result of testing the biopreparation on triticale seeds, positive results were also obtained on the mass of triticale roots and seedlings (Figure 4).
With the increase in the concentration of the metabolite solution from 0.25 % to 1.0 %, a gradual increase in the dry mass index of the plantlets was obtained from 90.56 % to 107 %, compared to the blank. A stimulation of the dry root mass index was also obtained with an increase in the metabolite concentration from 0.25 % to 0.5 % from 101.56 % to 132.81 % compared to the control, but further increasing the metabolite concentration to 1 %, a decrease of this index up to 129.69 % compared to the control was observed.

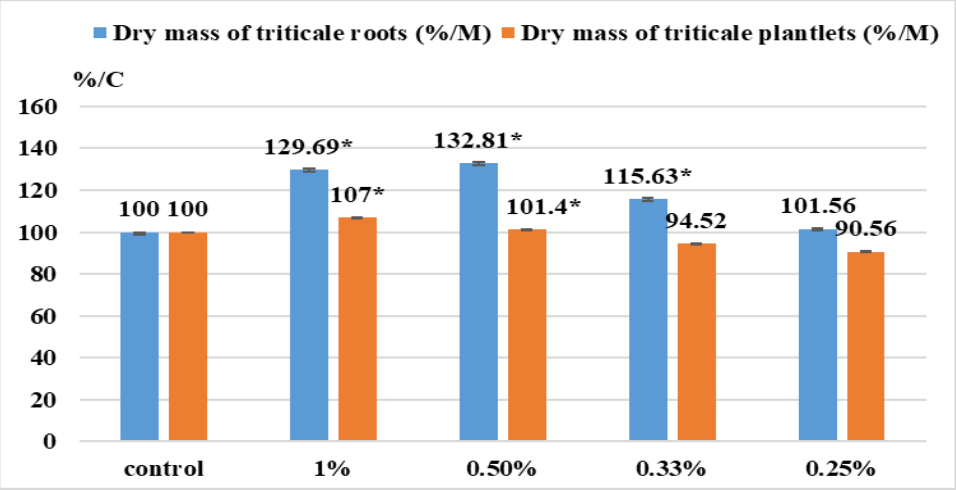

Figure 4. *Action of the biopreparation T. atrobruneum CNMN FD 25 on the dry mass of triticale seedlings and roots, *p ≤ 0.05*

The results obtained are consistent with those obtained by other authors [30, 31], who have demonstrated that *Trichoderma* is the genus of symbiotic, opportunistic and virulent micromycetes, which colonize not only the soil of the rhizosphere but also the root surface, mainly in the peak and elongation area of the root and stimulate plant growth by mechanisms similar to those used by mycorrhizal fungi. In addition, these fungi are able to improve the absorption of water and nutrients, thereby contributing to the more

vigorous development of the root system. *Trichoderma* also produces a wide range of bioactive metabolites, such as hydrolytic enzymes and antifungal compounds, which provide additional protection against soil pathogens. Thus, the use of *Trichoderma* strains in biological agriculture is an effective and sustainable strategy to stimulate plant growth and reduce dependence on chemical treatments [7, 30 – 32].
Based on the results obtained, we can find that the *T. atrobruneum* strain CNMN FD 25 can be considered as potential for obtaining phytostimulatory biopreparations for agricultural plants, especially for *Gramineae.*

CONCLUSIONS

Testing of the biopreparation, obtained based on the *Trichoderma atrobruneum* CNMN FD 25 strain, in concentrations of 0.5 % and 0.33 %, applied to wheat grains (Moldova variety) and triticale (Costel variety), revealed a significant stimulatory effect on germination parameters and early development of seedlings. The tested biopreparation demonstrated an increase in the following indices: seed germination by 6-16 %; average seedling length by 9.8 - 12.6 %, dry mass of roots by 15-32 %.
These results demonstrate the biostimulatory potential of the metabolites produced by *T. atrobruneum* CNMN FD 25 and support its applicability in pre-sowing treatments, in order to optimize plant growth and ensure uniform and healthy development in the early stages of vegetation.

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