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Fungal suspension in combination with compost leads to changes in soil microbiota for fertility improvement

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Abstract

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Soil formation processes occurring in the environment are greatly dependent on the activities of microorganisms that inhabit it – bacteria, actinomycetes and microscopic fungi. Having in mind the well known biocontrol potential of *Trichoderma viride* against a wide range of soil phytopathogens, the study presents the effect of addition of a microbiological preparation – *Trichoderma viride* suspension to compost (residue from methane production) as a bioorganic improver. It was applied to alluvial meadow and cinnamon forest poor soils to evaluate how the quantity of ammonifying and cellulose degrading microorganisms, actinomycetes, bacteria, utilizing mineral nitrogen and microscopic fungi changes. The number of ammonifying microorganisms, actinomycetes and bacteria, utilizing mineral nitrogen and cellulose degrading microorganisms in the alluvial meadow soil with the addition of compost and fungal suspension increased greatly and to a lower extent for the cinnamon forest soil, but the amount of microscopic fungi decrease for both types of soil. Soil fertility improvement was followed, when tomatoes (*Solanum lycopersicum* L.) were grown in pure soil, soil, supplemented with compost and soil with compost and *Trichoderma viride* suspension, where the last treatment proved enhanced growth, estimated by the stem and leaves size and surface.

Keywords: Trichoderma viride suspension; compost; soil fertility improvement

Introduction

The future of clean food production and environmental safety are issues of utmost importance, concerning human health. Unnecessary use of pesticides and synthetic fertilizers expose living organisms to the toxic chemical compounds. However, treatments are necessary, as there are many species of fungi that cause diseases of crops and plants known worldwide (Jain et al., 2019). Furthermore, pesticides could have a detrimental effect on some beneficial organisms, soil microbiomes, and on the general life conditions in an ecosystem (Alizadeh et al., 2020). The continuous application of these chemicals for plant disease management have in some cases created pathogen resistance, so the not pathogenic microbes appeared as a promising tool in this fight. Introduction of biotechnologies in plant cultivation, that are based on microorganisms that favor growth and development, avoiding soil pathogens becomes a subsequent element of sustainable agricultural practice (Lanzuise et al., 2022). In search of environmentally friendly alternatives to agrochemicals, Hozzein et al., 2019 evaluated the potential of naturally occurring actinomycetes for better soil properties, plant growth and photosynthesis improvement. A good solution to overcome these

problems is application of biological control by application of *Trichoderma spp.* in agricultural production (Zin & Badaluddin, 2020). It was reported by Contreras-Cornejo et al., 2015 that secondary metabolites secreted by *Trichoderma spp.* have proven its role in suppressing growth of pathogenic microorganisms and stimulating plant growth.

Another issue, such as improper management of agricultural waste also pollutes the environment. Sustainable development is a political priority of many countries. In the field of agriculture, this implies a reasonable and sparing use of natural resources. Composting is a good way to recycle organic waste and an effective method of maintaining and increasing soil fertility (Ho et al., 2022). Compost contains many essential nutrients, improves the physical and chemical properties of the soil and accelerates plant growth, together with reducing waste and carbon emissions.

The aim of the present study was to evaluate the effect of adding compost and compost, together with suspension of *Trichoderma viride* on the distribution and quantity of soil microbiota and estimate the fertility improvement after such treatments.

Materials and Methods

Effectiveness monitoring after addition of soil improvers – compost (sample 2) and compost and fungal suspension (sample 3) was carried out on two types of soils: 1- leached cinnamon forest soil, Sofia region, Bulgaria and 2- alluvial meadow soil, Plovdiv region, Bulgaria. Sample 1 represents the first and second type of pure soil.

For preparation of the fungal suspension *Trichoderma viride* strain was taken from the collection of fungal species of the "Stephan Angeloff" Institute of microbiology – Bulgarian Academy of Sciences.

For conidial germination and mycelium formation beer agar was used (Fig. 1).

Inoculum preparation was carried out in the following medium: Glucose – 3%, Corn extract – 4%, KH_2PO_4 – 1%, Peptone – 1%, $MgSO_4.7H_2O$ – 0,05%, pH = 4,8–5,0. Flasks (V = 100 ml) using single colonies from agar plates. It was left on a rotary shaker at 120 rpm for 36 h and 28°C. Then 30 ml were transferred in 300 ml medium, containing: Glucose – 6%, Corn extract – 4%, pH = 4,8–5,0 and again put on a rotary shaker at 120 rpm for 120 h and 28°C to reach the necessary volume for inoculation.

Inoculation with 10 ml of *Trichoderma viride* suspension from the culture obtained to 100 g of soil was performed.

The compost used was taken as a residue and represents a waste product after methane (renewable energy carrier) production.

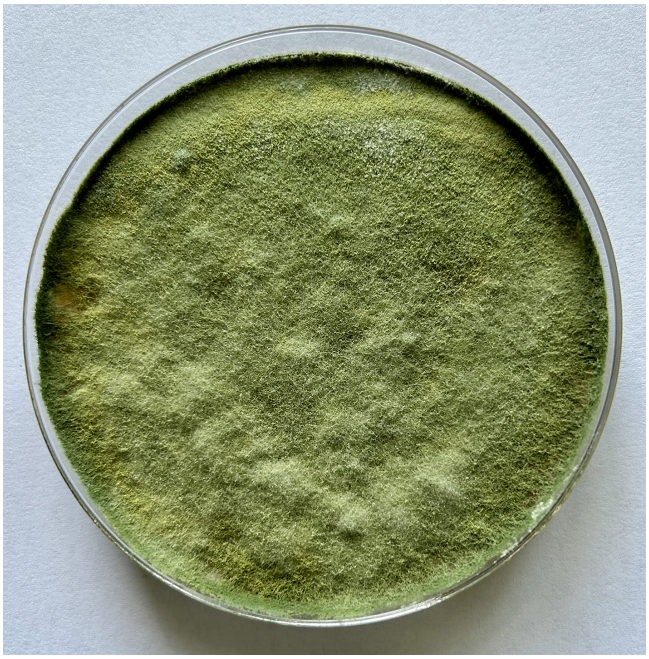

Fig. 1. ***Trichoderma viride***

Development of soil microorganisms was assessed in all treatments. Colony forming units CFU 10^6 were determined for 1g of soil using selective media, accordingly (Goushterov et al., 1977; Alef & Nannipieri, 1998).

Tomatoes (*Solanum lycopersicum* L.) were grown in pure soil, soil, supplemented with compost and soil with compost and *Trichoderma viride* suspension for testing soil fertility improvement. Was followed, estimated by the stem and leaves size and surface.

Results and Discussion

Microbiological analysis of soil samples taken from the pots under the tomatoes grown in which soil improvers had been added were carried out. Application of compost leds to increase in the populations of different groups of microorganisms. It appears as a factor that has a direct impact on entire soil microbiome. This phenomenon had already been reported (Choudhury et. al., 2019). The total number of viable bacteria showed definite changes between the treatments applied, most pronounced for the alluvial meadow soil (Fig. 2). All types of tested microorganisms increased compared to the control (without incorporation of additives). The most significant increase was found in the treatment with compost, together with the *Trichoderma viride* suspension.

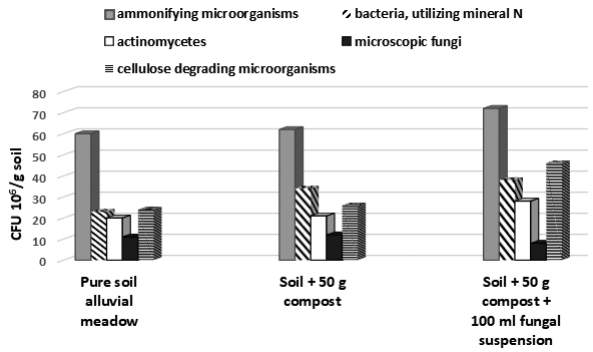

Fig. 2. Changes in the microbial content with the addition of soil improvers to alluvial meadow soil

The results revealed the content of soil fungi decreased and was significantly lower than in the control variant for both types of soils. It had already been reported that rhizosphere-competent fungi of the genus *Trichoderma,* are widely used as biofertilizers and biopesticides in different formulates because of the multiple beneficial effects they have on plant growth and disease resistance (Tyskiewicz et al., 2022). Organic products, such as *Trichoderma* products were reported to help optimizing soil health by reduction of the total content of fungal colonies (Georgieva & Karadzhova, 2020). A little less noticeable were the changes in the microbial content with soil improvers introduced to the cinnamon forest soil (Fig. 3).

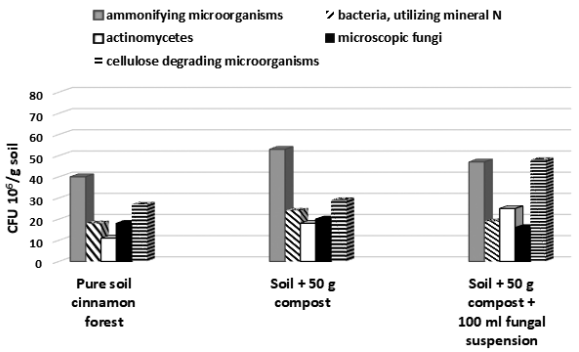

Fig. 3. Changes in the microbial content with the addition of soil improvers to cinnamon forest soil

However, for the two types of soils with improvers addition accordingly, the growth of *Actinomycetes* became abundant. It is not unexpected, as *Actinobacteria* were shown as biological control agents and a source of pathogenic fungi destroyers over the last two decades. In general, organic fertilization had a stronger stimulating effect on the development of actinomycete's populations than mineral fertilization. They play major roles in cycling of organic matter, inhibit the growth of some plant pathogens in the rhizosphere and decompose complex mixtures of polymers of plant, animal and fungal material by production of many extracellular enzymes (Bhatti et al., 2017). In some studies, plant growth promoting activities of actinomycetes on tomatos had been demonstrated (Goudjal et al., 2013). In addition, actinomycetes promote plant growth and development by the synthesis of phytohormones, atmospheric nitrogen fixation, and mineral solubilization (Mitra et al., 2022).

The introduction of *Trichoderma* suspension to low mineral background soils created favorable conditions for the development of microorganisms of all major groups to guarantee the functional stability in the relationships between the main ecological and trophic groups of microorganisms (Rudakov, 2006). The three types of soils – without treatments, with compost only and with compost and fungal suspension were used to test the degree of growth of tomatoes (*Solanum lycopersicum L.*). Growth was visualized 25 days after seeding in pure alluvial soil (Fig. 4 A) and another two weeks after transferring the plants in the pure soil, soil with compost and soil with compost and *Trichoderma viride* suspension (Fig. 4 B).

Evident difference was registered for the plants after two weeks of growth, in favor of the growth of *Solanum lycopersicum* L. in alluvial soil supplemented with compost and with compost and *Trichoderma viride* suspension. The positive effect was registered after measurements of stem height

Fig. 4. Growth of *Solanum lycopersicum* L. in pure soil – beginning -A, alluvial soil, soil with addition of compost and with compost and *Trichoderma viride* suspension – B

Table 1. Plants dimentions after two weeks of growth

Samples	Stem height, cm^2	Leaf area, cm^2
Sample 1 – control – pure soil	18.8	28
Sample 2 – soil and compost added	21.2	30
Sample 3 – soil, compost and *Trichoderma viride* suspension	23.6	34

and leaf surface after two weeks (Table 1) as another proof for the effect of this treatment.

Conclusions

The present research showed the possibility of developing a new biological formulation based on the combination of compost obtained as a residue from methane production, which improves soil properties and a microbial inoculum containing *Trichoderma viride,* which lead to enhancement in the abundance of microorganisms of almost all major groups while the number of microscopic fungi decreased. This may serve as a promising solution to overcome pathogenic microbes' action by application of biological control and together with the cost-effective solution by utilizing waste to obtain healthy plants with good yield for pure food and sustainable agriculture.

Acknowledgements

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