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The role of *Trichoderma* and *Gliocladium* fungi in the soil biocenosis of greenhouse cucumbers

Значението на гъбите *Trichoderma* и *Gliocladium* в почвената биоценоза на оранжерийни краставици

Nataliya KARADZHOVA, Olga GEORGIEVA (✉)

Maritsa Vegetable Crops Research Institute (MVCRI), 32 Brezovsko shosse, 4003 Plovdiv, Bulgaria

✉ Corresponding author: olgaizk@abv.bg

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ABSTRACT

The analysis was conducted on the general biogenicity and phylogenetic structure of fungi in the soil microbiome of greenhouse cucumbers grown as a monoculture and after the precursors of pepper and lettuce. The relationship between the following indicators has been proved: the total biological activity of the soil, the species composition of micromycetes, the development of *Fusarium* root rot, and yield. The effect of biological preparations based on strains of *Trichoderma viride* and *Gliocladium virens* antagonistic fungi on the development of *Fusarium* root rot, nematodes and yield of cucumbers was studied. The results of the experiment show that when cucumbers are grown after the precursor pepper and lettuce, the development of *Fusarium* root rot and nematodes does not exceed critical values. The introduction of *Trichoderma* and *Gliocladium* fungi into the soil in the form of a dry preparation with a titre 2.0 x 10^{10} conidia/g when transplanting plants to a permanent place at a consumption rate of 40 kg/ha increases the yield of greenhouse cucumbers by 20-23%. The experiment is part of the study of the "soil exhaustion" syndrome and the possibilities of overcoming it. The obtained results will serve as bioindicators that can be used for preliminary diagnostics of the sanitary condition of degraded soils, selection of agrotechnical, breeding and protective measures of plants.

Keywords: rhizosphere, micromycete complex, species, frequency of occurrence, biological products, suppressive effect

АБСТРАКТ

Извършен е анализ на общата биогенност и филогенетична структура на гъбите в почвения микробиом на оранжерийни краставици, отглеждани като монокултура и след предшествениците пипер и маруля. Доказана е връзката между следните показатели: обща биологична активност на почвата, видов състав на микромицетите, развитие на фузариозно кореново гниене и добив. Изследвано е влиянието на биологични препарати на базата на щамове антагонистични гъби *Trichoderma viride* и *Gliocladium virens* върху развитието на фузариозно кореново гниене, нематоди и добива при краставици. Резултатите от опита показват, че при отглеждане на краставици след предшественици пипер и маруля развитието на фузариозно кореново гниене и нематоди не надвишава критичните стойности. Внасянето на гъбите *Trichoderma* и *Gliocladium* в почвата под формата на сух препарат с титър 2.0 x 10^{10} c/g при разсаждане на растенията на постоянно място при разходна норма 40 kg/ha повишава добива при оранжерийни краставици с 20-23%. Експериментът е част от изследването на синдрома на "уморената почва" и възможностите за неговото преодоляване. Получените резултати ще послужат като биоиндикатори, които могат да се използват за предварителна диагностика на санитарното състояние на деградирали почви и избор на агротехнически, селекционни и растително-защитни мерки при отглеждане на растения.

Ключеви думи: ризосфера, микромицетен комплекс, видове, честота на поява, биологични продукти, супресивен ефект

INTRODUCTION

In recent decades, soil degradation processes, including in greenhouses, have increased worldwide, leading to the loss of their biocenotic and biosphere functions, as well as to a significant decrease in biodiversity (Murtazina et al., 2020; Paulitz and Belanger, 2001; Santhanam et al., 2019; Svistova, 2004). Intensive cultivation of vegetables in greenhouse conditions as monocultures, the use of genetic homogeneous varieties and hybrids contribute to changes in the soil microbiome (Grosch et al., 2007). When the process of self -regulation of species in the soil and crops is disturbed, the aggressive strains of pathogens outweigh the development and reproduction of useful strains and species (Satton et al., 2001). This leads to the development of dangerous diseases on plants and the loss of planned yield. The dynamics of development and the number of individual types of microbes in greenhouse are determined by both abiotic and anthropogenic factors: tillage, number, and forms of plant residues of the precursor culture, as well as root exudates of vegetative species (Adams, 1990; Kostyuchenko and Lyakh, 2017; Rose et al, 2003). The influence of these factors can lead to changes in the dominant species, the parameters of the structure of soil biocenosis and the formation of specific functional rows of micromycetes, including toxigenic or pathogenic for cultivated plants (Estifanos et al., 2018; Chernov et al., 2017).

Fusarium root rot of cucumbers are one of the major diseases in greenhouses. Cultivation of cucumbers as monoculture is a prerequisite for the accumulation of pathogens. The lack of drainage systems and a worsening temperature regime in greenhouses contribute to their mass development. The effectiveness of plant protection measures depends on the choice of appropriate chemical, physical, genetic and agrotechnical methods (Habig and Swanepoel, 2015; Papavizas, 1985; Kovrigo et al., 2016). Suppression or complete elimination of *Fusarium oxysporum* using natural mechanisms to maintain the balance in the soil biota may be a main principle in the development of technology for biological control of this pathogen (Bonfante and Genre, 2010; Contreras-Cornejo et al., 2009; Katoh et al., 2019; Li et al., 2015; Metwally et al., 2020). Best known in this respect are fungi of the *Trichoderma* and *Gliocladium* genus. Microbiological products based on antagonistic strains of the *Trichoderma* and *Gliocladium* genus are the most biologically active substances applied to major soil-borne pathogens (Leonetti et al., 2017; Whipps, 2001; Woo et al., 2014).

Trichoderma species are versatile filamentous ascomycetes which are found in nearly all environments. They live in soil, grow on wood as saprophytes, or feed on fungi, plants, animals and insects as parasites (McGonigle,1990; Mukherjee et al., 2014; Sternshis, 2022; Stoma et al., 2020). The importance of saprophytic species of the *Trichoderma* spp. and *Gliocladium* spp. in the microbiome of plant is large. It is believed that these fungi are responsible for the effect of biological control of pathogens in suppressive soils (Groten et al., 2015; Vargas et al., 2014). They affect the structural composition of the soil microbiome, suppressing the development of pathogenic species. In this aspect, the determination of species diversity (the wealth of the number of species and the uniformity of their distribution) or groups of microorganisms can be called a measure of stability of the tested soil ecosystem (Ventorino et al., 2016; Ventorino et al., 2018). The high sensitivity of the biotic complex allows the early diagnosis of any negative and positive changes in soil ecosystems and uses biological parameters as criteria for bio monitoring of their condition. The microbial community is the most sensitive bio indicator for the soil conditions that can give an integral assessment of the condition of the soil cover and the ecosystem (Zhang et al., 2020; Zhatova, 2018).

The purpose of the study was to determine the influence of antagonistic fungi *Trichoderma viride* and *Gliocladium virens* on the structural composition of soil microorganisms in the mycocenose of greenhouse cucumbers grown as a monoculture and after pepper and lettuce; the use of the indices "microbiological structure" and "biological activity of the soil" as bioindicators of the "soil exhaustion" syndrome and exploring the possibilities to overcome it.

MATERIAL AND METHODS

The experiments were conducted in 2019-2021, on the territory of the Maritsa Vegetable Crops Research Institute in a steel-glass greenhouse with the cucumber variety Palermo F_1. The plants were grown as a seedling culture in a sterile peat-perlite mixture (in 3:1 ratio, enriched with 1.200 kg of triple super phosphate, 0.500 kg ammonium nitrate, 0.500 kg potassium sulfate and 0.200 kg magnesium sulfate per m^3 mixture) and were planted in a permanent place after predecessors' cucumber (monoculture), pepper and lettuce (crop rotation). The plants were grown as soil culture with a planting density of 1.79 plants per m^2 (experimental design (200 + 80/35 cm), three replicates, and ten plants per replicate).

Two biological preparations based on antagonistic strain *Trichoderma viride* No 12, and *Gliocladium virens* No 30, produced by the technology for solid-phase production in the laboratory of the institute, were imported into the soil as dry substance with titer 2.0 x 10^{10} CFU/g at 40 kg/ha during transplanting plants in a permanent place. The titer of bio products (not less than 2.0 x 10^{10} CFU/g) was defined under a microscope in the Fuchs-Rosenthal chamber. As a standard, we used prophylactic treatment of plants with methyl thiophanate in a concentration of 0.1% - after transplanting plants and two weeks after firth application. The amount of the working solution is 500 ml per plant.

The experiments were conducted on a standard mineral background - $N_{16}P_{20}K_{22}$. The following indicators were reported: Total yield (kg/plant), percentage of diseased plants of *Fusarium* root rot and nematode (percent), Total soil biogenicity CFU/g, number of fungal colonies in 1 g of dry soil (percent). Microbiological analysis of soil samples was conducted at the beginning, the middle, and the end of the growing season – after harvest. The collection of soil samples was conducted according to the accepted method: the test for analysis was selected at the site at five points, according to the envelope principle, from a depth of 5-15 cm (Borovikova, 2003). Soil samples were mixed to obtain an integrated soil test. Immediately after sampling, the content and composition of ecological and trophic groups of microorganisms were determined in soil samples. A part of each soil test was dried to an air-dry state and was used to analyze the number of soil microorganisms, as well as to study the structural composition of soil microorganisms. The soil biogenicity was determined by the accepted microbiological methods for cultivating microorganisms – Zvyagintsev's (1991) and the taxonomic affiliation of the fungi – by Pidoplichko (1977).

Statistical methods

Calculations and statistical results processing were performed according to generally accepted methods. Parametric criteria were used for normal distribution, arithmetic mean, and standard deviation for significance level less than 0.05 (Ivanter, 2010).

RESULTS AND DISCUSSION

As a result, the microbiological analysis of soil samples established that biological product increased the total biogenicity of the soil. In the experiment with monoculture, the total number of microorganisms in the soil treated with *Trichoderma* spp. and *Gliocladium* spp., 18-22% is higher than in the control (Table 1).

The total number of microorganisms (fungi, bacteria and actinomycetes) in the soil treated with *Trichoderma* spp. is 3.85 million/g, *Gliocladium* spp. – 3.66 million/g, in non-decontaminated soil – 2.16 million/g, and in the variant with soil application of the fungicide methyl thiophanate - 3.48 million/g dry soil.

In an experiment with crop rotation, the total biogenicity of the soil treated with antagonists is twice as high as in untreated soil – 47-54% (Table 2).

The total number of microorganisms in the soil treated with *Trichoderma* spp. is 6.99 million/g, *Gliocladium* spp. - 6.11 million/g, in non-decontaminated soil - 3.25 million/g, and in the variant with soil application of the fungicide methyl thiophanate - 3.83 million/g dry soil.

Useful information about the dynamics of fungal communities in agricultural value, depending on the predecessor and the introduction of antagonists, supplies an analysis of the species composition of microorganisms (Druzhinina and Kubicek, 2016; Murtazina et al., 2020). In experiment with monoculture the total biogenicity of the soil increases significantly due to the increase in the number of actinomycetes and bacteria (Table 3).

The number of fungal colonies decreases compared to untreated controls. The structural analysis of the fungal colonies in the soil samples shows that among them there are predominate representatives of Fungi Imperfecti - *Fusarium*, *Penicillium*, *Aspergillus*, *Mucor*, *Cladosporium* - saprophytes and necrotrophs, which take part an active part in the process of humus formation. The increase in microorganisms that stimulate plant development is significantly higher than in the control variant: the increase in the number of antagonists in the rhizosphere of plants is six times, in actinomycetes - twice. In the variant with the introduction of *Trichoderma viride*, the number of pathogenic fungi is 2-6 times less than in the control variant.

In variants where plants are grown after the predecessors of peppers and salad, a saprotrophic complex of dominant and subdominant species formed by *Penicillium*, *Fusarium*, *Aspergillus* and *Trichoderma* was distinguished (Table 4).

It was assumed that the strong adaptive ability of *Penicillium* and *Aspergillus* both to abiotic environmental factors, and to nutritious substrates, led to their dominance in the structure of these cenoses. At the same time, the presence of the *Trichoderma* genus among dominant species shows an elevated level of fungistasis in this version. The development of *Trichoderma* spp. is a determining factor limiting the development of phytopathogenic micromycetes on plant residues. Under such conditions, the presence in the microcenosis of phytopathogenic species *Verticillium*, *Botrytis*, *Alternaria* in a slight or rare rank did not determine its level of suppression.

Table 1. Total soil biogenicity in the rhizosphere of greenhouse cucumbers (soil layer 5-15 cm in the active phase of mass flowering-fruiting) in an experiment with monoculture

Variants	Number of microorganisms, million/g dry soil				Percentage to Control
	Total	Fungi	Bacteria	Actinomycetes	
Sample taken before starting the experiment	2.155±0.39	0.055±0.002	2.000±0.68	0.100±0.015	-
Control without Biologics	2.980±0.58	0.880±0.19	1.100±0.35	1.000±0.19	-
Application of *Trichoderma viride*	3.850±0.59	0.540±0.15	1.330±0.33	1.980±0.33	22.60
Application of *Gliocladium virens*	3.660±0.59	0.730±0.17	1.550±0,02	1.380±0.11	18.58
Application of methyl thiophanate	3.478±0.58	0.008±0.005	2.82±0.18	0.650±0.17	14.32

Differences significant at ($P \leq 0.05$) level

Table 2. Influence of biological preparations to the number of microorganisms in the rhizosphere of greenhouse cucumbers in an experiment with crop rotation. Soil layer 5-15 cm in active phase of flowering-fruiting

Variants	Number of microorganisms, million/g dry soil				Percentage to Control
	Total	Fungi	Bacteria	Actinomycetes	
Sample taken before starting the experiment	5.050±0.59	0.030±0.001	4.990±0.11	0.030±0.01	-
Control without Biologics	3.250±0.39	0.200±0.02	0.500±0.017	2.550±0.11	-
Application of *Trichoderma viride*	6.990±0.68	0.230±0.02	1.620±0.17	5.140±0.15	53.51
Application of *Gliocladium virens*	6.110±0.58	0.120±0.01	1.120±0.16	4.870±0.12	46.80
Application of methyl thiophanate	3.830±0.41	0.180±0.02	0.880±0.12	2.770±0.14	15.14

Differences significant at ($P \leq 0.05$) level

Table 3. Influence of the introduction of the fungus *Trichoderma* on the development of some of the main types of microorganisms in the rhizosphere of greenhouse cucumbers (the depth of the soil layer is 5-15 cm, the active phase of mass flowering-fruiting) in experiment with monoculture

Action	Microorganisms	Number of microorganisms, thousands/g	
		Control	Application of *Trichoderma*
Suppression	*Fusarium*	3.00	1.00
	Mucor	1.60	1.30
	Rhizoctonia	2.00	0.00
	Penicillium	4.50	2.00
	Aspergillus	7.90	4.70
	Cladosporium	6.00	3.00
	Alternaria	3.00	0.50
	Verticillium	2.00	0.50
Stimulation	*Trichoderma*	1.00	3.00
	Actinomycetes	1000000	1980000

Table 4. Influence of the introduction of the fungus *Trichoderma* on the development of some of the main types of microorganisms in the rhizosphere of greenhouse cucumbers (the depth of the soil layer is 5-15 cm, the active phase of mass flowering-fruiting) in experiment with crop rotation

Action	Microorganisms	Number of microorganisms, thousands/g	
		Control	Application of *Trichoderma*
Suppression	*Fusarium*	6.00	1.00
	Rhizoctonia	2.00	0.00
	Penicillium	10.00	2.00
	Aspergillus	10.00	4.70
	Cladosporium	6.00	3.00
	Alternaria	10.00	8.00
Stimulation	*Trichoderma*	1.00	6.00
	Actinomycetes	2550000	5140000

A comparative analysis of the experimental data using biological preparations on greenhouse cucumbers grown as a monoculture and after predecessors showed a difference in the degree of infection of the culture with the main diseases - *Fusarium* wilt and nematode (Table 5). In the experiment with growing cucumbers as a monoculture, the development of *Fusarium oxysporum* against the background of severe damage to plants by the nematode (up to 62%), reaches 26% in the control. Epiphytotic growth of the nematode reported in all variants of the experiment. Damage to the roots by the nematode in the experiment with the alternation of crops: pepper, lettuce, cucumber does not exceed 8%. The development of *Fusarium* wilt and root rot in cucumber crops ranges from 3% in the variant with the introduction of methyl thiophanate to 7% in the control variant.

Table 5. Influence of biological products on the sanitary status and productivity of greenhouse cucumbers grown as monoculture, and after predecessors

Variants	Indexes					
	Monoculture		Crop rotation		Yield per plant, kg	
	Mi	*Fus*	*Mi*	*Fus*	Mono-culture	Crop rotation
Control	65.22	26.09	6.60	6.60	1.39	4.65
Methyl thiophanate	62.63	21.05	6.10	3.00	1.56	6.25
Effect, (%)	-	19.32	7.57	54.55	12.23	25.60
Trichoderma spp.	70.70	1.72	7.80	4.70	1.84	6.02
Effect, (%)	0.00	93.41	0.00	28.79	32.37	22.76
Gliocladium spp.	65.00	15.00	4.70	0.00	1.46	5.64
Effect, (%)	0.00	42.31	28.79	100.00	5.03	17.55

The effectiveness of antagonists against the pathogen *Fusarium oxysporum* in slightly contaminated soil is extremely high. When using a biological product based on *Gliocladium virens*, it reaches 100%. Monitoring of the indicator "yield per plant" over the next two years confirms the effectiveness and stability of the biological products on base *Trichoderma viride* and *Gliocladium virens* strains when used in cucumbers grown on the site with "soil exhaustion" (Table 6). The increase in yield in the variants with the introduction of biologics is due solely to the effectiveness of preparations against *Fusarium* wilt. The effect of biological product on yield increase in cucumber is 35% in the variant with the introduction of *Trichoderma viride*, and 47% in the variant with *Gliocladium virens*, respectively. The results of the experiment show that when growing cucumbers as a monoculture, the effect of tired soil manifests itself - the number of infections with nematodes and pathogenic microflora increases. On the background of mass development of nematodes, saprophytic forms of *Fusarium* spp. become pathogenic and cause secondary damage on the root system of plants.

Table 6. Influence of biological products on the development of *Fusarium* root rot and yield in greenhouse cucumbers after the predecessors pepper and lettuce

Variants	Indexes			
	Fusarium root rot, (%)	Effect, (%)	Yield per plant, kg	Effect, (%)
Control	6.60	-	4.65	-
Methyl thiophanate	3.00	54.55	6.25	25.60
Trichoderma	4.70	28.79	6.02	22.76
Gliocladium	0.00	100.00	5.64	17.55

CONCLUSIONS

The results of the experiments conducted give grounds to come to the following conclusions:

- Soil application of biological products based on antagonistic fungi *Trichoderma viride* and *Gliocladium virens* during transplantation of plants in a permanent place gives a stable effect on the development of *Fusarium* root rot and wilt in greenhouse cucumbers. The effect of the introduction of antagonists is 35-47%.
- Microbiological analysis of soil samples showed a significant increase in antagonists in the rhizosphere of plants from the time of application to the flowering phase. This is one of the main factors contributing to the restoration of the soil biome in the "soil exhaustion" crop.
- The results of microbiological analysis of the soil in the most active phase of plant development can serve as an indicator test for soil infectivity in crops.

CONFLICT OF INTEREST

There is no actual or potential conflict of interest in relation to this article.

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