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EFFECT OF THE NEW BIOLOGICAL PRODUCT CREATED ON THE BASIS OF TRICHODERMA ON SOIL MICROORGANISMS AND PLANTS OF DIFFERENT TAXONS

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Abstract

The environmental assessment of a new biocomposite preparation based on Trichoderma sp. L-6, L-3, and D-11 strains for the biodegradation of crop residues was carried out under the conditions of model experiments. The effect of biocomposite (titer not less than 10^9 cells/mL) on the microbial complex of turf soil was evaluated when applied in quantities corresponding to the recommended (2 L/ha), reduced (1 L/ha) and increased (4 L/ha) hectare doses. After 45 days the number of the main ecological-trophic and taxonomic groups of microorganisms involved in the nitrogen and carbon transformation cycles was taken into account. The calculation of ecological coefficients of mineralization and pedotrophy, which characterize the direction of microbiological processes, did not reveal significant changes in the structure of the soil microbial community in comparison with intact soil. Triticum aestivum, Sinapis alba, and Trifolium pannonicum were used as test cultures to study the phytotoxicity of Trichoderma spp. The initial preparative form of all the studied strains suppressed the vital activity of plants. When diluted with 1:10 and 1:100, phytotoxicity decreased, and the growth-stimulating properties of the strains were manifested. The highest level of phytotoxic effects was found in the cruciferous culture, growth-stimulating effect – in the legume culture. Based on the results obtained, recommendations are made for the practical use of a biocomposite based on Trichoderma fungi.

Keywords: Trichoderma, biological product, soil microorganisms, phytotoxicity.

Microscopic soil fungi Trichoderma spp. are one of the most widely used bioagents in modern agriculture. The popularity of these fungi is due to their ability to produce several hundred secondary metabolites, some of which are antimicrobial; induce local and systemic resistance of plants to damage by pests and pathogens [1]; increase the efficiency of nutrient use (especially nitrogen), stimulate plant growth and impart resistance to abiotic stresses [2]. However, in greenhouses, Trichoderma fungi are widely and successfully used instead of chemical fungicides [4]. The synergy of peptaibols with cell wall degradation enzymes inhibits the growth of mycopathogens and increases plant resistance to pathogens [2].

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Toxins from Trichoderma fungi can cause soil toxicosis [5]. Viridin, viridiol, gliotoxin, coningin A and B, trichoviridin and others are examples of phytotoxic substances produced by Trichoderma spp. [6]. The phytotoxicity of viridiol has been studied to a greater extent than the toxicity of other secondary metabolites synthesized by Trichoderma species. Viridiol is adsorbed in the soil and exhibits maximum toxicity towards annual plants. Typical symptoms are low seed germination and insufficient development of the root system. Strains that produce toxins are used as herbicides [7]. Variability in the levels of antimicrobial compounds and herbicides produced by fungi affects the ability of Trichoderma species to be used for the biocontrol of phytopathogens.

Fungi of the genus Trichoderma are active cellulolytics. In agriculture, Trichoderma cellulases were first used in the production of feed. Technologies for microbiological bioconversion of waste from agriculture, food and grain processing industries into high-quality carbohydrate and protein feed additives and compound feeds have been developed [2]. Experimental batches of composts based on waste using micromycetes of the genus Trichoderma, isolated on the territory of the Republic of Tatarstan, were obtained [7]. Recently, there has been a trend towards new use of Trichoderma spp. – as a basis for biopreparations for more efficient transformation of lignocellulose-containing plant polymers into humus components. When the fungus is introduced into the soil, root, stubble and other post-harvest residues contribute to its improvement, as they are an excellent trophic substrate for the growth of Trichoderma [8]. In particular, a new biocomposite preparation is being developed (Microbiotics LLC, Republic of Belarus) based on Trichoderma strains L-6, L-3 and D-1, which, along with a biocontrol and growth-stimulating effect, has enzymatic activity and may, when applied to the soil over stubble or other post-harvest residues, be a means of increasing soil fertility. The preparative form of the biocomposite is a suspension concentrate - a colloidal solution in the form of a liquid creamy mass of an auxiliary substance and biomass of separately grown strains, combined in an equal volume ratio immediately before use.

Despite some studies stating the positive effects of Trichoderma spp. In relation to the soil microbial complex and the productivity of individual agricultural crops [9], it must be taken into account that each strain has a unique spectrum of secondary metabolites with multidirectional effects. The negative effects of Trichoderma metabolites on soil microbial systems and plant growth may limit the use of fungi as biotechnological agents. To exclude possible undesirable consequences of using commercial strains of Trichoderma spp. An appropriate environmental assessment is required for plants and soil.

The purpose of the work was to study the effect of a new biocomposite of fungi of the genus Trichoderma on the soil microbial complex and the growth of seedlings of crops of different taxonomic affiliations.

The effect of a new biocomposite preparation based on Trichoderma fungi (titer of at least 1 billion cells/ml) was studied in a model experiment. 425 g of air-dry (air-dry) turf soil were placed in containers with a volume of 1.0 dm3 and moistened to 80% of the total soil moisture capacity. The

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biological product, depending on the option, was added to the soil at the rate of 1.5 x 10^5; 0.75 • 10^5 and 3.0 • 10^5 cells/g, which corresponded to the recommended (2 l/ha), reduced (1 l/ha) and increased (4 l/ha) hectare doses, taking into account the volumetric weight of chvy (1.3 g/cm^3). Intact soil served as a control. Each option was performed in three repetitions. Containers with soil were incubated for 45 days at 20 ± 2 °C.

To characterize the ecological state of the soil, the abundance of various ecological-trophic groups of microorganisms (MO) was determined. Assimilating organic forms of nitrogen (ammonifying) MOs were taken into account on meat-peptone agar (MPA), assimilating mineral sources of nitrogen (amylolytics) - on starch-ammonia agar (CAA), cellulolytics - on Hutchinson agar (HA) with filter paper, oligotrophic – on soil agar (SA), microscopic fungi – on Czapek agar (CA) [10]. To characterize changes in the structure of the soil microbial community, the coefficients of mineralization (CAA/MPA) and pedotrophy (PA/MPA) were calculated [11]. The micromorphology of fungi was studied on living specimens at a magnification of ×200 and ×400, using a Leica DM 2500 microscope (Germany).

Phytotoxic effects were assessed by testing strains of Trichoderma sp. L-6, L-3 and D-11 separately. Spring wheat (Triticum aestivum L.) Priokskaya, Pannonian clover (Trifolium pannonicum L.) Snezhok, and white mustard (Sinapis alba L.) were used as test crops. The seeds were soaked for 20 hours in dilutions of the drug with water 1:10, 1:100 and without dilution. Seeds soaked in distilled water served as a control. The treated seeds were germinated for 6 days at room temperature (20±2 °C). Germination, shoot height, root length and dry biomass of seedlings were taken into account.

Statistical processing of the results was carried out using standard methods using the built-in Excel software package.

Determining the number of microorganisms using the method of sowing from dilutions onto solid nutrient media showed that intact turf soil, in accordance with the approximate scale of soil enrichment with microflora [11], can be classified as “very rich” in terms of ammonifying agents (14 million CFU/ g per MPA), amylolytic (28 million CFU/g per CAA) and oligotrophic (72 million CFU/g per PA) MO (Table 1).

Table 1 The number of microorganisms of different ecological-trophic and taxonomic groups depending on the dose of the drug applied to the soil

Dose, L/ha	Total number on the ..., thousand CFU/g air dry soil				Trichoderma, % / Percentage of overgrowth of HA by fungus, %
	MPA	SAA	SA	ChA	
0	13939	28044	71770	130	56,7±20,2
1	9944	31876	94320	259	68,3±7,6
2	21795	35782	130410	87	58,3±17,6
4	14027	12013	43540	83	65,0±13,2
HCP_{095}	7742	5012	63440	148	–

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Table 2 Coefficients characterizing the direction of soil microbiological processes

Dose, L/ha	Coefficients of	
	mineralizationKAA / MΠA SAA / MPA	pedotrophyΠA / MΠA SA / MPA
0	2,01	5,15
1	3,21	9,49
2	1,64	5,98
4	0,87	3,10

As a result of introducing different doses of biocomposite into the soil, the number of these MO groups did not change significantly. At the same time, the number of fungal propagules counted in NA and GA did not exceed hundreds of thousands of CFU/g, which corresponds to the gradation of "very poor" soil. By direct microscopy of colonies on FA, it was established that the proportion of colonies formed by micromycetes of the genus Trichoderma in the soil of the control variant was 5%, while in variants with the addition of a biological product, the proportion of Trichoderma in the fungal complex varied from 22 to 38% depending on the dose.

On GA, Trichoderma also dominated among cellulolytics, but the proportion of its coverage of the cellulose disk changed insignificantly among the variants (Table 1).

Additional information about the ecological state of the soil microbial complex was provided by the calculation of environmental coefficients (Table 2). Values of the mineralization coefficient (MC) close to unity are considered to be an indicator of the balance of decomposition processes of nitrogen-free and nitrogen-containing organic matter (OM) in the soil; values exceeding one indicate an increased intensity of OM mineralization processes and the provision of soil with mineral forms of nitrogen; KM values of 2–3 may indirectly indicate an increase in the rate of humus decomposition. The introduction of a biological preparation into the soil had a regulatory effect on the processes of mineralization of soil OM, as indicated by the CM value changing according to the variants (Table 2).

Treatment of the soil with a biological product at a dose of 2 l/ha (recommended) led to a slight decrease in CM from 2.0 in the control to 1.6 in the experimental variant. When the recommended dose was doubled (4 l/ha), the initial value of CM decreased to 0.87, which may indicate inhibition of mineralization processes compared to the control and the variant with a reduced dose of the drug (1 l/ha), in which CM, on the contrary, it increased to 3.2. It was previously noted that the addition of T. asperellum to the soil had a positive effect on the mineralization of organic substrates and changed fungal populations [9].

Depending on the dose of the drug, the values of the pedotrophy index, which reflects the degree of representation in the soil of microflora associated with the formation of humus, also changed differently compared to the control. When applying a reduced dose (1 l/ha), it increased (to 9.4), and with an increased dose (4 l/ha), on the contrary, it decreased to 3.1. It is believed that soils with high values of this index are closer to the natural cenoses of the studied soil-climatic zone and are more resistant to negative impacts [13]. The effects of the biological product noted in the model

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experiment, expressed in changes in the ratio of individual groups of microorganisms and the direction of transformation processes of soil organic matter, can, to a certain extent, be extrapolated to real soil conditions. Therefore, the field dose of the drug should be adjusted depending on the degree of enrichment of MO and the intensity of the processes of transformation and humification of OM in a particular soil.

One of the important indicators of the environmental safety of a biological product is its interaction with a cultivated plant, that is, the degree of phytotoxicity of the introduced plant. The initial preparative form of the studied strains suppressed the germination of seeds by 76–89% and the linear growth of seedlings of all test cultures by 38–62%.

Thus, the phytotoxicity and effect on the soil microbial complex of a new biocomposite based on Trichoderma spp. was studied in laboratory experiments. The results show that the reaction of soil MOs may depend on the amount of introduced introduced species, and also be determined by the initial state of the resident microbial community. The concentrate suspension of the studied strains had a phytotoxic effect on plants, the degree of manifestation of which depended on the taxonomic affiliation of the test culture. With dilution, phytotoxicity decreased, and the growth-stimulating properties of Trichoderma spp. The results obtained indicate sufficient environmental safety of the biocomposite preparation, provided that the recommended doses are observed and such soil characteristics are taken into account as the reaction of the soil solution and the degree of enrichment of MOs involved in the processes of transformation of OM.

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