ANTIMICROBIAL AND PHYTOSTIMULATORY PROPERTIES OF THE FUNGAL STRAIN *Trichoderma longibrachiatum*

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Abstract. Food security is a fundamental problem worldwide. Ecological alternatives to pesticides and chemical fertilizers are microorganisms with a strong potential to produce compounds with high biological activity for agriculture. *Trichoderma* is the most promising microorganism for the development of sustainable agriculture due to their proven role as a biofertilizer and plant biological control agent. The *T. longibrachiatum* CNMN FD 27 strain, was used as the study object. Antifungal and antibacterial activities were tested on test cultures which cause losses for numerous agricultural crops, and the solution of exometabolites, were tested on sunflower seeds (length of the seedlings, dry mass of the seedlings and dry mass of the roots were evaluated). According to the present study *T. longibrachiatum* CNMN FD 27 provides essential activity against *A. alternata* and *F. oxysporum*. The obtained results had shown that, before sowing, the treatment of sunflower seeds with the aqueous solution of metabolites of the *T. longibrachiatum* strain (variant M 3) has a positive effect on the germination of the seeds, which constituted by 10% more than M 1 (H_2O). The tested metabolites also had a positive effect on seedling growth, stimulating their length by 9.7% in comparison with M 1 variant. The dry mass of seedlings and roots per 100 seedlings from variant M 3 exceeded them by 31.2% in comparison with M 1. Metabolites of the fungal strain *T. longibrachiatum*, used in the treatment of sunflower seeds before sowing, can contribute to seed germination and stimulate the growth of the crop.

Key words: antimicrobial activity; inhibition zone; metabolites; nutrient medium; phytopathogens; stimulation.

INTRODUCTION

The excessive use of pesticides, and chemical fertilizers has contributed to the destruction of the ecological environment of agricultural lands, the occurrence of various diseases in crops and the accumulation of chemical residues in soil and water [2]. A new biological control strategy for crops is needed to mitigate losses caused by various diseases and guarantee food production. Beneficial organisms and their products are used as an alternative to chemicals for plant disease control. This method also allows obtaining ecological products [8, 23].

Biological control offers a safe alternative for controlling plant pathogens due to the ability of biocontrol agents to establish in the ecosystem. An effective biocontrol agent is *Trichoderma* spp., which is effective against a wide range of pathogens and possesses diverse mechanisms of action with a direct impact on plant development and crop productivity. *Trichoderma* spp. is found in various environments and is actively involved in the management of biotic and abiotic stress in plants, aimed at their normal growth and development [61]. In addition, *Trichoderma* is able to destroy fungal phytopathogens through antibiotics, as well as through mycoparasitism (assimilation and degradation of phytopathogens by direct contact). Thus, the mechanisms used by *Trichoderma* spp. as a biofungicide include antibiosis, mycoparasitism, competitive advantage in the rhizosphere as well as the priming of self-defense mechanisms of crops [5, 33, 55, 66, 67, 70]. The action of *Trichoderma* strains against phytopathogens can be direct, through anabiosis, promoting plant growth and defense mechanisms, or indirectly through changing environmental conditions, competing for space and nutrients. These mechanisms can act coordinated, and their action in the biocontrol process depends on the *Trichoderma* strain and several abiotic parameters such as soil pH and temperature, water retention, nutrients, presence of heavy metals in the soil, soil acidity or alkalinity, soil microbial community, and crop plant. Activation of each mechanism involves the production of specific compounds and metabolites, such as antibiotic, hydrolytic enzymes (glucanases, chitinases, and proteases), plant growth factors (phytohormones), siderophores, and carbon and nitrogen permeases [6, 11, 42, 64, 66].

The culture *Trichoderma* is a low-cost, effective, environmentally friendly, and safe biocontrol agent for various crops. It interacts complexly with plant pathogens by parasitizing them, secreting antibiotics, and producing hydrolytic enzymes (glucanase, chitinase, protease) [22, 34].

Several species of the genus *Trichoderma* act as bioagents against a wide range of phytopathogens such as: *Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium graminearum*, *Fusarium culmorum*, *Rhizoctonia solani*, *Phylaspora rhodia*, *Diaporthe citri*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, *Pseudocercospora* spp., *Pythium* spp., *Colletotrichum*, *Aspergillus niger* [4, 7, 15, 16, 25, 36, 59, 60, 63, 68]. *Trichoderma*-based products are used to accelerate germination and rooting, fruiting capacity and reduce the ripening period, resistance to diseases and adverse factors, stimulate the quantity and quality of the harvest in agricultural plants, such as: cotton (*Gossypium hirsutum*), peanut (*Arachis hypogaea*), banana (*Musa acuminata*), citrus (*Citrus* L.), wheat (*Triticum aestivum*), sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), soybean (*Glycine max*), sugar cane (*Saccharum officinarum*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), cabbage (*Brassica oleracea*), pepper (*Capsicum annuum*), potato (*Solanum tuberosum*), onion (*Allium cepa*), sugar beet

(*Beta vulgaris*), pea (*Lathyrus oleraceus*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), tea (*Camellia sinensis*), coffee (*Coffea arabica*) etc. [3, 10, 24, 28, 30, 32, 39, 43, 44, 46, 51, 53].

Some species of *Trichoderma* spp. play an important role as nematicides [13, 19, 26, 58, 67]. They can also increase resistance against herbivorous insects [26, 47].

Most species of the genus *Trichoderma* produce phytohormones and the enzyme 1-aminocyclopropane-1-carboxylate deaminase, which stimulate plant growth, sustainability and various crop nutrient supply mechanisms. *Trichoderma* spp. has been shown in various studies to produce plant hormones such as auxins, harzianic acid, harzianolide, indole acetic acid, which are responsible for root development [1, 31, 38, 50, 61, 62, 65]. Cai *et al.* (2013), demonstrated that *T. harzianum* SQR-T037 strain synthesizes the secondary metabolite called harzianolide, which significantly induces the growth of tomato seedlings at early stages by increasing root length and root tips [12]. Contreras-Cornejo *et al.* (2014), found that *T. virens* and *T. atroviride* produce auxin-related substances and indole acetic acid, which have a beneficial effect on *Arabidopsis* roots [14]. Through signaling pathways such as heterotrimeric G protein, MAP kinase, and the cAMP pathway. G protein and MAPK are mainly involved in the secretion of antifungal metabolites and forming infection structures. The cAMP pathway helps the condition and enrollment of *Trichoderma* mycelium on pathogenic fungi and inhibits their proliferation [42].

Currently, *Trichoderma*-based products are used to biologically control gray mold and other plant diseases in tomatoes, cabbage, melons, peppers, potatoes, tobacco roots, and soybeans [3, 10, 28, 32, 39, 44, 46, 51].

Thus, it was demonstrated that *Trichoderma longibrachiatum* is a very useful bioagent that can reduce the severity of tomato wilt disease by 24.8% [53].

Fungi of the genus *Trichoderma* also have the role of biofertilizer, because they improve the absorption of macro and micronutrients by plants from the soil. In addition, the increase in the activity of soil enzymes (urease, phosphatase, and catalase) improved nutrient absorption and tolerance to environmental stress, quality and production increase of crops [20, 27, 29, 37, 49, 54, 71].

Based on the above, the aims of this study were evaluation *in vitro* of antifungal and phytostimulation abilities of the *Trichoderma longibrachiatum* CNMN FD 27 strain.

MATERIALS AND METHODS

The *T. longibrachiatum* CNMN FD 27 strain from the National Collection of Nonpathogenic Microorganisms (NCNM) of Institute of Microbiology and Biotechnology of Technical University of Moldova, which possesses significant antimicrobial properties against a wide spectrum of phytopathogens, was used as the study object. Phytopathogens were used as test strains: *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani*, *Fusarium oxysporum*, *Fusarium sporotrichiella*, *Sclerotinia sclerotiorum*, *Erwinia carotovora* (*Pectobacterium carotovorum*), *Corynebacterium michiganense* (*Clavibacter michiganensis*), *Xanthomonas campestris*, *Agrobacterium tumefaciens* (*Rhizobium radiobacter*). Antifungal and antibacterial activities were tested on these test cultures, resulting from the fact that these pathogens cause severe diseases and cause colossal losses for numerous agricultural crops in the Republic of Moldova and throughout the world. Phytopathogenic fungi test cultures were grown on wort agar medium of 5.0ºB (pH 5.8-6.0), and pathogenic bacteria on nutrient agar medium (pH 7.0-7.5), respectively [52].

For the selection of the optimal medium for submerged cultivation of the *T. longibrachiatum* strain, 3 nutrient media were tested:

- M 1 (g/L): glucose - 30.0; corn extract - 20.0; $CaCO_3$ - 1.0; $MgSO_4 \times 7H_2O$ - 1.0; $NaNO_3$ - 1.0; KH_2PO_4 - 1.0; tap water up to 1 liter; pH 6.0-6.2;
- M 2 (g/L): beet molasses - 20.0; glucose - 10.0; $MgSO_4 \times 7H_2O$ - 1.0; $NaNO_3$ - 2.0; KH_2PO_4 - 2.0; tap water up to 1 liter; pH 6.0-6.2;
- M 3 (g/L): glucose - 30.0; yeast extract - 10.0; $MgSO_4 \times 7H_2O$ - 1.0; $NaNO_3$ - 1.0; KH_2PO_4 - 1.0; tap water up to 1 liter; pH 6.0-6.2.

The submerged cultivation of strain in the mentioned media lasted for 7 days, with continuous agitation (160-180 r/min), at a temperature of 28-30°C. The solutions of exometabolites were obtained by separating the culture supernatant from the biomass by filtration, which were later tested on the phytopathogenic fungi studied in dynamics, after 3, 4, 5, 6 and 7 days of cultivation of the strain.

In the next step, the solution of exometabolites, obtained in variant M 1 containing corn extract, which demonstrated the highest antifungal activity, was tested on sunflower seeds. Sunflower seeds (100 seeds) were soaked in exometabolic solution at a concentration of 0.33% for 1 hour, then sown in pots with soil, 25 seeds each. Three variants were prepared: 1) M 1 (control) – seeds soaked in tap water; 2) M 2 – seeds soaked in Fitosporin M solution (2 g per l liter of H_2O, 150 mL solution per 100 seeds); 3) M 3 – seeds soaked in exometabolic solution (0.33%), 150 mL of solution per 100 seeds).

After 10 days of growing the seedlings of the sunflower in room conditions (20-22°C), the following indicators were calculated: the average length of the seedlings, the dry mass of the seedlings and the dry mass of the roots per 100 seedlings.

RESULTS

Determination of the antimicrobial activity of the *T. longibrachiatum* strain, cultivated on wort agar medium, against phytopathogens: *A. alternata*, *B. cinerea*, *F. solani*, *F. oxysporum*, *F. sporotrichiella*, *E. carotovora*, *C. michiganense*, *X. campestris*, *A. tumefaciens* showed different sensitivity. Thus, the diameter of the inhibition zones against phytopathogenic fungi varied within the limits of 26.7-35.0 mm, and against phytopathogenic bacteria a non-significant sensitivity only against *C. michiganense* (zone of 11.7 mm) and against *X. campestris* (zone of 15.3 mm). The studied strain did not affect growth and development of *E. carotovora* and *A. tumefaciens* (Fig. 1).

To obtain the solution of metabolites with high antifungal activity, the *T. longibrachiatum* strain was cultivated in different nutrient media (M 1, M 2, M 3) and the optimal media for cultivation was selected. The metabolic solutions obtained in these media after 3, 4, 5, 6 and 7 days of strain cultivation were tested against mentioned phytopathogenic fungi.

The obtained results demonstrated that the metabolite solution obtained during strain cultivation in the nutrient medium M 1, which contains corn extract, showed the highest sensitivity after 4 days of cultivation against the tested phytopathogens. Thus, after 4 days of cultivation, the metabolite solution showed the highest sensitivity against 4 out of 6 strains of pathogenic test cultures, the diameter of the inhibition zones varied from 32.3 mm (against *S. sclerotiorum*) to 41.7 mm (against *B. cinerea*). A lower sensitivity was recorded against *F. solani* and *F. sporotrichiella* (with inhibition zones of 27.7 mm) (Fig. 2 and 4). Further cultivation of the strain did not significantly contribute to the antifungal activity of the metabolic solution.

The metabolic solution of the *T. longibrachiatum* strain, obtained during cultivation in the M 2 medium, which contains beet molasses, after 4 days of cultivation showed a reduced sensitivity in comparison with obtained in the medium with corn extract. The diameter of the phytopathogen inhibition zones varied from 21.3 mm (against *F. solani*) to 40.3 mm (against *B. cinerea*). Further cultivation of the strain in this medium registered an insignificant increase in the antifungal activity of the metabolic solution, and after 7 days of cultivation of the strain, a higher sensitivity against *A. alternata* and *F. solani* was registered (with zones of 29.3 mm) and *S. sclerotiorum* (zones of 40.7 mm) (Fig. 3 and 4).

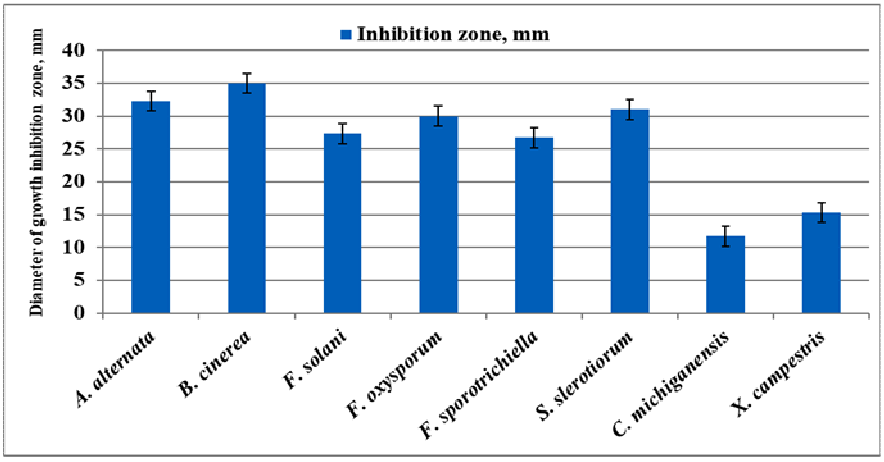

Figure 1. The diameter of phytopathogen inhibition zones under the action of *T. longibrachiatum* strain metabolites

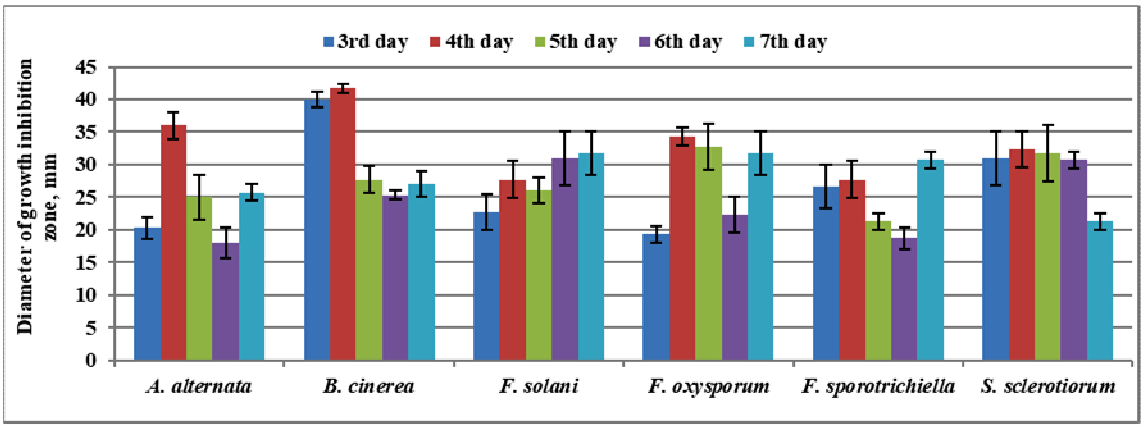

Figure 2. Antifungal activity of the metabolic solution obtained as a result of the cultivation *T. longibrachiatum* strain in the nutrient medium (M 1) containing corn extract

The metabolic solution obtained in variant M 3 (with yeast extract) showed an antifungal activity against the tested phytopathogens growing from the 3rd to the 6th day of cultivation. Thus, the maximum antifungal activity of the metabolic solution against 4 out of 6 strains of phytopathogens tested was manifested after 6 days of cultivation, with inhibition zones varied from 28.0 mm (against *A. alternata*) to 35.0 mm (against *B. cinerea* and *F. oxysporum*) (Fig. 5, 6).

Based on the obtained results, as optimal medium for the submerged cultivation of the *T. longibrachiatum* strain, the M 1 medium was selected, containing corn extract, which shown a high activity against majority of the phytopathogens tested after 4 days of cultivation.

In the next step, the metabolic solution obtained from the submerged cultivation of the *T. longibrachiatum* strain in M 1 medium (which contains corn extract) was tested on sunflower seeds.

Preparations based on *Trichoderma* are considered as active stimulators of crops. According to several reports the treatment of seeds with biopreparations based on *Trichoderma* accelerates germination, rooting, the ripening period, contributes to the improvement of the resistance on adverse factors (drought, diseases, etc.) and to the stimulation of the quantity and quality of the harvest of agricultural crops [48, 56, 57].

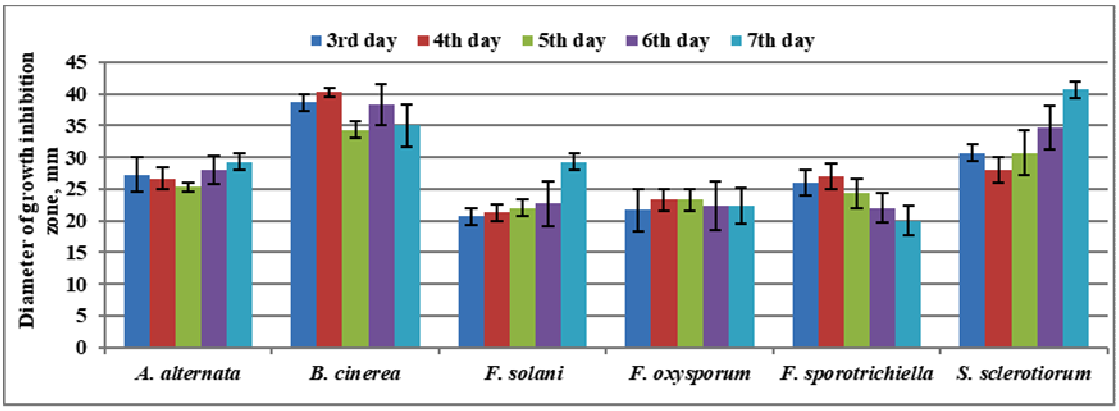

Figure 3. The antifungal activity of the metabolic solution obtained as a result of the cultivation *T. longibrachiatum* strain in the nutrient medium (M2) containing beet molasses

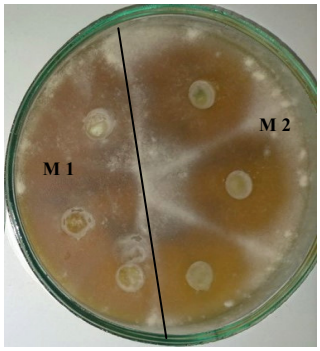

Figure 4. The antifungal activity of *T. longibrachiatum* strain cultivated in the nutrient media M 1 and M 2 against *B. cinerea*

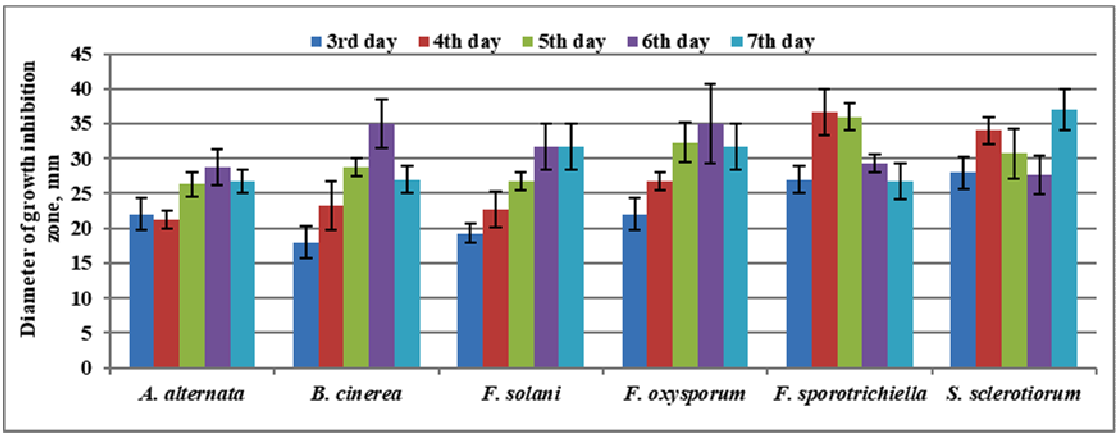

Figure 5. The antifungal activity of the metabolic solution obtained as a result of the cultivation *T. longibachiatum* strain in the nutrient medium (M3) containing yeast extract

Three variants were mounted: 1) M 1 (control) – seeds soaked in tap water; 2) M 2 – seeds soaked in Fitosporin M solution; 3) M 3 – seeds soaked in metabolic solution for 1 hour, then placed in soil and cultivated for 10 days. After 10 days of cultivation at room temperature of 20-22°C, the indices were calculated: germination, seedling length, dry mass of 100 seedlings, root dry mass per 100 seedlings (Fig. 7).

The obtained results had shown that, before sowing, the treatment of sunflower seeds with the aqueous solution of metabolites of the *T. longibrachiatum* strain (variant M 3) has a positive effect on the germination of the seeds, which constituted 110%, while in the variant M 2 (with Fitosporin M) was 102% in comparison with control (M 1). The tested metabolites also had a positive effect on seedling growth, stimulating their growth in length by 9.7% in comparison with control variant – M 1 (H_2O) and by 12% in comparison with M 2 variant (with Fitosporin M). The dry mass of seedlings and the dry mass of roots per 100 seedlings from variant M 3 (with metabolites) exceeded these indices obtained in variant M 1 (H_2O) by 31.2% and 5.4%, respectively. Stimulation of the dry mass index of the seedlings by 33.4% and a decrease of the dry mass index of the roots by 20% was also obtained in comparison with M 2 variant (with Fitosporin M). On the development of the root system, however, the metabolite solution acted less, surpassing the control variant by only 5.4%. However, the Fitosporim M solution (variant M 2) acted on this index more beneficial, which stimulated the development of the root system by 25.4% in comparison with control variant M 1 and by 20% in comparison with M 3 variant (with metabolites) (Fig. 7, 8).

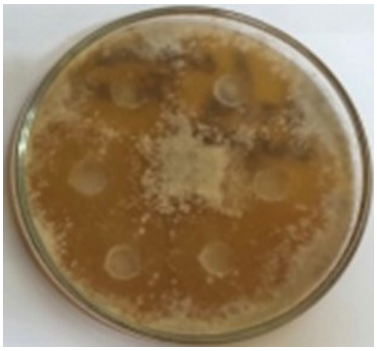

Figure 6. The antifungal activity of *T. longibrachiatum* strain cultivated in the nutrient medium M 3 against *B. cinerea*

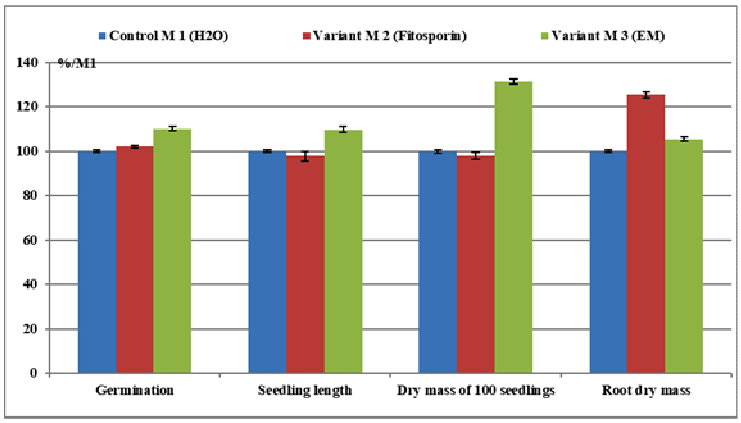

Figure 7. The action of metabolic solution of *T. longibrachiatum* strain used at pre-sowing of sunflower seeds

Figure 8. Fresh sunflower seedlings treated with: a) Control M 1 (H_2O) and b) Variant M 3 (EM)

Thus, according to the results, the solution of metabolites of the fungal strain *T. longibrachiatum*, used in the treatment of sunflower seeds before sowing, can contribute to seed germination and stimulate the growth of the crop.

DISCUSSION

According to the present study *T. longibrachiatum* CNMN FD 27 provides essential activity against *A. alternata* and *F. oxysporum*. Research performed by Díaz-García *et al*. (2024) [17] had shown that this fact happens due to synthesis of phenolic compounds with antifungal activity which can cause damage to these phytopathogenic fungi. Their chromatographic analysis of *T. longibrachiatum* revealed the presence of some phenolic compounds, which have not been previously identified in studies on *Trichoderma*. The identified phenolic acids, especially ferulic, chlorogenic, and p-coumaric acid, significantly reduced mycelial growth and spore germination of *A. alternata* by 33.9% and *F. oxysporum* by 24.8% [17]. The highest results for the strain *T. longibrachiatum* CNMN FD 27 against *A. alternata* were obtained with M 1 medium (Ø 36.0 mm – 40.0%), and against *F. oxysporum* with M 3 medium (Ø 35.0 mm – 38.8%). The strain shows individual cases of higher activity due to use of certain nutrient media.

Another significant result was registered against such phytopathogen as *B. cinerea*, inhibition zones were more than Ø 40.0 mm – 44.4%. Investigations provided by Du *et al.* (2020) showed that *T. longibrachiatum* is capable to synthesize terpenes. According to the results, novel norsesquiterpene can inhibit growth of mentioned phytopathogen ranging from 8 to 64 µg/mL [18]. While Hajji-Hedfi *et al.* (2023) discovered that culture filtrate from *T. longibrachiatum* is able to suppress growth of *B. cinerea* by 79.63% (almost 2 times higher than obtained data), which encourages to continue experiments with identification of suitable nutrient medium composition with best result [21].

In report to cultivation conditions, antifungal activity of *T. longibrachiatum* against *S. sclerotiorum* ranged between Ø 30.0-40.0 mm (33.3-44.4%). According to literature analysis, chitinases and glucanases are enzymes responsible for the cell-wall degradation of this phytopathogen [45]. Results obtained by Matroudi *et al.* (2009) had shown that growth inhibition zones against *S. sclerotiorum* by applying chitinases and β-1,3-glucanases complied almost 70.0% [40]. Results pay attention to the necessity of synthesising certain substances capable of suppressing targeted phytopathogen growth.

Against *F. solani*, *F. sporotrichiella* and *C. michiganensis*, *T. longibrachiatum* CNMN FD 27 strain was less active (zones did not exceed Ø 30.0 mm – less than 33.3%). By literature analysis, two strains of *T. longibrachiatum* (TL6 and TL13) had shown good results against *F. solani* 54.0-59.0% [9]. Modrzewska *et al.* (2024) had reported that activity of *T. longibrachiatum* against *F. sporotrichiella* ranged between 25.0-49.0% [41]. The activity of *C. michiganensis* could be suppressed by using huge concentration of ethyl acetate extract (up to 80.0%) from *Trichoderma* spp. [35]. These literature data confirm that *T. longibrachiatum* is less active against the pathogens mentioned above.

By growth-promoting activity were not found data with similar effect of *T. longibrachiatum* on sunflower seeds. Still, such research was provided on wheat with positive results, and showed a clear perspective in this direction of agriculture [69]. Also detailed results obtained by Boakye *et al.* (2022) suggest that the pretreatment of snow pea seeds with *T. longibrachiatum* TL13 increases snow pea seedling growth and activates plant defense mechanisms [9].

The input of biological preparations based on *Trichoderma* spp. for agriculture is enormous. They are an indisputable alternative to chemicals in pathogen control and plant growth-promoting, as a nematicide and insecticide. The results presented in this study demonstrated that the *T. longibrachiatum* CNMN FD 27 strain possess a high antifungal potential against a wide spectrum of phytopathogens, it can also stimulate seed germination, growth and development of crops so that it could be used in agriculture as a control biological agent.

At the same time, in further studies to obtain positive results, especially in antimicrobial activity, attention should be paid to such areas as: the composition of the culture medium; the synthesis and isolation of certain substances with inhibitory effects; as well as the study of other antimicrobial strains in addition to *Trichoderma* spp. that can suppress better the growth and development of certain phytopathogenic strains, about which the results of these studies were more modest.

Considering the above, the findings of this study allow the broadening of the spectrum of interest towards the strains of the genus *Trichoderma*, capable of producing substances of interest that can be used as phytosanitary products.

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